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Plenary lectures

PL-01

Approaches and progress toward bioactive leads from Fungi

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Of the over 250,000 compounds that have been described from Nature, only about 15,000 of these have been from fungi. This is somewhat surprising, since fungi are ubiquitous and found throughout the world, able to thrive in almost any environment. Estimates predict between 1.5 and 5 million species of fungi, and there are likely even more. Yet, barely 100,000 have been studied in any detail. Fungi are prodigious chemists, and research suggests that each fungus can produce unique metabolites. Thus, the question is not "can fungi produce unique metabolites"; rather it is, "how does one sort the most promising fungi from those that produce known chemistries". We probe this question daily, and my research team focuses on examining unique niches for under explored fungi, with the thought that unique biodiversity may reveal unique chemistry. Moreover, we prioritize samples by dereplicating them in situ, using techniques to analyze the chemistry of fungal cultures directly from Petri dishes. The process for sampling the surface of culture is based on an amalgamation of instrumentation, including a droplet-liquid-microjunction-probe coupled with UPLC-HRMS. For recent leads, the dereplication and scale up experiments have afforded some of the more pragmatic results, as they have provided materials for further pharmacological evaluation and medicinal chemistry development, even for bioactive fungal metabolites that were considered scarce.

PL-02

3D feature-based pharmacophore models: efficient tools for profiling natural products

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Pharmacophore-based virtual screening and activity profiling has become one of the most popular in silico techniques for supporting medicinal chemists in their hit finding, hit expansion, hit to lead, and lead optimization programs. [1]

At Inte:Ligand GmbH, we developed the program LigandScout [2] as an integrated software solution containing rapid and efficient tools for automatic interpretation of ligand-protein interactions and subsequent transformation of this information into 3D chemical feature-based pharmacophore models. Additionally, pattern recognition-based algorithms were developed for ligand-based pharmacophore modelling in the absence of a target 3D structure, as well as for establishing novel accurate virtual screening procedures. As an extension of this approach, parallel pharmacophore-based screening has also been introduced as an innovative in silico method to predict the potential biological activities of compounds by screening them with a

multitude of pharmacophore models, and made available as a LigandScout extension workflow node within the KNIME platform. [3]

In the presentation, Prof. Langer will give an overview of the pharmacophore technology developed over the last decade and will then present the results of several success stories: Examples range from proof of concept studies employing a set of antiviral compounds that were submitted to in silico activity profiling using a subset of the Inte:Ligand Pharmacophore Database (4) to in silico fragment-based discovery of novel enzyme inhibitors. Additionally, several medicinal chemistry application examples yielding clinical candidates will be highlighted.

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[2] Wolber, G., Langer, T. ; LigandScout: 3D Pharmacophores Derived from Protein-Bound Ligands and their Use as Virtual Screening Filters, J. Chem. Inf. Model. 2005, 45, 160-169.

[3] KoNstanz Information MinEr, available from KNIME.COM AG, Zurich, Switzerland (http://knime.org)

[4] The Inte:Ligand Pharmacophore Database is available from Inte:Ligand GmbH, Vienna, Austria (http://www.inteligand.com)

PL-03

African perspectives on natural product research based on ethnobotanical and ethnopharmacological knowledge

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Africa is a hotspot of botanical and cultural diversity – at least 5400 species are used in traditional medicine but much work remains to record the fragile oral-traditional knowledge about plants and their uses. Commercial phytomedicines in world markets are dominated by plants of European and Asian origin. Africa is a rich potential source of new crops and new products and progress towards a larger share of the international phytopharmaceutical and nutraceutical markets seems inevitable.

Examples of commercialised African products, each with its own unique ethnobotanical and ethnopharmacological history, include *Adansonia digitata, Agathosma betulina, Aloe ferox, Aspalathus linearis, Boswellia papyrifera, Bulbine frutescens, Catha edulis, Catharanthus roseus, Coffea arabica, Commiphora myrrha, Cyclopia genistoides, Griffonia simplicifolia, Harpagophytum procumbens, Harungana madagascariensis, Hibiscus sabdariffa, Hoodia gordonii, Hypoxis hemerocallidea, Kigelia africana, Lessertia frutescens, Mondia whitei, Pausinystalia johimbe, Pelargonium sidoides, Physostigma venenosum, Prunus africana, Sceletium tortuosum, Synsepalum dulcificum, Warburgia salutaris* and*Xysmalobium undulatum*.

Africa is sometimes viewed as a rich source of research materials for Western scientists and that, despite seemingly active collaboration, the transfer of skills and knowledge has been

limited. Perceptions of exploitation have resulted in international treaties such as the 1992 Convention on Biological Diversity and the 2014 Nagoya Protocol on Access and Benefit-sharing. The development of local intellectual and technical capacity is an urgent priority. There is a need to reach critical mass and to add value to raw materials if Africa is to maximise the opportunities presented by her rich cultural and botanical wealth.

PL-04

The Cannabis plant and the endocannabinoids: how an ancient medical plant helps uncovering of a major signaling system in our body

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Cannabis is one of the first plants used for medical purposes and for its euphoric effects in human history. Its beneficial effects has been demonstrated in numerous diseases, but its chronic use has also several adverse effects. Intense research during the last decades identified the endocannabinoid system, a chemical messenger system in our body, which underlie most consequences of cannabis use and abuse. In the present lecture, I will summarize the molecular and physiological logic of endocannabinoid signaling as well as will provide examples for its pathophysiological significance. In addition, I will also highlight recent efforts, which aim to exploit the therapeutic potential of cannabis without triggering unwanted side effects.

PL-05

Natural substances and neurodegenerative diseases: from molecular mechanisms to clinical effects

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Symptoms of Alzheimer's disease (AD), the most common form of dementia and neurodegenerative disorders, begin with insidious loss of memory which progresses to involve all aspects of cognition, including confusion and mood swings.

The dilemma we face today is that the five drugs approved for Alzheimer's only partially treat some of the symptoms. None of them can slow or stop the progression of the disease itself. A vast array of published data, however, shows that proper use of nutrients, hormones, and other natural drugs may dramatically reduce one's risk of developing AD. Several natural compounds have ample research behind them demonstrating their ability to take aim at multiple steps in the development of neurodegenerative disorders. Among dietary antioxidants curcumin and *Ginkgo biloba* were extensively studied for their neuroprotective effects in AD. The rationale for this alternative therapeutic approach was based on several pre-clinical studies which suggested the neuroprotective effects especially for *Ginkgo biloba*, curcumin and resveratrol, due to either a free radical scavenging activity or stabilization of mitochondrial functions. Moreover, several lines of evidence show that fish oil supplementation could be one other preventive strategy we have against the disease. Furthermore, a plausible link between neurosteroids and neurodegenerative disorders, like AD, has been discussed. Endogenous

neurosteroids (steroids that are synthesized within the nervous system) might have important roles in cognitive functions and normal aging is associated with several alterations in neurosteroid production and secretion. Particularly the neurosteroid allopregnanolone has recently shown promise in alleviating cognitive and neuronal sequelae of AD.

The aim of this lecture is to summarize the main pharmacologic features of natural substances as well as to underlie the main outcomes reached by clinical studies designed to demonstrate the efficacy of them in patients.

PL-06

Computational approaches to natural products-based lead discovery

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Innovative bioactive agents fuel drug discovery and the development of new medicines. Future success in chemical biology and pharmaceutical research will fundamentally rely on the combination of advanced synthetic and analytical technologies that are embedded in a theoretical framework that provides a rationale for the interplay between chemical structure and biological effect. A driving role in this setting falls on computer-assisted molecular design and engineering, by providing real-time access to a virtually infinite source of novel tool compounds and lead structures, and guiding experimental screening campaigns. We will present concepts and ideas for the representation of molecular structure, predictive models of structure-activity relationships, the de-orphaning of bioactive compounds, and discuss *de novo* design approaches that have proven their usefulness in drug discovery. Emphasis will be put on bioactive natural products as templates for computational lead discovery. We will showcase new methods for natural-product inspired molecular design and macromolecular target prediction.

Selected references:

[1] Miyao T, Reker D, Schneider P, Funatsu K, Schneider G. Chemography of natural product space. Planta Med 2015, in press

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PL-07

Limitations of *in vitro* assessments of the drug interaction potential of botanical supplements

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Botanical medicines are frequently used in combination with therapeutic drugs, imposing a risk for potentially harmful botanical-drug interactions (BDIs). Among the existing BDI evaluation methods, clinical studies are the most desirable, but due to their expense and protracted time-line for completion, conventional *in vitro* methodologies remain the most frequently used BDI assessment tool. However, many predictions generated from *in vitro* studies are inconsistent with clinical findings. Although there are inherent and well-recognized limitations of *in vitro* screening methodologies assessing conventional drug interactions, additional limitations are appreciated which are unique to the evaluation of botanical products. Among the larger issues faced are the uncertainty in assigning hepatic concentrations of multiple constituents and their potential metabolites, accounting for oral bioavailability, distribution, first-pass metabolism and active metabolites. Accordingly, this lecture is intended to discuss the problems and challenges in evaluating BDIs using *in vitro*methodologies.

PL-08

From traditional uses of Mesoamerican medicinal plants to modern phytotherapy in Guatemala

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Mesoamerica is a region of high biological and cultural diversity, where several cultures have flourished. Since 1976, the non-governmental organization Center for Mesoamerican Studies on Appropriate Technology (CEMAT) started a project for detection, production and validation of medicinal plants for primary health care (PHC). Until 1990, its activities consisted of several ethnobotanical surveys conducted among ten Guatemalan ethnical groups. From these surveys, more than 650 plant species used for medicinal purposes were detected. Complementary to this detection, production (collection or cultivation) started of some of the promising species. Initially in cooperation with multidisciplinary teams in USAC, and later with other academic institutions in Brazil, Costa Rica, Italy, Mexico, Panama, Spain and United States, in vitro and in vivo validation activities were performed, particularly about biocidal, anti-inflammatory, spasmolytic, immunomodulatory, antioxidant and other activities. After 15 years as a development project, it became the family business Farmaya natural products laboratories. Based on traditional utilization and preclinical or clinical evidence several national and international projects were conducted. The most interesting results include anti-candidal activity of *Solanum nigrescens*, anti-warts activity of *Jatropha curcas*, anti-menopause activity by *Piper hispidum*, and further prospection of this genus. With this information specific formulas were prepared for the treatment of different pathologies, particularly anti-infective, sedative, analgesic, diuretic and others, leading to twelve products that were registered as phytotherapeutical products in Guatemala and are presently sold as OTC products in the form of tisanes, tinctures, elixirs, syrup and capsules at a national level. Some of the best-sold products based on native plants include Bronquiol, Jacameb and

Tincalmol. The information and products generated has helped other sectors, in one hand the technology transfer to community group to develop products and rural laboratories for PHC, and in the other, commercial groups that request from Farmaya the design and production of new natural products.

PL-09

Helichrysum italicum: back to medicine from the tinsel of luxury

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Helichrysum italicum (Roth.) Don. (Asteraceae), an iconic plant of the Mediterranean area, has now become also an icon of luxury because of its use in glamorous perfumes and personal care products [1]. H. italicum is, however, also an important medicinal plant, and recent studies have provided the basis for a veritable Helichrysum Renaissance, rationalizing the fascinating ethnopharmacology of the plant in the light of molecular investigations on its constituents and their pharmacological targets [2]. Extracts from H. Italicum have the potential to be developed as a novel ingredient for medicinal and healthfood products just like its essential oil has been in perfumery and aromatherapy, but awakening this sleeping giant of the Mediterranean herbal medicine will required a multidisciplinary joint effort. One of the major challenges to be addressed is the establishment a reliable supply chain to overcome the plight of spontaneous harvest that is threatening the wild population of the plant in Sardinia and Corsica, where the most valuable chemotype of the plant grows. A second issue that needs to be addressed is the high variability of the phytochemical profile of the plant, where the concentration of heterodimeric pyrones, the major bioactive constituents of the plant, can range from undetectable to almost 1%.

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Short lectures

SL1A-01

Ion mobility spectrometry for detection of *Cannabis sativa* L. metabolites from exhaled breath

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Smoking *Cannabis* and driving is an illegal offense and causes severe accidents including a high death rate world wide. As an attempt to increase safety on roads a mobile and rapid analytical device was developed for the detection of secondary natural compounds from *Cannabis sativa* after consumption in exhaled breath. Ion mobility spectrometry (IMS) is a fast and sensitive bioanalytical tool for the detection of volatile compounds in gas phases. IMS has been used in the past in food industry and civil safety areas for the detection of flavors and fragrancies or explosives, respectively. Beyond this recent research IMS has been studied for the detection of volatile natural product after consumption like psychoactive drugs.*Cannabis sativa* L. is a widely used drug that is inhaled and consumers administer besides of cannabinoids other secondary compounds like terpenoids as well. In a in vitro and in vivo study IMS was studied to explore limitations for the detection of *C. sativa* L. metabolites after smoking standardized dried flowers (*Cannabis* flos) to determine biomarkers from exhaled breath. Based on pharmacokinetic data consumption of *Cannabis sativa* can be traced back for 3 hours significantly and obtained data allow discrimination between oral use and legal use of hemp based food products. Second, this contribution will report on the development of algorithms and statistical methods based on bioinformatics for the development of a software to detect *Cannabis* related pattern and to operate a mobile, non invasive and fast analytical device.

SL1A-02

Pre-clinical pharmacokinetics of the hERG blocking iboga alkaloid voacangine from *Voacanga africana*

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The bark of *Voacanga africana* Stapf ex Scott Elliot and related species is not only used in African folk medicine, but also as legal high with increasing popularity in Europe. Voacangine (**1**), its major iboga-type alkaloid, was recently identified as potent hERG channel blocker *in vitro* which might point towards possible cardiotoxicity [1]. Since there is no *in vivo*characterisation of **1**, the aim of this study was to assess its pharmacokinetics after oral administration of both, the pure compound and an ethanol extract of *V. africana* bark (VABE) containing 9.71% of **1**, to allow for a critical discussion of its potential cardiotoxic risk *in vivo*. A precise and sensitive LC-MS/MS method was developed and validated according to FDA

guidelines to detect **1** in plasma of male Wistar rats. Within the investigated drug concentration, **1** showed a high plasma protein binding of 98.7 ± 0.29%. Pharmacokinetics was evaluated by comparing four groups (G1-G4; n=5-7/group). G1 received a single 5 mg/kg *i.v.* bolus of **1**; G2 and G3 received single 25 mg/kg or 50 mg/kg doses of **1** *p.o.*, resp.; G4 received a single oral dose of 500 mg/kg of VABE. Non compartmental analysis showed a volume of distribution (Vz) of 6.1 ± 3.4 L/kg after *i.v.* dosing, a clearance (CL) of 1.4 ± 1 L/h/kg, and an average half-life ($t_{1/2}$) of 6.0 ± 2.0 h. No statistical differences were observed in CL, Vz and $t_{1/2}$ after oral application of **1**, and indicated linear pharmacokinetics. Oral bioavailability (F) of **1** was 12% when given as pure compound. In VABE, a decrease of F was observed for **1** (8%). These findings provide first data to estimate the *in vivo* hERG channel related cardiotoxic risk of **1**.

Acknowledgement: Supported by EU-FP7-PEOPLE-IRSES Marie Curie: hERGscreen, 295174

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SL1A-03

High-throughput prediction of passive intestinal absorption of natural products and plant extracts with the PAMPA assay

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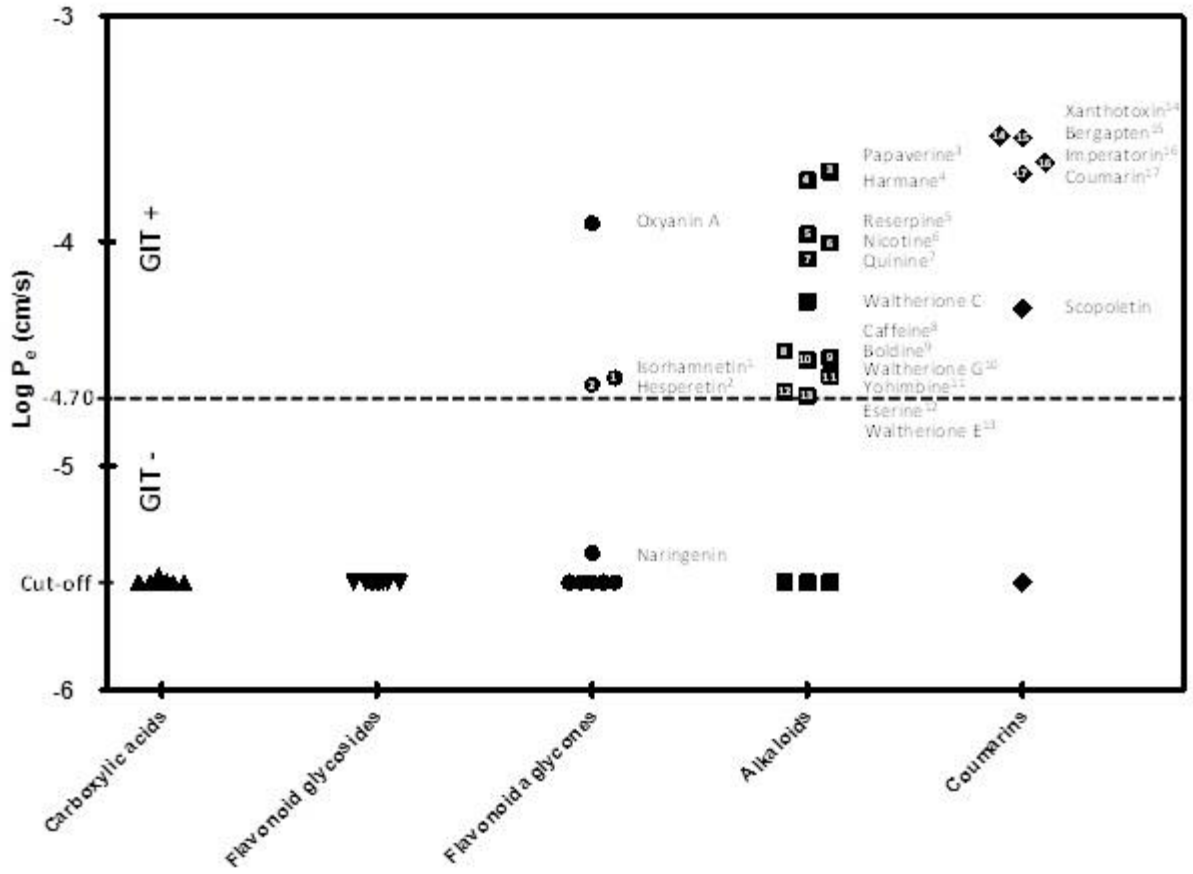
At early drug discovery stage, the high-throughput Parallel Artificial Membrane Permeability Assay (PAMPA) is one of the most frequently used *in vitro* model to predict transcellular passive absorption. While thousands of new chemical entities have been screened with PAMPA, in general permeation properties of natural products (NPs) have been scarcely evaluated. In this study, the Hexadecane Membrane (HDM) PAMPA was used to predict the passive intestinal absorption of a set of NPs. Alkaloids, coumarins and methoxylated flavonoid aglycones showed favourable passive intestinal absorption potential while flavonoid gylcosides and acidic NPs were found to have low passive intestinal absorption. To maintain a relatively high-throughput assay, a generic UHPLC-UV detection method was used and allowed easy calculation of the effective passive permeability value P_e (cm/s) of each NP without the need for specific compound dependent detection.

Since NPs are usually ingested in medicinal use as components of complex extracts in traditional herbal preparations or as phytopharmaceuticals, the applicability of HDM-PAMPA to screen crude extracts was further investigated based on three extracts containing chemically diverse active NPs. The first extract was composed of lipophilic furanocoumarins (*Angelica archangelica*), the second extract included alkaloids (*Waltheria indica*) and the third extract contained polar flavonoid glycosides (*Pueraria lobata*). The effective passive permeability

values P_e of major NPs in the extracts were rapidly estimated based on UHPLC-UV profiles and were found not to be affected by the presence of other constituents in the extracts.

HDM-PAMPA/UHPLC-UV is a cost-effective and high-throughput method to evaluate transcellular passive intestinal absorption of plant active principles. Early evaluation of absorption is important to predict if NPs showing interesting activities *in vitro* may have a chance to reach their target *in vivo*.



SL1A-04

Herbal medicinal products: Synergy in pharmacology and toxicology

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Medicinal plant extracts typically comprise hundreds or thousands of constituents, none of which would have a therapeutic effect if applied alone at the same dose.

In general, agonists act on the same target/signaling pathway in an additive manner. Their individual potency is expressed using relative potency factors (REPs) which are multiplied by the abundance of the compound in order to estimate its contribution to the overall effect (dose addition).

Combination of compounds with independent action can lead to an over-additive adversity. Currently, this approach is applied if each individual constituent is already present at an adverse level. A contrasting approach is under intense discussion, as regulatory bodies have suggested dose addition of constituents with independent or unknown mode of action as a default approach for the risk assessment of multiple constituents. since it does not presume dose adversity of each constituent.

In case of the lack of safety information of all constituents and due the difficulty to assess combination effects by studying individual compounds, testing of the combination may therefore be superior to separate testing of combination partners. Especially in the case that these partners are of low toxicity, also the latter strategy is considered to give sufficient information for a safety assessment. The toxicological programs, which were conducted, e.g., for some combination phytomedicines in the market, as STW 1 and STW 5, therefore were based on the combinations, and predicted well the benign safety profile of these medicines shown in their large scale clinical use.

Guidelines for the toxicological testing of herbal medicines, as e.g. the EMA guidelines for the genotoxicity assessment of herbal medicinal products, are based on the approach of testing the whole extract as a specific combination of plant constituents, as this has the advantage to indicate also potential cumulative toxicity.

SL1B-01

Learning from plants - Microbial production of plant specialized compounds

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Glucosinolates (GLs) are specialized bioactive compounds characteristic of plants in the order Brassicales, including the model plant *Arabidopsis thaliana*. GLs are key players in the natural defense system of plants against herbivores and microorganisms. Additional biological functions range from flavor compounds to bio-pesticides. Particularly, glucoraphanin (GRN), the major glucosinolate in broccoli has been associated with cancer-preventive properties of broccoli [1].

GRN is derived from methionine that first undergoes a series of four enzymatic reactions to form the chain-elongated dihomo-methionine (DHM). Subsequently, DHM is converted into GRN by a cytosolic seven-step pathway [2]. We have demonstrated the feasibility to engineer the 13-step pathway into the non-cruciferous plant species *Nicotiana benthamiana* by transient expression [3].

The goal is to ultimately transfer the GRN pathway into a microbial host organism for sustainable production. We successfully transferred the pathway for the tryptophan-derived indole GLs by stable integration into the genome of *S. cerevisiae* [4]. Recently, we succeeded in producing DHM, the precursor for GRN, in *E. coli*. We are currently implementing the full pathway into our two expression systems to evaluate the most promising production host.

[1] Juge, N., et al., Molecular basis for chemoprevention by sulforaphane: a comprehensive review. Cellular and Molecular Life Sciences, 2007. 64(9): p. 1105-1127.

[2] Sonderby, I.E., F. Geu-Flores, and B.A. Halkier, Biosynthesis of glucosinolates--gene discovery and beyond. Trends Plant Sci, 2010. 15(5): p. 283-90.

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SL1B-02

DNA Authentication of Raw Herbal Drugs for Industrial Quality Assurance

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Many plant-based medicines are still prepared from plants collected from the wild, requiring routine testing to ensure their correct identity. Quality control of wild-harvested plant materials has typically involved morphological and chemical analysis, but both approaches have their limitations. DNA-based authentication assays could complement these techniques and are currently under development for incorporation into industrial quality assurance procedures for a number of commercial products. A general strategy for the design of a robust DNA authentication assay for routine testing has been established. Specimens of the commercial plant species and its potential adulterants are collected and a DNA barcode sequence library created. PCR primers are designed to informative sequence strings that can be used to distinguish a target species from others in the dataset. Primers designed to generate short amplicons can be optimized for multiplex PCR, quantitative PCR and high resolution melt curve (HRM) assays.

Rhodiola rosea is a one such target for DNA test development. Raw material for *R. rosea* containing products still derives mainly from collection in the wild, with almost a dozen closely related species growing in the same habitat. Positive identification of the correct plant species is not obvious and misidentifications or even adulterations are common. Although rosavines are considered to be characteristic constituents of *R. rosea*, there is some doubt about their use as chemical markers. Informative sequence differences between the DNA barcodes of different Rhodiola species have been used to design a specific qPCR assay. This allows the quantitation of the target species DNA, but cannot detect unknown adulterant species. Conversely, an HRM assay can detect unknown species in mixed samples, but only at relatively high levels of contamination. Lessons learned from these and other examples will be discussed.

SL1B-03

Relative transcript analysis of a UDP glycosyltransferase and salidroside content in response to biotransformation of precursors in *Rhodiola rosea* L. callus culture

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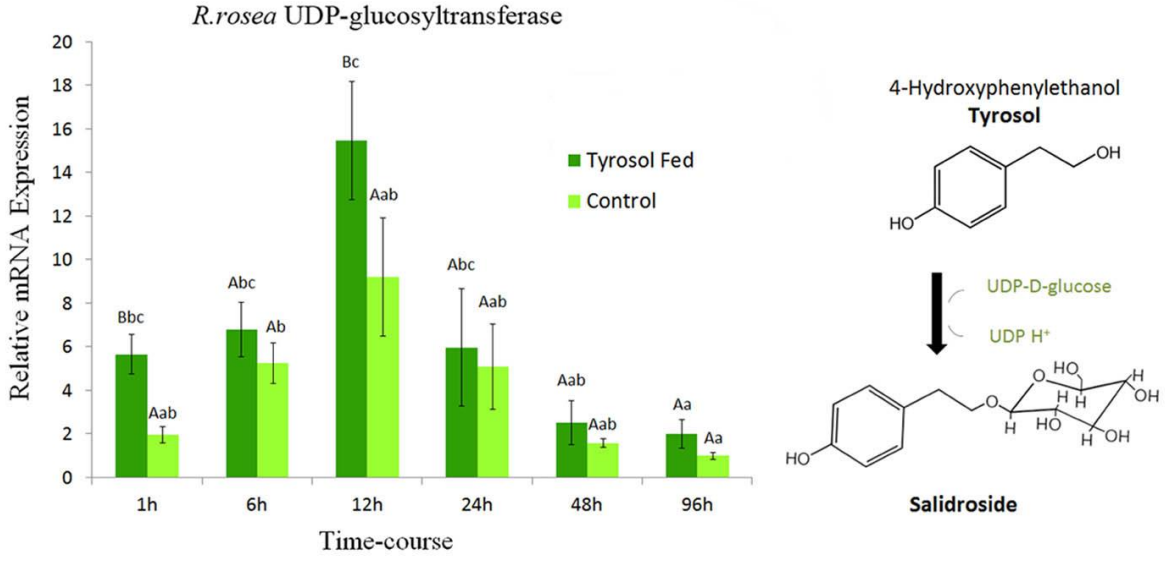
Rhodiola rosea is a medicinal plant with adaptogenic properties and various health-promoting effects. The compounds responsible for its medicinal effects are believed to be the phenylethanol derivatives (tyrosol & salidroside) and phenylpropanoids (rosin, rosavin & rosarian) which are mostly missing in its *in vitro* cultures. Roseroot is difficult to cultivate and grows slowly. Therefore, new methods for production of its pharmaceuticals are of interest. In this study, a full length cDNA encoding a UDPG gene was identified, cloned and characterized. Its ORF (1425 bp) was transformed and expressed in *E.coli* (BL21) and the expression of the recombinant enzyme was confirmed by SDS-PAGE analysis. To monitor the enzyme activity *in vivo*, 3 precursors (tyramine, 4-hydroxyphenylpyruvate & tyrosol) of salidroside biosynthesis pathway [1] were added to roseroot callus cultures and samples were harvested after 1, 6, 12, 24, 48 & 96h. Along with the controls (without the precursors feeding), each sample was subjected to HPLC and qRT-PRC for phytochemical and relative UDPG gene expression analysis, respectively. The HPLC analysis showed that the salidroside content significantly increased; reaching 0.5% of the callus dry weight (26 fold higher than the control) 96h after 2mM tyrosol was given to the media. The expression of a UDP-glycosyltransferase (Figure); a gene responsible for glycosylation of tyrosol to salidroside also significantly increased with highest being at 12h after the feeding. The effect of tyramine and 4-hydroxyphenylpyruvate was not as pronounced as of tyrosol. Here, we introduce for the first time a *R. rosea* specific UDPG gene and an alternative biotransformation method to increase the salidroside content in *in vitro* roseroot cultures.



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SL1B-04

Using xanthohumol as a tool to reveal an AMPK-mediated boost of the Nrf2/HO1 signalling axis

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Xanthohumol (XN) is a natural prenylated chalcone and exerts several bioactivities in mammalian cells including activation of AMP-activated kinase (AMPK) and nuclear factor E2 related factor 2 (Nrf2) signaling. AMPK is a master hub in the cellular energy homeostasis, and Nrf2 is a central transcription factor involved in detoxification and redox balance. As both share overlapping cellular responses upon activation we hypothesized that AMPK and Nrf2 crosstalk within their signalling networks.

To elucidate the influence of AMPK on Nrf2 signaling we made use of XN and examined the Nrf2-mediated up-regulation of heme oxygenase 1 (HO-1) in wildtype (WT) and AMPK-deficient (AMPK-/-) fibroblasts. XN (5 µM) induced HO-1 expression in AMPK-/- cells, however, to a markedly lower extent than in WT cells. The AMPK-mediated enhancement of the Nrf2/HO-1 signalling axis became evident on the mRNA and protein level of HO-1, as seen by immunoblot and qPCR. As recently published, we could reveal reduced endoplasmic reticulum (ER) stress upon AMPK activation as one contributor to the observed AMPK boost [1]. In this presentation we further disclose AMPK-mediated alterations in fatty acid synthesis, availability of acetyl-CoA and protein acetylation as an additional signal merging into enhanced Nrf2-signalling by AMPK.

Using XN as small molecular probe we revealed signaling events at the interface between AMPK and Nrf2 signalling. Our work underlines the inherent power of natural products with polypharmacology for dissecting the complex cellular signalling networks and may inspire the development of novel combinatorial drugs, such as dual AMPK and Nrf2 activators for a more effective prevention/treatment of age-related redox stress.

[1] Free Radical Res Biol *in press*; doi: 10.1016/j.freeradbiomed.2015.03.030.

SL1C-01

Investigation of euphrasianins as novel chemotaxonomic lipid markers of *Euphrasia* species

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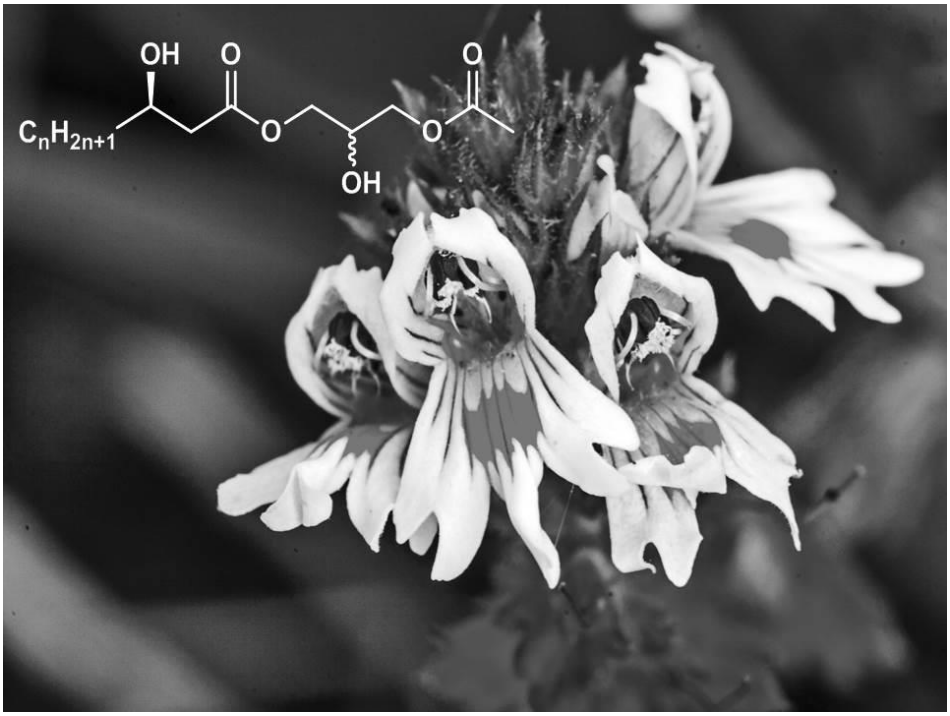
Eyebright (*Euphrasia rostkoviana* Hayne) is a medicinal plant belonging to the *Orobanchaceae* family. Extracts such as decoctions of *Euphrasia* have long been used for the treatment of inflamed eyes, conjunctivitis, stye and blepharitis. GC-MS and HPLC-APCI-MS^n analyses revealed the presence of five homologous acetyl monoacylglycerols of 3-hydroxyfatty acids (C14-C18) in CH_2Cl_2 extracts of *E. rostkoviana*. These compounds were named euphrasianins A-E, being members of a novel class of lipid constituents. For structure verification, a three-step total synthesis of one homologue (euphrasianin A) was performed which was characterized by 1D- and 2D-NMR. After alkaline hydrolysis of an *E. rostkoviana* CH_2Cl_2extract and methylation with BF_3/MeOH the 3-hydroxy fatty acid methylesters thus obtained, were analyzed by chiral GC. By comparison with reference compounds the absolute configuration was found to be '*R*'. Thus, the novel compounds were identified as 1-O-acetyl-3-[(3*R*)hydroxyfatty acid]-glycerols. While euphrasianins A and C were predominant in *E. rostkoviana*, another species, *i.e. E. tetraquetra* (seacliff eyebright), only revealed the occurrence of euphrasianins C and E, thus indicating that these target compounds may be utilized as chemotaxonomic markers.

[1] Lorenz P., Knittel D. N., Conrad J., Lotter E. M., Heilmann J., Stintzing F. C., Kammerer D. R. 1-O-Acetyl-3-[(3*R*)-hydroxyfatty acid]-glycerols from cuticular waxes of *Euphrasia rostkoviana* Hayne and *E. tetraquetra* (Brébiss.) Arrond., manuscript in preparation.



SL1C-02

Epicatechinoceanothic acid A and B, novel catechin-bonded ceanothane-type triterpenoids isolated from roots of *Ziziphus jujuba*

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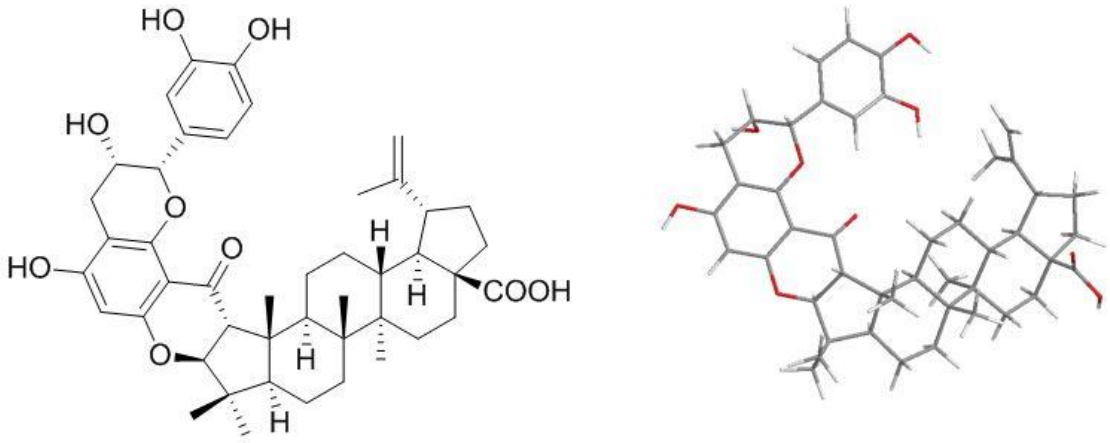
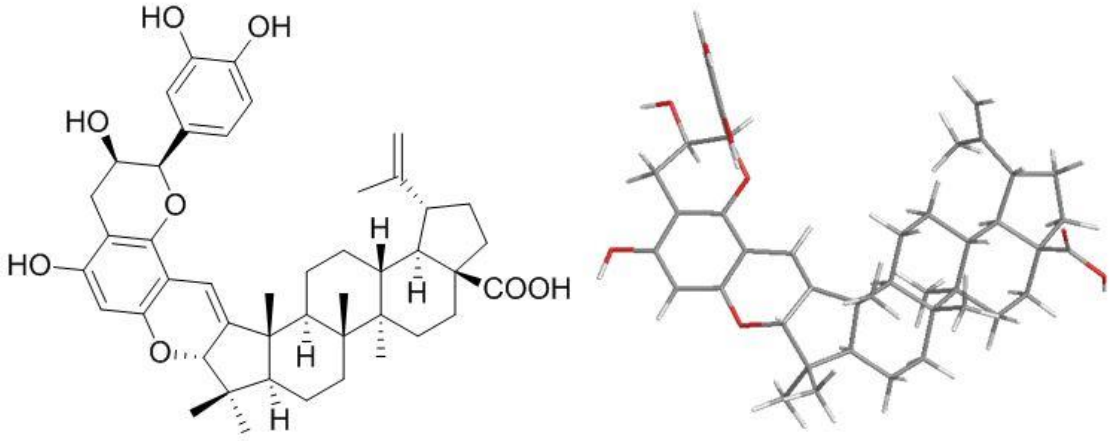
Ziziphus jujuba Mill. (Rhamnaceae) is a 5m tall deciduous tree, widely cultivated in southern Europe and Asia including India, Russia, the Middle East and China. In Korea, fruits and seeds of this plant have been used as food and traditional remedies for insomnia. Hundreds of compounds including triterpenic acids, flavonoids, phenolic acids and cyclopeptide alkaloids have been isolated and reported from *Z. jujuba*. However, chemical constituents of the roots have been rarely studied, compared to ones of other plant parts of this plant. In our investigation for novel bioactive constituents from the roots of *Z. jujuba*, we separated two structurally novel ceanothane-type triterpenoids. These two triterpenoids were containing an epicatechin moiety in their molecular structures, so they were named epicatechinoceanothic acid A (**1**) and B (**2**), after their structural characters. Additionally, three known compounds, ceanothic acid (**3**), epiceanothic acid (**4**) and (-)-epicatechin (**5**) were also separated and referred for the structural elucidation of **1** and **2**. Planar structures of **1** and **2** were determined by 1D, 2D NMR and MS spectra. Configurations of these compounds were suggested by ROESY NMR spectra, but there were still unsolved issues, such as the configurations of the epicatechin moieties. For configurational elucidation, molecular modelling method was applied. By comparing computational optimized structures of possible candidates with the ROESY NMR spectra data, the configurations of two epicatechinoceanothic acids were confirmed. **1**, **3**and **4** were subjected to bioactivity screening (**2** was excluded because of its small quantity), and **1** was evaluated to be a potent inhibitor of the signal transducer and activator of transcription-3 (STAT3) pathway in the human malignant glioma U87-MG cells.



1



2

SL1C-03

Distribution and HPLC-DAD-ESI-MS characterization of diarylheptanoids in several Central European species of the Fagales

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Diarylheptanoids are a group of phenolics bearing a 1,7-diphenylheptane skeleton. Due to the anti-inflammatory and antitumor activities of diarylheptanoids, the search for new plant sources is gaining significance.

The aim of our work was to study distribution of diarylheptanoids in diverse tissues of several species of the Fagales.*Corylus colurna* L., *Corylus maxima* Mill., *Corylus avellana* L., *Alnus glutinosa* (L.) Gaertn., *Betula pendula* Roth. and*Carpinus betulus* L. (Betulaceae), as well as *Juglans regia* L. (Juglandaceae) were evaluated. Leaf, bark, catkin, involucre and green

pericarp samples were investigated by HPLC-DAD-ESI-MS/MS and HPLC-DAD-ESI-TOF methods, in negative ionization mode. Linear gradient elution with acetic acid in water (0.2%, v/v) and methanol as eluents was applied.

Hirsutenone and its xylopyranoside derivative oregonin were detected as main diarylheptanoids for the *Corylus* species investigated. Additionally, their quantities were determined and compared in different samples [1-2]. Beside of hirsutenone, the presence of other phenolic linear type diarylheptanoids was proved for both *A. glutinosa* and *B. pendula*. Diarylheptanoids in leaves of silver birch and a non-cyclic type diarylheptanoid with a 1,7-bis-(3,4-dihydroxyphenyl)-4,6-dien-3-one structure in leaves and green pericarp of *J. regia* were detected for the first time.

The increasing scientific interest towards diarylheptanoids necessitates studies revealing valuable new sources of these natural products.

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SL1C-04

Chemistry and biological activity of molecules derived from *Isatis tinctoria*

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Isatis tinctoria is a well known medicinal plant in east asia. It is yielding many indole type heterocycles.The product tryptanthrin is known for its wide range of activities, e.g. antiplasmodial activity, but its poor solubility has undermined its development as potent pharmocological agent. The aim of this work was to present analogues of T. and to evaluate their antiplasmodial activity against the asexual and sexual blood stages of *P. falciparum*. Our results [2] suggest that most tryptanthrin analogues retained their antiplasmodial activity against chloroquine-sensitive and -resistant malaria parasite in the nanomolar range (30-100 nM). In the most active compound, synthetic NT1 3-Chloro-8-nitrotryptanthrin (IC_{50}: 30 nM; SI: 155.9), the antiplasmodial activity was similar in both strains and close to chloroquine (IC_{50}: 20 nM). In addition to their activity on asexual stages, tryptanthrin and derivatives prevented the formation of gametocytes at their IC^{90}concentrations, indicating their potential as transmission blocking candidates. The results of this study confirm that tryptanthrin and derivatives are potential candidates for further development. The same derivatives are active as anti-leucaemic agents [1]. In an overview, T.-derivatives were tested against several clones of pediatric ALL or AML. It specifically induced apoptosis in BJAB, Nalm6, Reh, Sup-B15 and p388 cells, as evidenced by DNA fragmentation, Annexin/PI staining, dissipation of the

mitochondrial membrane potential by activation of caspase-3. Together with the well known activity of indirubinsulfonate or its oxime, this data set sheds a light on how the mother compounds in Isatis might enable their century long known pharmacological activity.

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SL2A-01

Never-ending cranberry story: *In vivo, ex vivo* and *in vitro* studies indicate that tannin-depleted extracts from *Vaccinum macrocarpon* inhibit bacterial adhesion of uropathogenic *E. coli* by blocking FimH adhesin

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Inhibition of adhesion uropathogenic *E. coli* (UPEC) to bladder cells by *Vaccinium macrocarpon* should prevent urinary tract infection. Proanthocyanidin (PA) enriched extract (V.m.extract) and PA-free preparation (V.m.extract≠PAC) were investigated against T24 bladder cells and UPEC strains A2980 and NU14, characterized by different virulence factors.

V.m. extract evoked *in vitro* for both strains an increase of bacterial adhesion due to agglomeration of bacteria. In contrast V.m. extract≠PAC reduced the adhesion of NU14 significantly at 100 µg/mL (-30%) but did not influence the adhesion of A2980.

Adhesion of A2980 to bladder cells was increased by V.m.extract as PAs led to agglomeration of bacteria and induced a 2fold upregulation of the expression of the adhesins PapGII and FocG as a reaction to binding of PapGII to PA. Treatment of UPEC strain A2980 with V.m. extract≠PAC did not change the expression rate of adhesins. Decreased adhesion of NU14 after incubation of bacteria with V.m. extract≠PAC was presumably due to interaction of extract against FimH (not expressed by A2980) which was 2fold upregulated by feedback mechanism. V.m. extract significantly inhibited invasion into the bladder cells.

Both extracts had no influence on biofilm formation of both and did not interact with formation of curli on the bacterial surface.

Within an *in vivo*/*ex vivo* study (n=4 male volunteers) over 7 d and treatment with 600 mg/d V.m. extract, it was shown that urine collected after cranberry intake exhibited significant antiadhesive effects up to 50 % against UPEC strain NU14.

From these data it is concluded that PA do not contribute to the antiadhesive effect of cranberry extract. Significant antiadhesive effects are detected in urine of volunteers treated with cranberry. Pinpointing of the active compounds in urine by metabolomics approaches is just ongoing.

SL2A-02

Sabal serrulata - an assessment of clinical studies in LUTS

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The therapeutic usefulness of extracts of *Serenoa repens* has been a matter of discussion in the past. Therefore it was relevant to assess the results of the randomized, double-blind, controlled clinical studies with different extracts of *Serenoa repens* in the treatment of lower urinary tract symptoms (LUTS).

The study duration is relevant with respect to a chronic fluctuating disease. Of the studies conducted for up to 6 months a benefit was seen in 3 of 3 studies with ethanolic, in 8 of 9 studies with hexane and in 1 of 2 studies with CO_2 extracts. Of the studies conducted for more than 6 months a benefit was seen in 2 studies with hexane and in 1 study with CO_2 extracts, whereas 1 study with an ethanolic, 2 studies with hexane and 1 study with CO_2 extracts did not show positive results. So, the best evidence of clinical efficacy is available for hexane as well as for ethanolic extracts, supporting the well established use of these extracts.

As lower urinary tract symptoms are dynamic conditions with strong spontaneous fluctuation over time, the majority of patients might expect improvement of single symptoms and thus of quality of life, especially as the extracts are well tolerated even in long term treatment.

SL2A-03

Quality, bioavailability and clinical application of a new lecithin delivery system of *Boswellia serrata*extract

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The anti-inflammatory properties of *Boswellia serrata* extracts (BSEs) have been demonstrated in vitro and in animal studies [1-2]. Clinical trials covering a wide spectrum of disorders like asthma, osteo- and reumathoid arthritis, Crohn's disease and collagenous colitis have shown encouraging but not compelling results, which are mostly explained by the low quality and variability of BSEs [3]. In addition, pharmacokinetic studies have evidenced low and erratic systemic absorption of the putative active principles, the boswellic acids (BAs) [2]. In this context , we are reporting here a survey of BAs contents (detected with LC-MS/MS method) and the label claims of 17 top selling products from USA and EU. This survey confirms the great variability in the compositions and label misleading. Furthermore, we will discuss an original way to ameliorate and improve the clinical potential of BAs through the

natural delivery system of lecithin phospholipids. This formulation has been shown to significantly improve the absorption of BAs and tissue distribution in rats [4]. In the wake of this promising result, we have carried out a comparative pharmacokinetic study in 12 healthy volunteers on weight-equivalent (500 mg) dosages of the lecithin phospholipids formulated BSE and the non-formulated BSE extract.

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SL2A-04

Ficus carica (fig) paste supplementation in patients with multiple sclerosis associated constipation; a double blind randomized clinical trial

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Constipation is one of the most prevalent complications in patients with multiple sclerosis affecting up to 36% of these patients. Fig paste is used in traditional Persian medicine for the treatment of constipation. This study aimed to evaluate the efficacy of this natural product for patients suffering multiple sclerosis associated constipation.

The study was designed as a two-arm, double-blind randomized placebo-controlled clinical trial using a parallel design. Forty patients with multiple sclerosis (based on McDonald criteria) and constipation (based on ROME III criteria) were randomly allocated to receive 10 gram of the *Ficus carica*(fig) paste or placebo (1:1 allocation ratio) three times a day for three months. The patients were evaluated before and after the intervention in terms of frequency of spontaneous bowl movement, hard stool, straining during defecation, sensation of incomplete evacuation and need for manual maneuvers to facilitate defecations per week and any reported adverse events. The study protocol was approved by the Local Medical Ethics Committee of Tehran University of Medical Sciences.

Comparing to placebo, patients in fig paste group experienced a significant higher reduction in the frequency of spontaneous bowl movement ($p<0.001$), straining during defecation ($p=0.019$), sensation of incomplete evacuation ($p=0.013$) and need for manual maneuvers to facilitate defecations ($p=0.045$) per week. The mean reductions in frequency of hard stool in

intervention group showed no significant difference (p=0.518) with the value in placebo group (table 1). One patient in fig paste group reported nausea after taking the supplement.

This study provides a preliminary evidence for *Ficus carica* (fig) paste supplementation of patients suffering multiple sclerosis related constipation.

Table 1. Mean changes in constipation related outcomes in fig and placebo paste groups in patients with multiple sclerosis

Outcomes/week	Ficus carica paste (means±SE)	Placebo paste (means±SE)	P value
Mean increase in frequency of **spontaneous bowl movement**	2.5 ±0.47	0.70 ±0.16	<0.001
Mean reduction in frequency of **hard stool**	0.70 ±0.19	0.52 ±0.15	0.518
Mean reduction in frequency of **straining during defecation**	0.95 ±0.18	0.35 ±0.17	0.019
Mean reduction in frequency of **sensation of incomplete evacuation**	1.00 ±0.16	0.41 ±0.17	0.013
Mean reduction in frequency of **need for manual maneuvers**	0.90 ±0.23	0.23 ±0.16	0.045

SL2B-01

The impact of birch bark triterpenes on keratinocytes derived from diabetic donors

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Diabetes mellitus is known to cause major health problems and affects a high number of people. One of these problems is impaired wound healing and efficient remedies are urgently needed. Phytomedicines could be an interesting alternative for the treatment of this known subsequent damage of diabetic patients. Recently, a betulin-enriched triterpene extract (TE1) from birch bark (*Betula alba ssp.*) could show promising results regarding wound healing

efficacy in a clinical phase II study [1]. This beneficial effect can be explained on the molecular level. TE1 and betulin transiently upregulate pro-inflammatory mediators in epidermal keratinocytes and promote their migration by altering the actin cytoskeleton [2]. Continuing our research we now studied the effects of TE1 and betulin on keratinocytes derived from diabetic patients. TE1 and betulin treatment also led to promising effects on factors which are essential in the inflammatory and the reepithelialization phase of wound healing. mRNA levels of several cytokines, chemokines and other molecular mediators, e. g. IL-6, TNF-α, IL-8, IP-10 and Rantes were altered. Studies on the protein level confirmed these results. Moreover, triterpenes from birch bark affected the organization of the actin cytoskeleton. Proteins like RhoGTPases and p38-MAP-kinase that are known to be involved in this shape change [2] were activated indicating an influence of birch bark triterpenes on the migration of keratinocytes. Therefore, it can be stated that birch bark and its triterpenes do not only have an impact on keratinocytes derived from healthy donors, but also from diabetic donors. Clinical studies with diabetic patients have to confirm these promising results.

Acknowledgments: Funding by Aif and providing of TE1 and the triterpenes by Birken AG is gratefully acknowledged.

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[2] Ebeling S et al. PLoS One 2014; 9(1): e86147

SL2B-02

Glycosylated compounds from immature okra fruits inhibit the adhesion of *Helicobacter pylori* to gastric cells

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Infection of human stomach with *Helicobacter pylori* leads to severe gastrointestinal diseases such as gastritis, and gastric cancer. Due to the increasing resistance to antibiotic treatments new cytoprotective strategies against *H. pylori* infection are required. Since the adhesion of the bacteria to gastric cells displays the first step in the development of its pathogenicity, the specific inhibition of the bacterial adhesion to host cells is assessed to be an innovative tool for future treatments. The strong antiadhesive effect of an aqueous extract of immature Okra fruits against *H. pylori* has recently been shown [1]. For identification of the compounds responsible for this effect aqueous extracts from pulp and seeds were prepared and fractionated by ammonium sulfate precipitation with saturation levels of 30%, 60% and 90%. Analysis of precipitated polymers from the pulp extract indicated that fractions with saturation levels of 60% and 90% exert high dose-dependent antiadhesive effects against *H. pylori* adhesion (inhibition of 67% and 75% for 1 mg/mL). Structural analysis of these fractions revealed presence of pectin-like polysaccharides with rhamnogalacturonan I-backbones and short galactose side chains. Esterification of the polysaccharides was shown to be a prerequisite for antiadhesive activity. By dot-blot overlay assay specific interactions of the polymers with bacterial adhesins BabA and SabA were shown. The aqueous extract of Okra seeds shows strong antiadhesive effects in the range of 0.1-0.5 mg/mL, whereas a concentration of 1.0 mg/mL leads to a complete inhibition of *H. pylori* adhesion. Purification of the raw extract

yielded in different fractions with each showing both antiadhesive properties and hemagglutination activity against human erythrocytes (1 HU=2.7 µg). Analytical characterization by advanced MS studies and protein sequencing revealed that the polymers consist of highly O-glycosylated proteins.

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SL2B-03

A phytomedicine approach to stem cell modulation for heart regeneration

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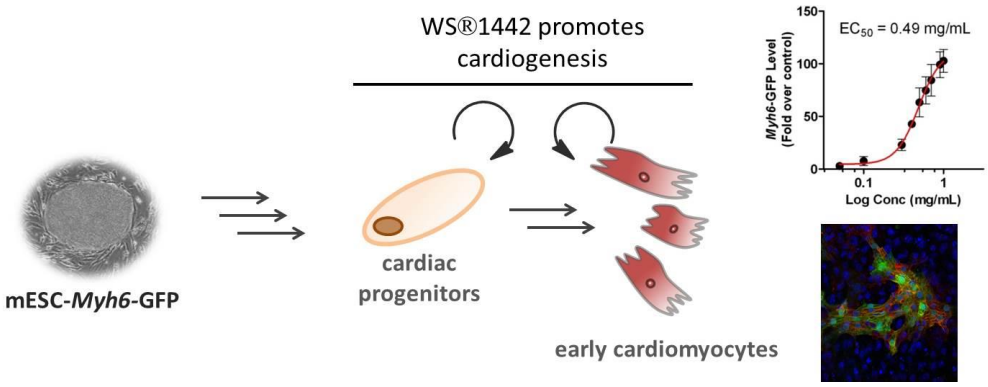
There is a great medical need for innovative therapies of heart disease that ask for paradigm shifts in pharmacotherapy. In this regard, the low and insufficient ability of the adult human heart to regenerate after myocardial infarction represents a great opportunity to identify chemical modalities, biological factors and mechanisms that improve this process. Owing to milestone discoveries in stem cell biology and regenerative medicine, several attractive technologies and molecules have emerged [1,2].

Based on the protective activity of *Crataegus* ssp. on the cardiovascular system and its positive effects on the myocardium after ischemic injury [3], we questioned whether mechanisms of cardiac regeneration could also contribute to the pharmacological profile of hawthorn extracts.



We have established a platform of murine and human stem cell-based phenotypic assays to probe differentiation and proliferation of cardiomyocytes (and progenitors). Using an array of readouts, including high-content fluorescence microscopy, flow cytometry and RT-qPCR, we found and validated that extract WS®1442 efficiently stimulated cardiomyocyte differentiation from murine (and human) stem cells in a dose-dependent manner after mesoderm was formed. First bioassay-guided fractionations of the extract suggested that this activity is reserved for specific compound classes.

We hypothesize that distinct *Crataegus* ingredients target multipotent progenitors, stimulate their differentiation towards the cardiac lineage but also expand their pool, thus addressing one of the mainly discussed potential sources for endogenous heart regeneration. Future studies will have to identify the active ingredient(s) and decipher the underlying cellular and molecular

mechanisms which may reveal novel, potentially druggable targets for *in vivo* heart regeneration.

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SL2B-04

Exploring for Bioactive Secondary Metabolites from the Chinese Medicinal Mangroves

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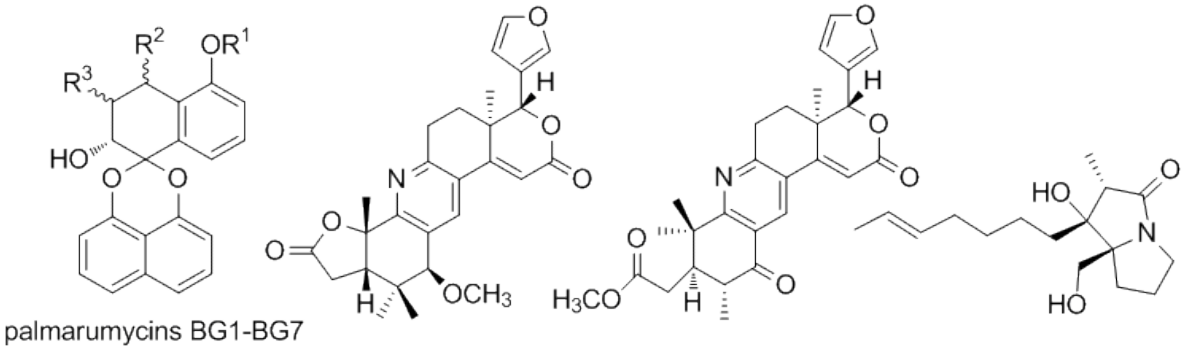
Mangroves comprise a large number of various salt-tolerant plants growing in tropical and subtropical intertidal estuarine zones. Historically, many mangrove plants were used to treat various diseases in traditional Chinese medicine. Currently, the secondary metabolites found in mangroves represent an extremely rich source of novel chemical diversity for academic drug discovery and chemical biology programs. It is particularly true that the mangroves from Southern Coast of China are very prolific producers of bioactive natural products [1].



Our group at SIMM has been long engaged in searching for novel secondary metabolites with pharmacological potential from Chinese mangrove medicinal plants [2]. In collaboration with biologists and pharmacologists at SIMM, numerous novel isolates were pharmacologically screened for activity in a variety of cell-based and pure enzyme assays, which led to the identification of numerous bioactive derivatives exemplified by cytotoxic palmarumycins [3a], xylogranatopyridines with PTP1B activity [3d] and the pyrrolizidine alkaloid penibruguieramine A [3e].

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[3] (a) Y.-S. Cai, T. Kurtán, Z.-H. Miao, A. Mándi, I. Komáromi, H.-L. Liu, J. Ding, Y.-W. Guo, J. Org. Chem., 2011, 76,1821. (b) X.-L. Chen, H.-L. Liu, Y.-W. Guo, Org. Lett., 2011, 13,5032. (c) J. Chen, C.-S. Jiang, J. Li, J.-X. Gong, Y.-W. Guo, Bioorg. Med. Chem Lett., 2013, 23, 5061-5065. (d) Z.-F. Zhou, W. Zhang, T. Kurtan, A. Mandi, A. Bényei, J. Li, O. Taglialatela-Scafati, Y.-W. Guo, Tetrahedron, 2014, 70, 6444-6449. (e) Z.-F. Zhou, T. Kurtan, X.-H. Yang, A. Mandi, B.-P. Ye, O. Taglialatela-Scafati, Y.-W. Guo, Org. Lett., 2014, 15, 1390-1393.

SL2C-01

A novel anti-chemoresistance agent designed from natural products by targeting GSTO

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The biological function of glutathione s-transferases (GSTs), a key member of phase II drug-metabolizing enzymes, plays an essential role in both cell detoxification and oxidative stress regulation through redox-mediated mechanism. Overexpression of GSTs in tumors is frequently associated with high capacity of detoxification, thereby endowing cancer cells with an increased capacity of drug detoxification even in the presence of chemotherapeutic agents. The correlation between GSTs and malignancy has been widely discussed, but the role and function of GSTs in tumorigenesis, metastasis, and drug resistance are not completely understood. Recently, we found that overexpressing GSTO1 is highly associated with tumor malignance. Protoapigenone (WYC02), first isolated from *Thelypteris torresiana*, show the anticancer activity. However, moderate effects of WYC02 impinged drug development. Recently, we have successfully synthesized the new generation of anticancer agents based on natural product protoapigenone for targeting GSTO in cancer. Two straightforward strategies including structure-based and ligand-based drug design were employed for best optimization. We conducted a structure-based designing strategy to modify natural product by introducing functional side chains mimicking GSH, a substrate of GST. Our team has also constructed a well-established pharmacophore for GSTO inhibitor. The newly design GSTO inhibitor shows the promising anticancer activity against chemoresistance. Furthermore, at least three classes of chemotherapeutic agents exhibit strong synergistic effects with the GSTO inhibitor. Altogether, we provide a potential therapeutic way for GSTO via naturally occurring compounds. Most importantly, this finding hopes to inspire further drug development.

SL2C-02

Specific role of oxidized species in the bioactivity of two antioxidants: apigenin and 20-hydroxyecdysone

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Antioxidants are generally considered as „double-edged swords": various health benefits are attributed to them since they can decrease oxidative stress caused by reactive oxygen species (ROS), but they can also increase it by directly or indirectly contributing to the formation of ROS [1]. On the other hand, a necessarily existing third face of antioxidants is generally overlooked, namely the specific bioactivities of their oxidized metabolites that are formed after scavenging ROS, and, as such, that must represent amounts proportional to the oxidative stress.

In the present work, a phenolic (apigenin; Ap) and a non-phenolic (20-hydroxyecdysone; 20E) antioxidant was studied for the biological importance of their oxidized metabolites. Protoapigenone (Pa), a p-quinol B-ring containing protoflavone known for its strong anticancer properties [2] was obtained from Ap upon oxidation with PIFA. *In silico* studies revealed the favorable formation of Pa from Ap when scavenging OH radicals, which was confirmed by HPLC-DAD when subjecting Ap to Fenton-reaction. Moreover, incubation with GSH yielded Ap from Pa, revealing an Ap-Pa redox cycle of outmost biomedical interest. 20E was subjected to base-catalyzed auto-oxidation, yielding B-ring modified derivatives including calonysterone and its desmotrope pair. *In vitro* activity of the compounds was tested on the phosphorylation of Akt (playing an important role in cell survival/apoptosis), and much stronger activities than that of 20E were observed. All major metabolites were detected from the Fenton-reaction of 20E.

Based on these examples, our results demonstrate that oxidized metabolites, forming when antioxidants scavenge ROS, can play specific role in the bioactivity of these compounds, and that such metabolites worth the attention concerning related drug discovery initiatives.

Acknowledgement: COST Action CM1407.

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SL2C-03

Phytohormone derivatives targeted to several cancer hallmarks

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As purines play vital roles in many biological processes the effects of modifying substituents on the purine ring on diverse biological activities warrant close examination, to elucidate the structure-activity relations of their interactions with proteins involved in tumour growth, cancer cell survival, angiogenesis and metastasis. Purines are highly amenable to the chemical manipulations required, and we have exploited this feature to prepare massive libraries of purine derivatives with wide arrays of types and combinations of functional groups at all seven reactive centres on the exterior of the purine heterocycle. The success of olomoucine, roscovitine (licensed to Cyclacel Pharmaceuticals; it is currently in phase IIb clinical trials - see www.ClinicalTrials.gov) and related drugs based on the purine scaffold has prompted and directed parallel advances in the preparation of related heterocyclic systems. We are currently trying to develop (sub)nanomolar inhibitors and rigorously characterise their effects on hallmark features of tumorous cells and systems/cells representing other diseases. In the design and optimization of kinase inhibitors, a key challenge is to elucidate the highly complex interactions involved in targeted processes to ensure that candidate agents have the desired selectivity. This will require comprehensive screening of the activities of a wide range of inhibitors against kinomes of selected cells, detailed in vitro analyses and, the synergistic application of advanced HTP technologies, using both traditional and novel strategies.

SL2C-04

Cytotoxic xanthones isolated from the stem bark of malaysian bintangor trees, *Calophyllum* spp.

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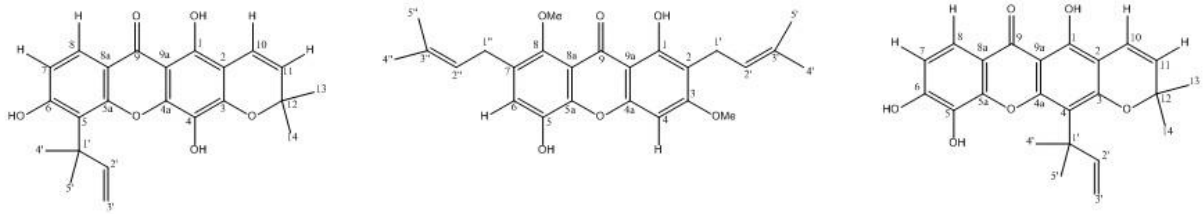
Bintagor trees, *Calophyllum* spp. is well known for its medicinal uses and the presence of secondary metabolites such as xanthones, coumarins and triterpenoids contributed to their various bioactivities, anti-HIV, anti-cancer and antimicrobial activities [1-2]. The detailed studies on two species of Malaysian Bintangor trees, *Calophyllum soulattri* and *Calophyllum inophyllum* lead to the isolation of a series of natural xanthone derivatives with the attachment of various moieties such as prenyl groups, pyrano and furano rings. Soulattrin and phylattrin were isolated from *C. soulattri* while inophinnin and inophinone from *C. inophyllum*. The other xanthones present in both species were macluraxanthone, caloxanthone C, rheediaxanthone A, trapezifolixanthone, brasixanthone B, pyranojacareubin and 4-hydroxyxanthone. The structures of these natural constituents were determined on the basis of spectroscopic analyses such as 1D and 2D-NMR, GCMS, IR and UV. The cytotoxicity of these natural constituents against four cancer cell lines, Raji, LS174T, IMR-32 and SK-MEL-28, were determined in vitro by MTT assay. Soulattrin was the most cytotoxic towards the cell lines with low IC_{50} values of 1.25 μg/mL. It is followed by phylattrin and macluraxanthone

with IC_{50} values lower than 7.81 μg/mL. The structure-activity relationships for the xanthone derivatives were predicted according to the cytotoxic effect of the existence of the substituent moieties on their main skeleton. It was found that the prenyl moeity, pyrano and furano ring present on the xanthones resulted significant effects on their cytotoxicity.



Soulattrin Phylattrin Macluraxanthone

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SL3A-01

A comprehensive platform for the identification and mode of action characterization of bioactive natural products from complex libraries

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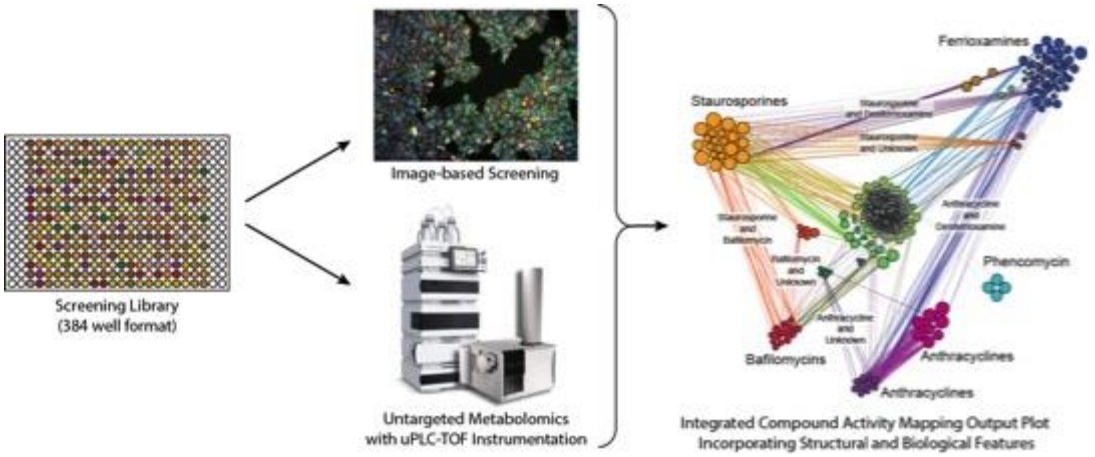
Despite the widespread use of natural products as inspirations for existing drugs, the rate of development of new natural product-based drugs has slowed in recent years. This is due in part to an increase in the rates of rediscovery of existing scaffolds, as well as limited early characterization of biological functions of hits from primary screens. Recent whole genome sequencing efforts suggesting that there exists a vast reservoir of potential new drug leads from underexplored natural sources. We have developed a new platform for integrated natural products discovery that uses image-based screening and high-resolution mass spectrometry to permit 'function-first' annotation of natural products libraries and provide a comprehensive annotation of the identities and biological attributes of all bioactive constituents.



This new platform, termed Compound Activity Mapping, integrates image-based phenotypic profiling data from our recently reported cytological profiling platform with untargeted metabolomics data incorporating uPLC separation and qTOF MS2 mass spectrometric analysis. Using a custom informatics platform, these two datasets are integrated to identify candidate molecules that are consistently positively correlated with specific phenotypes. Using network display, the bioactive metabolome from the natural product library is then displayed as an annotated network diagram that identifies all sets of bioactive molecules from within this set, allowing the selection and development of high priority lead compounds.

Natural products libraries suffer from a number of technical limitations for high-content analysis, including the presence of complex mixtures, constituents of unknown titer, and compounds with widely varying chromatographic properties. In order to address these issues we have developed a method to integrate biological and metabolomics data for the direct annotation of bioactive mixtures. Our new system uses these data to refine predictions about which constituents are responsible for the observed phenotypes in image-based whole cell screening data. This combination of multimode high-resolution untargeted metabolomic profiling and multiparametric biological annotation provides an opportunity to highlight even very minor bioactive constituents from natural product extracts that cause strong and reproducible phenotypes. These data give the natural products chemist direct information about the accurate mass, molecular formula, retention time and UV spectrum of active constituents, therefore obviating the need for bioassay-guided fractionation, and providing a method for the direct isolation of novel compounds. This technology therefore provides natural products chemists with a new mechanism to convert complex metabolomics profiling of extract libraries into a Compound Activity Map, which clusters extracts and metabolites based on common chemical and biological properties and highlights those compounds predicted to be responsible for the observed phenotype of a particular extract.

To demonstrate the power of this approach, we examined a library of 234 actinobacterial extracts in both the cytological profiling assay and the untargeted metabolomics system. Analysis of the cytological profiling results revealed the presence of 20 distinct phenotypic clusters. Subsequent incorporation of the metabolomics data reduced the >30,000 molecular features to ~700 features that were positively correlated with bioactive clusters. Subsequent validation of these bioactive candidates revealed the presence of 10 classes of known natural products, as well as one new class of compounds with unique structural and biological features.

SL3A-02

Metabolomics and dereplication strategies in the discovery of natural product derived drugs

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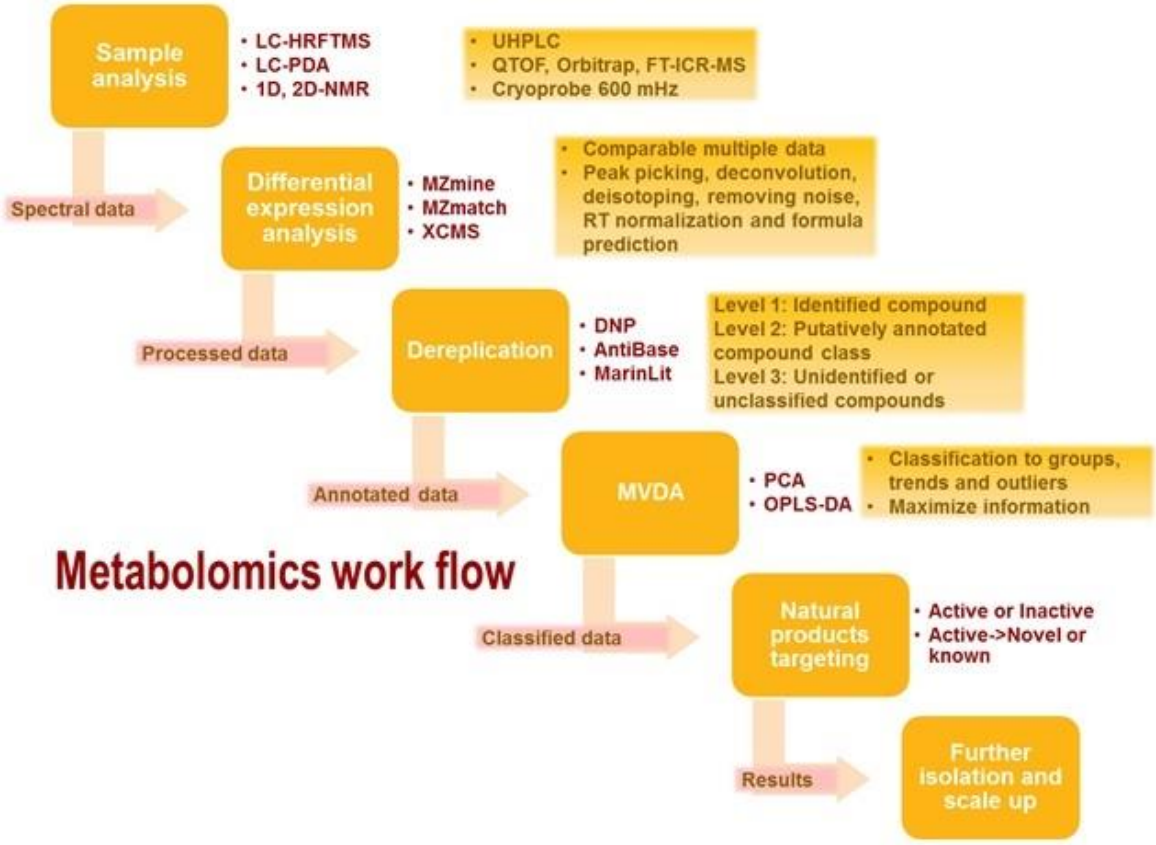
[3] *Faculty of Pharmacy, Helwan University, Cairo, Egypt*



Metabolomics is the technology designed to provide general qualitative and quantitative profile of metabolites in organisms exposed to different conditions. Metabolomics is applied in many aspects of natural drug discoveries, particularly in bioactivity screening to improve dereplication and identification procedures. Fast dereplication of known compounds and identification of lead bioactive metabolites is important in the primary stages of metabolomics profiling prior to an intensive isolation work. Two levels of metabolomics were used in this study. First was metabolites fingerprinting which aimed for rapid classification of samples by comparing the metabolites patterns or fingerprints. Second was metabolites profiling and dereplication study for class of compounds related to a specific pathway in order to individually identify and quantify these metabolites. This study involved isolation of endophytic fungus *Aspergillus aculeatus* from Egyptian medicinal plants, *Terminalia laxiflora*. Identification of the strains has been achieved through molecular biological methods. Metabolomic profiling, using NMR and HR-MS were done at different stages of the growth phase for both solid and liquid culture media. Dereplication studies were accomplished by utilizing the MZmine 2.10 software with aid of the AntiBase and DNP databases. By end of the dereplication process metabolites were sorted out into three levels; level 1: identified compounds, level 2: putatively annotated compound class and level 3: completely unidentified

and unclassified compounds. Multivariate data analysis was employed by using PCA in order to classify samples into groups, trends and outliers, which maximize the information, can be obtained from spectral data. OPLS-DA was used to correlate the chemical profile with tested biological activity. Metabolomics has been shown to be a powerful facilitator in the discovery of natural products, which are considered an excellent source for novel leads.

SL3A-03

A metabolomic and phytochemical based study of *Rhodiola* species sourced from Asia and Europe

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Rhodiola rosea L. has a long history of use in Europe as an economically valuable medicinal plant. It is used for the treatment of stress-induced fatigue,anxiety and high-altitude sickness [1]. With the increasing demand and over-collection of *R. rosea*, some adulterated products have been found. According to our investigations, approximately 25% of products claiming to be *R. rosea* on the European market are adulterated. Adulteration may occur at different stages along the value chain. Therefore the authenticity and quality of raw materials of *R. rosea* is an important area for investigation.The aim of the project is to investigate the metabolite differences of *Rhodiola* spp. collected from China and Europe and assess their phytochemical variation. Approximately 45 crude root dried plant specimens were sourced from different suppliers. HPTLC and ^{1}H-NMR analysis was used to evaluate the samples phytochemical variation and metabolites differences. R. crenulata was found to contain no rosavin (a marker compound for *R. rosea*) but another marker compound, particular to R. crenulata, was identified. Approximately 30 % of R. rosea and 27% of R. sachalinesis crude root dried specimens were adulterated with *R. crenulata* on account of containing no rosavin. *R. sachalinesis* contains a low concentration of rosavin and salidroside compared with *R. rosea*. Variations in secondary metabolites of *Rhodiola* spp. were identified and can be used to distinguish different species of *Rhodiola*. A marker compound was identified for *R. crenulata*. Adulteration among these species appears to be commonplace. The method of combining HPTLC and ^{1}H-NMR is shown to be a robust quality control method.

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SL3A-04

Metabolomics and chemoinformatics approaches to identify natural compounds of interest from Asteraceae

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The generation of extracts and pure compounds libraries has been shown to be an important strategy to obtain compounds of interest. Many strategies have been used to generate natural

products libraries, especially considering the Lipinski rule. However, the combination of powerful analytical methods, chemical structure databases and chemoinformatics tools can certainly provide additional information. We describe herein the generation of an extract library consisting of *ca*. 300 extracts from species from the family Asteraceae and *ca*. 150 pure compounds that have previously been isolated from these species as well as chemoinformatics strategies used for dereplication of medicinal plants extracts [3]. The extracts were obtained using an optimized and standardized method. Afterwards, the extracts and the pure compounds were analyzed by UHPLC-UV-HRMS. The same chromatographic system (Kinetex C18, 1.7 μm, MeCN-H_2O) was used to obtain metabolic profiles of selected extracts whose dereplication would be performed. Two-dimensional chemical structures and their analytical data (accurate masses and retention times) were used to build a QSRR model for retention time prediction of compounds that were not included in the library. Using PCA it was possible to verify matches of chemical structures with those present in chromatograms of medicinal plants extracts. As a result, several compound matches were identified; for example, compounds from *Lycnophora ericoides* (a medicinal plant known as "Brazilian arnica") were dereplicated, like the flavonoid chrysin 6,8-di-C-glucopyranoside and the sesquiterpene lactones 4β,5-dihydro-15-deoxygoyazensolide and 4β,5-dihydrolychnopholide, which were identified with 80-90% of confidence. Based on the results, we conclude that focused libraries combined with chemoinformatics tools are important to accelerate the identification of known compounds and also to improve the possibility of finding new compounds.

SL3A-05

High-resolution fungal plasma membrane H^+-ATPase profiling combined with hyphenated HPLC-HRMS-SPE-NMR for identification of antifungal constituents in plants

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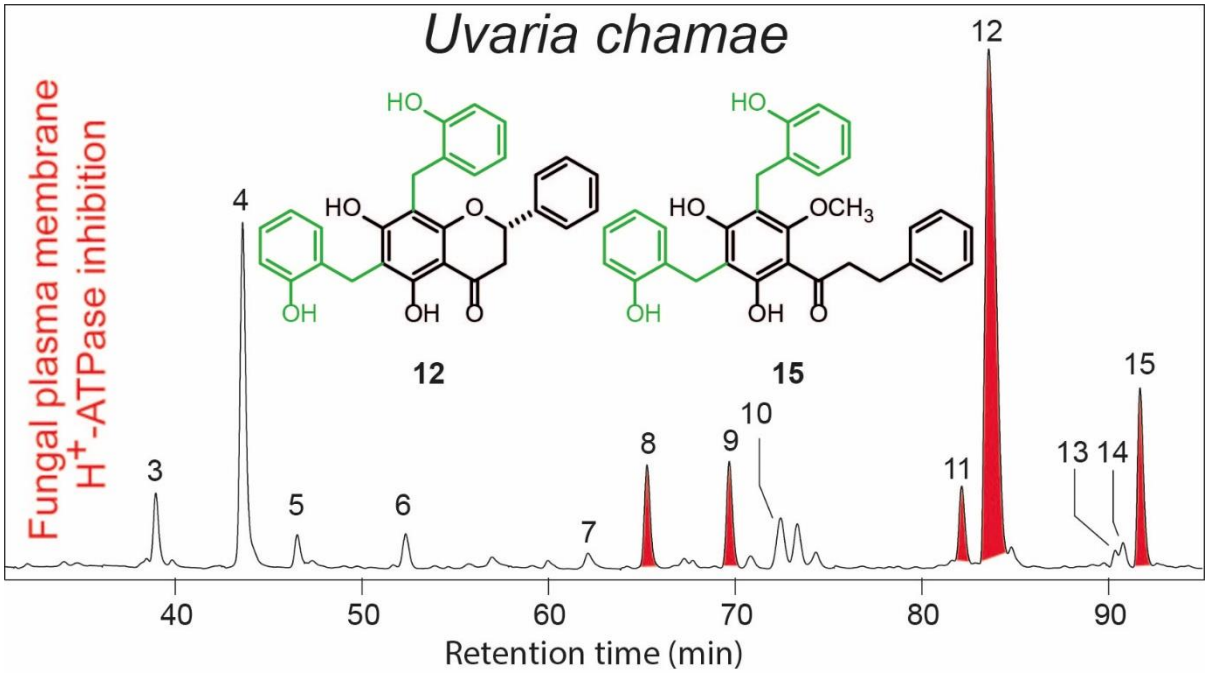
Plants are constantly exposed to fungal attacks, and development of a biosynthetic machinery for production of antifungal compounds might have been many plants' evolutionary counterattack. We are therefore using plant extracts in our search for inhibitors of the fungal plasma membrane (PM) H^+-ATPase - a promising target for antifungal compounds because *i)* it plays a pivotal role in the trans-membrane electrochemical proton gradient necessary for nutrient uptake, and *ii)*, the *PMA1*gene encoding the PM H^+-ATPase is highly conserved among fungi, but having only 32% sequence identity to its counterparts in plants. To advance identification of fungal PM H^+-ATPase inhibitors, we combine high-resolution fungal PM H^+-ATPase inhibition profiling with hyphenation of analytical-scale high-performance liquid chromatography, high-resolution mass spectrometry, solid-phase extraction, and nuclear magnetic resonance spectroscopy, *i.e.*, HR-bioassay/HPLC-HRMS-SPE-NMR [1]. The HR-bioassay profiles allow pinpointing of HPLC-peaks representing fungal PM H^+-ATPase inhibitors, and thus allow subsequent HPLC-HRMS-SPE-NMR analysis being targeted towards bioactive constituents only. In this talk, the use of HR-bioassay/HPLC-HRMS-SPE-NMR for identification of PM H^+-ATPase inhibitors as part of a large screening program will be presented. This lead to identification of bioactive *C*-hydroxybenzylated flavanones and chalcones in *Uvaria chamae*, hederagenin glycosides in *Lecaniodiscus cupanioides*, and biflavonoids in *Lophira lanceolata*.



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SL3B-01

Redefining natural product screening and characterization using MS technologies

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Natural products chemical ingredient profiling is a challenging task because of the sample complexity and the analyses required.

For any natural products related study, regardless the research focus and the final end goal, ingredient profiling (to elucidate the active compounds from a particular plant), sample comparison (to understand point of origin and to prove authenticity), quantification and identification of target compounds for quality control purpose are all routinely required. With the constant evolution in analytical technologies, the application of high resolution LC/MS instrumentation such as UPLC/QTof MS has been gaining popularity in chemical ingredient analyses. Application of these technologies can help to shorten analysis time, increase separation efficiency, and obtain results with high confidence. However, these technologies generate large and complex datasets, data analysis and interpretation can be the rate limiting step in chemical ingredient profiling.

Here, we present various botanical studies to demonstrate how to effectively use different analytical system solutions to natural product related problems. All the studies shown utilizes

UPLC/QTOF MSE and novel informatics tools for complex sample comparison, to solve problems such as point of origin, plant authentication, and commercial product authentication. The presentation also includes a simple and novel informatics tools with a natural product database that has been specifically developed for natural product chemical ingredient profiling.

SL3B-02

Xanthones from the bitter plants *Gentiana lutea*, *Centaurium erythraea*, and *Frasera caroliniensis*(Gentianaceae) inhibit vascular smooth muscle cell (VSMC) proliferation

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Excessive proliferation of vascular smooth muscle cells (VSMC) plays a major role in cardiovascular disease and greatly contributes to restenosis, the recurrence of blood vessel constriction after surgical treatment of stenosis. Due to unfavourable side effects of available pharmaceuticals, the discovery of new inhibitors of VSMC proliferation is of great importance.

A natural product screening approach using a resazurin conversion assay led to the identification of a xanthone derivative as inhibitor of VSMC proliferation. In order to potentially find further active compounds and to gain more information about the structure activity relationship (SAR), 13 additional xanthone derivatives isolated from three bitter plants from the Gentianaceae family (*Gentiana lutea*, *Centaurium erythraea*, and *Frasera caroliniensis*) [1] were also tested. Whereas some compounds showed no or moderate activity when applied at 30 µM, the four most active xanthones showed IC_{50}values between 7.8 and 12.5 µM. Their anti-proliferative effect was confirmed by measuring DNA synthesis in VSMC by quantification of 5-bromo-2′-deoxyuridine (BrdU) incorporation into DNA. In this model, the four xanthones exhibited IC_{50}values in the range 5.7- 24.5 µM. Cell death quantification (LDH assay) revealed that they are not cytotoxic in the tested concentration range.

In conclusion, our study identifies xanthones as new scaffold VSMC proliferation inhibitors that might be of relevance as a starting point for the development of new therapeutic applications to combat restenosis.

Acknowledgement: Supported by the TWF, the FWF (NFN-S10703, NFN-S10704, P25971-B23, and P23317-B11), the EU-FP7 Marie Curie Fellowship 252881, and the University of Vienna “Back-to-Research Grant”.

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SL3B-03

Neuroactive alkaloids from *Psychotria* (Rubiaceae) are SIRT inhibitors

Claudia A. Simoes-Pires[1], Carolina S. Passos[1], Lucie Ryckewaert[1], Lionel Sacconnay[1], Maria Cristina Guerra[2], Lucilia Kato[3],Cecilia Maria A. Oliveira[4], Alessandra Nurisso[1]

[1] *School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland*

[2] *Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*

[3] *Laboratório de Bioatividade Molecular, Instituto de Química, Universidade Federal de Goiás, Goiânia, Brazil*

[4] *Laboratório Farmacognosia, Departamento de Produção de Matéria-Prima, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*

Epigenetic enzymes such as histone deacetylases (HDACs) play a crucial role in the development of aging-related diseases. Among human HDAC isoforms, class III HDACs, also known as sirtuins (SIRTs), have been considered as promising targets for treating neurodegenerative conditions [1]. *Psychotria* alkaloids have been reported for their inhibitory properties against central nervous system (CNS) cholinesterases and monoamine oxidases [2]. Given the multifunctional profile of these alkaloids in the CNS, we hypothesized that they could also interact with SIRTs. Here, we show the SIRT inhibition by alkaloids previously isolated from *Psychotria* spp. first by *in silico* methods, followed by enzymatic and cell assays. Five alkaloids, namely, vallesiachotamine lactone, E-vallesiachotamine, Z-vallesiachotamine, prunifoleine, and 14-oxoprunifoleine showed an inhibitory profile comparable to that of sirtinol, used as reference compound, in a dose response manner from 10 to 150 μM (Figure 1). The cytotoxicity on rat astrocytes and human cells was also evaluated and correlated to the pharmacokinetic profile of tested compounds.

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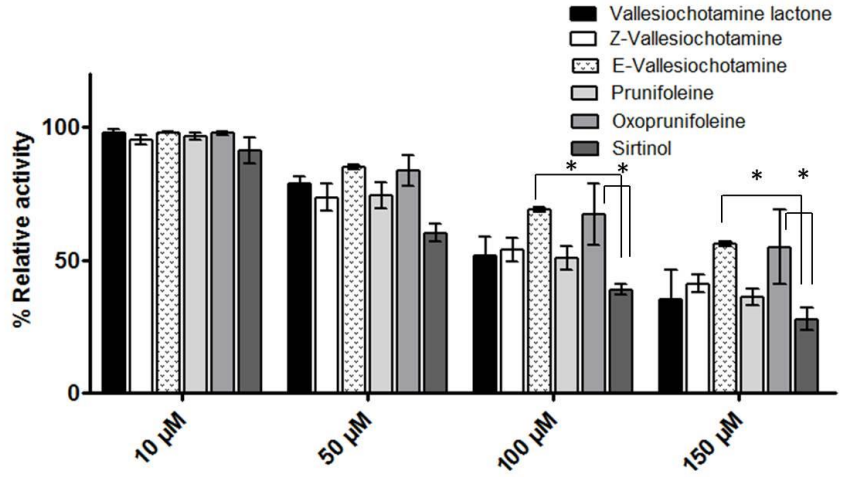

Fig. 1. Enzymatic SIRT1 activity measured for *Psychotria* alkaloids and sirtinol. *Significantly different ($p \leq 0.05$) in the post hoc Dunnett's test.

SL3B-04

Ursolic acid and 12-*O*-methylcarnosic acid from the leaves of *Rosmarinus officinalis* L. suppressed melanin production with downregulation of tyrosinase expression in HMV-II melanoma cells

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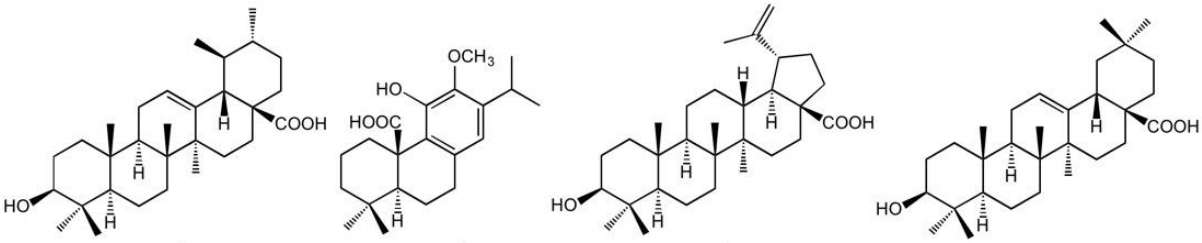

1 **2** **3** **4**

Horticultural therapy involves horticultural activity for elderly or disabled individuals, with the aim of improving quality of life (QOL). Experimental data showing improved QOL from horticultural plants would indicate objective benefits from horticultural therapy. We evaluated the skin-whitening effects of horticultural therapy plants on melanogenesis in HMV-II human melanoma cells.

A total of 15 samples of extracts obtained from horticultural herbal therapy plants were examined. Based on IC_{50} values, the highest inhibitory effect on melanogenesis was observed with extracts from the leaves of *Rosmarinus officinalis* L. (rosemary). This extract showed stronger activity than the flowers of *Erica vulgaris* (heath), which include abundant levels of the major skin whitening compound, arbutin (positive control). Next, we isolated active compounds from rosemary by melanogenesis inhibitory activity-guided fractionation, identifying ursolic acid (**1**) and 12-*O*-methylcarnosic acid (**2**) as the main bioactive compounds. Compared with **1** and its isomers (betulinic acid (**3**) and oleanic acid (**4**)) at the concentration of 7.5 μM, inhibitory effects of **1** and **3** were 67.2±9.4% and 0.5±0.5% (% to control), respectively. Compound **3** showed high cytotoxicity (viability, 7.4±1.4%), while **4** had no effect inhibiting melanogenesis. Tyrosinase is one of the key enzymes involved in melanogenesis. We therefore studied whether these compounds would suppress expression of tyrosinase on HMV-II cells. Compounds **1**, **2**, and **3** showed downregulation of tyrosinase expression, according to the results of western blotting.

The anti-melanogenesis activity of **1** on B16 mouse melanoma cell has been reported. However, these findings offer the first report of the anti-melanogenetic activity of **1** and **2** on HMV-II human melanoma cells and their mechanism. Anti-melanogenetic compounds isolated from rosemary are currently under investigation.

SL3B-05

Enriching the biologically relevant space sampled by natural products through biotransformations: speeding up the natural-product based drug discovery process

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Natural products have been evolutionary selected to occupy the part of the chemical space that is of biological relevance [1]. Unfortunately, their inherent scaffold complexity has limited their fruitful integration in the drug discovery pipeline. We have exploited and established novel chemoenzymatic tools to manipulate in a very rapid and biologically targeted way the natural product core [2-5]. We will illustrate that rapid product screening, transformation efficacy and regioselectivity can be readily monitored in situ and in real time as also that evaluation of binding with putative drug targets can be conducted in a cost and time effective way [2-5]. The functionalized natural products have been then accessed for their bioactivity in traditionally thought "undruggable" targets [5-7]. Different examples from our research on natural product based molecular hybridization [4,6] as also natural product scaffold sculpting and associated biological evaluation in in vitro cancer cell lines and other therapeutic directions will be presented [6-10].

Acknowledgement: This project has been co-financed by the European Union (European Regional Development Fund- ERDF) and Greek national funds through the Operational Program "THESSALY-MAINLAND GREECE AND EPIRUS-2007-2013" of the National Strategic Reference Framework (NSRF 2007-2013).

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SL3B-06

α-glucosidase inhibiting compounds from *Justicia secunda* VAHL (Acanthaceae) – from HPTLC bioautography screening to isolation and structure elucidation

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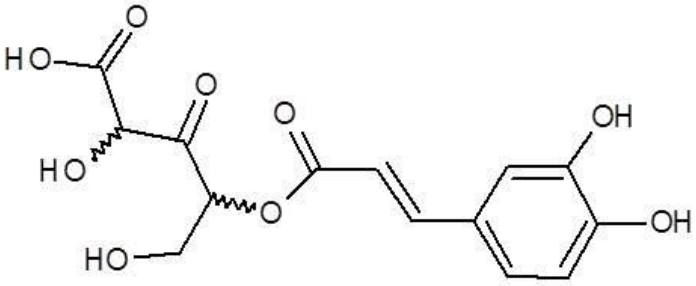
Justicia secunda VAHL belongs to the family of Acanthaceae and is a barely examined species. In Ecuador, the leaves of *J. secunda* are applied in ethnomedicine against indications related to diabetes [1]. The aim of this study was to screen *J. secunda* for its potential α-glucosidase inhibiting effect performing a bioautographic HPTLC assay [2]. A methanol extract of leaves was first partitioned successively between solvents of increasing polarity (light petroleum, CH_2Cl_2, BuOH, H_2O). The aqueous fraction showed distinct activity. It was subjected to column chromatography on polystyrene for further fractionation and sugar removal, yielding two subfractions with α-glucosidase inhibiting effects. For final isolation and characterization of pure active compounds, preparative HPTLC and semipreparative HPLC on RP-18e were conducted. MS and extensive NMR measurements led to the structure elucidation of the unknown diastereomers 4RS-{[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-2RS,5-dihydroxy-3-oxopentanoic acid (**1**) and the recently published diastereomers secundarellone B and C (**2**) [3].



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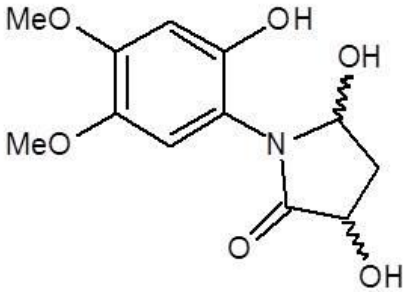

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SL3C-01

Medical efficacy of Ayurvedic herbs in reducing SCC of dairy cattle under Dutch conditions

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High somatic cell count (scc) –which is an indication of an incipient or chronic mastitis- is one of the major problems in dairy farming. To investigate the effects of an Ayurvedic remedy a pilot experiment was performed with 40 dairy cows, selected based on an increased scc (>250,000/ml) for a maximum of 3 subsequent milk recording dates. All animals were treated for 5 days after milking in the morning and the evening by rinsing and massaging the udder with lukewarm water. Half of the cows were anointed with a herbal mixture, whereas the control animals received no treatment. The herbal remedy consisted of fresh *Aloe vera* (L) Burm. f., *Curcuma longa* L. powder and calcium hydroxide, mixed with water and prepared fresh every day. Milk samples were taken from every quarter before and 14 days after treatment, and scc and bacteriological status was determined.

In the herbal remedy group a significant reduction in cell count ($p<0.003$) could be determined as compared to baseline data. However most cell counts were not reduced to below 250.000. Concerning the bacteriological status there were no clear differences between the groups.

The herbal treatment leads to a reduction of scc but less than expected compared to Indian experiences. This might be explained with the daily repetition of treatments. In India farmers are instructed to treat the animals 10 times a day with the remedy. For practical reasons we choose to treat two times per day. Another difference might be the composition of the constituents of the *Aloe vera*. Here we used young plants cultured by a breeder in a green house. In India the plants are older and kept outside. The preparation of the remedy and the treatment of the cows were very time consuming and not suitable for Western circumstances. The positive effects on the scc however warrant further investigation.

SL3C-02

Flavonoids present in ruminant diet affect the contractility of bovine isolated abomasum specimens

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Ruminants are constantly exposed to flavonoids present in feed but it is not clear if those phytochemicals have the potential to affect the activity of gut smooth muscle. Therefore, the

aim of the study was to verify the effect of three flavonoids on bovine isolated abomasum smooth muscle.

The study was conducted on abomasum smooth muscle collected from healthy dairy cows that underwent routine slaughter. The effect of apigenin, luteolin and quercetin (0.001-100 μM) applied in a non-cumulative manner on acetylcholine (ACH)-precontracted abomasum specimens was tested.

The results obtained in studies aimed at testing dose-effect dependence revealed that: (i) apigenin caused dose-dependent myorelaxation; (ii) luteolin acted as a contractile agent, if used in low concentrations and showed a tendency to increase the force of ACH-evoked reaction, if applied at higher doses; (iii) quercetin used in low concentrations enhanced the response to acetylcholine but if it was used in high doses the flavonoid caused clear myorelaxation.

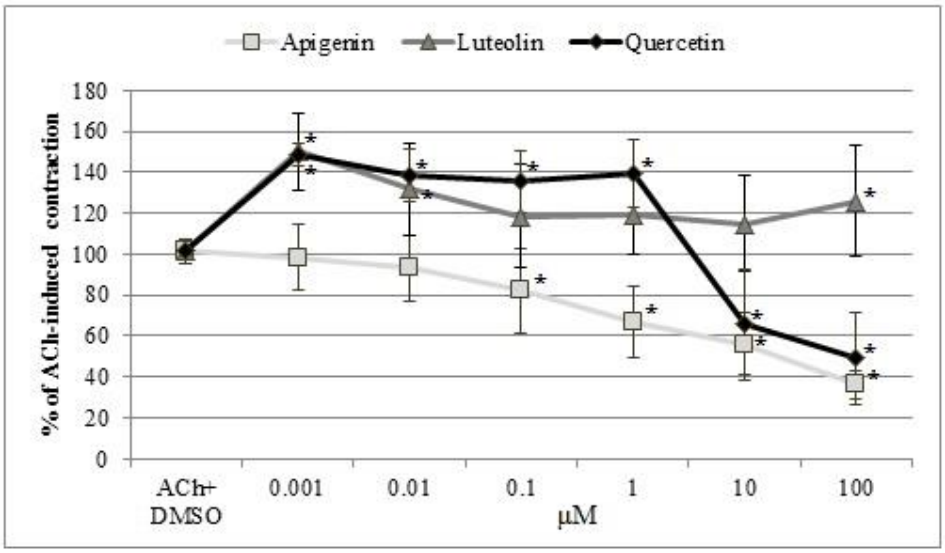

Figure 1. The effect of apigenin, luteolin and quercetin on ACH-induced contraction of bovine isolated abomasum circular smooth muscle strips.

The results are expressed as % of the contraction caused by acetylcholine applied in the reference dose of 10μM. The results are expressed as mean of 5-6 independent experiments (±SD), *p≤ 0.05 vs. ACh (10 μM) followed by DMSO (0.5%) application.

Interestingly, if flavonoids were used directly in high concentrations the effects were comparable with those evoked by the same phytochemical but applied in the lowest dose. It suggests a non-specific, dose-independent mechanism of action and the possibility that smooth muscle might get used to flavonoids impact and do not respond correctly to a gradual increase of the applied doses in one experiment. Besides, the multiple application of the same flavonoid in the lowest dose revealed that after several treatments the evoked reactions are different from the first one.

Luteolin and quercetin in concentration up to 0.1 μM seem to be potent myocontractile agents. They show the potential to be used as a diet supplement in dairy cattle. Additional to many beneficial effects on animal health, the use of luteolin- and quercetin-rich feed might be used as a method to reduce the likelihood of abomasum dysmotility.

SL3C-03

Impetus of phytogenics on broiler performance during periods of infectious stresses

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Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, Kolkata, India

Efficacy of the inclusion of a phytogenic feed additive (PFA) based on a blend of essential oils of *Mentha piperita*,*Pimpinella anisum* and *Syzygium aromaticum* compared to an antibiotic growth promoter (AGP) on broiler performance, faecal shedding and caecal colonisation pattern of certain bacterial species and humoral immune response (HIR) against Newcastle Disease (ND) during infectious stresses in presence of a real pathogen challenge were considered in this study.

A 38 d experiment was conducted with 120 male one-day-old Cobb 400 broiler chicks (3 treatments, 8 replicates). The dietary treatments included feeding a corn-soybean based control diet without added growth promoters and treatment diets containing either bacitracin methylene disalicylate (225 mg/kg) or the PFA (150 mg/kg).

Supplementation of diet with PFA improved body weight gain and feed conversion ratio ($P<0.05$) despite oral challenges with pathogenic *S. enteritidis* and *E. coli* compared to the control group, the AGP gave intermediate performance.

Faecal Salmonella indicated a lower count ($P<0.05$) at 0 h after challenge on d 28 in the PFA group compared to Control. At 24 h past challenge *Salmonella* and *E. coli* numbers were lower in the AGP and PFA groups than Control ($P < 0.01$).

Enumeration of bacteria in the caecal content at the end of the experiment on day 38 indicated significant reduction of Salmonella, E. coli and Clostridium numbers in the AGP and PFA supplemented groups ($P<0.01$) compared to the Control. However, the number of *Lactobacillus* increased in PFA group in contrast to Control and AGP groups ($P<0.01$).

HIR against ND was identical across the diets at 7 d, increased with age ($P<0.01$) irrespective of dietary treatments. At 21 d, the ND hemagglutination inhibition (HI) titre was better in the AGP and the PFA groups than Control.

It may therefore be concluded that PFA may be utilized as an effective tool for enhancing broiler performance especially during periods of infectious stresses.

SL3C-04

Controlling gastro-intestinal nematodes of goats with plant extracts

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Gastro-Intestinal Nematodes (GIN) impact on health of goats. In response to increasing problems with anthelmintic resistance, farmers opt for other strategies to control GIN. Amongst these, the use of plant preparations receives renewed interest. In the south of France where the production of medicinal plants is economically important, goat farmers traditionally use commercially available plant essential oils (EO) to cure and alcoholic plant extracts (AE) to prevent infection with GIN. We performed a series of on-farm trials with adult goats where the effect of (i) a specific EO mixture and (ii) an AE of various plants (Table 1) were tested on GIN. Three goat farms, each with>40 adult animals were enrolled in the study. Faecal egg count (FEC) were performed for every animal individually and all goats with EpG>1000 were selected for the study. For each farm, the study animals were randomly distributed to either a treatment or a control group, which resulted in 10 to 12 animals per group and farm. EO was dissolved in paraffin (7 ml per treatment unit and day) and administered to all animals of the treatment group per os for 3 consecutive days (Table 1). Control animals received paraffin only. AE treatment was done on only one goat flock. In order to examine the preventive potential of AE, all animals were treated with a short acting anthelmintic in order to assure GIN naïve conditions. The experimental animals were then treated for 10 consecutive days with AE and were allowed to pasture from the beginning of the treatment procedure. FEC were performed at day 0, 7 and 14 for the EO treatment and at day 0, 23 and 33 of the AE treatment. Compared to controls, EO treatment did result in a significantly reduced FEC in one herd 7 days after treatment onset (Figure 1). No further effects of EO treatment were found for the other farms, neither at day 7 or day 14 after EO treatment. AE treatment did not lead to FEC differences between the control and the treatment group.

Table 1. Composition of essential oil (EO) and alcoholic (AE) extracts and administered doses

Essential oil	Daily dose (ml)	Total dose(ml)*	Plant part
Cinnamomum cassia	0.1	0.3	-
Origanum compact	0.1	0.3	-
Eugenia aromaticum	0.1	0.3	-
Thymus vulgaris	0.1	0.3	-
Peumus boldus	0.17	0.5	-
Laurus nobilis	0.43	1.3	-
$EtOH/H_20$**			
Artemisia absinthium	0.5	5	Aerial part
Juglans regia	0.5	5	Leaves
Rubus fructicosus	0.5	5	Leaves
Allium sativum	0.5	5	Bulb
Tanacetum vulgare	0.5	5	F lowers

*Total treatment period was 3 days for EO and 10 days for AE

**Ethanol contents of different plant extracts varied between 20% and 60%

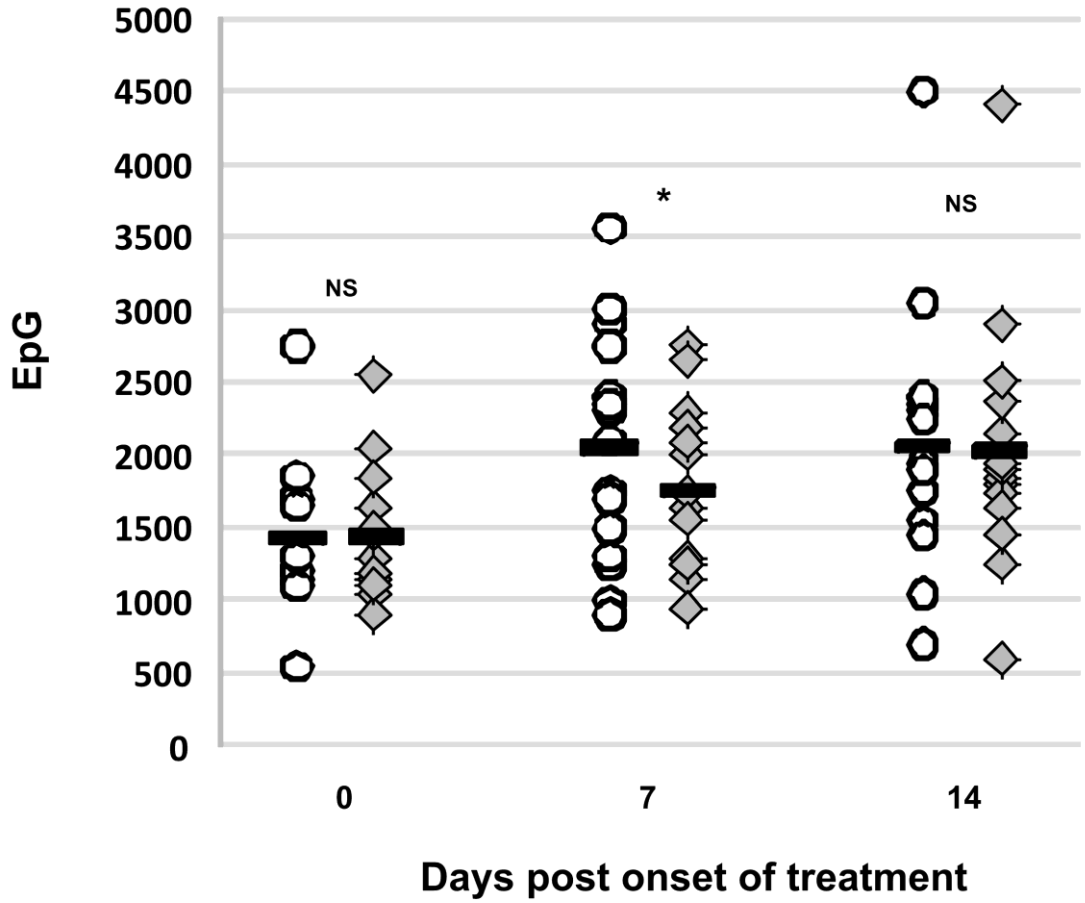

Figure 1. Efficacy of essential oil mixture on FEC as measured by eggs per gramm faeces (EpG), before and 7 and 14 days post treatment. Grey squares represent treated animals (n=12), open circels represent control animals (n=11), black bars indicate group means. NS = not significant, *= significant at $\alpha = 0.05$

SL3C-05

Role of Ethno-veterinary Practices (EVP) in reducing antimicrobial resistance in livestock production systems: a field experience

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Abstract text (including references) A high disease incidence in Indian cross-bred dairy cows leads to high economic losses. Widespread use of antimicrobials and other veterinary drugs in dairy animals cause (a) residues in animal products and (b) a rising number of antimicrobial resistances, both related to consumer's health hazards. The traditional health care approaches have effective solutions for humans, animals and agriculture. The Ethnoveterinary Program of the Trans-disciplinary University of Bangalore documented local ethnoveterinary practices (EVP) in cooperation with the Tamil Nadu Veterinary and Animal Sciences University. Using the rapid assessment method [1] 353 out of the 460 documented ethnoveterinary formulations could be assessed as safe and efficacious. One forty medicinal plants were used in these

formulations They subsequently mainstreamed in to the livestock primary health care through field veterinarians and farmers. Veterinarians (154) were trained for using EVP to manage 15 clinical diseases in livestock. They were given practical training for identification of the plants and raw drug, preparation of the formulations and the application under field conditions. We have also trained 2700 farmers for managing and preventing certain clinical diseases in dairy animals using EVP. The Field study using the fresh EVP formulation for mastitis on 314 cases in three states (Kerala, Karnataka and Tamil Nadu) indicated 97 & efficacy. 240 milk sample collected from the farmers of different milk cooperative from Kerala and Karnataka before and after intervention showed 18 to 49% residue in the milk which indicative EVP-based natural products is an effective alternative to synthetic chemicals in dairy farming. [1]

SL3C-06

Selected oral presentation from the 9th Young Researchers Workshop

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SL4A-01

Haemanthus coccineus extract shows profound anti-inflammatory actions *in vitro* and *in vivo* due to its main alkaloid narciclasine

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Extracts of the bulb of blood lily (*Haemanthus coccineus* L., Amaryllidaceae) have been used in the traditional South African medicine for the treatment of inflammation-associated pathologies, such as febrile colds or asthma. Since there is still a great demand for new therapeutic options against inflammatory conditions, we aimed to pharmacologically characterize the anti-inflammatory potential of an *H. coccineus* extract (HCE) *in vitro* an *in vivo* and to identify the underlying molecular mechanism.

Dried bulbs of *H. coccineus* were extracted using 60 % (w/w) ethanol. The ethanol was largely removed and the remaining solution was partitioned with ethyl acetate. The organic phase was separated and dried (DER 50:1). The resulting HCE contained 2.2 % narciclasine. *In vivo*, HCE (450 mg/kg orally or 2 mg/kg intraperitoneally) reduced edema formation, leukocyte infiltration, and cytokine synthesis in two murine models of inflammation (dermal edema induced by arachidonic acid or croton oil; kidney injury upon unilateral ureteral obstruction). *In vitro*, HCE (100-300 ng/ml) inhibited the interaction of leukocytes with endothelial cells (ECs) and effectively blocked the activation of both cell types (EC adhesion molecule expression; cytokine synthesis and proliferation of leukocytes). At the applied concentrations, HCE did not exert cytotoxicity. Interestingly, HCE suppressed NFκB-dependent gene transcription, but did neither affect the canonical NFκB activation cascade (degradation of IκB,

translocation of p65, DNA-binding activity of NFκB), nor MAPK signaling. Moreover, we could identify the main alkaloid of HCE, narciclasine, as the responsible bioactive ingredient.

In summary, this study highlights HCE and its main alkaloid narciclasine as interesting, novel approach against inflammatory conditions.

SL4A-02

Non-cellulosic glucans as novel initiators for human keratinocyte differentiation: a new approach for wound healing

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The epidermis is a layered tissue consisting particularly of keratinocytes and undergoes continuous regeneration. During a stringently regulated process of terminal differentiation, which is a crucial step during wound healing, keratinocytes move upward to the *stratum corneum* and undergo complex morphological and biochemical alterations.

A recent study revealed that the β-D-glucans xyloglucan (β-1,4/1,6-glucan with xylose-galactose and xylose side chains) from *Tropaeolum majus* L. (XG) and lichenan (β-1,3/1,4-glucan) from *Cetraria islandica* (L.) ACH. (LI) act as inductors for keratinocyte differentiation. Demonstrated by qPCR analysis the expression of the differentiation marker genes involucrin, cytokeratin 10, transglutaminase, loricrin and filaggrin is increased by treatment of primary keratinocytes with XG and LI (100 µg/mL each) in a time dependent manner. The same effect is also detectable on protein level (western blot- and immunofluorescence-studies). These findings could be confirmed by "Whole Human Genome Microarray"-analysis which revealed that most of the upregulated genes during treatment of the cells with XG or LI are related to differentiation processes. For identification of the target molecules of XG and LI, different gel electrophoresis-experiments with membrane preparations of keratinocytes were carried out followed by blotting of the separated proteins on a membrane and incubation of the latter with FITC-labelled XG or LI. Subsequent mass spectrometric analysis amongst others resulted in peptide fragments for EGFR and integrins. Additionally, a human phospho-kinase array for XG-treated cells was carried out which revealed a decreased phosphorylation of EGFR and the transcription factor CREB. Both molecules are known to affect cellular proliferation when activated by phosphorylation. Thus, XG could exert its effect on the cells by blocking the EGFR-signaling serving as an impulse for the initiation of the differentiation process.

SL4A-03

Gastroprotective activity of the rhizome ethanol extract of *Zingiber simaoense* Y. Y. Qian in rats

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Zingiber simaoense Y. Y. Qian belongs to Zingiberaceae family [1,2]. Its rhizome is used in Thai traditional medicine to treat gastric disorders. Since the scientific evidences for its pharmacological activities have not yet been reported, the present study aimed to investigate the gastroprotective activity of its rhizome ethanol extract in rats. The gastric ulcer models induced by ethanol/hydrochloric acid [3], indomethacin [4], and restraint water immersion stress [5] were used to test its activity. After oral administration, the extract at the doses of 7.5, 15, and 30 mg/kg as well as cimetidine (100 mg/kg) significantly inhibited gastric ulcer formation in all models. These findings indicate that the rhizome ethanol extract of *Zingiber simaoense* possesses gastroprotective activity which may relate to the cytoprotective activity and the inhibition of gastric acid secretion.

Acknowledgement: This work was supported by the Center for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education and the Faculty of Medicine Research Fund, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

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SL4A-04

Anti-inflammatory stilbenoids and cannabispiradienone derivatives from *Tragopogon tommasinii* Sch.Bip.

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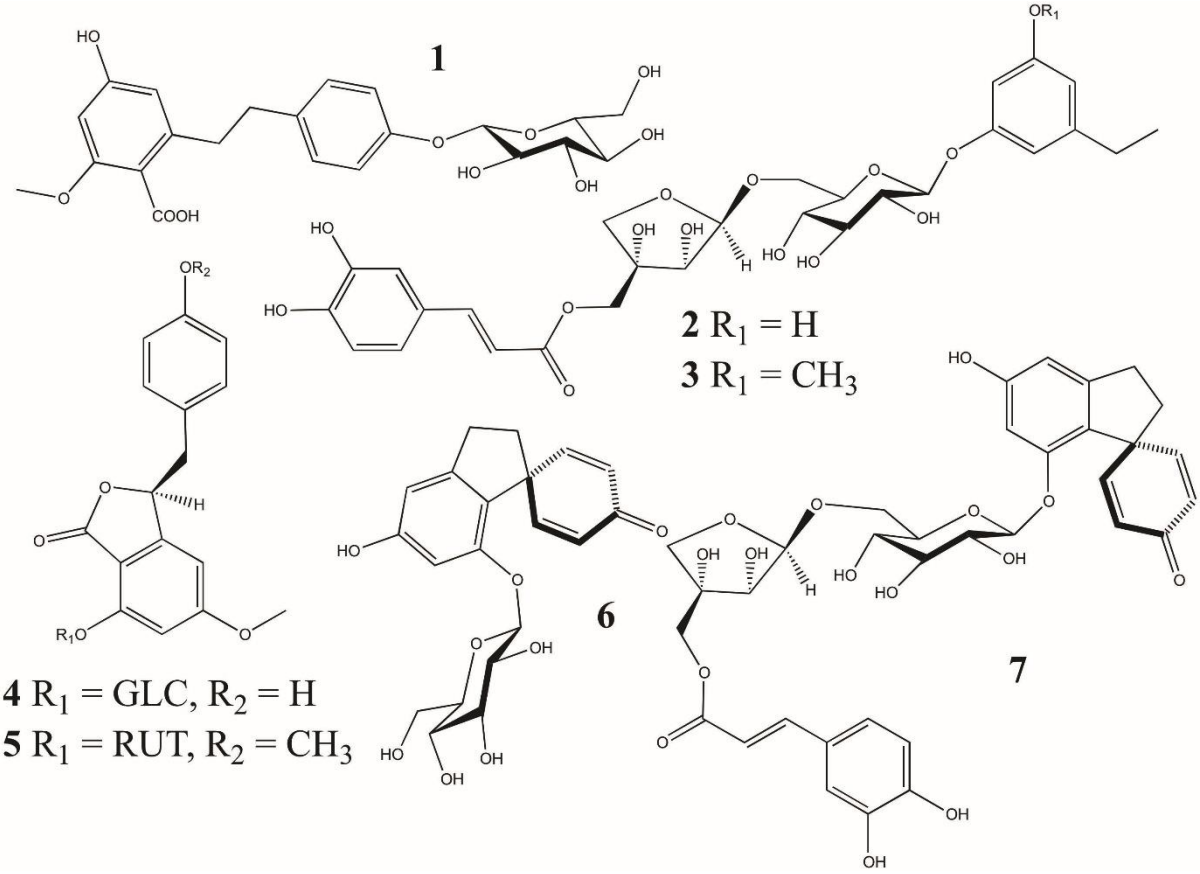
Tragopogon tommasinii Sch.Bip. is a perennial plant with large yellow flowering heads, endemic to the Illyrian floristic region. Previous phytochemical studies Tragopogon taxa yielded flavonoids, phenolic acids, sterols, triterpenes, triterpene glycosides, and triterpene saponins.

An extract prepared from aerial parts of *T. tommassinii* was fractionated using column chromatography with different media followed by the preparative HPLC to yield seven new natural products comprising three simple bibenzyls [2-carboxyl-5-hydroxy-3-methoxy-4'-β-glucopyranosyloxybibenzyl (1), 3-caffeoyl-(9->5)-β-apiosyl-(1->6)-β-glucopyranosyloxy-5,4'-dihydroxy-3'-methoxybibenzyl (2), 3-caffeoyl-(9->5)-β-apiosyl-(1->6)-β-glucopyranosyloxy-4'-dihydroxy-5,3'-dimethoxybibenzyl (3)], two phthalides [7-β-glucopyranosyloxy-(S)-3-(4-hydroxybenzyl)-5-methoxyphthalide (4), and 7-(1->6)-α-rhamnosyl-β-glucopyranosyloxy-(S)-3-(4-hydroxybenzyl)-5-methoxyphthalide (5)], and two cannabispiradienone derivatives [3-O-β-glucopyranosyldemethoxycannabispiradienone (6) and 3-caffeoyl-(9->5)-β-apiosyl-(1->6)-β-glucopyranosyloxydemethoxycannabispiradienone (7)] (Fig. 1). All compounds were tested as potential anti-inflammatory agents using the LPS-stimulated human neutrophils model. The influence of compounds at a concentration of 50 μM on the production of IL-8, IL-1β, TNF-α, and MMP-9 was investigated. Compounds 3, 5, and 7 had the strongest effect and were able to decrease IL-8, IL-1β, and TNF-α production by 27-62 %, 7-29 %, and 70-83 %, respectively. Additionally, compounds 3 and 4 were able to lower the level of MMP-9 by 20-30%. Thus, the isolated compounds could be interesting lead structures for further bioactivity studies.

Acknowledgements: This work was supported by the Fonds zur Förderung der wissenschaftlichen Forschung (FWF, grant P201278-B16 to CZ), the Polish National Science Centre (DEC-2013/08/T/NZ7/00318, PhD fellowship to SG), and the Foundation for Polish Science (START to SG).



SL4A-05

Bronchipret® film-coated tablets exert potent anti-inflammatory activity in a rat model of bronchoalveolitis

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A fixed combination of thyme and primula extract (Bronchipret® film-coated tablets [BRO]) is clinically used for the treatment of acute bronchitis. Anti-inflammatory properties are expected to substantially contribute to its efficacy in patients. We aimed to provide better understanding of the therapeutic potential of BRO on inflammatory key parameters using an *in vivo* model of LPS-induced bronchoalveolitis.

Bronchoalveolitis in male Wistar rats was induced by intratracheal LPS instillation (100 µg/animal). BRO was given daily at 1-10-fold equivalents of the human daily dose (68-680 mg/kg) for up to three days. Animals were sacrificed at 24h intervals up to 72h post LPS challenge to analyze cell infiltration and mediator production in bronchoalveolar lavage fluid (BALF) and myeloperoxidase activity in lung tissue.

BRO significantly reversed the LPS-induced increase of granulocyte numbers and the levels of all analyzed prostaglandins (PGD_2, PGE_2, TXA_2) in BALF at 48h post LPS instillation. It

furthermore significantly normalized MPO tissue activity at 48 and 72h post LPS. All effects ranged in a similar magnitude as those of the positive control dexamethasone (5 mg/kg).

The fixed combination of thyme and primula dry extract contained in Bronchipret® TP exerts anti-inflammatory effects in an *in vivo* model of bronchoalveolar inflammation. The anti-inflammatory activity of BRO should most likely contribute to its clinically proven efficacy as treatment of acute bronchitis in humans.

SL4B-01

UHPLC-qTOF-MS, and SFC-qTOF-MS, combined with traveling wave ion mobility, enables a more comprehensive and reliable analysis, of plant metabolites.

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Metabolomics and lipidomics studies involve targeted and untargeted analysis, of various endogenous biomolecules in complex samples, whereas 5000-20,000 estimated metabolites in a single plant.

NMR, GC/MS, UHPLC/MS, are currently used for metabolome analysis and lately advancements in ion mobility separation, namely Traveling-Wave Ion Mobility (TWIM), have been used to provide additional analyte selectivity in metabolome studies of plants and other organisms.

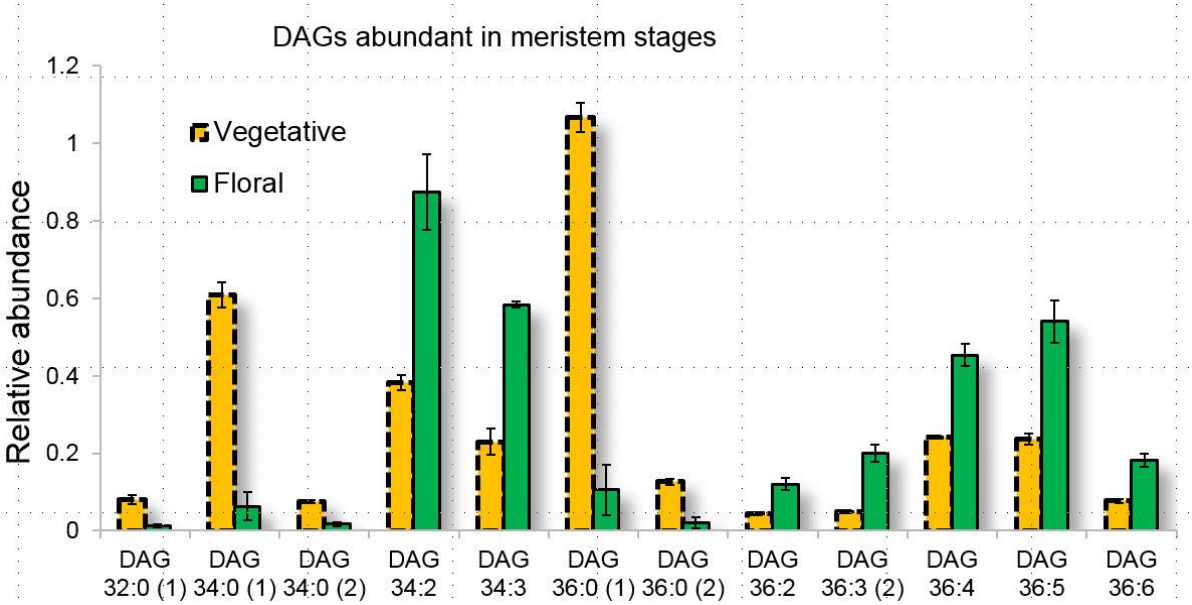
We describe the use of UHPLC-TWIM-MS, and SFC-TWIM-MS, modes for the polar, semi polar and non-polar plant metabolites study. Both modes used to generate the following five parameters per metabolite: accurate mass, isotope ratios, retention time, mass fragments, and the rotationally averaged collision cross-section (CCS) parameters.

A dedicated software tool capable of using all these five parameters, generates data sets with better reliability, for searching our own database and external databases, such as, Traditional Chinese Medicine(TCM), METLIN, ChemSpider, etc.

The addition of ion mobility parameter to the marker criteria and the available software tool backed by manual evaluation, enable us to find many more metabolites in our samples, in less time and better reliability compared to our previous methods. We hope to verify some more of these in the near future

In the study of floral and vegetative meristem from tomato (*Solanum lycopersicum*): Out of 13,347 mass signals and approximately 2500 metabolites found, we have identified 267 lipids so far.

Comparison of the lipids abundance in tomato vegetative meristems versus the floral meristems shows that DAGs with no double bonds have significantly higher abundance in the vegetative as compared to the floral meristem and might indicate an age-related decrease in expression [1].



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SL4B-02

Plant-associated fungi in *Plantago lanceolata* L.: effects on plant drug quality and the bioactive natural products

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The natural products of plant-associated fungi and endophytes is an intensively researched field. Research data on the direct effects of these fungi on the plant bioactive metabolite matrix are, however, rare. In this contribution, we present the main results from our recent work in this topic.

In this work, quality changes of improperly stored *Plantago lanceolata* L. leaves were quantified. The used high relative humidity air resulted in severe reduction of plant drug quality. Alterations in color, microbiological quality (CFU count) and bioactive metabolite content (iridoid glycosides and phenylethanoid glycosides) were quantified. Quantification of the natural products was accomplished using a newly developed capillary electrophoresis (CE) technique, and GC-MS.

Unsurprisingly, storage of the plant material in high humidity air resulted in massive fungal growth accompanied by the decomposition of the bioactive iridoid glycosides (aucubin, catalpol) and acteoside. However, during the modeling of the metabolite decomposition in sterile water extracts of the plant infested with the fungal strains isolated from the plant material, a surprising phenomenon was found: while some fungi increased the decomposition rate of acteoside, several others inhibited its spontaneous decomposition in the aqueous plant extract. These species were shown to secrete a high amount of phenolic acids into the medium,

which acted as antioxidants. These antioxidants were shown to protect acteoside from spontaneous breakdown.

We can therefore conclude, that fungi in medicinal plant materials can enrich the plant metabolite matrix with many metabolites originally linked to the plant, and under some circumstances, the activity of fungi can increase the stability of bioactive natural products.

SL4B-03

The (complete) species composition (plants and fungi) of two commercial samples of Salviae officinalis folium

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The advent of next-generation DNA sequencing (NGS) offers completely new possibilities like the identification of whole communities, termed 'DNA-metabarcoding'. Here we present an assessment to identify plant and fungal species of two commercial samples of sage (*Salvia officinalis* L., Lamiaceae) by a standard DNA barcoding region, the ribosomal internal transcribed spacer (ITS). DNA was extracted and ITS1 and ITS2 pre-amplified. The two samples were barcoded with a short sample recognition oligonucleotide and submitted to next-generation sequencing (Illumina MiSeq). The sequences were 'collapsed' (identical sequences combined into one) and sequences with a coverage of more than 9 submitted to identification. The main species in both samples was (correctly) identified as Salvia officinalis. The spectrum of accompanying plant and fungal species, however, was completely different between the samples. The species composition was also depending on the primer set used what clearly demonstrates the need for primer standardization. This NGS approach for quality control is suitable for routine analysis and gives deeper insight into the real species composition of biological contaminations. Therefore, it would allow a better knowledge-based risk-assessment than any other method available today. However, the method is economically feasible in routine analysis only if a high sample throughput can be guaranteed.

SL4B-04

Bryophyllum pinnatum, a herbal product used in obstetrics and gynecology: New insights into its pharmacological actions and chemical constituents

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Bryophyllum pinnatum (Syn. *Kalanchoe pinnata*, Crassulaceae) is used in anthroposophic medicine to treat restlessness, and as a tocolytic agent to prevent premature labour. In clinical studies, treatment with *B. pinnatum* revealed comparable tocolytic efficacy and less side effects than standard therapy. We investigated the effect of press juice from leaves (the active

ingredient in products), and of extract fractions, on the contractility of human myometrial strips. All tested samples reduced the amplitude and the area under the curve (AUC) of the contractions. Most active were the undiluted press juice and a flavonoid fraction. In addition, the press juice prevented oxytocin-induced increase of intracellular [Ca^{2+}] in human myometrial cells in a concentration-dependent manner.

We also investigated the effect of press juice on detrusor contractibility to explore the potential of *B. pinnatum* as a treatment option in overactive bladder syndrome. At a concentration of 5%, the press juice inhibited detrusor contractibility in muscle strips from porcine bladders by 74.6%. A significant reduction of contractibility to 21% was also observed with the flavonoid fraction.

Besides flavonoids, the plant is also known to contain bufadienolides. A UHPLC-ESI-MS/MS method was validated and used for quantification of the main bufadienolides, in order to provide quantitative data about these constituents of potential toxicological relevance in leaves and manufactured products. The total bufadienolide content ranged from 16.3 to 40.5 mg /100 g dry weight (DW) in leaves from plants grown in Brazil, and was significantly lower in plants cultivated in Germany (3.8-12.5 mg/100 g DW). In press juices obtained from plants cultivated in Germany and Brazil total bufadienolide contents were 0.09-0.16 mg/100 mL, and 0.89-1.16 mg/100 mL, respectively. When analyzing single leaves from individual plants, the content of bufadienolides was markedly higher in young leaves.

SL4B-05

Enzymatic bioautography on HPTLC: combined phytochemical and activity screening tool for quality assessment and *in vitro* cultivation bioprocess control for selected medicinal plants from the Balkan region

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HPTLC is a tool of long term tradition in medicinal plants analysis. Separated compounds are fixed on the solid silica phase like a compound library. By direct performance of visualizable health relevant enzyme reactions on the dried plate, similarities and differences in the phytochemical and activity fingerprints can be identified.

In this work, enzymatic bioautographic inhibition assays, using xanthine oxidase (XOD) [1]; lipase [2] and acetylcholinesterase (AchE) [3] have been optimized and applied for screening to show challenges and pitfalls.

Merck HPTLC plates were used on CAMAG equipment. Flavonoid fingerprints, derivatized with Neu's/PEG reagent were detected at 366nm. Validated, robust procedures have been established. False positive results are avoided by detection at different wavelength.

Fingerprints of less studied, *in vitro* cultivated medicinal plant species (see Table 1: Detection and number of enzyme inhibition zones for the in vitro cultivated species from the Balkan region using bioautography) from the Balkan region showed the following enzyme inhibtory results:

	XOD	Lipase	AchE
Hypericum sp.	+ (1-2)	-	(+)
Pulsatilla sp.	+ (2-4)	+ (1)	(+)
Inula britannica	+ (1-2)	(+)	-
Sideritis scardica.	-	+ (1)	+ (2-4)
Artemisia alba	+ (1)	-	-
Clinopodium vulgare	+ (2-3)	++ (1)	+(1)

Legend: + inhibition, (+) faint inhibition, – no visual inhibition, (number) number of inhibition zones

A binary answer for the activity of the separated zones appears visually on the HPTLC chromatogram. Application of positive controls on the silica layer as a concentration ladder allows for intensity benchmarking as a qualitative control activity equivalent.

It can be concluded, that bioautography offers a rapid and simple tool for screening of secondary metabolite profiles combined with enzymatic inhibition screening by HPTLC, either for quality control purposes or bioprocess control for *in vitro* cultivated medicinal plant biomass. Such assays can complement sophisticated assays to reduce the number of samples.

Acknowledgements: BSRP, grant No. IZEBZ0, 142989; DO2-1153

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SL4C-01

Antiadhesive effect of *Agropyron repens* L. rhizome extract against uropathogenic *E. coli* and pinpointing (*E*)-hexadecyl 3-(4-hydroxyphenyl) acrylate as active ingredient

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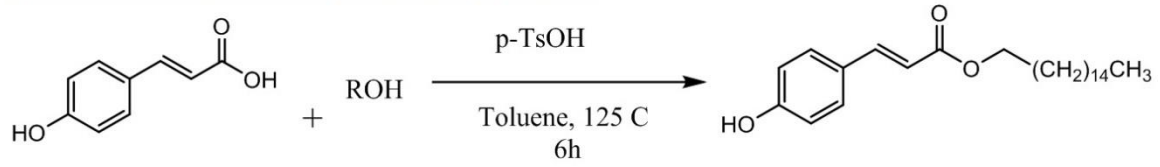
The initial step for colonization and development of urinary tract infections, commonly caused by uropathogenic*Escherichia coli* (UPEC), is the adhesion to bladder cells. Therefore, inhibition of bacterial adhesion at early stages is an alternative approach for specific

prevention. In this study we investigated the antiadhesive activity of extracts from the rhizomes of *Agropyron repens* L. against UPEC.

Extracts with different polarity (water, EtOH 50%, EtOH 90%, acetone, methanol) were prepared and direct cytotoxicity of the extracts was screened against T24 bladder cells and UPEC A2980 strain by MTT assay. None of the extracts inhibited the UPEC growth or influenced cell viability of T24 cells. Influence of extracts on bacterial adhesion was analyzed by *in vitro* flow cytometric assay. Especially the acetone extract significantly decreased the bacterial adhesion (40 % at 1 mg).This extract was fractionated by FCPC and an active compound was identified by ^{1}H-NMR and LC-MS as (*E*)-hexadecyl 3-(4-hydroxyphenyl) acrylate (HHA). HHA was synthesized by esterification of *p*-coumaric acid with the respective C16 alcohol (Fig. 1). Modification of the synthesis resulted in different derivatives, indicating that the hydroxylation of the phenyl part of the molecule is essential for antiadhesive effects, while the length of the alkyl side chain has only minor influence. HHA significantly reduced (50% and 70% respectively at 1 mMol) both adhesion and invasion of UPEC.

Fig.1
Synthesis of alkyl cinnamate HHA from p-coumaric acid and C16 alcohol



SL4C-02

Antifungal activity of quinoline alkaloids from *Waltheria indica*

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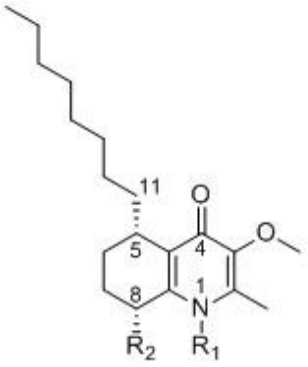
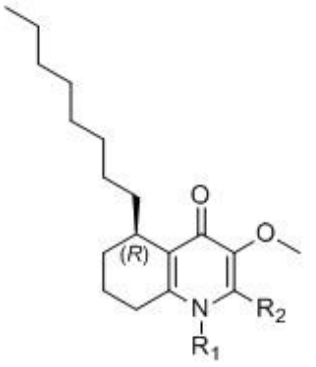
The incidence of infections caused by Candida species (candidosis) has increased considerably over the past three decades, mainly due to a high number of immunocompromised patients (AIDS), an increasingly aged population, and the more widespread use of indwelling medical devices [1]. The increasing number of clinical isolates resistant to azole drugs and the limited number of alternative options (polyenes and echinocandins) have driven the search for new antifungal agents. As part of our project focused on the discovery of new natural antiparasitic compounds from *Waltheria indica* [2], extracts from this plant were screened against *Candida albicans* (CA). From the active alkaloid extract, sixteen 4-quinolone derivatives, i.e. antidesmone (**1**), 8-deoxoantidesmone (**2**), vanessine (**3**) and waltheriones E-Q (**4**-**16**) were isolated and characterized by NMR including 1H-, 13C-NMR, HSQC, HMBC, COSY and NOESY experiments, ECD, UV, IR, and MS. Among these, five compounds **12**-**16** have not yet been described in the literature and vanessine (**3**) is reported for the first time in *W. indica*. All these compounds showed significant *in vitro* inhibition of CA biofilm metabolic activity

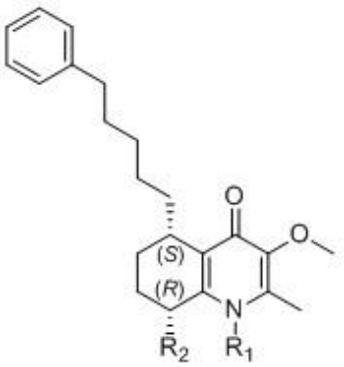
(MIC between 8 and 32 µg/ml), except waltherione O (**14**) with a MIC superior to 64 µg/ml. Moreover, alkaloids **1**, **2**, **4**-**6**, **9**, **13**and **16** inhibited CA planktonic cell growth at pH 4.6 with MIC values between 4 and 32 µg/ml. These promising results encourage further investigations to determine the range of activity towards other Candida species.



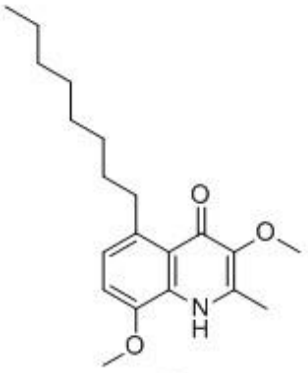
	R_1	R_2
1	H	=O
4	H	OCH_3
9	OCH_3	H
10	OCH_3	OCH_3
16	H	OH



	R_1	R_2
2	H	CH_3
3	H	CH_2OH
8	OCH_3	CH_2OH



	R_1	R_2
6	OCH_3	H
7	OCH_3	OCH_3
12	H	H
13	H	OCH_3
15	H	=O



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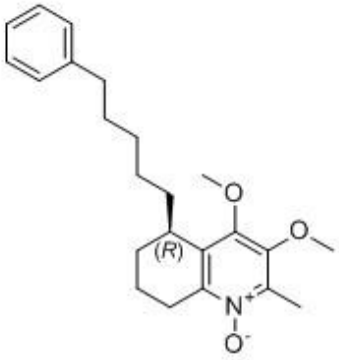

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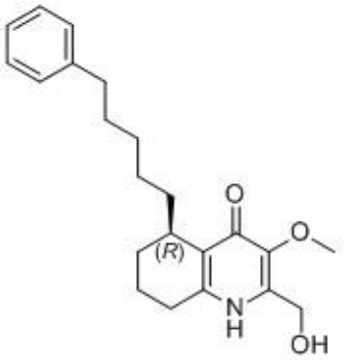

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SL4C-03

Callyaerins, cyclic peptides from the indonesian marine sponge *Callyspongia aerizusa* with potent and selective antitubercular activity

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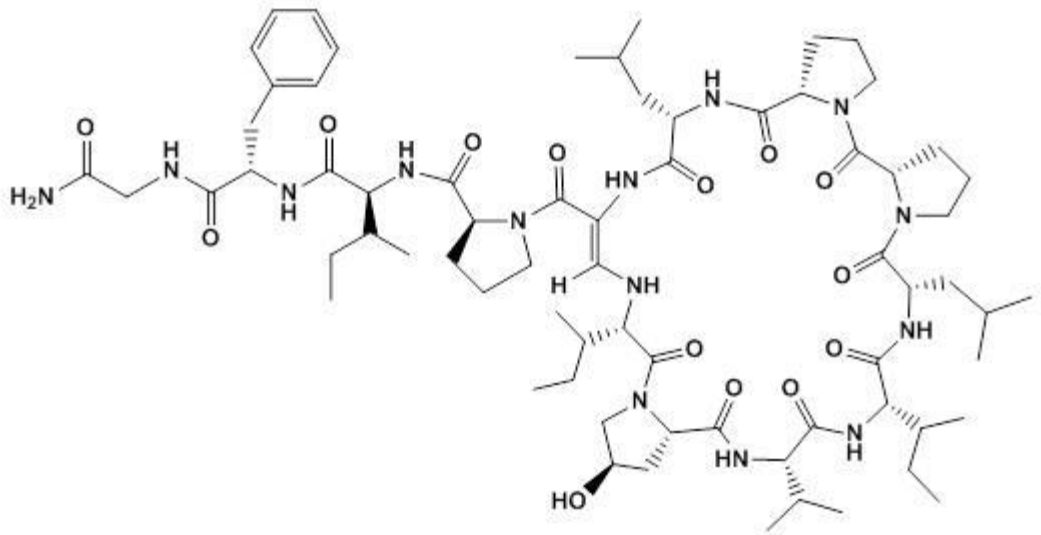
Chemical investigation of the Indonesian sponge *Callyspongia aerizusa* afforded thirteen callyaerin derivatives. The structures of the isolated compounds were unambiguously elucidated on the basis of 1D and 2D NMR spectroscopic data and MS interpretation. The basic structural unit of the callyaerins comprises a cyclic peptide with a linear peptide side chain, both of variable size, linked through a non-proteinogenic (*Z*)-2,3-diaminoacrylic acid (DAA) functional group. The peptides are unusual in containing a considerable number of proline residues, of which one proline is always positioned at the beginning of the side chain, while all others are found in the ring system. The remaining residues are predominantly hydrophobic amino acids, such as Ile, Leu, and Phe. All compounds were investigated *in vitro* against *Mycobacterium tuberculosis*, as well as against THP-1 (human acute monocytic leukemia), and MRC-5 (human fetal lung fibroblast) cell lines in order to assess their general cytotoxicity. Callyaerin A followed by callyaerin B were found to inhibit *M. tuberculosis* at low micromolar concentrations making these compounds interesting candidates for further studies.



Structure of Callyaerin A

SL4C-04

Search for antifungal compounds using a susceptible strain of *Candida albicans* and *in vivo* activity with the *Galleria mellonella* model

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Invasive fungal infections have dramatically increased over the last 20 years. They became a major cause of nosocomial infections in developed countries making urgent the need of new antifungal drugs [1]. In this context, NPs have a high potential to become interesting leads for drug discovery. In our search for new antifungal compounds, four South American plants extracts were selected based on their *in vitro* antifungal activity. These extracts presented interesting activity against*Candida albicans* in a bioautography assay using hypersusceptible engineered strain [2]. This mutant strain was used in order to isolate the minor active constituent which would not have been detected otherwise. Bioassay-guided microfractionation was undertaken using HPLC to localize the active compounds. Different zones of the HPLC-UV chromatogram were linked to antifungal activities. In parallel to this HPLC-based activity profiling, UHPLC-TOF-HRMS was used for the early identification of some of the compounds present. The targeted isolation of the active compounds was performed by MPLC-UV and further semi-preparative HPLC steps. Structures of the isolated compounds were elucidated by spectroscopic methods including UV, NMR, MS and HRMS. Their absolute configuration was elucidated by ECD. Some of them were new NPs. Belong the fourteen isolated compounds, three saponins, two terpenes and two anthraquinones were specifically active against *Candida* spp with MIC below 32 µg/ml. The antifungal properties were also evaluated against biofilms of *C. albicans* and *in vivo* activity was assessed using the *Galleria mellonella* model.

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SL4C-05

Antiparasitic neolignans from leaves of *Nectandra leucantha*

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Previous studies performed with *Nectandra leucantha* Ness & Mart (Lauraceae) afforded three neolignans with significant activity against *Leishmania donovani* [3]. In continuation of our studies with this specie, the n-hexane and MeOH extracts from leaves displayed activity against *Leishmania (L.) infantum* and *Trypanossoma cruzi*. Using a bioactivity guided fractionation, were obtained five neolignans: dehydrodieugenol (**1**), dehydrodieugenol B (**2**), 1,2-dimethoxy-6-[2'-methoxy-4'-(8'-propenyl)phenoxy]-4-(8-propenyl)benzene (**3**), (R)-1-hydroxy-2-methoxy-6-[2'-methoxy-4'-(8'-propenyl)phenoxy]-4-(7-hydroxy-8-propenyl)benzene (**4**), and (S)-1,2-dimethoxy-6-[2'-methoxy-4'-(8'-propenyl)phenoxy]-4-(7-hydroxy-8-propenyl)benzene (**5**), including two new metabolites (**4** and **5**). The structures of isolated compounds were determined by analysis of NMR, UV, IR, HRESIMS, as well as CD spectral data. The *in vitro* antiparasitic activity of the isolated compounds against *L. (L.) infantum* and *T. cruzi* as well as mammalian cytotoxicity was evaluated. Compounds **2** and **3**were effective against the intracellular amastigotes of *L. (L.) infantum* (IC_{50} values of 85.5 and 19.7 μM, respectively) while compound **4** displayed activity against trypomastigote forms of *T. cruzi* (IC_{50} value of 20.5 μM). The mammalian cytotoxicity (CC_{50}) was evaluated against peritoneal macrophages. Compounds **1, 3 – 5** were not toxic up to 200 μM, whereas compound **2** demonstrated a CC_{50} value of 154.2 μM. Therefore, considering the effective antiparasitic activity of the neolignans **3** and **4**, the obtained results suggest these compounds as candidates for future experimental pre-clinical assays against visceral leishmaniasis and Chagas disease.

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SL5A-01

Semi-synthetic preparation, structural elucidation and pharmacological study of nitrogen-containing ecdysteroid analogs

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Ecdysteroids are specific hydroxysteroids that exert a wide a range of biological functions in the flora and the fauna. Our group has recently described that less polar ecdysteroid derivatives can strongly sensitize various cancer cell lines to certain chemotherapeutics, which can be

observed both in multi-drug resistant (MDR) and drug susceptible cancer cells [1-2]. Recent studies also revealed that oxime groups can increase the antitumor activity of certain steroid derivatives [3], but ecdysteroid oximes have previously not been studied from this point of view.

In our current work, we aimed to synthesize C6 ecdysteroid oximes of 20-hydroxyecdysone 2,3;20,22-diacetonide, optimizing the previously available methods [4]. Rotational planar chromatographic, analytical and preparative RP-HPLC and centrifugal partition chromatographic methods were developed for the purification. The diacetonides of two 14,15-anhydro ecdysteroid oxime isomers (E/Z-oximes) were obtained, and Beckmann rearrangement of the new 14,15-anhydro E-oxime yielded another new derivative containing a seven-membered lactam B-ring [5].

The ecdysteroid oximes were tested for their activity against L5178 mouse T-cell lymphoma cells (non-MDR) and their sub-cell line transfected with pHa MDR1/A retrovirus overexpressing the human ABCB1 efflux pump (MDR cell line). Each oxime was found to be a promising modulator of resistance on the MDR cell line, and the E-oxime could also sensitize the non-MDR cells to doxorubicin. Our preliminary SAR study also revealed, that the antitumor activity of ecdysteroid diacetonides follow the ecdysteroid < Z-oxime < E-oxime order.

Acknowledgement: Szeged Foundation for Cancer Research.

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SL5A-02

Charting biological activity in chemical property space using ChemGPS-NP

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First developed in 2007 the chemographic model of natural products chemical property space, ChemGPS-NP, has proven useful for a series of applications [1-3]. In the more recent work, one focus has been charting and interpreting the distribution of 'neighbourhoods' corresponding to various biological activities.

Implementing such a map may serve as a tool for rapid interpretation, selection of potential 'Compounds Of Interest' (COIs) – or a similar de-selection of compounds that appears to lack the combination of properties required to take up residency in a particular neighbourhood.

Furthermore, when successively described in more detail, this map could be used to provide a first insight into the potential biological activity of a novel compound – tuned to provide support for the often structurally complex natural products.

In this study we will demonstrate how ChemGPS-NP can be used to rapidly evaluate datasets ranging from hundreds to thousands of molecules, characterizing their neighbourhoods, and subsequently validate this map by attempting prediction of a control-set of compounds with experimentally determined activities. For the purpose of describing these neighbourhoods, multi-dimensional volumes, discriminant analyses, Euclidean distances and structure-fragment based indices will be employed.

[1] Larsson et al. (2007) ChemGPS-NP: Tuned for navigation in biologically relevant chemical space. J. Nat. Prod. 70: 789-794.

[2] Muigg et al. (2013) In silico comparison of marine, terrestrial and synthetic compounds using ChemGPS-NP for navigating chemical space. Phytochem. Rev. 12: 449-457.

[3] Rosén et al. (2009) ChemGPS-NP mapping of chemical compounds for prediction of anticancer mode of action. QSAR Comb. Sci. 28: 436-446.

SL5A-03

2, 3-dehydrosilybin: A new potent inhibitor of Glyceraldehyde-3-phosphate-dehydrogenase from*Trypanosoma brucei* identified by in silico screening and an enzyme inhibition assay

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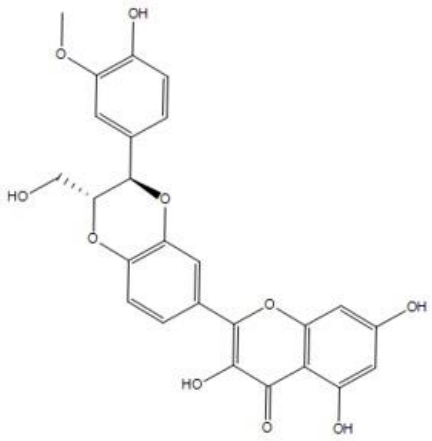
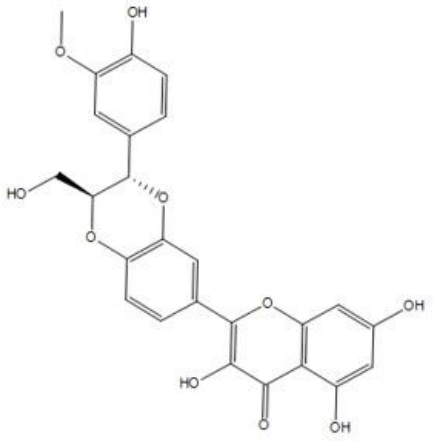
In continuation of our screening [1] aimed at the identification of natural inhibitors of Glyceraldehyde-3-phosphate-dehydrogenase from *Trypanosoma brucei* (TbGAPDH), a commercial natural product database (Phytolab GmbH) was examined *in silico* by pharmacophore based virtual screening and molecular docking. Experimental validation of the *in silico*hits was carried out by a photometric enzyme inhibition assay with recombinant TbGAPDH [1]. Virtual screening of the mentioned database (1100 molecules) against GAPDH of various parasites resulted in the identification of 18 potential hits. Noteworthy, six silybin-related flavolignans occurring in *Silybum marianum* (Asteraceae) were among the *in silico* hits. The enzyme assay proved one of them, 2, 3–dehydrosilybin A **1** to inhibit the enzyme quite efficiently (≈50 % inhibition at 75 µM) while most others (silybin B, isosilybins A and B as well as silychristin) did not show any inhibition. The fact that **1**, and only to a lesser extent its enantiomer **2** (Fig. 1), showed inhibitory activity, promises insight into a highly specific mechanism of inhibition. The EC_{50} values for **1** and **2** were determined at 31.6 and 43.7 µM. Maximum inhibition by higher concentrations of **1** was about 60% but only about 25% by **2**, due to poor solubility. 2,3–dehydrosilybin A may thus be a potential lead structure for further inhibitor design and mechanistic studies. Evaluation of the antitrypanosomal activity in cellular tests is in progress.



2, 3 – dehydrosilybin A

1



2, 3 – dehydrosilybin B

2

Fig. 1: Structures of dehydrosilybins A and B

Acknowledgments: Test compounds were kindly donated by Phytolab GmbH, Vestenbergsgreuth, Germany. Bacteria containing the expression plasmid for TbGAPDH were kindly provided by M.L. Bolognesi and S. Piretti (Bologna, Italy) and P. Michels (Edinburgh, U.K.). Support of CCG, Montreal, Canada, is gratefully acknowledged.

This work is part of the activities of ResNetNPND: http://www. ResNetNPND.org/

[1] Herrmann F , Schmidt T J. Planta Med. 2013; 79 1132

SL5A-04

Availability and quality of biomass as key factor for large scale isolation of natural products

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TEVA Czech Industry, Opava, Czech Republic

The pharmaceutical factory Galena in Opava (since 2006 a part of TEVA Group) is a traditional producer of natural products: all ergot alkaloids isolated from field ergot, some morphinan alkaloids, some other natural drugs isolated from herbal material (galanthamine, silymarin and paclitaxel) and some products of fermentation (cyclosporine, tacrolimus). The successful manufacture of all these products depends on availability and quality of the biomass used for the large scale isolation. This claim will be demonstrated on examples from the whole our history: why we were successful in ergot alkaloids, why we were not successful in cardenolides, what is actual situation in manufacture of silymarin, galanthamine and paclitaxel. Mentioned will be also some actual problems with availability of plant material e.g. biomass of *Podophyllum hexandrum* for isolation of podophylotoxine which is the starting material for

anticancer drug etoposide. And last but not at least, actual situation in manufacture of Ingenol angelate will be discussed in the lecture.

SL5A-05

Separation and purification of nine platycosides from Platycodi radix by pHPLC and a flash-freeze treatment

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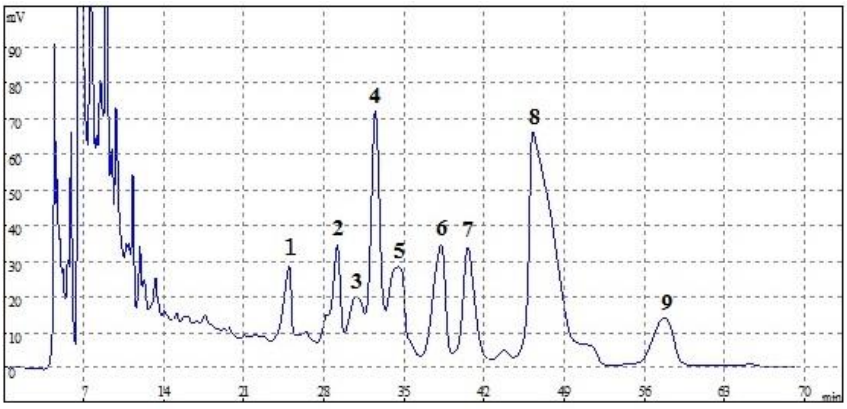
Platycodi radix, a frequently used herb and food in East Asia, mainly contains platycosides. Acetyl platycosides present in this herb readily undergo transacetylation. The activation energy and enthalpy of the reaction are respectively 63.01 and 7.48 kJ/mol, which indicates that these acetylated compounds are highly unstable and difficult to purify [1]. Herein, a fast and simple method to purify these unstable components as well as other platycosides is described.

Nine platycosides including four acetyl platycosides were separated by pHPLC. The separation was carried out on a preparative RP-C_{18} column (250 x 30 mm, 10 μm) eluted with 25% acetonitrile (V/V) at a flow rate of 10 ml/min (Fig 1). The eluted fractions were immediately frozen using a flash-freeze device, in order to prevent acetyl migration. The frozen fractions were subsequently dried by freeze-drying to give the pure compounds without acetyl transfer reaction. Separation of 300 mg of sample gave amounts of pure platycosides ranging from 2 ~ 40 mg. This pHPLC method showed higher separation efficiency and a more convenient sample preparation, compared with high speed counter current chromatography (HSCCC) previously reported [2-3]. It represents also to the best of our knowledge the first report of the isolation of unstable acetyl-platycosides from Platycodi radix by pHPLC.

Fig.1 Representative pHPLC chromatogram of *Platycodi Radix*



1: platycoside E; 2: platycodin D3; 3: deapioplatycodin D; 4: platycodin D; 5: polygalacin D; 6: 3″-*O*-acetylplatycodin D; 7: 2″-*O*-acetylplatycodin D_2; 8: 2″-*O*-acetylplatycodin D; 9: 3″-*O*- acetylplatycodin D_2

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[2] Ha YW, Kim YS. Preparative isolation of six major saponins from Platycodi Radix by high-speed counter-current chromatography. Phytochem Anal 2009; 3: 207-213

[3] Ha IJ, Kang M, Na YC, Park Y, Kim YS. Preparative separation of minor saponins from Platycodi Radix by high-speed counter-current chromatography. J Sep Sci 2011; 34: 2559-2565

SL5A-06

Selected oral presentation from the 9th Young Researchers Workshop

SL5B-01

Anthelmintic effects of tannin-rich plants and oligomeric proanthocyanidin clusters against parasitic and free-living nematodes

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Based on the results of an ethnopharmacological study in Ghana, a leaf extract (EtOH 50%) of *Combretum mucronatum*was investigated by bioassay-guided fractionation which resulted in condensed tannins as the active compounds. Further components identified were flavonoids, including a biflavanoid with an unusual biphenyl linkage. Therefore, extracts from other tanniferous plants (*Paullinia pinnata* roots (EtOH 50%; acetone), *Rhododendron ferrugineum* leaves (H_2O), *Rumex acetosa* herb (ac. 70%) and *Crataegus* spp. leaves (EtOH 45%) were included in this follow-up study. Extracts (1–1000 µg/mL) and purified oligomeric procyanidin (OPC) clusters (1–1000 µM) with a degree of polymerization from 2 to 10 showed a lethal activity against larval stages of *Toxocara cati* and *Trichuris vulpis*. The effect was dependent on the molecular size of the compound, with the exception of one tetrameric cluster mainly composed of Procyanidin D1 (LC_{50} 18 µM). Extracts from *P. pinnata* (ac.) and *C. mucronatum* were most active with LC_{50} 22 µg/mL and LC_{50} 46 µg/mL. Levamisole (1 mM) served as a positive control. The best selectivity was shown by the *Crataegus* extract: LC_{50} 179 µg/mL vs. a low cytotoxicity against Caco-2 cells (IC_{50} 918 µg/mL). The stability of the active compounds during a simulated gastric passage (pH 2, 37 °C, 2h) was confirmed using *C. mucronatum* extract followed by an anthelmintic assay against *Caenorhabditis elegans*. The worms' response to xenobiotic and oxidative stress during the incubation with a flavonoid-rich fraction from *C. mucronatum* was investigated in transgenic *C. elegans* expressing a glutathione-S-transferase-4-GFP transcriptional fusion, but no increase in the expression was observed. Microscopic observations of dead larvae of *T. cati* revealed disruptions of the cuticle. In summary, OPCs exert a strong *in vitro* anthelminthic activity which is currently being further investigated in controlled animal studies as well as concerning the underlying molecular mechanism.

SL5B-02

Osteoprotective activity from *Pholidota articulata* Lindley (Orchidaceae): A traditional plant used for healing fractures in Uttarakhand Himalaya, India

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Pholidota articulata Lindley (PA) family Orchidaceae is commonly used for healing fractures in folk traditions of Uttarakhand Himalaya, India. Bone remodeling is a constant and dynamic process that requires the involvement of osteoblasts, which helps in bone formation and mineralization. Present study was aimed to characterize the fracture healing properties of PA to validate the folklore claims of healing fractures. The osteogenic potential of ethanolic extract of PA was evaluated using Ovariectomized (Ovx) estrogen deficient adult female Balb/c mice (an animal model for post-menopausal osteoporosis). Subsequently, three phenanthrene derivatives isolated from ethyl acetate fraction of PA and evaluated through alkaline phosphatase assay (ALP) and mineralization assay. The ethanolic extract of PA exhibited significant restoration of trabecular micro architecture in both femoral and tibial bones, better bone quality and devoid of any uterine estrogenicity. One of the isolated phenanthrene derivatives, oxoflavidin enhanced ALP activity (a marker of osteoblast differentiation), mineral nodule formation and mRNA levels of osteogenic markers like BMP-2, Type 1 Collagen, RUNX-2 and osteocalcin. These results supported and validate the bone healing properties of PA and demonstrate the therapeutic potential for osteogenic activities during post-menopausal osteoporosis.

SL5B-03

Rationalization of the traditional use of *Antidesma ghaesembilla* to treat hormone related disorders

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[2] *Technische Universität Dresden, Faculty of Mathematics and Natural Sciences, Department of Biology, Dresden, Germany*

Antidesma ghaesembilla, a tropical Asian plant also known as black currant tree, is widely used as medicine to treat headaches, to stimulate menstrual flow and breast milk production but also as purgative. Antibacterial activity, antioxidant capacity, sedative and anxiolytic potentials of the methanolic extract are already described in literature. Part of the described activities might be attributed to interactions with estrogenic receptors. Thus, the aim of this study was to investigate crude extracts of *A. ghaesembilla* for estrogenic effects and to identify potential active principle(s).

Experimentally, extracts and single constituents were tested using estrogen receptor subtype specific transactivation assays in human bone-derived U2OS cells. Cytotoxicity was measured

by using MTT assay in U2OS cells. Both DCM and MeOH bark extracts exhibited appreciable estrogenic but also subtype specific anti-estrogenic effects. Three compounds, a novel aristolochic acid derivative, 6β-hydroxy-stigmast-4-en-3-one, and asperphenamate were isolated from the DCM extract. Asperphenamate, showed a high, dose-dependent estrogenic activity comparable to the positive control 17β-estradiol. Asperphenamate displays moderate cytotoxic activity against several cancer cell lines but to our knowledge the estrogenic activity is so far unknown. The aristolochic acid derivative showed a weak subtype specific agonistic effect. No estrogenic activity was observed for 6β-hydroxy-stigmast-4-en-3-one. Five single compounds could be isolated from the methanolic extract, but no significant estrogenic effects were detected for those compounds.

This study on estrogenic properties of *Antidesma ghaesembilla* extracts and their active constituents should contribute to a better, science based understanding of traditionally known medicinal uses of plants.

SL5B-04

The preliminary study searching a potential activity of *Lamium album* extracts as a background for their traditional use

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Preparations from aerial parts or flos of *Lamium album* (white dead nettle, Lamiaceae) were used in rash in children, as well as catarrh and uterus or vagina inflammations [1]. Considering that neutrophils are cells of immune system involved in the inflammation, we used them to determine the potential anti-inflammatory activity of dead nettle extracts.

The aim of the study was a phytochemical analysis and a comparison of activity of ethanolic extracts from *L. album* flos and aerial parts using model of human neutrophils.

The composition of extracts was determined with HPLC-DAD-MS/MS method. The inhibition of neutrophils oxidative burst was analyzed after receptor-mediated (f-MLP) and non-receptor-mediated (PMA) stimulation, using luminol- or lucigenin- dependent chemiluminescence. The effect of extracts on cytokines, such as IL-8 and TNFα, secretion after LPS-induced stimulation was established with ELISA assay.

The most abundant compounds of ethanolic extracts of both flos and aerial parts of dead nettle have been iridoid glucosides (lamalbide), phenylpropanoids (lamalboside, echinacoside, verbascoside), phenolic acids (chlorogenic acid) and flavonoids (quercetin and kaemferol derivatives). The effect of ethanolic extracts on ROS production and cytokines secretion in human neutrophils has been presented in table 1.

Table 1

Neutrophils functions	*L. album* flos 25 µg/ml	*L. album* aerial parts 25 µg/ml	Stimulated control	Unstimulated control
ROS (f-MLP)	44.7 ± 8.8%	65.4 ± 7.2%	100.0 ± 12.6%	23.3 ± 13.5%
ROS (PMA)	60.9 ± 13.0%	54.3 ± 8.0%	100.0 ± 12.6%	9.4 ± 3.0%
IL-8	76.7 ± 25.8%	105.4 ± 12.4%	108.2 ± 10.8%	9.5 ± 8.5%
TNFα	105.8 ± 43.3%	104.4 ± 20.0%	97.1 ± 14.1%	34.3 ± 16.9%

In conclusion, the ethanolic extracts of both *L. album* flos and aerial parts have shown particularly antioxidant activity, probably due to the presence of phenolic compounds, what has partly explained the traditional use of these plant materials.

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SL5B-05

Anti-hyperuricemia natural products from medicinal plants used by local tribes in Indonesia

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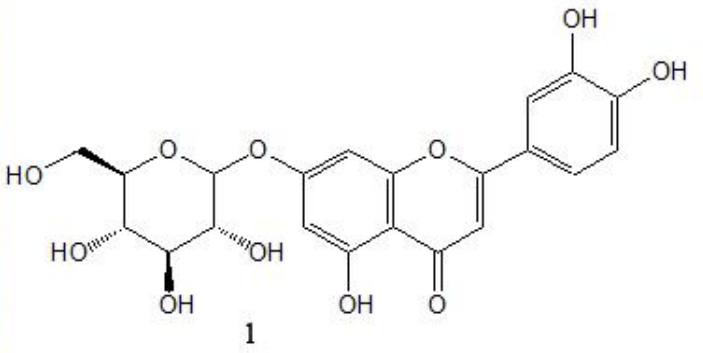
Hyperuricemia, results from the overproduction or underexcretion of uric acid. During the last step of purine metabolism, xanthine oxidase (XO) catalyzes the oxidation of xanthine and hypoxanthine into uric acid [1]. XO inhibitor has the potential to be a therapeutic agent for hyperuricemia and ROS-induced diseases [2]. Allopurinol, an XO inhibitor that has been used clinically for several decades possesses some unwanted negative effects such as hepatitis, allergies and toxicity of 6-mercaptopurine [3]. In our search into natural antihyperuricemia agents, extracts of 30 medicinal plants from 19 families were evaluated by the XO inhibitory assay. Of the 30 extracts assayed, extracts from *Sonneratia caseolaris* (Sonneratiaceae) leaves and *Zingiber purpureum* (Zingiberaceae) rhizome displayed potential to inhibit the XO with activity greater than 50% at 50 mg/ml. Bioassay-guided fractionation of the leaves extract of *S. caseolaris*, led to the isolation of compound 1, identified as luteolin-7-O-glucoside. Simultaneously, XO inhibitory assay-guided isolation of the extract of *Z. purpurea* rhizome gave compound 2, identified as 3-(3,4-dimethoxyphenyl)-3,4-dimethoxystyryl-cyclohex-1-ene. Compounds 1 and 2 inhibited the activity of XO with the IC_{50} values of 12 mg/ml and 27 mg/ml, respectively. Allopurinol showed IC_{50} value of 6 mg/ml in our XO inhibitory activity assay.



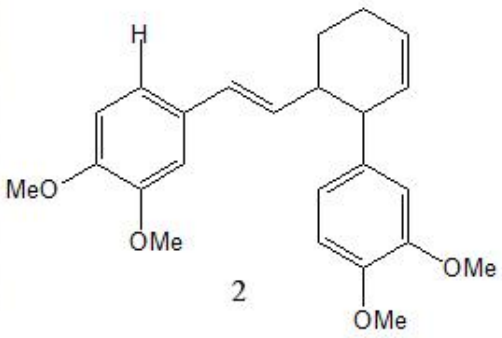


Fig. *Sonneratia caseolaris* leaves (upper left) and *Zingiber purpurea* rhizome (lower left) and the isolated xanthine oxidase inhibitory compounds.

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SL5B-06

Kew's Medicinal Plant Names Services (MPNS) enable effective information retrieval and communication

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Royal Botanic Gardens, Kew, Richmond, United Kingdom

MPNS (www.kew.org/mpns) is enhancing the safety, quality and effectiveness of global plant-based medicine research and practice. We are achieving this by offering a range of Information Services enabling individuals and organisations to communicate reliably when using plant

names. The users of MPNS's services include professionals working in public health, legislation and research, the pharmaceutical industry, herbal trade, and conservation.

The Royal Botanic Gardens, Kew, manages the most comprehensive and authoritative global plant name and taxonomic resources: The Plant List (TPL), International Plant Names Index (IPNI) and World Checklist of Selected Plant Families (WCS). These resources satisfy the needs of taxonomists but have limitations for those working in pharmaceutical research or public health: notably the lack of pharmacopoeia and other non-scientific names.

Knowing all possible names used to refer to a plant is necessary to search literature comprehensively, to find all patient records, to achieve unambiguous legislation and to safely prescribe herbal products. Searching PubMed with one scientific plant name will retrieve only a fraction of the publications that actually refer to that plant.

MPNS has created a new resource which records all names for herbal substances used in pharmacopoeias or the medicinal literature and maps these onto the current scientific botanical nomenclature within Kew's comprehensive resources. The MPNS resource is used to offer services for name validation, training, controlled vocabularies (e.g. a recent ISO standard for health regulators globally), consultancies and web services for those with their own IT systems. The MPNS portal enables users to search for individual names, resolve nomenclatural confusions, and locate data published in other online resources that use synonyms.

We now look to extend MPNS to cover plants used in food supplements, nutraceuticals and medical devices, and plants of known toxicity.

SL5C-01

Effects of a *Melissa officinalis* special extract on mood and cognitive function

Sybille Buchwald-Werner, Itxaso Vazquez

Vital Solutions GmbH, Langenfeld, Germany

In the present study a *Melissa officinalis* special extract was investigated for its mood modulating effects. Literature data have shown that selected *Melissa officinalis* breeding lines support new applications on cognitive performance based on cholinergic receptor binding properties [1].

In our in vitro study the Melissa officinalis special extract was tested at 200 µg/ml for its ability to inhibit MAO-B and PDE4 enzyme activities which are relevant enzymes for memory and mood modulation. The extract showed an MAO-B inhibition of 60% compared to the positive control pargyline (100% inhibition; 0.159 ng/ml). The extract showed a PDE4 inhibition of 30% compared to the positive control rolipram (63% inhibition; 2.753 µg/ml).

Furthermore, positive effects on cognition and mood were confirmed in 3 independent double-blind, randomized, placebo-controlled, balanced-crossover, monocentric studies involving 25 healthy people each. Effects were evaluated using the Cognitive Drug Research (CDR) core battery, a computerized cognitive assessment system, 1 and 3 hours after intake. Our study results demonstrated significant improvements in cognition and mood by intake of 300 mg lemon balm extract after 1 and 3 hours compared to base-line and placebo. The present data

are results from the first human study carried out for the*Melissa officinalis* special extract in a functional beverage formulation [2].

In conclusion our data demonstrate that a *Melissa officinalis* special extract has beneficial cognitive and mood modulating effects.

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SL5C-02

The blood-brain barrier permeability properties of plant *N*-alkylamides

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N-alkylamides (NAAs), a large group of secondary metabolites occurring in more than 25 plant families, are potential drug candidates as several biological and medical functions are known such as central nervous activities [1].

Our general goal was to investigate if NAAs with a different structure, *e.g.* spilanthol and pellitorine, are able to pass the blood-brain barrier (BBB) and if so, to what extent.

A blood to brain (multiple time regression (MTR)), as well as a brain to blood (efflux) transport experiment were conducted in an *in vivo* mice model to investigate the initial BBB rate kinetics. The mice were anesthetized, after which the NAA dose solution was injected. Blood was obtained at regular time points after injection, and thereafter, the mice were decapitated and the brains collected. Quantification of the NAAs was done using a bio-analytical LC-MS method.

The MTR experiment indicated that spilanthol was able to cross the BBB in mice. A rapid but highly significant influx of spilanthol into the brains was observed with an unidirectional influx rate of 217.0 µl/(g×min). The curve reached a plateau-phase after about 10 minutes exposure time and can be explained by efflux of spilanthol out of the brain or distribution or elimination of spilanthol. The efflux transfer constant calculated for spilanthol was 0.1 min^{-1}. This equals a brain half-time disappearance of 6.4 min. The comparative results of on-going BBB studies, including the permeability properties of pellitorine, will be reported as well.

Seen the different possible pharmacological targets of these plant NAAs and their pharmaco-ethnological use, our BBB results may trigger further exploration into the medicinal use of these important plant metabolites.

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SL5C-03

Agrimonia pilosa Ledeb. prevents cognitive dysfunction and energy and glucose dysreguation in rats with experimentally induced memory loss

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We evaluated whether long-term oral consumption of cinnamon (CCB), *Lonicera japonica Thunb.*(LJT), or *Agrimonia pilosa Ledeb.* (APL) improves cognitive dysfunction and glucose homeostasis in rats with dementia.

Male rats received hippocampal CA1 infusions of β-amyloid (25-35) (AD) or β-amyloid (35-25) (Non-AD, normal-control), at a rate of 3.6 nmol/day for 14 days. AD rats were divided into 4 groups receiving either 3% lyophilized water extracts of CCB, LJT, or APL or 3% dextrin (AD-C) in high fat diets. Cognitive function was measured by water maze and passive avoidance tests, and metabolic function and gene expression were measured.

Hippocampal β-amyloid (25-35) deposition was increased as was the phosphorylation of tau by impaired hippocampal insulin signaling and decreasing the expression of TNF-α and iNOS (neruoinflammation markers) in AD-C, but β-amyloid (35-25) accumulation was not detected in Non-AD. CCB, LJT and APL prevented the attenuation of hippocampal insulin signaling by decreasing the β-amyloid accumulation. AD-C exacerbated short-term and spatial memory loss whereas CCB, LJT and APL protected it and APL had a similar cognitive function to Non-AD. AD-C rats gained less weight than Non-AD due to decreased energy intake and increased energy expenditure whereas only CCB had weight gain up to Non-AD and APL did not gain weight due to markedly increased daily energy expenditure. AD-C decreased fat oxidation and used carbohydrate more than fat, but that was reversed by CCB, LJT and APL. APL-treated rats had less visceral fat than AD-C rats by marked increase of fat oxidation. AD-C rats exhibited impaired insulin sensitivity and increased insulin secretion during OGTT in comparison to Non-AD whereas CCB, LJT and APL prevented the impairment.

APL was most effective for preventing memory loss and the disturbance of energy and glucose homeostasis in β-amyloid infused rats. APL is a potential therapy for Alzheimer's disease.

SL5C-04

Preclinical evidence for rational use of bergamot essential oil in pain trials

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The essential oil (EO) of bergamot is used in aromatherapy to minimize stress-induced anxiety, mood disorders and chronic pain. Preclinical data show that for systemic administration bergamot EO is endowed with behavioural and EEG effects being a functional reflection of the phytocomplex interfering with synaptic plasticity (see Bagetta et al., 2010, Fitoterapia 81, 453-461). Here we now report experimental evidence demonstrating that bergamot EO is effective in controlling neuropathic pain. Peripheral nerve damage causes neuropathic pain manifested by hyperalgesia and allodynia. Bergamot EO (1 ml/kg s.c. given once daily/7 days) attenuates mechanical allodynia in the spinal nerve ligation model of neuropathic pain in mice ($n=10$; $p<0.05$ vs control) and this has been attributed, at least in part, to the anti-inflammatory action of linalool at the spinal level. In partial sciatic nerve injury model, injection of this EO in the injured paw (20 μg; $n=10$) induces a local anti-allodynic effect ($p<0.01$ vs control); administration of the EO in the controlateral hindpaw does not attenuate nociceptive response. Similar results are observed after local injection of linalool (10 μg/paw; $n= 10$) though the effect induced by the monoterpene is longer lasting. Neuropathic pain is often resistant to opioids and neither systemic nor i.t. morphine is effective. In mice, a large dose of morphine (256 μg) is required to achieve an anti-allodynic effect similar to that obtained with a much lower dose in the nociceptive, capsaicin test. In neuropathic mice, intraplantar injection of very low doses of morphine (4.0-64 μg) induces a dose-dependent analgesic effect when combined with inactive doses of bergamot EO (5.0 μg) or linalool (2.5 μg). The latter originates from interactions with primary afferent neurons and is important since unwanted side effects in the CNS are avoided. In conclusion, our data form the rational basis for translation of EO of bergamot in pain clinical trials.

SL5C-05

Structural modification of xanthohumol C and the effect on inducing differentiation in neural precursor cells

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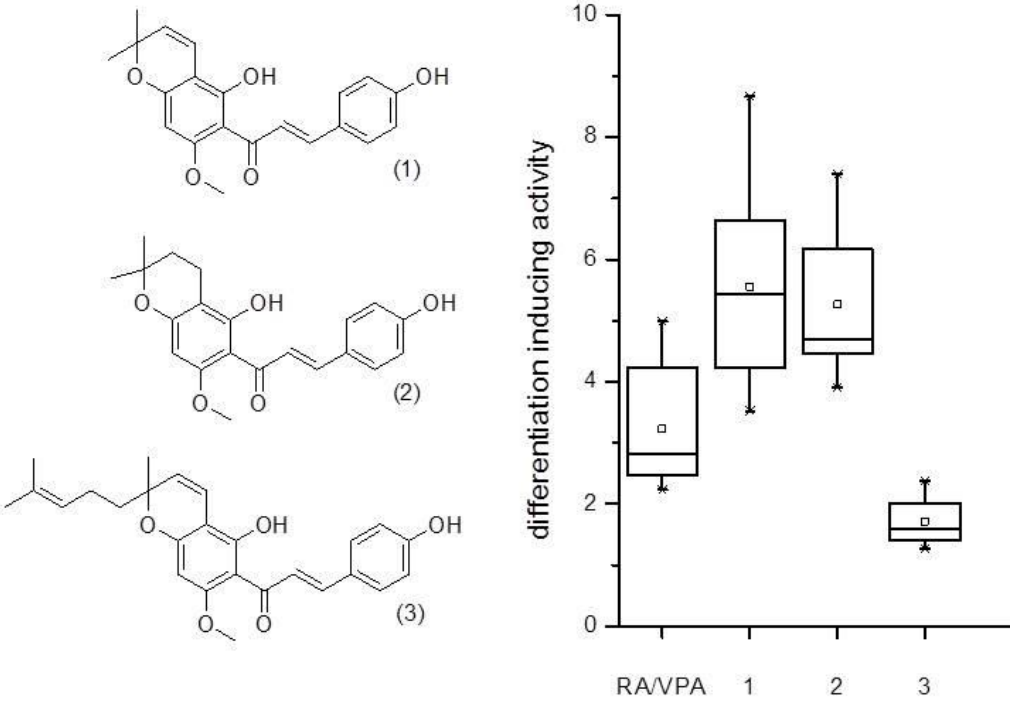
Destructive diseases concerning the nervous system are an increasing burden in our aging society. After the dogma of the non-regenerating nervous system had to be dropped, a new therapeutic approach arose – the regeneration strategy. In an optimal way, endogenous regeneration could be induced by small molecules using stimulation of differentiation of neural stem cells, without an invasive intervention.

Recently a pyranoflavonoid of *Humulus lupulus* L. was identified as potent inducer of differentiation in neuronal precursor cells [1]. To quantify the activity, a dual luciferase reporter gene assay based on doublecortin, a marker of early neurons, was used. The most active compound was the pyranochalcone, xanthohumol C.

Accordingly, we questioned if this compound can be used as lead structure for a further development in a regeneration approach. Hence, we compared side-chain open (prenylflavonoids) with side-chain closed flavonoids (pyranoflavonoids) which led to the assumption, that the pyrano ring is one structural characteristic beneficial for differentiation induction.

Consequently, the next step concerning a lead structure was the variation of the chromene structure. Firstly, the chain length of alky substituent and the degree of saturation was modified. Secondly, the oxygen in the pyrano ring was replaced by hetero atoms like sulfur or nitrogen, to reach a different chemical surrounding.

While the side chain prolongation caused a significant loss in activity, the level of activity wasn’t changed by the saturation of the chromene system compared to xanthohumol C.



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SL5C-06

Salicin from willow bark can modulate neurite outgrowth in human neuroblastoma SH-SY5Y cells

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Salicin from willow bark has been used throughout the last centuries in China and Europe for the treatment of pain, headache, and inflammatory conditions and is chemically related to aspirin (acetylsalicylic acid). Recently it could be demonstrated that salicin binds and activates the bitter taste receptor TAS2R16. Studies on rodent tissues showed the general expression of bitter taste receptors (TAS2Rs) in rodent brain. Here, we demonstrate the expression of hTAS2R16 in human neuronal tissues. Strong TAS2R expression was found in neuronal cells. Double stainings revealed in the neuroblastoma cell line SH-SY5Y the localization of TAS2R16. The functionality was analyzed in the neuroblastoma cell line SH-SY5Y after stimulation with salicin, a described TAS2R16 agonist. In our setting salicin induced in SH-SY5Y cells phosphorylation of ERK and CREB, the key transcription factor of neuronal differentiation. PD98059, an inhibitor of the ERK pathway, as well as probenecid, a TAS2R16

antagonist, could inhibit receptor phosphorylation as well as neurite outgrowth. These data show that salicin as bitter taste receptor agonists might modulate neurite outgrowth and therefore fulfill other functions beyond pain and inflammation.

9th Young Researchers Workshop

YRW-01

Antileukemic lanostanoids from *Poria cocos*

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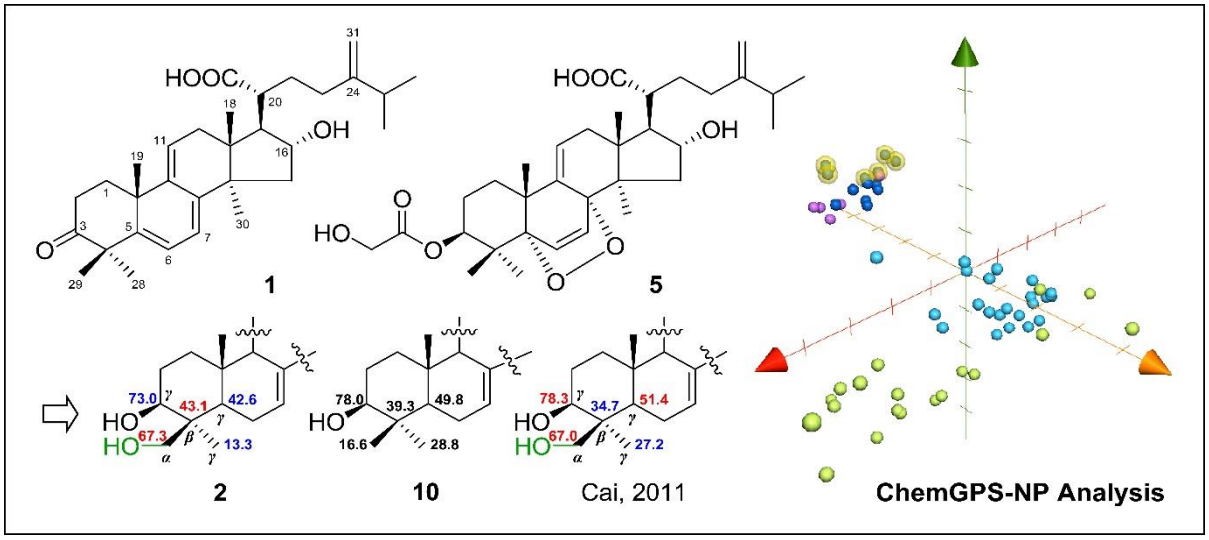
Seven new lanostanoids, isolated from the sclerotia of *Poria cocos*, were elucidated to be (20*ξ*)-16*α*-hydroxy-3-oxo-24-methyllanosta-5,7,9(11),24(31)-tetraen-21-oic acid (**1**), (20*ξ*)-3*β*,16*α*,29-trihydroxy-24-methyllanosta-7,9(11),24(31)-trien-21-oic acid (**2**), (20*ξ*)-3*β*,16*α*,30-trihydroxy-24-methyllanosta-7,9(11),24(31)-trien-21-oic acid (**3**), (20*ξ*)-3*β*-acetyloxy-16*α*,24*α*-dihydroxy-lanosta-7,9(11),25-trien-21-oic acid (**4**), (20*ξ*)-5*α*,8*α*-epidioxy-3-*O*-hydroxyacetoxy-3*β*,16*α*-dihydroxy-24-methyllanosta-6,9(11),24(31)-trien-21-oic acid (**5**), (20*ξ*)-3*β*,16*α*-dihydroxy-7-oxo-24-methyllanosta-8,24(31)-dien-21-oic acid (**6**) and (20*ξ*)-3*α*,16*α*-dihydroxy-7-oxo-24-methyllanosta-8,24(31)-dien-21-oic acid (**7**), based on the extensive spectroscopic analyses. The antileukemic activity of the new compounds (except **3** and **4**), along with the fifteen known lanostane-type triterpenoids, was evaluated against four leukemic cell lines (Molt 4, CCRF-CEM, HL 60 and K562). Dehydropachymic acid (**9**), dehydroeburicoic acid (**12**), pachymic acid (**14**) and lanosta-7,9(11),24-trien-21-oic acid (**20**) exhibited cytotoxic effect on CCRF-CEM cancer cell line with IC_{50} values of 1.43, 2.96, 2.61 and 5.96 μg/mL, respectively. Both dehydropachymic acid (**9**) and dehydroeburicoic acid (**12**) showed cytotoxicity against Molt 4 (IC_{50} 7.26 and 6.67 μg/mL) and HL 60 (IC_{50} 3.84 and 2.79 μg/mL) leukemic cell lines. ChemGPS-NP analysis on the active lanostanoids from *P. cocos* suggested that targets other than topoisomerases may be involved in the cytotoxic effect.



YRW-02

Vasorelaxant studies on stilbenoids and phenanthrene derivatives from *Brasiliorchis porphyrostele* Rchb.f. (Orchidaceae)

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Seven compounds were isolated from the South American orchid *Brasiliorchis porphyrostele* (formerly known as*Maxillaria porphyrostele* [1]) and identified as 1,2,6,7-tetrahydroxy-4-methoxyphenanthrene (**1**), 9,10-dihydro-2,7-dihydroxy-4,6-dimethoxyphenanthrene (**2**), 3,4'-dihydroxy-5,5'-dimethoxydihydrostilbene (**3**), 3,4'-dihydroxy-3',5'-dimethoxydihydrostilbene (**4**), shikimic acid, *p*-hydroxybenzenepropanoic acid and euphorbol; compound **1** has not been described previously. As similar structures are recognized as spasmolytic or vasorelaxing agents [2,3], as well as having been shown to be selectively cytotoxic [4], the aim of the present investigation was to assess firstly their vasoactivity and secondly their cytotoxicity in various cancer cell lines. Preliminary results indicate that compounds **2**, **3**, and **4** possessed vasorelaxing activity on *in vitro* rat aorta rings pre-contracted with either phenylephrine or high K^+. However, compound **1**was inactive. Furthermore, compound **4** inhibited, in a concentration-dependent manner (IC_{50} = 20.2 µM), Ba^{2+} currents through L-type Ca^{2+} channels of rat tail artery myocytes. Compounds **1** and **2** were tested at 10 µM against several leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines. The compounds did not meet activity criteria in the one-dose NCI59 cell test for further testing. In conclusion, *B. porphyrostele* may represent a source of vasoactive agents potentially useful for the treatment of vascular diseases such as hypertension. Further studies are needed, however, to clarify their mechanism of action.

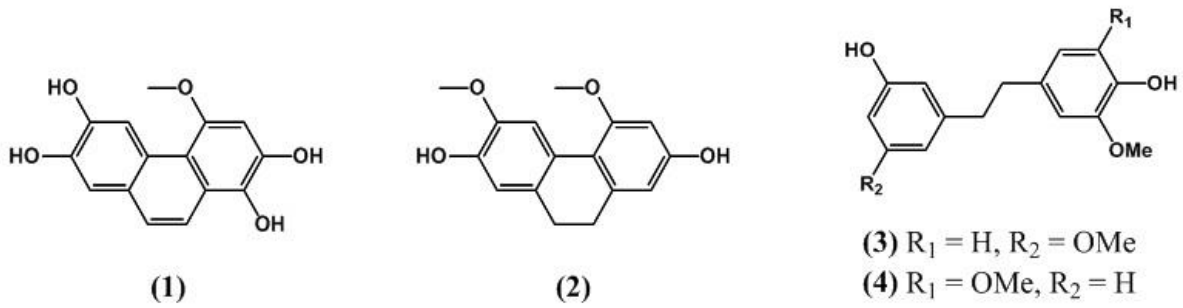

Figure 1 Compounds isolated from *B. porphyrostele*

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YRW-03

Discovery of natural products potentially active against myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is a genetically inherited muscle disorder that is characterised by progressive muscle wasting and weakening, cataracts, and cardiac conduction defects. At present there is no cure or effective treatment for this disabling disease. In this context, a collection of 70 pure compounds and 2100 extracts from different plants and fungal strains were screened with a novel DM1-based biochemical assay for their ability to inhibit the formation of the pathogenic complex formed between (CUG)n-RNA and the splicing-factor muscleblind-like 1 (MBNL1). As a result, eight extracts from different plant species were found to be active (≥50% inhibition at 100 µg/ml). Active constituents were tracked using HPLC-based activity profiling, an approach which combines bioactivity data, structural information from online HPLC-UV-MS and offline microprobe NMR analyses, and database searches. Methylenetanshinquinone and 1,2-dihydrotanshinquinone were found to be the most active compounds in *Salvia miltiorrhiza*. The β-carboline alkaloid harmine was responsible for the activity of *Peganum harmala*, and the iridoid-glycoside auroside was identified as the active constituent in *Lamium album*. The HPLC profiles suggested the presence of tannins in the remaining five active extracts. Retesting of these extracts after tannin removal by filtration over polyamide confirmed the nonspecific interaction of the original extracts with the protein-based screen. In addition, the protoberberine alkaloid berberine was identified as a potent hit from the library of pure compounds. Overall, this study identified several small molecules of

natural origin which are promising hit compounds in (CUG)n-MBNL1 complex inhibition. In a secondary cellular assay some of the identified small molecules partially reversed the splicing defects associated with DM1. Detailed secondary *in vitro* and *in vivo* investigations on these compounds are ongoing.

YRW-04

Phytochemical and pharmacological investigation of *Kickxia ramosissima*

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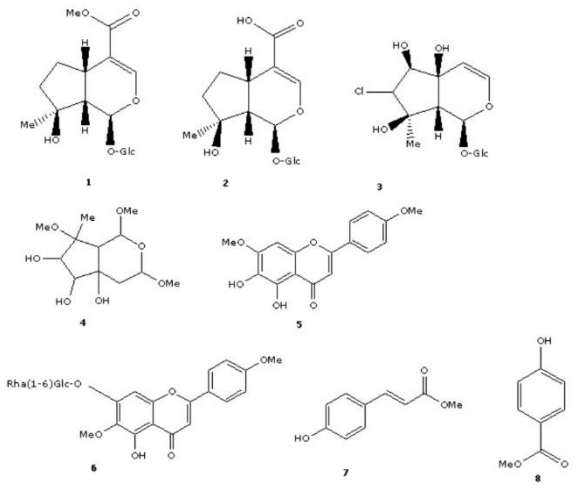
Kickxia ramosissima (Wall.) Janch.(Scrophulariaceae) is a small herb that is highly appreciated as a traditional medicine in the Indian subcontinent [1-2]. The scientific data reporting its constituents are poor and therefore the present investigation was undertaken to discover the main constituents. A double maceration was performed at room temperature with methanol 90%, followed by liquid-liquid partition with various solvents. Each fraction was then tested for claimed biological activities including antibacterial, antifungal, cytotoxic and antiglycation assays. The microorganisms used were *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Microsporum canis* and MRC-5 cells as a cytotoxicity control. The n-hexane fraction showed notable antibacterial (IC_{50} 8 µg/mL) and antifungal (IC_{50} 24.40µg/mL) activity. All other fractions were considered as inactive (IC_{50}>64 µg/mL).The ethyl acetate fraction showed the highest anti-glycation (Advanced Glycation Endproducts, AGEs) activity (IC_{50} 87.14 µg/mL), followed by the *n*-butanol (IC_{50} 144.62 µg/mL), methanol 90% (IC_{50} 167.16µg/mL) and chloroform (IC_{50} 175µg/mL) fractions. In order to provide adequate phytochemical information all extracts were further fractionated using repetitive flash chromatography and analysed by TLC and HPLC-DAD. For the isolation of major compounds, a semi preparative HPLC(RP)-DAD-MS system was used. Subsequently NMR and mass spectra were recorded to elucidate the structure of the isolated compounds, which could be identified as iridoids (**1-4**) flavonoids (**5-6**),p-hydroxy-coumaric acid methyl ester (**7**) and p-hydroxy-benzoic acid methyl ester (**8**). Compound **4** was a new iridoid. Biological evaluation of isolated compounds is in progress.



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YRW-05

Synthesis and SAR of ecdysteroid derivatives as adjuvant anticancer agents

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Ecdysteroids are known as regulators of moulting and reproduction in arthropods, and they can also be found in many plants as defensive agents against herbivores [1]. Our research group has recently reported their adjuvant antitumor activity in combination with various chemotherapeutics on several cancer cell lines, by studying fourteen 20,22-, seventeen 2,3;20,22- and two 2,3-dioxolane derivatives semi-synthesized from 20-hydroxyecdysone (20E) [2-4].

Previously obtained SAR data turned our attention to the selective dioxolane-formation at the 2,3-diol. The more reactive 20,22-diol was protected by phenylboronic acid, and subsequent reactions followed by de-protection yielded 4 new derivatives. In order to obtain corresponding analogues of poststerone (Ps), a known in vivo metabolite of 20E, oxidative side chain cleavage was applied on 20E at the gram scale [5]. Further six new dioxolanes of

Ps were obtained in consecutive reactions with various aldehydes and ketones, such as acetone, propanal, butanal, pentanal and methyl-isobutyl-

All compounds were tested for their anticancer activity in combination with doxorubicin on a multi-drug resistant mouse lymphoma cell line expressing the human ABCB1 transporter. Poststerone 2,3-dioxolanes possessed very low intrinsic cytotoxicity but could greatly potentiate the cytotoxic activity of doxorubicin. By means of SAR of altogether 43 semi-synthetic ecdysteroids, the new derivatives were found promising leads against multi-drug resistant cancer.

Acknowledgement: Szeged Foundation for Cancer Research.

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YRW-06

"Ligand fishing" in chemical space reveals new potential leishmanicidals

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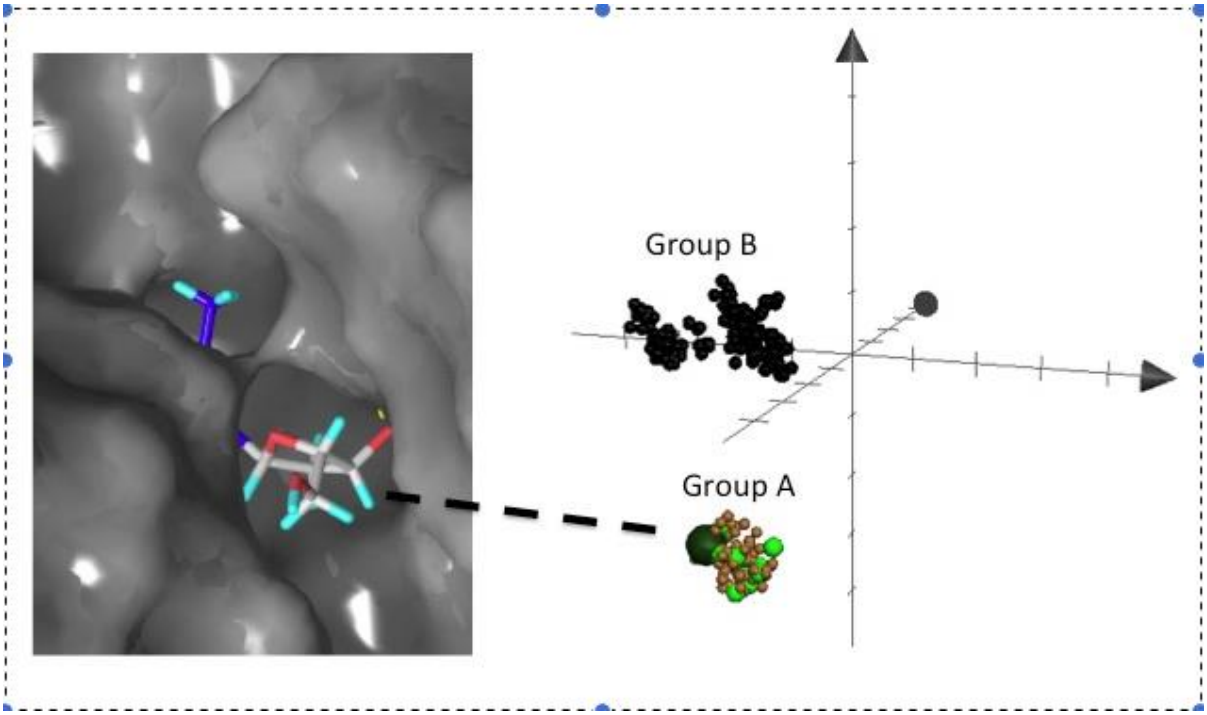
Pteridine reductase 1 (PTR1) is suggested to be a potential drug target in *Leishmania* parasites, because it is predicted to be essential for the pathogen's survival and it appears to lack human homologues [1].The aim of this study is to elucidate if "ligand fishing" in chemical space using ChemGPS-NP can be used to find new potential PTR1-inhibitors of natural origin. PTR1 in complex with 7,8-dihydrobiopterin (DHB), was obtained from the Protein Data Bank. Two sets of compounds, A and B, of natural origin were retrieved using ChemGPS-NP. ChemGPS-NP positions compounds in ch emical space according to their physical-chemical properties [2,3]. Group A included natural compounds that are positioned near DHB. Group B included natural compounds positioned far from all ligands in all crystalized structures of PTR1. The inhibitory effects of the compounds in group A and B, on PTR1 were assessed by predicting their affinity towards the enzyme using molecular docking. Thirteen of the 78 compounds in Group A were predicted to bind with a higher affinity than DHB to PTR1, and nine of these, interacted with the binding pocket of PTR1 in other ways than known ligands. None of the 191 compounds in Group B, were predicted to bind to PTR1 with the same or higher affinity than DHB. Hence, "ligand fishing" in chemical space using DHB as bait can be a successful path for finding new potential PTR1 inhibitors of natural origin.



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YRW-07

A phytochemical and biological study of *Juncus maritimus*, an extremophile plant from Tunisia

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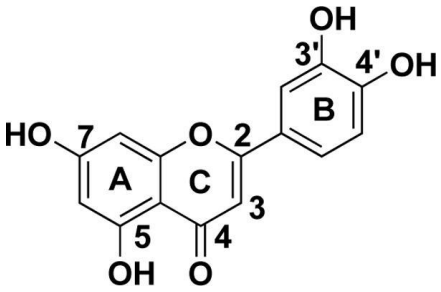
In many regions of Tunisia, plants are often subjected to severe environmental conditions that influence the production of some secondary metabolites involved in stress defence mechanism.

Some of them are phenolic compounds known for their biological activities.These plants can be promising sources of potential drug leads [1].

In this context, 8 extremophile plants have been collected in different areas in Tunisia. Crude methanolic extracts of different parts of these plants have been prepared, and then evaluated for their antiradical, antimicrobial (on 36 strains Gram + and Gram -) and antiviral activities (hepatitis C). Two plants showed the most interesting activities, *Limonium virgatum*Fourr. and *Juncus maritimus* Lam. The extract of *J. maritimus* rhizomes demonstrated a moderate antiradical activity (IC_{50}= 45.23 ± 2.38 µg/mL) and a specific antibacterial activity against *Streptococcus dysgalactiae* and *S. pyrogenes* (MIC = 39 µg/mL). In addition, this extract showed the highest activity against hepatitis C virus (relative infection < 10% at 50 µg/mL).

Bioactivity-directed fractionation of the *J. maritimus* rhizomes extract showed that the ethyl acetate partition exhibited the highest antiradical activity while the methylene chloride partition was most likely responsible for antibacterial and antiviral activities. The major compound of the ethyl acetate partition was isolated using Centrifugal Partition Chromatography (CPC). It is luteolin, a common flavone known for its antiradical activity.



The two major compounds of the methylene chloride partition were isolated by CPC followed by semi-preparative HPLC. According to preliminary NMR and mass analyses, these natural products are phenanthrene derivatives.

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YRW-08

Neuroprotective effects of xylopic acid on lipopolysaccharide-induced neuroinflammation

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Oxidative stress and neuroinflammation are implicated in several central nervous system (CNS) disorders. Xylopic acid has CNS effects including anti-neuropathic pain [1], anxiolytic and antidepressant effects [2] while other kaurene diterpenes have protective effect against MPP^+-induced neuronal death [3]. This study evaluates a possible neuroprotective effect of xylopic acid to help explain its myriad CNS effects. 8-week old mice received either xylopic acid (3, 10 or 30 mg/kg), fluoxetine (3, 10 or 30 mg/kg) or distilled water 10 ml/kg for 14 days.

Neuroinflammation was then induced by intraperitoneal injection of 830 µg/kg lipopolysaccharide (LPS) [4]. 24 h post LPS injection, sucrose preference test, forced swim and social interaction tests were performed to assess neurologic functions. Mice brain were remove 48 h after LPS injection for antioxidant enzymes assay and staining for degenerating neurons. Brain derived neurotropic factor was also measured using ELISA. Xylopic acid attenuated LPS-induced depressive-like symptoms by reducing immobility, increasing sucrose preference and enhancing social interaction ($F_{3, 35}$=56.14, P< 0.001). Oxidizing enzyme myeloperoxidase was significantly ($F_{7, 32}$=7.251, P< 0.001) reduced while antioxidant enzymes superoxide dismutase and catalase activity were elevated along with increased glutathione levels. Lipid peroxidation was also ameliorated as indicated by reduced TBARS in xylopic acid-treated mice. Xylopic acid potently (EC_{50}=1.72±1.65, Emax=93.92±12.18) increased brain derived neurotropic factor as well as reduced neurodegeneration. These results indicate neuroprotective effects of xylopic acid which may contribute to its myriad beneficial CNS effects.

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YRW-09

Stimulatory and depressant-like effects of the crude alkaloids of *Picralima nitida*

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Picralima nitida (Stapf) T.Durand & H.Durand (Apocynaceae) locally known in Ghana as Akuamma (Asante-Twi) is widely used in West Africa for various medicinal purposes including infections and pain [1]. The rich alkaloidal nature of the plant accounts for majority of its pharmacological actions [2-3]. Although a number of scholarly studies exist on the plant (eg. analgesia, anti-inflammatory) [3], its in-depth effect on the CNS has not been explored. The aim of our research wa to investigate the stimulatory and depressant effect of the crude alkaloids of *P. nitida*. Powdered seeds were de-fatted in petroleum ether, cold macerated in 10% v/v HCl, basified with 36% v/v NH_3 and solvent-solvent extracted with $CHCl_3$ to obtain the crude alkaloids (PNE). The effect of PNE (30 to 3000 $mgKg^{-1}$ *p.o.*) on behavioural and physiological functions was assessed using Irwin's model in male ICR mice [4]. The sleep effect was further investigated in pentobarbitone interaction test [5]. Male ICR mice were treated with PNE (100 to 1000 $mgKg^{-1}$ *p.o.*) and sleep induced with sodium pentobarbitone (50 $mgKg^{-1}$ *i.p.*), first after an hour and in another experiment, 30 min after pretreatment with PNE to assess the onset of drug action. In both cases, mice were observed for latency and duration of sleep. **Results:** Initially observed CNS stimulating effect of PNE marked by

hyperactivity progressed steadily into depressive action. PNE significantly enhanced sleeping effects ($P<0.001$, $F_{5,32}=13.86$) in a dose-dependent manner indicating a depressant-like effect Figure 1. LD_{50} of PNE was approximately 3000 $mgKg^{-1}$. PNE acts biphasically; an initial temporal CNS stimulation and a sustained sleeping effect.

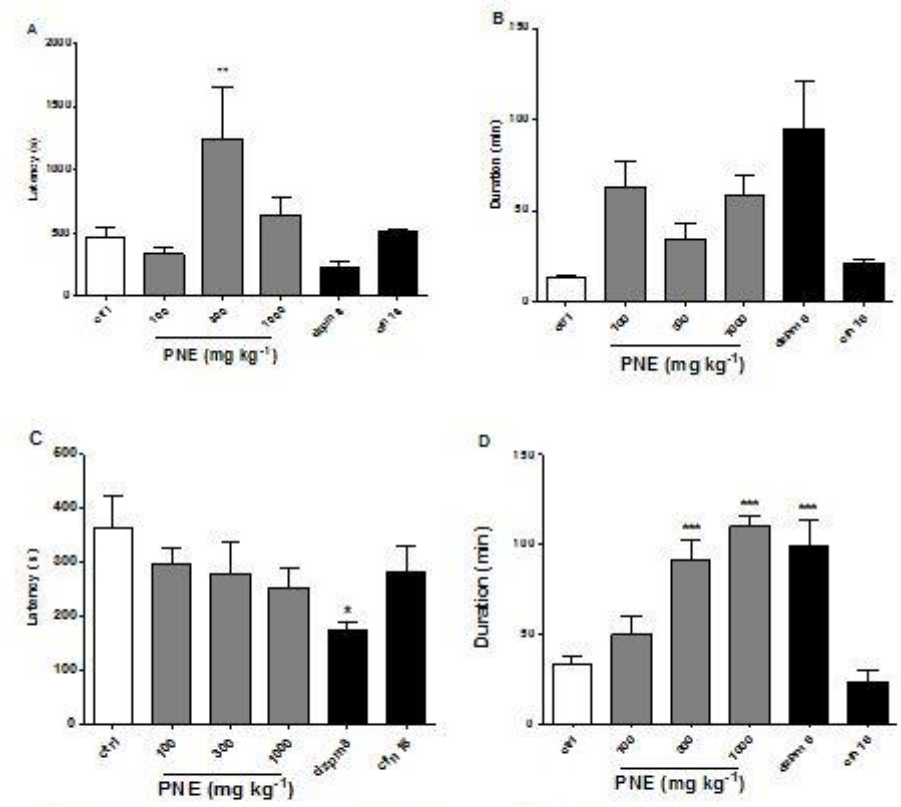

Figure 1: **Effect of PNE, diazepam and caffeine in pentobarbitone interaction test**

A= Latency to sleep (1 hour) **B**= Duration of sleep (1 hour)

C= Latency to sleep (30 minutes) **D**= Duration of sleep (30 minutes)

Data presented as group mean ± SEM ($n=6$); $*P<0.05$, $**P<0.01$, $***P<0.001$; compared to vehicle treated group, $P>0.05$ = not significant (One-way ANOVA followed by Newman-Keuls' test).

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YRW-10

Lepidotols and lepidotins: new phenylcoumarins from *Mesua lepidota* as promising inhibitors of endothelial immune responses and dysfunction

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[2] *INSERM, UMR1064, Nantes, France*
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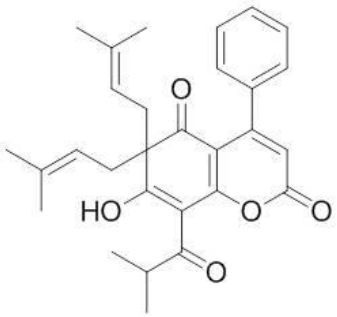
During organ transplantation, graft endothelium is the first barrier encountered by immune cells of the recipient. Endothelial cells surface presents inflammatory and immune proteins which are over-expressed after activation by pro-inflammatory cytokines, Damage Associated Molecular Patterns (DAMPs) or Advanced Glycation End Products (AGEs) [1]. Among natural products, several polyprenylated polyphenols have shown anti-inflammatory, immunomodulatory and anti-AGEs properties [2-3]. Such secondary metabolites are biosynthesized by *Calophyllaceae* species such as *Calophyllum* or *Mesua*species. In order to identify natural products able to prevent endothelial dysfunction, a dereplication analysis was conducted on various extracts from *Calophyllum* and *Mesua* species native to Malaysia. It appeared that the fruits of *Mesua lepidota*T. Anderson are a rich source of original phenylcoumarins named as lepidotols and lepidotins. The main compound, lepidotol A, was evaluated for its anti-inflammatory, immunomodulatory and anti-AGEs potential. Beside a potent inhibitory effect of the VCAM-1, class II HLA and HLA-E induced surface-expressions on human endothelial cells (52 %, 97 % and 66 %, respectively), lepidotol A exhibited an inhibition of AGEs formation five to thirty times higher than aminoguanidine (positive control). These results are consistent with the marked pharmacological activities of prenylated aromatic metabolites [4], and highlight a new approach to discover protective compounds against graft rejection.



Lepidotol A

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YRW-11

Plant derived natural products as novel fusion inhibitors against *Herpes simplex* virus type 1 (HSV-1)

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Herpesvirus infection and spread can be specifically blocked by preventing the fusion between the virion and the host cell membrane. The core fusion machinery of HSV-1 consists of the glycoproteins gD, gH, gL and gB. While gD mediates the interaction with various host cell receptors, gB executes the fusion of viral with cellular membrane after activation by a reaction cascade between the glycoproteins.

Based on this mechanism a virus-free *in vitro* screening assay was developed for direct identification of antiviral compounds with fusion-inhibiting capability. Vero cells are transfected with gD, gH, gL and gB (effector-cells) and seeded on untransfected Vero cells (target-cells). The formation of syncytia and thereby the amount of fusion is visualized through mCherry-labelled gB. To quantify fusion activity, effector cells transfer a transactivator into the target cells, which in turn switches on a reporter gene, e.g. luciferase [1]. The use of Tet-On 3G as transactivator reduced cytotoxicity, widened the measureable window and allowed selective induction of reporter gene expression.

Docosanol (5 mg/ml), a known entry inhibitor of enveloped viruses, and α-gB-2c, a neutralising, gB-specific monoclonal antibody, served as positive controls. Aescin from *Aesculus hippocastanum* was identified as a potent fusion inhibitor against HSV-1. Two different batches, characterized in detail by LC-MS, showed IC_{50} between 5 and 10 µM, depending on incubation time and serum concentration in the cultivation media. Aescin reduces also HSV-1 plaque formation. Within a broader screening of saponins as fusion inhibitors we identified 2 active oleanan glycosides: hederacoside C (IC_{50} about 200 µM) from *Hedera helix*, and esculentoside A (IC_{50} about 150 µM) from *Phytolacca esculenta*.

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YRW-12

A polyphenol enriched fraction of rose oil distillation water inhibits proliferation in HaCaT cells and induces apoptosis

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Water steam distillation of rose flowers (*Rosa damascena*) separates the essential oil from the polyphenol containing rose oil distillation waste water (RODW). While the essential oil represents the desired liquid for the cosmetic industry, the polyphenol containing RODW is in the center of our interest. Recently, a strategy was developed to separate RODW into a polyphenol depleted water fraction and a polyphenol enriched fraction [RF20-(SP-207)]. Polyphenols are known to have a wide spectrum of biochemical and pharmacological effects. In the present study, it was of interest to investigate possible antiproliferative effects of RF20-(SP-207) and fractions thereof F(I)-(IV) in immortalized human keratinocytes (HaCaT). The BrdU cell proliferation assay was used to measure cell proliferation. Cell migration was elucidated by time lapse microscopy. The data demonstrated that from all tested fractions only F(IV) revealed a dose dependent antiproliferative effect which is comparable to RF20-(SP-207) (IC50 of approx. 10 µg/mL). This effect is similar to both positive controls LY294002 (PI3K-inhibitor, 30 % inhibition) and NVP-BEZ235 (dual PI3K/mTOR-inhibitor, 30 % inhibition) and clearly exceeds the anti-proliferative action of quercetin (approx. 20 % inhibition). Time lapse microscopy revealed that cell migration was dramatically decreased under influence of RF20-(SP-207) and F(IV). This effect was comparable to LY294002 and NVP-BEZ235. Fluorescence microscopy images confirm the qualitative increase of apoptosis under influence of RF20-(SP-207) and (IV).

YRW-13

Preparation and analysis of nanocarriers for brain delivery of neuroprotective andrographolide

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Andrographolide (AG) is a major diterpenoid of *Andrographis paniculata* (Burm. f.) Nees, the clinical utility of which has been demonstrated in the treatment of inflammation-related neurodegenerative disorders [1]. Low bioavailability and poor water solubility limit the further development of the compound.To overcome these limitations AG was loaded into albumin based nanoparticles (HSA NPs) and polyethylcyanoacrylate nanoparticles (PECA NPs). NPs were prepared by coacervation using thermal cross-linking, and by emulsion-polymerization, respectively. Both NPs appeared as spherically shaped with an average diameter of 255,4 ± 8,9 nm, a polydispersity (PD) of 0,19 ± 0,02, and a zeta potential of -4,77 ± 0,18 mV for PECA NPs. HSA NPs showed a mean diameter of 202.15 ± 6.15 nm, with a PD of 0.17 ± 0.01, and

a zeta potential of -10.20 ± 0.15 mV. The average drug-entrapment efficiency (EE) and loading capacity (LC) were 94,6 ± 0,41% and 13,2 ± 0,36%, respectively, for PECA NPs, and 98.21 ± 0.01% and 8.50 ± 0.01 % for HSA NPs.The ability of free AG and AG-loaded NPs to cross the blood-brain barrier (BBB) was evaluated with two *in vitro* BBB models based on human hCMEC/D3 and murine bEnd5 endothelial cells. For that purpose, a quantitative LC-MS/MS method for AG in Ringer HEPES buffer, in the range of 10-2000 ng/mL, and with forskolin as internal standard was developed and validated according to FDA/EMA guidelines [2-3]. Apparent permeability coefficients (Papp) in apical-to-basolateral (A-B) direction across cell monolayers cultured on 24-well format will be discussed.

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YRW-14

Effects of urolithins on prostate cancer cells and activity of antiandrogens

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Herbal products are popular among cancer patients as elements of complementary and alternative medicine [1]. Some clinical studies provide evidence for positive effects of administration of ellagitannin rich preparations in prostate cancer. Urolithins, gut microbiota metabolites of ellagitannins, are readily absorbed from gastrointestinal tract [2]. In this study, we examined the effects of urolithin A, B and C (concentration 10-40 μM; 72h) on LNCaP and DU-145 prostate cells proliferation and interaction between urolithins and androgen receptor antagonists, bicalutamide and 2-hydroxyflutamide, used in prostate cancer therapy. Cell proliferation was determined by DNA-Hoechst 33285 stain complexes fluorescence intensity measurement in cell lysates. Cells were also double stained with Annexin V-FITC/propidium iodide and tested for apoptosis by flow cytometry. All tested urolithins dose-dependently inhibited prostate cells proliferation ($p<0.05$). Urolithin A was the most active against LNCaP cells, while urolithin C showed the greatest anti-proliferative effect in DU145 cells. Both urolithin A and B dose-dependently induced apoptosis in LNCaP cells. Urolithin A and 2-hydroxyflutamide additively inhibited LNCaP cells proliferation (combination index CI=1). Combinations of urolithin B and C with 2-hydroxyflutamide and urolithin A, B, and C with bicalutamide exhibited antagonism. These results suggest that ellagitannin rich products may be used in prostate cancer chemoprevention, but should be very carefully used during antiandrogen prostate cancer therapy.

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Regulatory Affairs of Herbal Medicinal Products Workshop

RAW-01

Contributions and visions of the Committee on Herbal Medicinal Products (HMPC)

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Medicinal plants have been used in health care since ancient times. Whereas therapeutic use of herbal medicinal products has a long tradition a formal regulation only started during last century. The European Union has established a common regulatory framework for (traditional) herbal medicinal products. Definitions and basic principles are laid down in Directive 2001/83 EC and its amendments. Quality, efficacy and safety of finished medicinal products have to be evaluated before being introduced into the market. Registration of traditional herbal medicinal products is simplified with respect to the proof of efficacy and data on safety. The Committee on Herbal Medicinal Products (HMPC) at the European Medicines Agency (EMA) in London was established in 2004 and has developed guidance on assessment of (traditional) herbal medicinal products. One of the major tasks of the HMPC is to establish harmonised community monographs on safety and efficacy of herbal substances or combinations. About 130 monographs have been finalised until 2014, representing a substantial progress of harmonisation in the European Union. The development of community monographs is a transparent process which is offering options for scientific input by interested parties. After adoption a complete package of monograph, assessment report, overview of comments and list of references is published at the website of EMA (www.ema.europa.eu).

Basically, the provisions of Directive EC 2004/24 offer also options for traditional herbal medicinal products originating from non-European therapeutic systems. Moreover, applicants may seek scientific advice preferably in early developmental stages of professional projects. The HMPC started in its working programme a pilot project on monographs on efficacy and safety of herbal substances of non-European origin. Questions & Answers on the European Union regulatory framework for (traditional) herbal medicinal products, including those from a 'non-European' tradition, have been adressed in a specific document. Communication, scientific research and regulatory dialogue are necessary to improve the options for a harmonised global usage of traditional medicines.

RAW-02

Pharmacopoeia Monographs for Chinese Herbal Drugs for Quality Assurance

Gerhard Franz

Chairman of the TCM-Working Party of the European Pharmacopoeia Commission Department of Pharmacy, University of Regensburg, Regensburg, Germany

The consistent request of European patients for a safe access to Chinese Materia Medica (CMM) was the basis, almost 10 years ago, to accept and implement the respective quality monographs for the European Pharmacopoeia (Ph Eur). The safe use of TCM herbal drugs

relies on the correct definition of the material in question and further a detailed pharmacognostic botanical and chemical identification. Good agricultural and collection practice (GACP) and all the necessary quality parameters based on Pharmacopoeia standards are an essential prerequisite. However, the existing quality standards of the Chinese Pharmacopoeia (Ch P), even in their English version, could not be directly adopted, due to significant differences between European- and the Chinese Pharmacopoeian legal requirements. Consequently, an experimental re-examination and modification, followed by corresponding validation procedures as well as in some cases, introduction of new analytical methods had to be applied to pre-existing Ch P TCM herbal drug monographs prior to an acceptance by the Ph Eur Commission .

In a first period, a working program, existing of 75 monographs was established by the Commission of the Ph Eur, out of which almost 50 new TCM herbal drug monographs have been implemented for the Ph Eur so far. Often occuring problems and new results of this work period will be provided and discussed in detail.

A specific problem will be the further highlighted, the equivalence of commercial TCM granules with the corresponding herbal drug and the respective aqueous extracts i.e. decoctions. It was found that in many cases the so-called phytoequivalence between commercial granules and genuine herbal drugs is questionable.

Finally, it will be highlighted that for the future a closer collaboration between Europe and China i.e. between the Ch P and the Ph Eur is still an essential prerequisite for a successful, and for the patient a safe utilization of CMM in Europe.

RAW-03

THMP from non-European countries – View of regulatory authorities

Emiel van Galen

CBG/MEB, Utrecht, Netherlands

RAW-04

THMP from non-European countries – View of industry

Mei Wang

Sino-Dutch Preventive and Personalized Medicine, TNO/Leiden University / SU BioMedicine, Zeist, Netherlands

The European Directive 2004/24/EC has introduced a simplified registration procedure for traditional herbal medicinal products which plays an important role in harmonizing the current legislation framework for all herbal medicinal products in the European Union. Although substantial achievements have been made under this directive, only a limited number of herbal medicinal products from non-European traditions commonly used in Europe have been registered. Identification of the obstacles, and determination of appropriate means to overcome

the major challenges in the registration of non-European traditional herbal medicinal products (THMP) are important for the EU herbal medicinal product market.

Traditional Chinese Medicine (TCM) is one of the major non-European THMP in the European market. Key for acceptance of Traditional Chinese Medicine (TCM) is the ability to provide scientific evidence combined with a quality control system based on the bioactive ingredients. Modern scientific technology tools are now available to accomplish standardization of TCM products in order to achieve a high level of efficacy and safety, enhancing the introduction into the international markets. Despite the complexity of ingredients and the aspect of synergistic bioactivities in TCM, so far the analysis for quality control was mainly limited to major components for each herb without evidence for a direct relationship with the bioactive components. In a Systems Biology approach, the multi-dimensional chemical and pharmacological approach enables linking of the complex metabolic profile of herbs with biological effects and is, therefore, a key for quality control of TCM material medica, while providing simultaneous scientific evidence for the underlying efficacy and worldwide acceptance of TCM products.

The root extract of *Dioscorea nipponica* is a well-known component in Traditional Chinese Medicine formula's. The bioactivity of this extract has been described in Chinese medicine as: (1) activating blood circulation to dissipate blood stasis (2) promoting qi circulation to relieve pain and muscle tension due to stress (3) relieving internal resistance of stagnant blood.

In order to translate the explanatory system-level functionalities in Chinese medicine into a Western biochemical, functional and clinical understanding, an extensive series of pharmacological experiments was used to obtain insights in various effects of prevention and treatment in the cardiovascular domain. Moreover, the active component profile consisting of saponins was studied and found to be of a system synergetic nature. Integrating this information with the outcome of clinical trials in which over 16.000 patients were involved together with results from over 100 million people treated, yielded a systems pharmacological view on the working mechanism and insights in the effectiveness and safety profile of this product produced and controlled under GALP and GMP. This product was the first tHMP herbal medicine product registered in the EU that was produced in China. It underpins a new view on evaluation of herbal medicine products in general and creates an important systems pharmacology concept applicable in a wider sense.

RAW-05

THMP from Europe in non-European countries – view of industry

Bernd Roether

Bionorica SE, Neumarkt/Opf., Germany

Directive 2004/24/EC has opened a new regulatory category for Traditional Herbal Medicinal Products in the European Community. The requirements to be fulfilled simplify access for pharmaceutical companies since a proof of efficacy/safety is deferred or reduced to a an expert statement/evidence of a 30 years use respectively.

Similarly these products often do not inevitably meet requirements stipulated by competent authorities abroad, all the more defining criteria of a tradition might be different. In consequence many of the non-EU-regulators do not know what to do with that.

On the EU side open access to products coming from the ayurvedic and traditional Chinese medicine - if these have been placed for at least 15 years on the EU market - is given under the THMP regime.

Market liberalization throughout the world subsists only on mutual acceptance of rules for different approaches of authorizing THMPs and needs therefore a common understanding.

Poster session 1.

Clinical and observational studies with herbal products

PM-01

Regulatory restrictions in prokinetic drugs: Phytotherapy to fill the gap left by cisapride, metoclopramide and domperidone in the therapy of functional gastrointestinal diseases

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After the prokinetic cisaprid, an important therapeutic option in functional gastrointestinal (GI) diseases, had been withdrawn from the market due to rare severe side effects earlier, in 2014 metoclopramid (MCP) and domperidone have likewise been restricted from the use in these indication, so leaving a therapeutic gap.

A systematic review was conducted for identifying herbal medicinal products with therapeutic equivalence to MCP.

A herbal medicinal combination product, STW 5, was shown to be a therapeutically equivalent therapeutic option, with efficacy proven by several RCTs and a very benign safety profile, due to a multi-target effect on multiple etiological factors.

This allows a long term treatment of functional dyspepsia and irritable bowel syndrome also in the future.

PM-02

Immunotoxicological safety and observational studies of homeopathic preparations from *Aspergillus niger*and *Aspergillus ruber*

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The biotechnological produced biomass of *Aspergillus niger* van Tieghem DSM 6563 and *A. ruber* Thom & Church DSM 1965 (syn. *Eurotium rubrum* W. Bremer) is purified from culture medium and then mechanically opened through a cell mill, respectively. After different purification steps, the water-soluble filtrate undergoes sterile filtration and is freeze-dried. The resulting lyophilisate is named e volumine cellulae (lyophil., steril.) = evc. Acute toxicity and possible immunotoxic effects after repeated oral and rectal, intradermal/dermal or

subcutaneous application were tested in various studies with GLP compliance in genetic defined mice and guinea pigs. These studies include general immunotoxicity, mitogenic effects of naive T-cells, proliferation of antigen-stimulated T-cells, delayed type hypersensitivity reactivity, antigen-specific antibody production, acute systemic anaphylaxis induction, and skin sensitisation studies. *Aspergillus niger* evc (Nigersan, Vetokehl Nig) can be regarded as immunotoxicological safe in potency D5, D4 and D3 as well as *Aspergillus ruber* evc (Ruberkehl) in potency D5 and D3. These data are valid only for the investigated fungi strains as well as for the specific, GMP controlled manufacturing process. An observational study for the application of the aqueous D5 dilution of*Aspergillus niger* evc with 104 patients (recurrant respiratory effects) was carried out. The aqueous D5 dilution of*Aspergillus ruber* evc is authorised in Switzerland for the homeopathic treatment of allergic respiratory diseases. This is supported by an observational study with 70 patients (hay fever and allergic rhinitis). Furthermore, the D5 solution for injection is known as a homeopathic remedy against hay fever (observational study with 40 patients). All application studies show a very good safety profile.

PM-03

Capsaicin Heat Plaster in the treatment of muscular back pain. Results of a non-interventional observational study.

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Topical capsaicin formulations are widely used for management of various pain conditions with safety and efficacy supported by numerous studies and meta-analysis [1,2,3]. To confirm the therapeutic effect a medicated capsaicin plaster (ABC Wärmepflaster Capsicum 11 mg) was investigated in patients with muscular back pain in routine clinical practice.

A total of 232 patients with mild to moderate back pain were enrolled in the prospective, multicenter, non-interventional observational study with 11 general practitioners (GPs) in Germany. Data was collected before and after the 3-5 day treatment. Main outcome measures included changes in pain intensity using a visual analog scale (VAS) and global assessment of effectiveness and tolerability by patients and physicians.

Data of 229 patients were eligible for analysis. Mean average pain intensity considerably improved by 5.1 points from initial scores of 6.6 to 1.7 at visit 2 (VAS). 93.4% of patients reported improvement or being free of pain. Physicians (86.8%) rated effectiveness as good or very good. During treatment only two adverse effects were reported. Physicians assessed local tolerability as good or very good (81%). Patients reported fast and lasting pain relief and heat sensation. Questionnaires confirmed the practical and convenient use of the plaster. Consequently, patients (83.1%) were satisfied or very satisfied with the treatment and 86.5% plan to use the plaster in future.

The observational study in patients with back pain in routine medical care confirmed findings from previous clinical trials. Physicians and patients perceive the capsaicin plaster as an efficacious topical treatment. The plaster was generally well tolerated and associated with high therapy satisfaction.

[1] O'Neill, J et al. (2012) Pharmacol Rev 64(4):939-71.

[2] Keitel, W et al. (2001) Arzneimittelforschung 51(11):896-903.

[3] Frerick, H et al. (2003) Pain 106(1-2):59-64.

PM-04

Clinical trials of locally produced herbal tincture “Limonidin”

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Basic elements of the national security include the development of own pharmaceutical industry. New drugs are currently created in Kazakhstan on the basis of local medicinal plants. One of them is tincture "Limonidin", obtained by the leading author from the roots of *Limonium gmelinii*, for which the technological scheme of production was developed at pharmaceutical company "Chempharm".

Safety and efficacy of tincture has been shown during its clinical trials at two leading hospitals in Almaty: complete relief of symptoms, improvement of the endoscopic picture in patients with chronic diseases of the gastrointestinal tract: esophagitis, gastritis, and duodenitis, as well as in patients with non-infectious nature of the disease, especially in patients receiving long-term non-specific anti-inflammatory drugs or glucocorticosteroids.

Tincture "Limonidin" was also tested on patients with antibiotic-associated diarrhea, caused by the combined seven-day administration of two antibiotics - amoxicillin and clarithromycin during eradication therapy for *Helicobacter pylori*-associated gastric diseases and 12 duodenal ulcer; in an outpatient setting at scientific and clinical diagnostic center (at the Institute of Cardiology and Internal Diseases under the Ministry of Health Care of the Republic of Kazakhstan). Positive clinical and bacteriological dynamics in the absence of side effects allows recommending the tincture "Limonidin" for the treatment of diarrhea.

Strong anti-inflammatory effect in the treatment of inflammatory diseases within postoperative period with absence of allergic reactions or other side effects has been shown during the clinical trials both on patients with acute odontogenic purulent inflammations of the maxillofacial area, and patients operated for sinusitis and jaw cysts.

On the basis of conducted clinical trials tincture "Limonidin" was recommended for introduction into medicine and for the industrial production as an antiinflammatory drug in 2011.

PM-05

Vaccinium macrocarpon (cranberry) reduces intake of antibiotics in the treatment of non-severe lower urinary tract infections: A drug monitoring study

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Urinary tract infections (UTIs) are among the most common bacterial infections affecting women. Due to the growing problem of antibiotic resistances there is an urgent need for alternative herbal medicinal products for the treatment of non-severe lower UTIs. *Vaccinium macrocarpon* Aiton is effective through various mechanisms of action without inducing resistances in microorganisms [1]. Thus, a drug monitoring study (n=48) was conducted on women older than 18 years with an increase in leukocytes and clinical symptoms typical for UTIs like dysuria, imperative strangury, increased or new onset of incontinence, hematuria and suprapubic pain. All subjects received one tablet of a standardized Cranberry extract (67 mg proanthocyanidines) combined with *Nasturtium officinale* W.T. Aiton, *Armoracia rusticana* G. Gaertn., B.Mey & Scherb., and vitamins, twice daily (morning and evening) during the first week and one tablet per day for the following four weeks. After 14 days (t1) and after 35 days (t2) taking the tablets a further survey and control check-up with a urine test have been conducted. Patients with persistent clinical symptoms received an antibiotic, for the others the intake of Cranberry tablets was continued. 34 patients (70.8%) could abstain from taking antibiotics within the first 14 days (t1) and 32 patients (66.7%) within 35 days (t2). 28 patients (87.5%) were without symptoms at t2. In conclusion, this drug monitoring study was the first study performed with a combination product (cranberry, nasturtium, armoracia), which furthermore supports the concept of using alternative medicine treating non-severe lower UTIs.

[1] Lavigne J-P, Bourg G, Combescure C, Botto H, Sotto A. In-vitro and in-vivo evidence of dose-dependent decrease of uropathogenic Escherichia coli virulence after consumption of commercial Vaccinium macrocarpon (cranberry) capsules. Clin Microbiol Infect 2008; 14:350-355

PM-06

Consumer experience with a solid extract preparation of *Althaea officinalis*

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Consumer experience is key to understanding the usefulness of a medicinal product. This is especially the case in self medication, which is predominant e.g. in cough and cold. Patient surveys at the point of sale are therefore important tools to generate data on these products. As the presence of a health professional is relevant for the quality assurance of such a survey, the pharmacy is the preferred place for its conduct.

In contrast to cough syrup from *Althaea officinalis* root, a solid dosage form of an extract of this drug has not yet been available in the market. Therefore it was relevant to conduct a survey on this new medicinal product (STW 42-H, Phytohustil Lutschpastillen), with a questionnaire for the acquisition of data handed out to the patient when buying the product. Patients were

recruited in a large, nationwide multi-centric approach by 136 pharmacies in Germany during the cold season, so excluding selection bias. Mean age of patients was 45 years and more than 70 % were female. About 90 % of patients reported that cough interfered with daily activities, and about 80 % complained the cough during nighttime. Less than 30 % treated their cough with co-medication. About 50 % of the patients started the treatment with the *Althaea officinalis* root product after two days of symptoms/coughing.

Acceptance of the survey in pharmacists and consumers was high, so allowing for the first time to document data for this new herbal medicinal product, which support the assumption that there is a high medical need for it. A survey in consumers therefore is an excellent tool for gaining new data in self medication.

From natural products toward potential drug leads

PM-07

Isolation and *in vitro* evaluation of biological activity of the anthocyanin fraction from petals of *Rosa damascena*

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Rosa damascena Mill. (Rosaceae) is an ornamental and medicinal plants and a source of fragrance. Rose water is traditionally known as *golab* in Iran, obtained by hydrodistillation from fresh petals of *R. damascena* and has been used in folk medicine for relief from toothache, aphthous lesions, also a remedy for gastrointestinal complications [1]. The aim of current research was the isolation of anthocyanin fraction from the waste water of *R. damascena* petals, after rose water production. Then, different methods of antioxidant activity including DPPH free radical scavenging activity, ferric reducing antioxidant power (FRAP) assay and nitric oxide assay and also quantitative analysis of total phenol, flavonoid and anthocyanin contents were evaluated according to standard procedures. Anthocyanin fraction were extracted from crude extract by using of Amberlite resin XAD-7 as stationing phase. Table 1 represents IC_{50} values for DPPH and FRAP antioxidant methods and percentage inhibition of NO radical at 400 µg/mL and also quantification of total phenol, flavonoid and anthocyanin contents. According to table 1 anthocyanin fraction showed a good antioxidant activity by all methods against quercetin as a standard. Determination of total phenolic showed, anthocyanin fraction had high phenol content and maybe responsible for a part of free radical scavenging activity. Total anthocyanin content after column chromatography was increased when compared to that before. Hence, ion exchange chromatography could be a good separation method for the anthocyanin fraction. According to present findings anthocyanin fraction isolated from the waste water of *Rosa damascena* after rose water production can be considered as a good candidate for production of new drugs based on natural products, and also for further investigation.

[1] Moein M, Zarshenas MM, Delnavaz S. Chemical composition analysis of rose water samples from Iran. Pharm Biol 2014; 52: 1358-1361.

Table1. In vitro antioxidant activity, total phenol, flavonoid and anthocyanin contents of anthocyanin fraction

Sample / Test	Anthocyanin Fraction	Quercetin
DPPH IC_{50} (µg/ml)	57.82±0.24	35.61±0.06
FRAP IC_{50} (µg/ml)	21.08±0.16	8.69±0.03
% of NO Inhibition in 400 µg/ml	47.22±1.52	75.33±1.95
Total phenol content (TPC) (mg gallicacid / g of fraction)	605.75±5	-
Total flavonoid conten (TFC) (mg quercetin / g of fraction)	151.78±2	-
Tota l anthocyanin content before column chromatography (TAC-B) (mg cyaniding glycoside / 100 g of extract)	782.35±6.8	-
Total anthocyanin content after column chromatography (TAC-A) (mg cyaniding glycoside / 100 g of fraction)	3782.35±7.9	-

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PM-08

Bioactive phenylethanoid glycosides from *Digitalis davisiana* Heywood

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In the Flora of Turkey, the genus *Digitalis* is represented by nine species [1]. In this research aerial parts of *Digitalis davisiana* Heywood was studied phytochemically on the basis of LXR (Liver x receptor) ligand activity, free radical scavenging and cytotoxic activities guided fractionation. Methanol extract applied to polyamide column chromatography for fractionation. Due to examination of mentioned bioactivity tests, active fractions were detected. Radical scavenging activity was tested against DPPH, NO, SO radicals. IC_{50} values of methanolic extract for tested radicals were found to be; 133.8, 309.9, 1560 µg/ml, respectively. Cytotoxic activity was tested against HEp-2, HepG2 and 3Y1 cell lines by MTT method [2]. IC_{50} values were 50.8, 211.4 and 94.7 µg/ml respectively. LXR ligand activity was tested by a LXRα luciferase reporter assay on HEK293 cells [3]. Since methanol extract showed cytotoxicity, LXR ligand activity couldn't be determined. When fractions tested in lower concentrations moderate activity was detected. After evaluating the bioactivity test

results, isolation studies started from active, phenylethanoid glycosides rich fractions. Studies resulted in the isolation of six phenylethanoid glycosides. Lugrandoside, maxoside and purpureaside E were identified by extensive NMR techniques. Structural determination studies on the other active compounds are in progress.

Acknowledgments: Vahap Murat Kutluay was supported by research grant from The Scientific and Technological Research Council of Turkey(2214/A,2211).

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[2] Saracoglu I, et al. Studies on constituents with cytotoxic and cytostatic activity of two Turkish medicinal plants Phlomis armeniaca and Scutellaria salviifolia. Biol Pharm Bull 1995; 18:1396-1400

[3] Kotani H, et al. Identification of a naturally occurring rexinoid, honokiol, that activates the retinoid X receptor. J. Nat. Prod. 2010;73:1332–1336.

PM-09

Cytotoxic activities of Amaryllidaceae alkaloids against gastrointestinal cancer cells

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Oncological diseases are one of the leading causes of death in the developed countries and the increase of its prevalence seems to be inevitable. In most cases oncological patients die due to resistance of cancer to therapy, metastasis and dissemination of cancer cells into vital organs. The standard treatment covers surgical intervention, radiotherapy and/or chemotherapy.

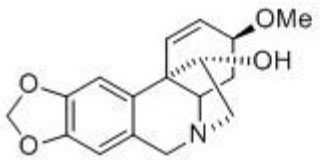
Additionally conventional anticancer treatments damage healthy tissue, resulting in a variety of side effects. Therefore, substantial efforts are being invested into identifying and developing compounds that would be able selectively target tumor cells while not damage healthy cells.

Among various natural sources that have been investigated for constituents with potential use in cancer treatment, plants of the Amaryllidaceae family have been particularly promising and fruitful. To date, about 50 of these alkaloids were tested against different cell lines.

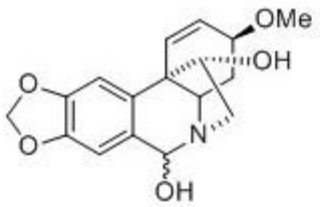
In current study we screened and determined IC_{50} *in vitro* growth inhibitory activity of 18 Amaryllidaceae alkaloids in cancer cell lines Caco-2 and HT-29 using the MTT colorimetric assay. A human normal intestine cell line FHS-74 was used as a control for the overall toxicity. All tested alkaloids have been previously isolated in our laboratory from three plant species *Nerine bowdenii*, *Zephyranthes robusta*, and *Chlidanthus fragrans*.

Among the tested compounds lycorine, haemanthamine, and haemanthidine exhibited the most potent cytotoxic potential against both tested cell lines with IC_{50} values of 0.99-3.28 μM for

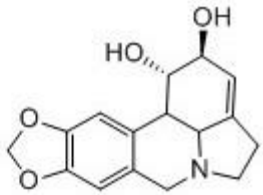
Caco-2, and 0.59-1.72 μM for HT-29. Lycorine and haemanthamine showed only moderate toxicity against normal cells (15 μM < IC_{50} < 30 μM) in comparison to used standard vinorelbine (IC_{50} 3.98 μM). Other tested alkaloids showed moderate or weak cytotoxic potential. Further step of the current study is the preparation of semisynthetic analogues by changing different parts of the structure of the most active compound haemanthamine.



Haemanthamine



Haemanthidine



Lycorine

PM-10

Meadowsweet (*Filipendula ulmaria*): LC-MS phenolic characterization and ameliorating effect on cisplatin-induced hepatotoxicity

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The liver plays a major role in detoxifying and clearing xenobiotics that may lead to liver damage and hepatic dysfunction. The present study was carried out to evaluate the effect of the methanolic extracts from aerial parts and roots of *Filipendula ulmaria* (L.) Maxim. (Rosaceae) (FUR, FUA) on cisplatin-induced hepatotoxicity. Wistar rats were treated for 10 days with FUA and FUR in three doses (100, 200 and 400 mg/kg body weight). Hepatotoxicity was induced on 5th day of treatment with a single injection of cisplatin (7.5 mg/kg, i.p.). Negative and positive control groups were also evaluated. The results show that the treatment with cisplatin (positive control group) increased the level of serum transaminases (ALT, AST). Treatment with both extracts, especially at concentrations of 200 and 400 mg/kg, significantly reduced the enzymatic activity of ALT and AST. Oxidative stress markers in liver tissue like superoxide-dismutase (SOD) and catalase (CAT) activities, as well as levels of reduced glutathione (GSH) and malonyl dialdehyde (MDA), indicated a high level of oxidative damage in the group treated with cisplatin only. The treatment with FUA and FUR significantly ($p<0.05$) and dose-dependently alleviated activities of SOD and CAT. Also, GSH level in hepatic tissue was dose-dependently increased due to extracts treatment. Lipid oxidation in livers of the rats treated with *F. ulmaria* extracts was considerably reduced regarding MDA levels. Based on presented results it can be concluded that the extracts of *F. ulmaria* possess hepatoprotective activity against CP-hepatotoxicity, which was demonstrated through regulation of oxidative stress markers in serum and hepatic tissue. The phytochemical profile of the extracts was evaluated by LC-DAD-MS^n. In FUR catechin as well as procyanidin dimers

and trimers and in FUA catechin, epicatechin, hydrolysable tannins (trigalloyl-hexahydroxydiphenol-glucoses), and the flavonoids spiraeoside and quercitrin could be identified.

PM-11

Metabolomics of fabaceous invasive plants from Colombia: an approach for lead finding from nature

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Fabaceae family is a big group of flowering plants. Several of them have been introduced in various countries for diverse purposes but some become a problem because of biological invasions [1]. In Colombia, two Fabaceous species were introduced from Europe for ornamental uses such as Ulex Europeaus and Genista monspessulana, and they have progressively dominated some native environments as well as altering many aspects of ecosystem functioning [2]. For invasion success, this kind of plants can produce novel and unique secondary metabolites [3]. Thus, as part of our research on chemoprospecting of invasive plants, 17 and 19 plant accessions of U. europeaus and G. monspessulana, respectively, from different invaded places in Bogotá plateu, were investigated through a comprehensive untargeted LC-MS-based metabolomics approach in order to observe the chemical variability between samples and its implication on DPPH• and ABTS+• radical-scavenging and ferric-reducing capacities. The MS analysis resulted in the identification of different metabolites belonging to varied chemical classes but most samples exhibited the presence of free and glycosilated genistein, daizein and luteolin. The thirty six materials also showed antioxidant capacity at different levels (2>IC50(μg/mL)>55). The PCA and PLS-DA-derived score plots indicated several differences between samples but clustered according characteristic chemical constituents. The supervised analysis indicated the existence of three non-common flavone-related compounds to be responsible of the FRAP and radical-scavenging capacities. The present untargeted metabolomics exploration of these invasive plants is an excellent approach for lead finding from nature.

Acknowledgement: The present work is a product derived by the Project IMP-CIAS-1567 financed by UMNG - Validity 2014.

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PM-12

Alantolactone, a sesquiterpene lactone isolated from *Inula helenium* L. selectively suppresses STAT3 activation and exhibits anticancer activity in MDA-MB-231 cells

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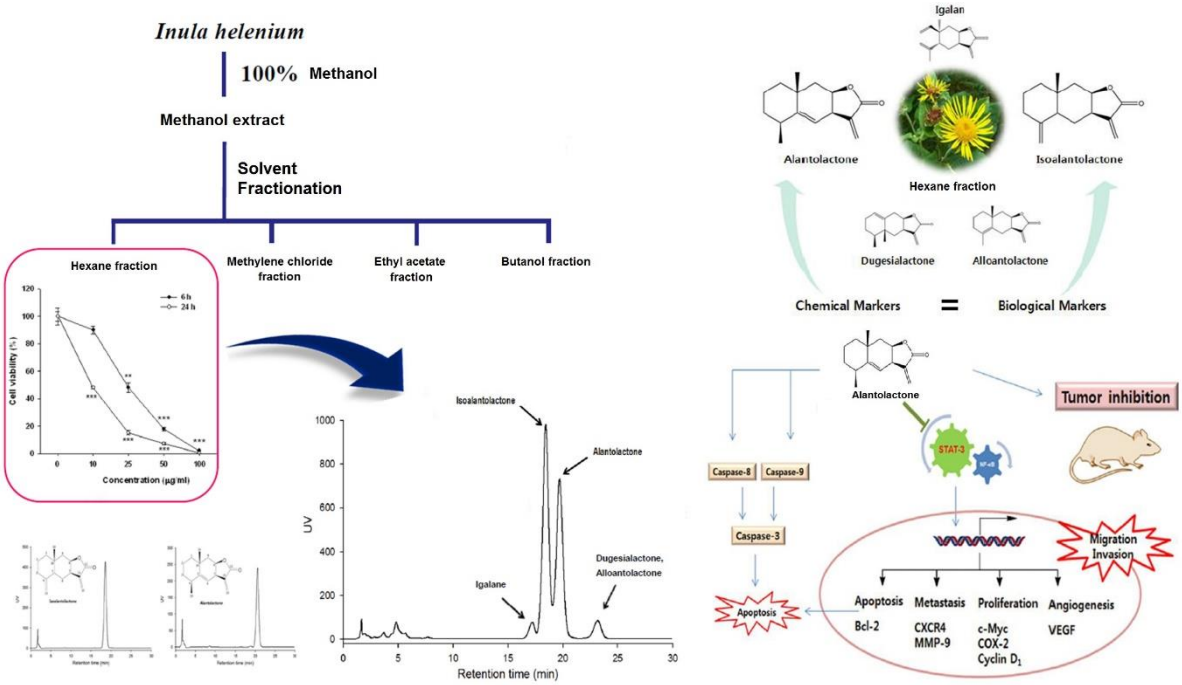
The important goal of cancer drug discovery is to develop therapeutic agents that are effective, safe, and affordable. In the present study, we demonstrated that alantolactone isolated from *Inula helenium* L. (Asteraceae), has potential activity against triple-negative breast cancer (TNBC) MDA-MB-231 cells by suppressing the signal transducer and activator of transcription 3 (STAT3) pathway. Alantolactone effectively suppressed constitutive and inducible STAT3 activation at tyrosine 705. Alantolactone decreased STAT3 translocation to the nucleus, its DNA-binding, and STAT3 target gene expression. Alantolactone significantly inhibits STAT3 activation with a marginal effect on MAPKs and NF-κB transcription; however, this effect is not mediated by inhibiting STAT3 upstream kinases. Although SHP-1, SHP-2, and PTEN, which are protein tyrosine phosphatases (PTPs), were not affected by alantolactone, the treatment with a PTP inhibitor reversed the alantolactone-induced suppression of STAT3 activation, indicating that PTP plays an important role in the action of alantolactone. Finally, alantolactone resulted in the inhibition of migration, invasion, adhesion, and colony formation. The *in vivo* administration of alantolactone inhibited the growth of human breast xenograft tumors. Sesquiterpene lactones-enriched hexane fraction, including alantolactone, isoalantolactone, igalan, dugesialactone, and alloantolactone, also has the potential to inhibit STAT3 activation. These results provide preclinical evidence to continue the development of alantolactone and *I. helenium* as a STAT3 inhibitor and as a potential therapeutic agent against breast cancer.



PM-13

Antibacterial activity of *Rumex aquaticus* and *R. thyrsiflorus* extracts and isolation of the biologically active compounds

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Nosocomial infection became a major problem not only in hospitals, but worldwide as it can cause endemic diseases. The uncontrolled usage of antibiotics led to the increased number of resistant bacteria strains. The aim of our study was to find new compounds from plants with potential antibacterial properties on several resistant strains.

The members of the genus *Rumex* (family Polygonaceae), which are distributed worldwide, comprise approximately 200 species. Previously, anthraquinones, naphthalenes, flavonoids, stilbenoids, triterpenes, carotenoids and phenolic acids have been isolated from Rumex species. The extracts of these plants, and compounds isolated from them, have been demonstrated to possess various pharmacological activities, including anti-inflammatory, antioxidant, antitumour and antimicrobial properties.

We have investigated the antimicrobial potency of the aerial parts and roots of two *Rumex* species, *R. aquaticus* and *R. thyrsiflorus* against different bacterial strains (methicillin-resistant *Staphylococcus aureus*, *S. aureus*, *Staphylococcus epidermidis*, *Moraxella catarrhalis*, *Streptococcus pneumonia*, *S. pyogenes* and *Bacillus subtilis*) with disc-diffusion method. The ethyl-acetate fraction of the aerial parts of *R. aquaticus* and the roots of *R. thyrsiflorus* and the *n*-hexane, chloroform and ethyl-acetate fractions of the roots of *R. aquaticus* possessed remarkable antimicrobial effects. With the combination of different chromatographic methods 14 compounds, among them flavonoids (quercetin- and kaempferol-glycosides, epicatechin, procyanidin B5), anthranoids (chrysophanol, emodin, torachryson-glucoside, citreorosein), naphtalenes (musizine- and nepodine-glucoside) and a monoacyl-glycerol were isolated, some of them responsible for the pharmacological activity.

Acknowledgement: This work was supported by the Hungarian Scientific Research Fund (OTKA PD101432) and a János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

PM-14

Antioxidant potentials of *Origanum majorana* leaves extract against reproductive toxicity and apoptosis-related gene expression resulted from methomyl exposure in male rat.

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pesticides have conferred immense benefits to mankind by improving health and nutrition. However, they have widely differing potential to produce adverse effects in living organisms, including reproductive toxicity. So, the present study is conducted to evaluate the protective effects of *Origanum majorana* leaves extract against methomyl-induced oxidative damage and testicular injures in male Wister rat.

Male rats were divided into six groups of six rats each: control group (I); extract groups (II&III) received extract at doses of 150 & 300 mg/kg body weight; methomyl group (IV) received methomyl (2.034 mg/kg bw, 1/10 LD50); groups (V & VI) simultaneously received methomyl along with the two doses of extracts. All the applications were administered via oral route for 28 consecutive days.

Exposure of rat to methomyl induced significant decreases in the activities of testicular SOD, CAT and GPx and in GSH level, while induced a significant increase in testicular LPO level accompanied by histopathological alterations. Furthermore, results revealed a significant up-regulation in the level of the expression for the activity of four key stress and apoptosis-related genes (CASP3, CASP9, Tp53 and Bcl2), in response to methomyl exposure in rats. However, Co-administration of *O. majorana* leaves extract to methomyl ameliorated the above-mentioned parameters and modulated the observed significant up-regulation in the expression level of apoptosis-related genes, indicative of a protective interfering role in signaling transduction process of methomyl-mediated toxicity. The ultimate effect was achieved by the highest dose of the extract.

These data suggested that co-administration of *O. majorana* leaves extract attenuated the testicular oxidative damage and apoptosis-related genes induced by methomyl exposure, which may be attributed to its antioxidant potential. So, the extract could be used as therapeutic option against testicular injuries.

PM-15

Styryllactones and bis-styryllactones from *Goniothalamus lanceolatus*

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Goniothalamus lanceolatus is an Annonaceae plant used by natives of East Malaysia to cure or prevent certain type of illnesses such as cancer, fever and skin infections. In search of scientific evidences to support the ethnopharmacological uses of *G. lanceolatus*, we have embarked on a comprehensive phytochemical study. The bark, leaves and root extracts showed significant *in vitro* cytotoxicity against two breast cancer cell lines, MCF-7 and MDA-MB231, with IC_{50} ranging from 0.08 µg/ml to 2.2 µg/ml, illustrating its potential anticancer properties. Through series of modern chromatographic techniques performed on the dichloromethane extract from the bark, thirteen of styryllactones including four bis-styryllactones were successfully isolated. Structure elucidation using various spectroscopic techniques led to identification of (+)-goniodiol, (+)-9-deoxygoniopypyrone, (+)-8-epi-9-deoxygoniopypyrone, (+)-8-epi-9-deoxygoniopypyrone acetate, (+)-goniothalamin, (+)-isogoniothalamin oxide, (+)-goniodiol-7-monoacetate, (+)-goniodiol-8-monoacetate, (+)-goniofupyrone A, (+)-deoxygoniopypyrone A, (+)-goniolactone G, (+)-digoniodiol and (+)-7-acetyl-digoniodiol.

PM-16

The evaluation of wound healing potential of acetyl alkannin isolated from *Arnebia purpurea*

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Medicinal plants containing alkannin/shikonin and their derivatives have been used for wound healing treatment for years.*Arnebia* (Boraginaceae) species have these compounds in high quantity. Previous studies showed the root extract of *Arnebia*spec. and alkannin derivatives have wound healing, antiinflammatory, antitumoral, antibacterial and antifungal effects [1,2]. In this study, acetyl alkannin, the major naphthoquinone isolated from the roots *n*-hexane extract of *Arnebia purpurea* S. Erik & H. Sümbül which is an endemic species in Turkey have investigated in terms of its wound healing activity. Acetyl alkannin in PBS suspension (120 mg/kg, 0.1%) applied topically on both excision normal wound model and hydrocortisone-induced impaired wound model [3-4]. When our results analyzed histopathologically (lymphocyte density, vascular proliferation, edema formation and fibrosis), acetyl alkannin

applied groups have a statistically significant effect on all type wound healing phases compared with the control groups ($p<0.05$, ANOVA test).

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PM-17

Phytochemical investigations on *Lotus aegaeus*

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The genus *Lotus*, which belongs to Fabaceae family has 17 species in the Flora of Turkey [1]. *Lotus aegeus* (Gris.) Boiss. is known as 'devre otu' in Anatolia and used as animal forage and antimicrobial agent for the treatment of animal injuries [2]. In this study, the chemical composition of *L. aegaeus* has been characterized for the first time.The *n*-butanol extract of aerial parts was chromatographed using various chromatography systems to yield 4 compounds. The structures of isolated compounds were established by spectroscopic methods. The isolated compounds were determined as flavonoids, quercetin-3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside, quercetin-3-*O*-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside, kaempferol-3-*O*-β-xylopyranosyl-(1→2)-α--L-rhamnopyranosyl-7-*O*-α-L-rhamnopyranoside, a saponin, wistariasaponin C and a cyanogenic glucoside, linamarin, based on literature data [3-6].

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PM-18

Small molecular reverses chemoresistance of breast cancer through inhibition of DNA damage response

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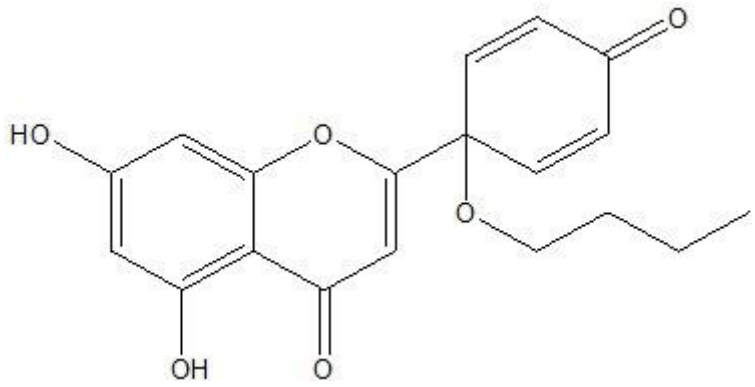
Targeting of DNA is a common and effective stratagem for cancer therapy, often seen in radiotherapy and DNA-damaging chemotherapy where lethal damage in DNA is inflicted upon treated cells. However, chemotherapy is often undermined by resistance in cancer cells. Because this issue remains unresolved, identifying ways to increase chemosensitivity in cancers is of critical importance. DNA-damaging chemotherapy treated cells activate the DNA damage response (DDR), which couples with activation of DNA repair processes. These processes are initiated and conducted by ATM/ATR sensor kinases which phosphorylate their downstream effector kinases Chk1/Chk2, and are known to counteract therapeutic efficacy. In order to identify ways to increase chemosensitivity in cancers, we tested a series of synthetic compounds with structural modifications of fern plant-derived protoapigenone for their ability to alter DDR in MCF-7 cell line. Among them, compound AT738 was the most potent on inhibition of cisplatin- or doxorubicin-induced ATM, Chk1, and Chk2 phosphorylation, and was therefore chosen for further study. As a result of DDR inhibition, AT738 increased chemosensitivity in doxorubicin-resistant MCF7-KCR cell line, which displays higher DDR related gene expression in ABCB1 independent mechanisms. Together, our findings suggest AT738 as a novel agent that, when used in combination with chemotherapy, can reverse multidrug resistance through inhibition of DDR.



PM-19

Euphrasianins A-E, novel acylglycerol lipids from *Euphrasia rostkoviana* and *E. tetraquetra*

Peter Lorenz, Diana N. Knittel, Florian C. Stintzing, Dietmar R. Kammerer

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Plant cuticles form a hydrophobic layer covering all aerial parts of terrestrial plants as a protective barrier against environmental impacts such as desiccation, extreme temperature changes, microbial infection and intense UV radiation [1]. We herein identified in CH_2Cl_2 extracts of *E. rostkoviana* by use of GC-MS and HPLC-APCI-MS^n methods a novel class of homologous acylglycerol lipids derived from 3-hydroxy fatty acids (C14-C18), henceforth named euphrasianins A-E [2]. Based on MS data, the structure of one representative homologue (euphrasianin A) was elucidated as 1-*O*-acetyl-3-(3-hydroxymyristoyl)-glycerol, which was verified by total synthesis of this compound. The absolute configuration of the 3-hydroxyfatty acid moiety of euphrasianins was found to be '*R*', based on alkaline hydrolysis and methylation experiments and subsequent chiral GC analysis. Interestingly, euphrasianins C and E were exclusively detected by GC-MS in extracts of*E. tetraquetra* (seacliff eyebright) collected in Ireland, suggesting potential chemotaxonomic relevance of these novel cuticular wax constituents.

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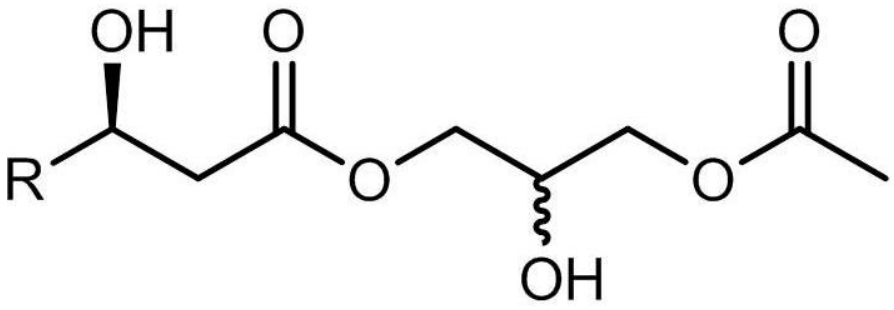

$$R = C_nH_{2n+1}\ (n = 11 - 15)$$

PM-20

New phenolics from the leaves and twigs of *Osteomeles schwerinae* and their inhibitory effects on AGE formation and RLAR *in vitro* and vessel dilation in larval Zebrafish *in vivo*

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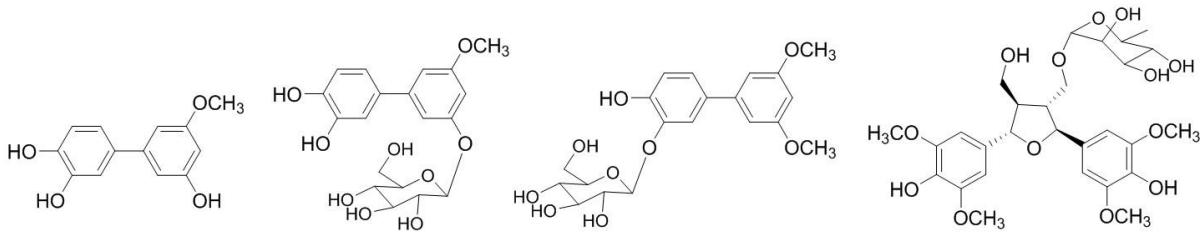
In our continuing efforts to identify effective naturally sourced agents for diabetic complications, three new phenolic biphenyls (**1**–**3**), 5'-methoxy-(1,1'-biphenyl)-3,4,3'-triol (**1**), 5'-methoxy-(1,1'-biphenyl)-3,4,3'-triol 3'-*O*-β-D-glucopyranoside (**2**), and 3',5'-dimethoxy-(1,1'-biphenyl)-3,4-diol 3-*O*-β-D-glucopyranoside (**3**), and one new lignan glycoside, icariol A2 9′-O-α-L-rhamnopyranoside (**4**), were isolated from the leaves and twigs of *Osteomeles schwerinae*. The structures of the new compounds were established by extensive spectroscopic studies and chemical evidence. The inhibitory effects of isolated compounds (**1**–**4**) on AGEs formation and RLAR in vitro were examined. Of the tested compounds, only phenolic biphenyls (**1**–**3**) markedly inhibited AGE formation with IC_{50} values of 39.5–169.1 μM, compared with that of a positive control, aminoguanidine (IC_{50}=975.9 μM). In the RLAR assay, consistent with the inhibition of AGE formation, phenolic biphenyls (**1**–**3**) exhibited considerable inhibition of RLAR with IC_{50} values of 3.7–13.8 μM, compared with that of a positive control, 3,3-tetramethyleneglutaric acid (TMG, IC_{50}=24.1 μM). In addition, the effects of these isolates on the dilation of hyaloid-retinal vessels induced by high glucose (HG) in larval zebrafish were also investigated. Of the tested compounds, only compound **1** significantly reduced the dilation of HG-induced hyaloid-retinal vessels. This compound reduced the diameters of HG-induced hyaloid-retinal vessels by about 95% and 99% at 10 and 20 μM, respectively, versus the HG-treated control group, whereas the positive control, VEGFR inhibitor, exhibited 77% inhibition at a concentration of 1 μM.



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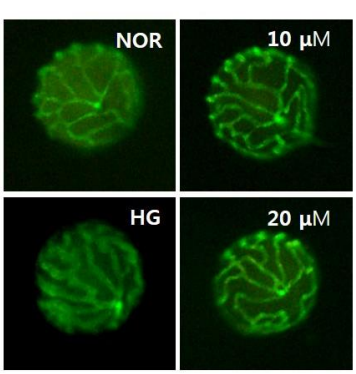

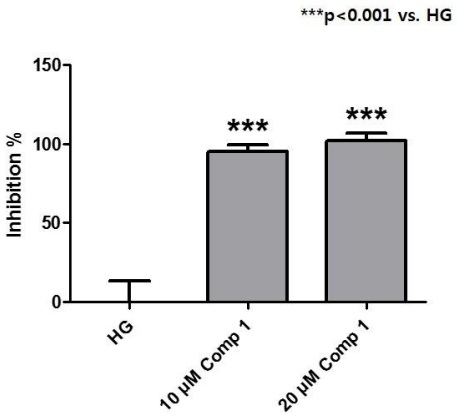

PM-21

Screening for diterpene alkaloids in the *Spiraea* genus

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Diterpene alkaloids are characteristic compounds of Ranunculaceae species, their presence in other taxa is a chemotaxonomical curiosity. The structural diversity of diterpene alkaloids and the recent results on their physiological effects have attracted considerable interest and motivation for screening the poorly examined genera for these compounds. Previously, some diterpene alkalkoids were identified in *Spiraea* species. While *S. japonica* and *S. fritschiana* have been widely examined, other species have not been studied so far in detail.

The aim of the present work was the screening of seven poorly investigated *Spiraea* species *(S. crenata*, *S. media*, *S. salicifolia*, *S. nipponica*, *S. vanhouttei*, *S. billardii* and *S. chamaedryfolia)* for the presence of alkaloids. The extracts were prepared according to the standard pH-gradient extraction procedure used for alkaloid isolation and investigated as described in ref. [1].

The presence of alkaloids were detected only in *S. chamaedryfolia*. The neutral, acidic and alkaline extracts of the roots of this plant were subjected to antibacterial and xanthine oxidase inhibitory activity examination. All extracts of *S. chamaedryfolia* exerted noteworthy xanthine oxidase inhibitory activity (>70%). The extracts exhibited antibacterial activity*in vitro* against *Staphylococcus aureus, Bacillus subtilis, Moraxella catarrhalis, Streptococcus pneumoniae* and*Staphylococcus aureus* (MRSA). Further studies will focus on the identification of

alkaloids in *S. chamaedryfolia* and the characterization of the compounds responsible for the antibacterial and xanthine oxidase inhibitory activity.

Acknowledgement: This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 'National Excellence Program'.

[1] Nostro A, Germano MP, D'Angelo V, Marino A, Cannatelli MA. Lett Appl Microbiol 2000: 30; 379–384

PM-22

Sinulariolide suppress human hepatocellular carcinoma cell migration and invasion by inhibiting matrix metalloproteinase-2/-9 through MAPKs and PI3K/Akt signaling pathways

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Sinulariolide is an active compound isolated from the cultured soft coral *Sinularia flexibilis*. In this study, we investigate the migration and invasion effects of sinulariolide in hepatocellular carcinoma cell HA22T. Sinulariolide inhibited the migration and invasion effects of hepatocellular carcinoma cells in a concentration-dependent manner (0~8 ug/mL). The results of a zymography assay showed that sinulariolide suppressed the activities of matrix metalloproteinase (MMP)-2 and MMP-9. Moreover, protein levels of MMP-2, MMP-9, and urokinase-type plasminogen activator (uPA) were reduced by sinulariolide in a concentration-dependent manner. Sinulariolide also exerted an inhibitory effect on phosphorylation of c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinases (ERK), phosphatidylinositol 3-kinase (PI3K), Akt, FAK, GRB2. Taken together, these results demonstrated that sinulariolide could inhibit hepatocellular carcinoma cell migration and invasion and alter HA22T cell metastasis by reduction of MMP-2, MMP-9, and uPA expression through the suppression of MAPKs, PI3K/Akt, and FAK/GRB2 signaling pathway. In present study, we investigated the potential anti-metastatic effects of sinulariolide on human hepatocellular carcinoma and the underlying mechanisms.

PM-23

Metabolomics and dereplication studies of some Egyptian medicinal plants and its associated endophytes

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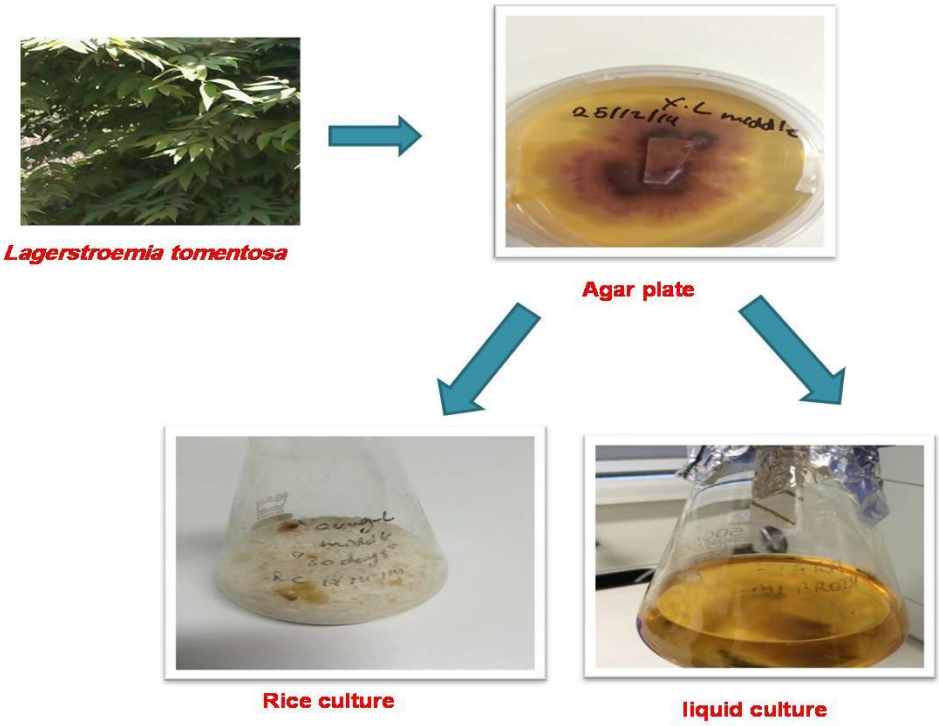
A major health problem is the increase of incidence of cancer and drug resistant bacterial infection cases which has become a leading cause of death worldwide. These facts emphasize the need for the search for new anticancer and anti- microbial agents. This work aims for the investigation of the secondary metabolites of *Lagerstroemia tomentosa* extract and associated fungal endophytes to search for new antimicrobial and anti-cancer natural products.



The ethyl acetate extract of the plant was prepared and fractionated on MPLC. Metabolite profiling and dereplication studies of the plant fractions identified known and new compounds [1]. Multivariate data analysis was employed efficiently to highlight the putative metabolites which were predicted to be responsible for the activity of active fractions. Metabolomics and bioassay-guided fractionation led to the isolation of five compounds. On the other hand; six endophytic strains were isolated from different parts of the fresh plant [1]. The fungal extracts were tested against lung and ovarian cancer cell line and five drug resistant bacteria (*MARSA16, MARSA106,* two strains of *Klebsiella pneumoniae* and *Mycobacterium marinum*). Investigation of the biological activity showed a significant anti-microbial activity and moderate anti-cancer activity. Metabolites fingerprinting using HR-MS and NMR was successfully used to choose the optimized culture media which yield the maximum amounts of the bioactive metabolites.

[1] Tawfike,A.F. and Viegelmann, C. and Edrada-Ebel, R. Metabolomics and dereplication strategies in natural products. In: Metabolomics Tools for Natural Product Discovery. Methods in Molecular Biology, 1055. Glasgow, UK; University of Strathclyde. 2013: 227-244.

PM-24

Phytochemical studies on *Galanthus gracilis* L.

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The genus *Galanthus* L. (Amaryllidaceae) is represented by 14 species (15 taxa) in Turkey. Among these species,*Galanthus gracilis* Ćelak. is mainly distributed in Western Turkey [1]. Galanthus species are known to produce Amaryllidaceae alkaloids which represent a diverse

group of isoquinoline alkaloids with a wide spectrum of biological activities [2]. Galanthamine, isolated from several Amaryllidaceae species, is a selective, reversible, long acting and competitive AChE inhibitor and therefore it is used for the treatment of Alzheimer's Disease [3]. Our previous investigations on *Galanthus gracilis* L. of Turkish origin afforded a number of new alkaloids with diverse structures indicating that it is a valuable source for chemically interesting alkaloids [4,5]. In the present study, *Galanthus gracilis* L. collected from a different locality (Odemis, Izmir), has been investigated for its alkaloidal profile. As a result, three known compounds namely, homolycorine, 8-*O*-demethylhomolycorine and *E*-feruloyltyramine, were isolated.

Acknowledgements: This study was financially supported by Ege University Research Fund (Project Number: 14/ECZ/032).

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[3] Ago Y, Koda K, Takuma K, Matsuda T. Pharmacological aspects of the acetylcholinesterase inhibitor galantamine. J Pharm Sci 2011; 116 (1): 6-17
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PM-25

Structures and bioactivities of withanolides from the leaves of *Solanum capsicoides*

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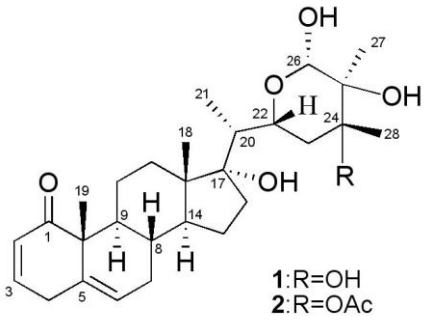
A known withanolide steroid cilistol G (**1**) [1] and six new related withanolides, capsisteroids A–F (**2- 7**), were isolated from the acetone extract of the leaves of *Solanum capsicoides*. The structures of compounds **1- 7** were elucidated by extensive spectroscopic analysis, including 2D NMR spectroscopy (COSY, HSQC, HMBC, and NOESY). The structure of **1** was further confirmed by a single-crystal X-ray diffraction analysis. It was found that **1**, possessing 26 *S* configuration, is not stable for prolonged periods of time, and was gradually epimerized to 26 *R* and finally reached to a 3:1 26 *S*/ *R* diastereomers. These compounds were evaluated for their cytotoxicity against the proliferation of a limited panel of cancer cell lines. Further, anti-inflammatory activity of compounds **1- 7** was studied by measuring their ability in suppressing superoxide anion generation and elastase release in fMLP/CB-induced human neutrophils.



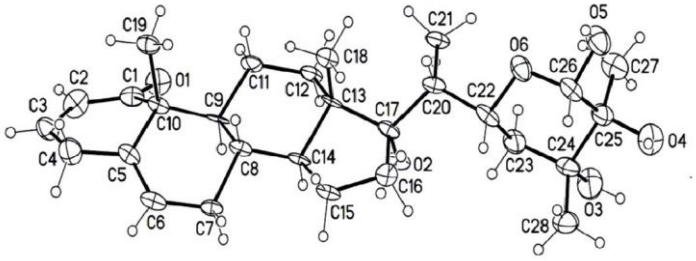


Figure 1. Molecular structure of **1** based on X-ray analysis.

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PM-26

Sinularioperoxide F, a new cytotoxic cyclic peroxide from soft coral *Sinularia erecta*

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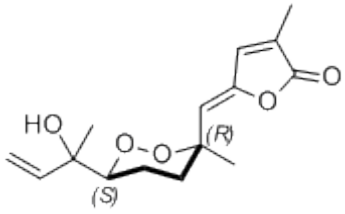
Soft corals belonging to the genus Sinularia have proven to be a rich source of diterpenes, sesquiterpenes, steroids, steroidal glycosides, sphingosine derivatives, glycolipids, and spermidine derivatives [1,2]. The acetone-solubles of the Formosan octocoral *Sinularia erecta* collected at Penghu island off Taiwan was found to exhibit cytotoxicity against P-388 (murine lymphocytic leukemia), HT-29 (human colon adenocarcinoma), and A-549 (human lung epithelial carcinoma) cells. Bioactivity-guided fractionation resulted in the isolation of a new cytotoxic compound, sinularioperoxide F (**1**). The structure of sinularioperoxide F (**1**) was determined on the basis of their spectroscopic analyses (^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, HMBC, NOESY, HRESIMS, UV and IR). Sinularioperoxide F exhibited significant cytotoxicity against P-388 cell and A-549 cell with ED_{50} of 2.2 and 3.6 μg/mL, respectively.



1

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PM-27

The influence of tannins on the human neutrophils' pro-inflammatory mediators.

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Medicinal products rich in tannins are popularly used as external remedies in the prevention and treatment of oral cavity diseases, which are a growing problem among patients. Neutrophils play a crucial role in the induction and progression of inflammatory response in the periodontal and gingival diseases.

The aim of this study was to establish if tannins occurring in externally used drugs can modulate the production of pro-inflammatory factors by LPS-stimulated neutrophils.

Five dimeric ellagitannins – oenothein B, salicarinins A, B, gemin A and gemin G –, five monomeric tannins – castalagin, vescalagin, casuarinin, stachyurin, pedunculagin and penta-O-galloyl-β-D-glucose (PGG) – all at concentrations of 1, 5 and 20 μM were tested as potential immunomodulatory agents using human neutrophils. The influence of pure tannins on the production and the release of reactive oxygen species (ROS), IL-8, IL-1β, TNF-α and MMP-9 was investigated.

The experiments showed that the investigated compounds could modulate the inflammatory response of human neutrophils. All five monomers and PGG were able to inhibit the ROS production at all tested concentrations, while dimeric compounds had pro-oxidative effect at 20 μM. Monomers and dimers at the concentration of 20 μM inhibited the release of IL-8. Monomers were also able to decrease the production of IL-1β while dimers induced the production of this cytokine at the highest tested concentration. Monomers and dimers enhanced the production of TNF-α at higher concentrations (5 and 20 μM). Only dimeric compounds gemin A, agrimoniin and salicarinin B at 20 μM were able to decrease the release of MMP-9.

The results show that tannins can modulate the inflammatory response of human neutrophils. The effect depends on the structure of the investigated compounds.

Acknowledgements: The project was financially supported by Polish National Science Center research grant PRELUDIUM- UMO-2012/07/N/NZ7/01136.

PM-28

An in-vitro evaluation of *Tinospora bakis* and *Curcuma longa* against *Madurella mycetomatis*

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Mycetoma is a chronic specific granulomatous subcutaneous inflammatory disease. It is caused by true fungi (eumycetoma) or by bacteria (actinomycetoma). Current medical treatment of eumycetoma by various antifungals seems to be inadequate and recurrence after a long treatment is very common [1].

An attempt was made to explore the potential antimycetomal activity of the root of *Tinospora bakis* (Menispermaceae) and the rhizome of *Curcuma longa* (Zingiberaceae). The root of *T. bakis* was extracted with chloroform and a mixture of methanol acetone, while the rhizome of *C. longa* was extracted with 70% ethanol followed by its concentration and fractionation with ethyl acetate. All the extracts and fractions were subjected to screening against *Madurella mycetomatis* using resazurin assay in sterile 96-well microplate [1].

The *T. bakis* chloroform extract, and methanol-acetone mixture exhibited MICs of 78.1 and 39.1 µg/mL, respectively. The crude ethanlic extract of C. longa showed inhibition with MIC of 39.1 which was far exceeding the MIC of 156 µg/mL of ethyl acetate fraction, while ketoconazole, the positive control showed MIC of 0.25 µg/mL.

The presence of alkaloid berberine and the diarylheptanoid curcumin provided evidence of the underline molecular mechanism of *T. bakis* [2] and *C. longa* [3], respectively.

[1] Khalid SA. Development of microtiter plate-based method for the determination of the MIC of antimycetomal agents against Madurella mycetomatis. ResNet NPND workshop on natural products against neglected diseases, Nov. 25-28th, 2014, Rio de Janeiro, Brazil.

[2] Dhamgaye,S., Devaux F., Vandeputte, P., Khandelwal NK. et al, Molecular mechanism of action of herbal alkaloid berberine, in Candida albicans. Plos One (2014) 9(8): e104554. Doi:10.1371/journal.pone.0104554

[3] Martins C V B., da Silva D L, Neres A T M, Magalhaes TFF, Watanabe G A, Modolo L Vet al. Curcumin as a promising antifungal of clinical interest, J. Antimicrob. Chemother. (2009) 63, 337–339.

PM-29

Protective impact of extract from *Aronia melanocarpa* berries against low-level exposure to cadmium-induced lipid peroxidation in the bone tissue: a study in a rat model

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Recently, using a rat model of low-level lifetime human exposure to cadmium (Cd), we have revealed that consumption of a polyphenol-rich extract from *Aronia melanocarpa* berries (AMPE) prevented gastrointestinal absorption and accumulation in the body of this toxic metal [1] and Cd-induced disorders in bone metabolism [2] as well as improved the antioxidative bone status [3]. In this study it was investigated whether AMPE may prevent Cd-induced lipid peroxidation in the bone tissue. F2-isoprostanes (F2-isoP) and lipid peroxides (LPO) were determined, as markers of lipid peroxidation, in the distal femur epiphysis (trabecular bone region) of female Wistar rats administered a 0.1% water AMPE (containing 61.24±0.01% of polyphenols and characterized by a potent free radical scavenging capability - 75.01±0.19%) or/and 1 mg Cd/kg diet for up to 24 months. Moreover, a correlation between Cd concentration and the extent of lipid peroxidation in the bone was evaluated. The exposure to Cd increased the bone concentration of F2-isoP (already after 3 months), but had no impact on LPO concentration. AMPE administration under the exposure to Cd completely prevented the Cd-induced increase in F2-isoP concentration. A positive correlation was noted between the bone concentrations of Cd and F2-isoP. Based on the results it can be concluded that consumption of AMPE may protect from lipid peroxidation in the bone tissue under low chronic exposure to Cd. The present study provides further evidence that *A. melanocarpa* berries seem to be good candidates for investigation of their possible prophylactic use in humans exposed to Cd.

Acknowledgement: This study was financially supported by the Grant (No. N N405 051140) from the National Science Centre (Poland).

[1] Brzóska MM *et al.* Curr Drug Targets, in press, doi: 10.2174/1389450116666150102121708.

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[3] Brzóska MM *et al.* Planta Med, 2014; 80: 1425.

PM-30

Pentacyclic Triterpenoids from Roots of *Lantana montevidensis* (Spreng.) Briq. Cultivated in Egypt

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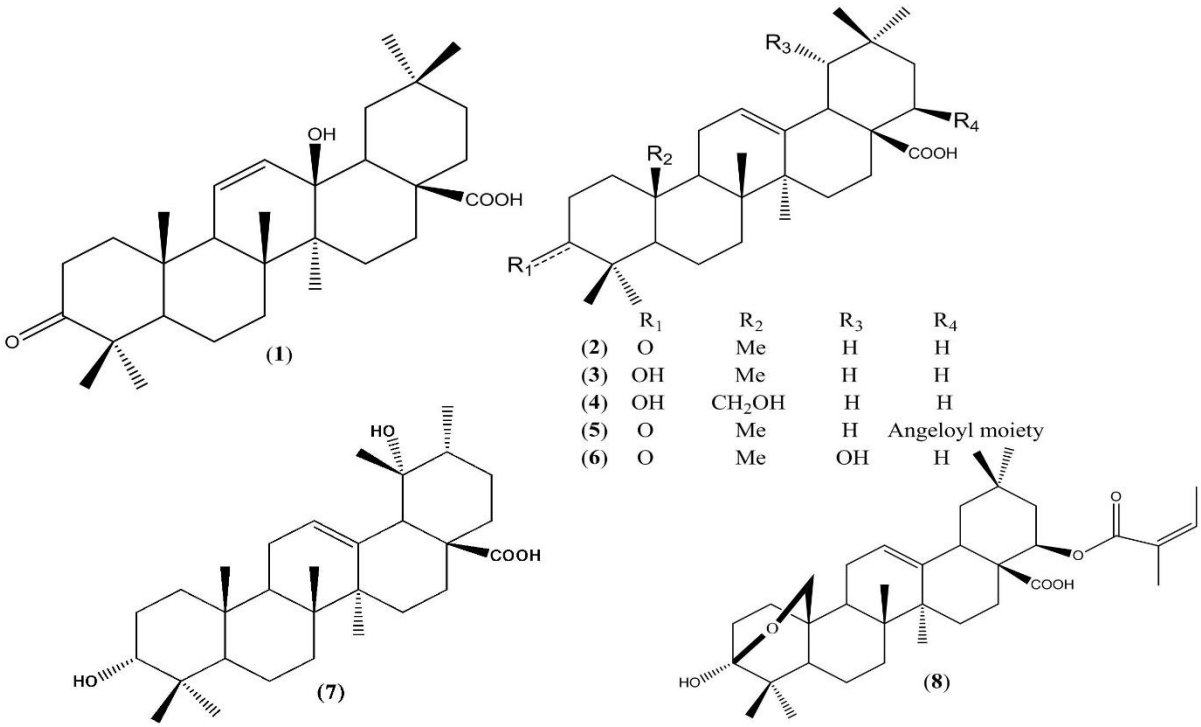
Eight pentacyclic triterpenoids have been isolated from the chloroform fraction of the roots of *Lantana montevidensis* (Spreng.) Briq. cultivated in Egypt. Compound (**1**), which is a new compound, established as 13β -Hydroxy-3-Oxo-Olean-11-en-28-oic acid (**1**) obtained as white amorphous powder with molecular formula $C_{30}H_{46}O_4$ as determined by the HRESIMS at m/z 453.3370 $[M\text{-}H_2O+H]^+$. The ^{1}H- and ^{13}C-NMR spectra showed the presence of seven quaternary methyls and two olefinic protons, an oxygen bearing quaternary carbon , one carbonyl group and a carboxylic group. The hydroxyl group at C-13 and the double bond at $\Delta^{11(12)}$ were located using HMBC experiment. The known compounds are: oleanonic acid (**2**), oleanolic acid (**3**), 3β,25β-dihydroxy-olean-12-en-28-oic acid (**4**), lantadene A (**5**), 19α-hydroxy-3-oxo-olean-12-en-28-oic (**6**), pomolic acid (**7**), camaric acid (**8**), together with β-sitosterol (**9**) and its glycoside (**10**). The isolated compounds were tested for antimicrobial activity and subjected to cannabinoid and opioid receptor binding assay. Compound **8** showed a good activity in Mu and delta opioid receptor binding assay with % of inhibition 48.8 and 46.0, respectively while it showed mild activity in CB2 cannapinoid receptor binding assay with % of inhibition 32.8. Compound **4** showed mild activity in CB1 and Delta opioid receptor assay with % of inhibition 32.1 and 27.9, respectively. Compound **8** showed potent antibacterial activity against *Staphylococcus aureus* and Methicillin resistant*Staphylococcus* with IC_{50} values of 4.95 and 4.58 μg/mL, respectively, while Compounds **2** and **4** showed moderate antibacterial activity toward Methicillin resistant *Staphylococcus* with IC_{50} 15.74 and 14.22 μg/mL, respectively (using Ciprofloxacin as a positive control with IC_{50} of 0.12 μg/mL, for both organisms). Compound **3** showed mild activity toward *Cryptococcus neoformance* with IC_{50} 19.84 μg/mL. (Using Amphotericin B as a positive control with IC_{50} of 0.28μg/mL).



PM-31

A new 2*H*-Pyran-2-one derivative and anti-inflammatory constituents from *Alpinia zerumbet*

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Alpinia zerumbet (Persoon) B. L. Burtt & R. M. Smith (Zingiberaceae), commonly known as shell ginger, is a perennial species of ginger native to East Asia. Flavonoids, monoterpenoids, kava pyrones, diterpenoids, and their derivatives are widely distributed in plants of the genus *Alpinia*. Many of these compounds exhibit diverse biological activities, including antihypertensive and antioxidant activities. In a preliminary screening, the methanolic extract of the rhizome of this species showed anti-inflammatory activities *in vitro*. The current phytochemical investigation of the rhizome of this plant has led to the isolation of a new 2*H*-pyran-2-one derivative, 4-hydroxy-6-(4-methoxyphenethyl)-2*H*-pyran-2-one (**1**), along with 4 known compounds. The structure of new compound **1** was determined through spectroscopic and MS analyses. (*E*)-Labda-8(17),12-diene-15,16-dial (**4**) and quercentin (**5**) exhibited potent inhibition, with IC $_{50}$ values of 3.18±1.13 and 1.17±0.13 μ g/mL, respectively, against formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP)-induced superoxide anion ($O_2^{\bullet-}$) generation. The structural elucidation of **1** and its anti-inflammatory property are described herein.

PM-32

Antineutrophilic inflammatory secondary metabolites from *Solanum macaonense*

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Eight new spirostanol saponins and ten new furostanol saponins, together with twenty-one known compounds including six spirostanols [1], four caffeic acid derivatives, two amides, seven flavonoids, one ligand, and benzaldehyde [2] were isolated from *Solanum macaonense*. All structures of compounds were determined from their spectroscopic data, and the compounds were tested for in vitro antineutrophilic inflammatory activity. It was found that both immediate inflammation responses including superoxide anion generation and elastase release were significantly inhibited by treatment with three of the newly isolated spirostanols and one caffeic acid derivative (superoxide anion generation: IC_{50} 7.0, 7.6, 4.0, 4.6 μM; elastase release: IC_{50} 3.7, 4.4, 1.0, 4.0 μM, respectively). However, two spirostanol saponins and one flavonoid exhibited effects on the inhibition of elastase release only, with IC_{50} values of 3.2, 4.2, and 4.0 μM, respectively, while one spirostanol and three caffeic acid compounds were active against superoxide anion generation only, with an IC_{50} value of 6.1, 3.3, 4.8, and 4.2 μM. Accordingly, spirostanols and caffeic acid derivatives may be promising lead compounds for further neutrophilic inflammatory disease studies, such as asthma, chronic obstructive pulmonary disease and acute respiratory distress syndrome.

[1] Lee CL, Hwang TL, Yang JC, Cheng HT, He WJ, Yen CT, Kuo CL, Chen CJ, Chang WY, Wu YC. Anti-inflammatory spirostanol and furostanol saponins from Solanum macaonense. J Nat Prod 2014; 77: 1770−1783

[2] Lee CL, Hwang TL, Peng CY, Chen CJ, Kou CL, Chang WY, Wu YC. Anti-inflammatory and cytotoxic compounds from Solanum macaonense. Nat Prod Commun 2015; 10: 345−348

PM-33

Evidence that the essential oil of bergamot modulates autophagy flux in human tumor cell lines

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The long standing folk use of the essential oil (EO) of bergamot (*Citrus bergamia* Risso et Poiteau) as antiseptic is consistent with the observed antifungal and antimicrobial activities in conjunction with the most recently described increase of oxidative metabolism in human polimorphonuclear leukocytes [1]. We have previously reported that in SH-SY5Y human neuroblastoma cultures the EO of bergamot causes concentration-dependent cell death [2]. Here we now report compelling evidence demonstrating that in these cell cultures bergamot modulates autophagy in a concentration-dependent fashion, not linked to effects on cell survival and via mechanisms mTOR-independent. Enhanced LC3 conversion and degradation of the autophagic substrate p62 are still observed following suppression of Beclin-1 expression by siRNA. The observed effects are not cell line specific because enhanced LC3 lipidation and reduced p62 levels were also observed in bergamot exposed MCF7 human breast cancer cells. In depth investigation on the principle responsible for autophagy activation shows that limonene, the main terpene of the EO, is responsible for such an important effect of the phytocomplex. Autophagy is a complex and finely tuned cellular process that plays fundamental roles under physiological as well as pathological conditions; in fact, it has been implicated in severe human diseases, including cancer. Accordingly, our original observation is of great importance for putative clinical translation in chemotherapy.

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[2] Berliocchi et al., 2011, Food Chem Toxicol 49: 2780-2792

PM-34

High-resolution hyaluronidase inhibition profiling combined with HPLC-HRMS-SPE-NMR for identification of anti-necrosis constituents in *Clausena excavata*

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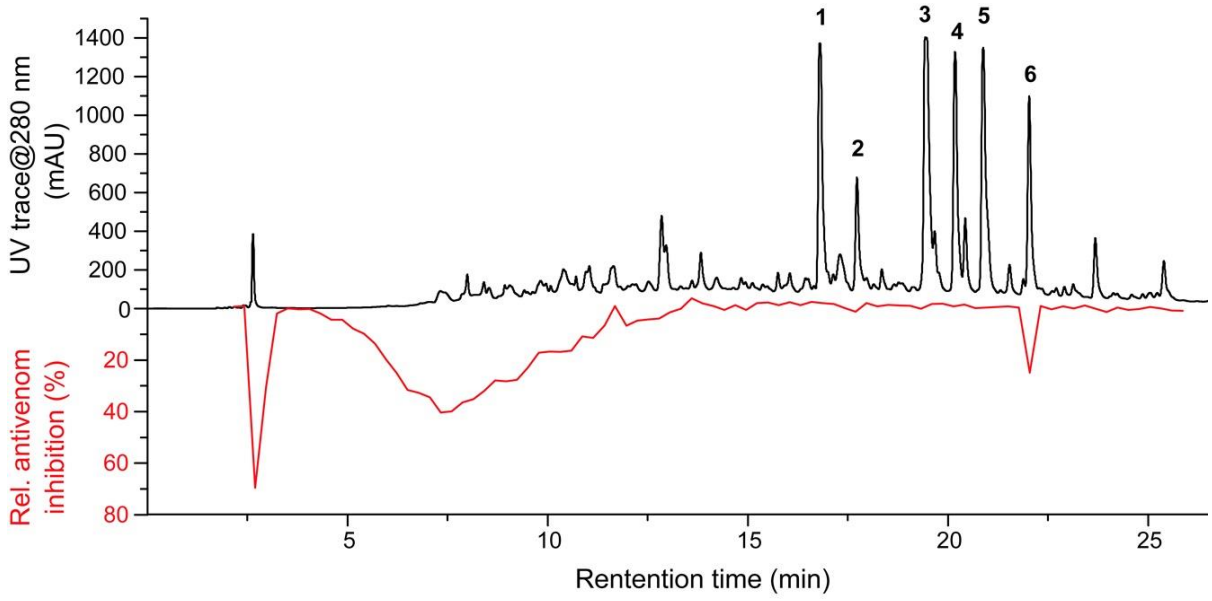
On average 100,000 persons are bitten by venomous snakes in China each year, with a mortality rate of 5-10% [1].*Gloydius blomhoffi brevicaudus, Deinagkistrodon acutus, Naja naja atra* and *Trimeresurus stejnegeri* bites are most common. Necrotizing enzymes phospholipase A_2, proteases, and hyaluronidase in the snake venom cause local tissue damage. *Clausena excavata* is a common plant used against snakebite in China. The aim of our study was to explore the anti-necrosis potential of plant *C.excavata* and identify non-tannin anti-necrosis constituents.

Extracts of *C. excavata* were tested at 1 mg/mL in hyaluronidase, phospholipase A_2, and protease inhibition assays [2] against *G.blomhoffi*, *D.acutus*, *N.naja* and *T. stejnegeri* venom. Extracts with over 90% inhibition were fractionated into microplates and biochromatograms were constructed (high-resolution profiling). Bioactive constituents from the biochromatograms were analyzed by HPLC-HRMS-SPE-NMR.

Analysis of HRMS and NMR data obtained via HPLC-HRMS-SPE-NMR led to identification of the metabolites as 2,3-dihydroxy-*N*-methyl-3-phenyl-*N*-[(*Z*)-styryl]propanamide (1), lansiumamide I (2), indicolactone (3), *N*-methyl-3-phenyl-*N*-[(*Z*)-styryl]oxirane-2-carboxamide (4), ξ-clausenamide (5), and lansiumamide B (6). The 2,3-trans double bond and 1',2'-cis double bond of 6 might be essential for the inhibition of hyaluronidase by comparing the structure with compound 1, 2, 4 and 5. Compounds 1-6 were purified by preparative scale HPLC and subjected to the activity test in the hyaluronidase assay against *D. acutus* venom. Compounds 1-5 showed no activity in the test. The IC_{50} value of lansiumamide B (6) was very close to the value of the standard hyaluronidase inhibitor aristolochic acid, which indicate lansiumamide B might be a promising inhibitor against snakebite of D. acutus.

[1] Nie HJ. Modern Clin Med 2007; 33: 234-236.[2] Molander, M et al. J. Ethnopharmacol 2014; 157: 171-180.



PM-35

Potential anti-dengue activity of three *Faramea* species (Rubiaceae) and their common active new flavanone glycoside

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Dengue virus infection is a neglected disease prevalent in most tropical and subtropical areas. Currently there is no vaccine or any antiviral drug indicated for the routine treatment of dengue patients. As a part of our ongoing search for potential anti-dengue virus agents we have investigated the MeOH extracts from the leaves of three Brazilian species of the genus*Faramea* (Rubiaceae). The *in vitro* cytotoxic and anti-dengue virus serotype 2 effects were measured in human hepatocarcinoma cell lineage (HepG2). The HepG2 cells were infected at a multiplicity of infection (MOI) of 1 with DENV-2 16681. After adsorption period, the cells were cultured in alpha-MEM medium, treated with 50 μg of the samples and then incubated for 48 h at 37 °C in 5% of CO_2 atm. Cell viability was assessed by MTT assay and viral replication was determined by virus titration by plaque assay using mouse fibroblast kidney cell line (BHK-21) of conditioned medium. The results were expressed as pfu/mL and normalized by the control. The extracts were non-cytotoxic and a marked reduction on viral load (ranged from 90% to 100%) was observed. Bioassay-guided fractionation of the bioactive crude extracts led to active flavonoid-rich MeOH/H_2O 9:1 fractions (reduction on viral load ranged from 77% to 100%). Sephadex LH-20 and reverse phase (C18) CC of these active fractions allowed isolation of a common bioactive new flavanone glycoside: 5-hydroxy-4'-methoxy-flavanone-7-*O*-ß-D-apiofuranosyl-(1→6)-ß-D-glucopyranoside (reduction on viral load of 83%). Structural determination was made by 1D and 2D NMR techniques, UV, OR and CD.

PM-36

Cytotoxic and apoptotic activities of 12,16-dideoxy aegyptinone B from *Zhumeria majdae* on MDA-MB-231, MCF-7 and NIH-3T3 cells

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Breast cancer is the most common cancer among women worldwide. Numerous chemotherapy agents are used in cancer treatment which exhibit significant effects on malignant cells. However, high side effects and resistance of cancer cells limit the use of these drugs. Therefore, searching for novel agents that are more efficient and selective on cancerous cells is still an important research line.

The present study was designed to determine the in vitro cytotoxic and apoptotic effect of an active compound from an Iranian native plant, *Zhumeria majdae* Rech. F. & Wendelbo [1-2].

MDA-MB-231(ER^-), MCF-7(ER^+) human breast cancer cell lines and NIH-3T3 (mouse embryonic fibroblast cell line) were incubated with the active compound, 12,16-dideoxy aegyptinone B, from Z. majdae. Cell viability was evaluated by MTT assay. The results were presented as 50%-inhibitory concentration (IC_{50}). Apoptosis was further measured by flowcytometry using an annexin V-PE/7-AAD apoptosis assay kit.

The results indicate that IC_{50} values of the active compound are 0.91±0.05, 0.722±0.06 and 1.08±0.02 µg/ml against MDA-MB-231, MCF-7 and NIH-3T3 cells, respectively. Cells treated with the active compound showed increasing number of apoptotic cells in a time dependent manner.

The results conclusively imply that 12,16-dideoxy aegyptinone B possesses apoptosis-based cytotoxicity against breast cancer cells. The high cytotoxic effects on both estrogen receptor negative cells (MDA-MB-231) and estrogen receptor positive cells (MCF-7), suggests that the compound could be a good candidate for further investigations.

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[2] Mohaddese M, Nastaran K. Antimicrobial activity of *Zhumeria majdae* essential oil against different microorganisms from Iran. Pharmacognosy Magazine 2009; 5:105.

PM-37

Targeting the antiapoptotic BCl-2 family proteins with flavonoid scaffolds

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Discovering small molecule inhibitors targeting protein-protein interactions (PPIs) is a very challenging issue mainly due to the large interface area implicated in the binding and the intrinsic difficulty to compete/dissociate with a small molecule. Anti-apoptotic proteins belong to one of the most important cancer drug targets since they neutralize pro-apoptotic protein counterparts and lead to immortality of cancer cells, thus development of compounds targeting these proteins is of immense therapeutic importance. Herein, we focused on flavonoid natural product scaffolds [1] in an effort to discover pharmacophores required to recognize BCL-2 protein and lead to apoptosis utilizing a multidisciplinary approach including biochemical and physicochemical assays.

Acknowledgement: This project has been co-financed by the European Union (European Regional Development Fund- ERDF) and Greek national funds through the Operational Program "THESSALY-MAINLAND GREECE AND EPIRUS-2007-2013" of the National Strategic Reference Framework (NSRF 2007-2013).

[1] Primikyri A, ACS Chem Biol. 2014 Dec 19;9(12):2737-41.

PM-38

In vitro susceptibility of *Madurella mycetomatis* to the leaves extracts of *Terminalia brownii*

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The present communication represents an attempt to investigate the antimycetomal activity of the leaves of *Terminalia brownii* (Combretaceae) and to define the chemical profiles of the active agents. The antifungal activity of a number of taxa of this family has been attributed to its tannin and saponin contents [1].

Air dried ground leaves of *T. brownii* were extracted using 80% methanol. The concentrated methanolic extract was sequentially fractionated with petroleum ether, chloroform and ethyl acetate. The aforementioned extract and its respective organic fractions were tested against *Madurella mycetomatis* employing 96-microplate-based anti-mycetomal assay incorporating resazurin as an indicator [2]. The plant methanolic crude extract showed activity against *M. mycetomatis* with an MIC of 78.1 µg/mL while the chloroform and ethyl acetate fractions exhibited significant activity with MIC<39.1 µg/mL.

Reverse phase HPLC coupled with tanden mass spectromentry led to the identification of a tetrahydro chalcone, p-coumaryl glucoside, and catechin 3–*O* gallate in the ethyl acetate fraction. Additionally, two flavonoids beside the C-glycosylated flavone, isoorientin, and myricetin 3- *O*-hexose gallate were also identified in this fraction. Combrestastin B5 *O*- hexose gallate stilbene, in addition to five other prenylated stilbenes were also identifiedt. Ellagic acid was the major compound in the chloroform fraction. Hydroxylated flavan-3-ol gallolcatechin was identified in this fraction along with two polymethoxylated flavonoids.

[1] Baba-Moussa F, Akpagana K, Bouchet P. Antifungal activities of seven West African Combretaceae used in traditional medicine. Journal of Ethnopharmacology 66 (1999) 335–338

[2] Khalid SA. Development of microtiter plate-based method for the determination of the MIC of antimycetomal agents against Madurella mycetomatis. II ResNet NPND workshop on natural products against neglected diseases, Nov. 25-28th, 2014, Rio de Janeiro, Brazil.

PM-39

Antibacterial compounds from *Cratoxylum* spp.

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The phytochemical investigations of *Cratoxylum* spp. including *C. cochinchinense*, *C. formosum* ssp. *prunifrolum* and *C. sumatranum* led to the isolation and identification of thirty eight compounds. Twelve, fourteen, and twelve compounds were isolated from *C. cochinchinense* stem barks, *C. formosum* ssp. *prunifrolum* roots and *C. sumatranum* twigs, respectively. Their structures were elucidated on the basis of spectroscopic methods, including UV, IR, NMR and MS. These isolated compounds can be divided into four types including xanthones, anthraquinones, benzophenones and isocoumarin. Some compounds show significant antibacterial activity against *Micrococcus luteus*, *Bacillus cereus*, *Staphylococcus epidermidis*and *Pseudomonas aeruginosa* with MIC values ranging from 1-8 μg/ml.

PM-40

Identification of the strictosamide isolated from *Nauclea latifolia* as the bioactive agent against *Madurella mycetomatis*

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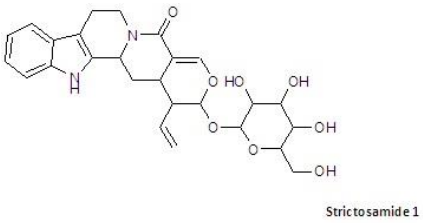
The decoction of different parts of *Nauclea latifolia* Smith (Rubiaceae) is commonly employed in the African traditional medicine for the treatment of several diseases, such as malaria, gastrointestinal tract disorders, and as antimicrobial [1]. It has already been demonstrated that *N. latifolia* is very efficient in the biosynthesis of β-carboline alkaloids besides other secondary metabolites [2].

In our quest to identify a new antifungal agent from natural source we screened the crude extract of the fruits, root bark, leaves and stem bark of *Nauclea latifolia in vitro* against *Madurella mycetomatis*, the most common eumycetoma causative organism.

Following bioactivity guided fractionation while employing the newly developed resazurin assay in 96-microplate [3] a promising inhibitory activity emerged against *M. mycetomatis* ranging between 625 and 39.1 μg/mL. This attempt has eventually resulted in the isolation of the β-carboline alkaloid, strictosamide (**1**), which exhibited a MIC of 3.91 μg/mL while ketoconazole, the positive control showed MIC of 0.25 μg/mL. It is pertinent to note that

strictosamide has already exhibited antiparasitic activity against a number of neglected diseases [2].



Strictosamide 1

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PM-41

Chemical constituents and their bioactivities from the stem of *Neolitsea konishii*

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Neolitsea konishii (Hay.) Kanehira & Sasaki (Lauraceae) is a small evergreen tree that is distributed in the Ryukyus and Taiwan. The methanolic extract of the stem of *Neolitsea konishii* has been shown with anti-eβG (*Escherichia coli* β glucuronidase) and anti-inflammatory activities. The anti-eβG activity of the stem of *N. konishii* has not yet been investigated; therefore, the aim of this study is to isolate its chemical constituents and evaluate their biological activities.

Bioassay-guided fractionation of the stem of *N. konishii* has led to the isolation of one new 1,3-diphenylbutanoid, (–)-konishibutanin and two new lignans, neokoninins A and B, along with 21 known compounds till now. The structures of these isolates were elucidated by spectral methods. Among the isolates, 13^2-hydroxy-(l3^2-R)-pheophytin a and *N-trans*-feruloyl-3',4'-dihydroxyphenylethylamine exhibited potent anti-eβG activity with inhibition ratio of 88% and 76% and with low inhibition of hβG (human β glucuronidase) respectively. In addition,

trans-9,9'-*O*-di-(*E*)-feruloyl-(–)-secoisolariciresinol showed potent inhibitory U46619 factor and fMLP-induced superoxide generation in human neutrophils activity with IC_{50} values of 7.89±0.35 and 2.43±0.03 μM.

PM-42

In vitro evaluation of cytotoxic activity of *Trigonella foenum-graecum* on human cervical cancer HeLa cells

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Trigonella foenum-graecum L. (fenugreek) is a plant of the Fabaceae family. The species contains steroidal saponins, flavonoids, polysaccharides and phenolic acids, which are pharmacological active compounds with anti-inflammatory, antioxidant and hypoglycemic properties. This study reports on the evaluation of cytotoxic effects of the plant methanolic and aqueous extracts, flavonoid fraction and isolated flavonoids -orientin, isoorientin, vicenin-1, -2, and -3- on human cervical adenocarcinoma cell line HeLa. The antiproliferative activity was estimated on the cells with MTT assay and the xCELLigence system (Roche), which allows continuous monitoring of cell viability. The obtained data show that both methanolic and aqueous extracts, as well as the flavonoid fraction have significant cytotoxic activity on the cells with IC_{50} values of 13.47±0.62 μg/mL, 17.43±0.30 μg/mL and 3.91±0.03 μg/mL, respectively. The flavonoids did not exhibit activity on the cells (IC_{50}>50 μg/mL). Additionally, HeLa cells treated with the fraction were stained with annexin V and analyzed on the Muse Cell Analyzer (Merck Millipore). The flow cytometry analysis shows that the fraction induces apoptosis in the tested cells in a dose-dependent manner. To confirm induction of apoptosis in HeLa cells incubated with the fraction, we stained the cells with Hoechst 33342 dye and observed the chromatin condensation and nuclear fragmentation in the cells.

This study indicates that the flavonoid fraction isolated from *T. foenum-graecum* could be a potential drug in the treatment of human cervical cancer.

PM-43

Bioactive phthalides derivative from the stem of *Pittosporum illicioides* var. *illicioides*

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Pittosporum illicioides var. *illicioides* (Pittosporaceae) is an evergreen shrub that grows in medium-to-high altitude forests throughout China and Taiwan. Sesquiterpene glycosides, carotenoids, tritepenoid saponins, phthalide, and their derivatives are widely distributed in plants of the genus Pittosporum. Many of these compounds exhibit diverse biological

activities, including cytotoxic and antimicrobial activities. In our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for *in vitro* anti-inflammatory activity, and *P. illicioides* var. *illicioides* has been found to be an active species. Phytochemical investigation of the stem of this plant has led to the isolation of two new phthalide derivatives, (*E*)-3-ethylidene-5,6,7-trimethoxyphthalide (**1**) and (*S*)-3-ethyl-5,7-dihydroxy-6-methoxyphthalide (**2**) and five known compounds. The structures of new compounds **1** and **2** were determined through spectroscopic and MS analyses. Among the isolated compounds, (*E*)-3-ethylidene-5,6,7-trimethoxyphthalide (**1**), (*S*)-3-ethyl-5,7-dihydroxy-6-methoxyphthalide (**2**), and (*Z*)-3-ethylidene-6,7-dimethoxy-phthalide (**3**) exhibitited inhibition (with IC_{50} values in the range of 0.64-6.66 μ g/mL) of superoxide anion generation by human neutrophils in response to formy-L-methionyleu-L-lckyl-L-phenylalanine/ cytochala-sin B (fMLP/CB).

PM-44

Isolation and structural elucidation of secondary metabolites obtained from the soft coral *Sinularia candidula* present in the Egyptian Red Sea

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The present study described the isolation and structural elucidation of five compounds (**1**-**5**) obtained from the Red Sea soft coral *Sinularia candidula*. Sinularia is a soft coral belonging to the phylum Cnidaria, class Alcyonaria, and family Alcyoniidae. Its taxonomic identification is largely based on the examination of the spicules, and a fairly reliable taxonomic key is available to guide species identification [1]. The presently investigated species yielded two sterols namely, Cholesterol (**1**), 24-methylene cholesterol (**2**), two oxygenated compounds namely chimyl alcohol (**3**), heptadecanoic acid (**4**), along with the ceramide (R)-2'-hydroxy-N-[(2S,3S,4R)-1,3,4-trihydroxypentacosan-2-yl] nonadecanamide (**5**). Their structures were elucidated by means of 1D and 2D NMR spectroscopic techniques. Cytotoxicity testing for *Sinularia candidula* extracts of different polarities was performed against HELA, HCT, MCF-7 and PC3 cell Lines [2], where the hexane-ethyl acetate (1:1) fraction revealed the highest percentage of inhibition by 73.77 % and 78.92 % against MCF-7 and PC3 cell lines, respectively, while the ethyl acetate fraction showed the highest percentage of inhibition by 67.29 % against HCT cell line. This study also includes the investigation of lipoidal matter of the Red sea soft coral *Sinularia candidula*, where the hydrocarbons as well as the fatty acid contents were identified in both the unsaponifiable and saponifiable fractions of the total extract using GC/MS.

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PM-45

Synergistic potentials of coffee on injured pancreatic islet and insulin action via K_{ATP} channel blocking in zebrafish

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Pancreatic islets (PIs) are damaged under diabetic conditions, which can cause decreases in PI size [1, 2, 3]. In this study, we examined the regenerative effects of coffee and its components (caffeine, CFI; trigonelline, TRG; chlorogenic acid, CGA) on zebrafish larval PIs and β-cells damaged via alloxan (AX) administration. In addition, we investigated the influence of coffee and its active components on K_{ATP} channels using diazoxide (DZ) as a K_{ATP} channel activator [4, 5]. PI size and fluorescence intensity were significantly increased in the coffee-treated group relative to the no-treatment group ($P<0.0001$). Coffee exerted significant regenerative effects on pancreatic β-cells ($P=0.006$). Furthermore, treatment with TRG and CGA resulted in recovery from PI damage, and the combination of TRG/CGA had a synergistic effect. In conclusion, our results show that coffee can exert beneficial effects on PIs damaged by AX, and furthermore that coffee has the potential to be used as a blocker of pancreatic β-cell K^+ channels.

[1] Prentki, M., & Nolan, C. J. Islet β cell failure in type 2 diabetes. Journal of Clinical Investigation, 2006, 116(7), 1802.

[2] Altobelli, E., Blasetti, A., Verrotti, A., Di Giandomenico, V., Bonomo, L., Chiarelli, F. Size of pancreas in children and adolescents with type I (insulin-dependent) diabetes. J. Clin. Ultrasound. 1998, 26(8), 391-395.

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PM-46

New pyrrolizidine alkaloid from *Heliotropium digynum*

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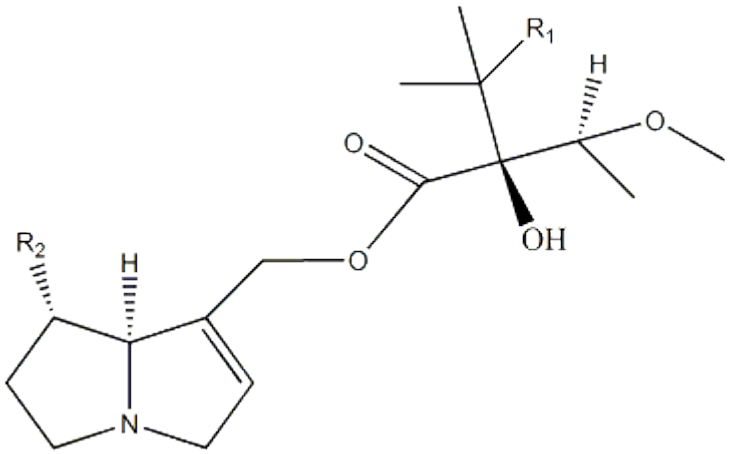
The phytochemical study of the ethanol extract of aerial parts of *Heliotropium digynum* Forssk family Boraginaceae growing in Egypt resulted in the isolation and identification of seven pyrrolizidine alkaloids: heliotrine **(1)**, heliotrine N-Oxide **(2)**, 7-angeloyl heliotrine **(3)**, 7-angeloyl heliotrine *N*-Oxide **(4)**, lasiocarpine **(5)**, europine **(6)** and europine *N*-Oxide (**7**). Compound **4** was isolated for the first time from natural source and its structure was determined using 1D and 2D NMR spectroscopic analysis. Although compounds **2** and **7** are known alkaloids but were isolated for the first time from this species. The ethanol extract at concentration 25 µg/mL showed a very comparable inhibition of nitric oxide generation (40%) to reference drug Dexamethasone at concentration 0.05 µg/mL (46%). It is also notable that the ethanol extract inhibits tumor necrosis factor alpha (TNFα) secretion by 66% in comparison to positive control lipopolysaccharide (LPS), in LPS stimulated RAW 264.7 murine macrophage.



	R_1	R_2
1	**H**	**OH**
2	**N-Oxide of 1**	
3	**H**	**angeloyl**
4	**N-Oxide of 3**	
5	**OH**	**angeloyl**
6	**OH**	**OH**
7	**N-Oxide of 6**	

PM-47

Phenolic profile, antioxidant and antinociceptive properties of *Syringa vulgaris*

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Syringa vulgaris L. (Common lilac, fam. Oleaeae) has been traditionally used in folk medicine to treat several ailments. In this study, we investigated the chemical composition, antioxidant and antinociceptive activity of *S. vulgaris* bark and leaf. For identification of the compounds, accurate molecular mass and formula, acquired by LC and electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS), and fragmentation patterns given by LC-ESI-MS/MS analyses were used. Altogether 29 phenolics were identified in the extracts, including 15 secoiridoids, 4 cinnamic acid derivatives, 4 flavonoids and 6 low molecular weight phenols. Based on the chromatograms, syringin and oleuropein are the main components of the bark, and rutin and oleuropein of the leaf. The radical scavenging activities of the extracts and the major constituents were also investigated by the DPPH and ABTS assays. Both extracts showed remarkable antioxidant activities in both tests. *In vivo* analgesic activity of the methanolic extract of Syringae folium and cortex was also tested using the hot-plate test on mice. Basal reaction time was determined, and two doses of each extract (100 mg/kg and 200 mg/kg body weight) were administrated to two groups. Aspirin (ASA) (200 mg/kg) was used as reference standard and vehicle served as the control. Our investigations show that both extracts present similar analgesic effect as ASA at a dose of 200 mg/kg [1].

The objective of this study was to provide molecular evidence for the antioxidant and antinociceptive effects of Syringae folium and cortex extracts, which could lay the scientific basis of future clinical perspectives.

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PM-48

Antihyperglycemic principles of *Caesalpinia sappan* L. wood, through *in vitro* inhibition of carbohydrate digestive enzymes: α-glucosidase and α-amylase

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One approach of diabetes treatment is controlling the postprandial blood glucose level, achieved by inhibiting carbohydrate digestive enzymes that include intestinal α-glucosidase and pancreatic α-amylase [1]. Indonesian indigenous plants are a source of compounds for use

as alternative antihyperglycemic agents. Based on 28 Indonesian plants screening experiment, *Caesalpinia sappan* L. wood showed potential α-glucosidase inhibition.

To identify α-glucosidase inhibitors, in particular intestinal maltase inhibitors contained in *C. sappan* L. wood using enzyme assays and in addition their inhibitory activity in α-amylase and intestinal sucrase.

The ethyl acetate-soluble layer from aqueous methanol extracts of *C. sappan* was fractionated successively by silica gel column chromatography and HPLC (ODS) to yield four phenolic compounds – brazilin (1, 0.03% of plant weight), sappanchalcone (2, 0.01%), protosappanin B (3, 0.5%) and protosappanin C (4, 0.12%).) – as intestinal maltase inhibitors. The intestinal maltase inhibitory activity was in the order of 3 (IC_{50} = 0.81 mM), 2 (0.96 mM), 4 (2.59 mM), and 1 (3.83 mM). The isolated compounds were also investigated for their intestinal sucrase and α-amylase inhibitory activity. Although 1 showed moderate inhibition of intestinal sucrase (IC_{50} = 1.12 mM) and PPA (IC_{50} = 1.22 mM), compounds 2, 3, and 4 did not significantly inhibit sucrase and PPA at the tested concentrations. Acarbose was carried as positive control in *in vitro* enzymatic assay (IC_{50} maltase = 0.5 μM; sucrase = 14 μM; α-amylase = 5 μM).

Caesalpinia sappan wood showed antihyperglycemic activity through the inhibition of digestive enzymes, α-glucosidase and α-amylase. This research may be useful for the alternative medicines and complementary therapies for diabetes prevention and management.

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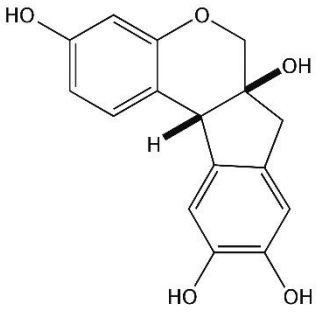
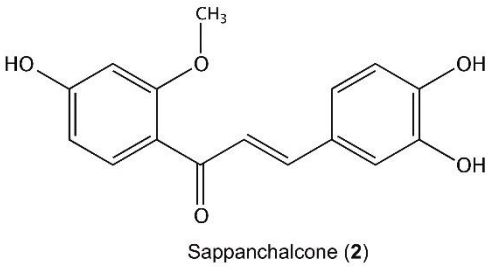
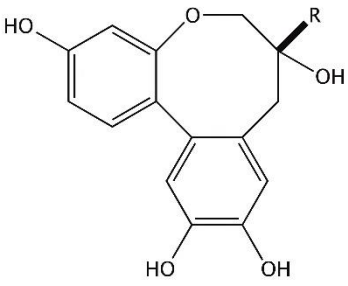


Brazilin (**1**)



Sappanchalcone (**2**)



R = CH_2OH: Protosappanin B (**3**)
R = CHO : Protosappanin C (**4**)

PM-49

Skeletal muscle protective effect of capillarisin from *Artemisia capillaris* on exercise-induced damage

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Capillarisin, separated from *Artemisia capillaris* is known to have anti-inflammatory, antioxidant and anti-cancer effects. In this present study, we investigated skeletal muscle

recovery after intense exercise (C57BL6 mouse; 13 m/min for 60 min downhill running) with or without capillarisin administration (ip injection). Muscle damaging exercise mainly results in oxidative stress and inflammation. Reactive oxygen species (ROS) produced during exercise may increase nuclear translocation of nuclear factor-κB (NF-κB), chemokine and cytokine expression, leading inflammation. C57BL6 mice were divided into rested control, exercised, and exercised with capillarisin treatment (20 mg/kg and 80 mg/kg) groups. Exercise increased nuclear p65 and cytosolic p- IκBα at protein level, the chemokine CINC-1 and MCP-1, and cytokine IL-6 at mRNA level in gastrocnemius muscle, whereas capillarisin limited these increase. From DCFH-DA antioxidant assay, exercise increased the level of ROS production (169±1.24 % of control), but these changes were attenuated by capillarisin-treated groups (109±4.67, 105±7.28 % of control respectively). Capillarisin-treated groups also improved the inflammation markers, such as creatinine phosphate kinase (CPK, peak 580±20 U/L reduced to the normal range 270±50, 210±10 U/L respectively) and lactate dehydrogenase (LDH, peak 3835±245 IU/L reduced to the normal range 1400±150, 1390±110 IU/L respectively) levels in plasma.

Overall, these results indicate that ROS produced by intense exercise involves in inflammation and capillarisin can attenuate such damage by exerting both antioxidant and anti-inflammatory effects. Thus, our results suggest that capillarisin is a potential candidate for the muscle protective agent in the exercise-induced muscle damage.

PM-50

Investigation of pharmacological properties of *Ananas comosus* extracts on uterine activity

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In folklore medicine *Ananas comosus* (L.) Merr. is reputed to act as an abortifacient and in expectant women as a means of inducing labor. Several reports have claimed abortifacient property of *A. comosus* fruit (ripe or unripe) [1-2]. Scientific evidences supporting the efficacy of pineapple extracts in inducing uterine contractions is clearly lacking. This study investigated the pharmacological effects of different fractions of pineapple extract to identify the most potent uterotonic fraction.The ethanolic crude extracts of pineapple (edible part) were prepared and fractionated through a series of liquid-liquid partitions. Fractions were separately tested on isolated rat uterine muscle from virgin and pregnant SD female rats and human pregnant myometrium, which were cut into strips along the longitudinal axis of uterus, mounted vertically in organ baths (37 °C) and exposed to cumulative addition of fractions (0.1-10 $mg.ml^{-1}$), serotonin (0.05-5 μM) and different blockers to delineate the mechanism of action of the bioactive compounds. Following our initial finding that aqueous fraction (F4) possesses uterine stimulant property which was blocked by verapamil but unaffected by indomethacin and prazosin, the uterotonic activity induced by F4 was further characterized using antagonists of 5-HT(2A and 2C) receptors. Notably, ketanserin (10 μM), diminished the maximal contractile response (% of 120 mM KCl contracture) induced by both F4 and 5HT by 74.3% and 92.1% respectively, implicating the presence of 5HT or 5HT-like compound and serotonergic pathways in uterotonic activity of aqueous fraction of pineapple extract (Fig. 1).

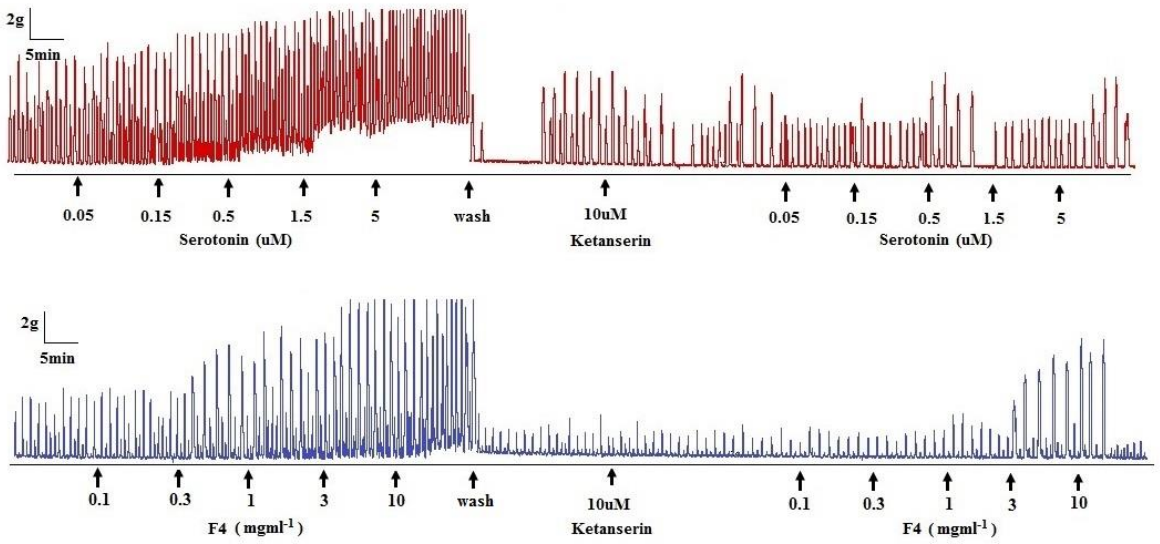

Fig. 1. Representative traces of serotonin and F4 on spontaneously contracting rat uterine strips (late pregnant) in the absence (control) and presence of ketanserin

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PM-51

Creation of medicinal products on the basis of *Limonium gmelinii*

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The expected advantage of herbal medicines lies in softness and complexity of their therapeutic action, low toxicity, absence of cumulative effects, addiction and rare induction of allergic reactions. One of the medicinal plants meeting those criteria is*Limonium gmelinii* (Willd.) Kuntze, family Plumbagenaceae, which has industrial resources on the territory of Kazakhstan.

Roots of *L. gmelinii* are introduced into the medicine in 2005 (Pharmacopeial Article 42-903-05) and as a monograph into the State Pharmacopoeia of the Republic of Kazakhstan in 2009 (Vol. 2, pp. 706-707). Unified by the source name "Limonidin" was given to all medicinal products, derived from the roots of *L. gmelinii*.

Substance "Limonidin" is obtained with 22-25% yield by extraction of roots with 50% ethanol followed by concentration of filtrate to dryness. The chemical study of this substance revealed that the largest group of biologically active compounds is comprised of polyphenols: flavonoids with dominance of aglycone myricetin and its glycoside and condensed tannins,

including (-)-epigallocatechine-(4β→8)-(-)-3,5,7,3',4',6'-hexahydroxyflavan and (+)-gallocatechine-(4α→8)-[(-)-epigallocatechine]5-(4β→8)-(-)-epigallocatechin gallate (Chemistry of Natural Compounds, 2006). As was shown by clinical trials its hepatoprotective activity is comparable with Silibor, its antiviral effect with amphotericin B, oxolinic ointment and ribavirin.

Tincture "Limonidin" was obtained by extraction of roots with 70% ethanol, filtration and sedimentation, whereas ointment and syrup "Limonidin" were created on the basis of the substance. Basis of the ointment are petrolatum and emulsifier T-2; other excipients include methyl and propyl parabens, 40% ethanol and purified water. Besides the substance and purified water syrup also includes 95% ethanol, glycerin and sugar. As was shown by clinical trials tincture and syrup display antiinflammatory effect, while ointment – antiviral and antiinflammatory.

PM-52

Five new anti-inflammatory active flavonoids from the aerial part of *Lindera akoensis*

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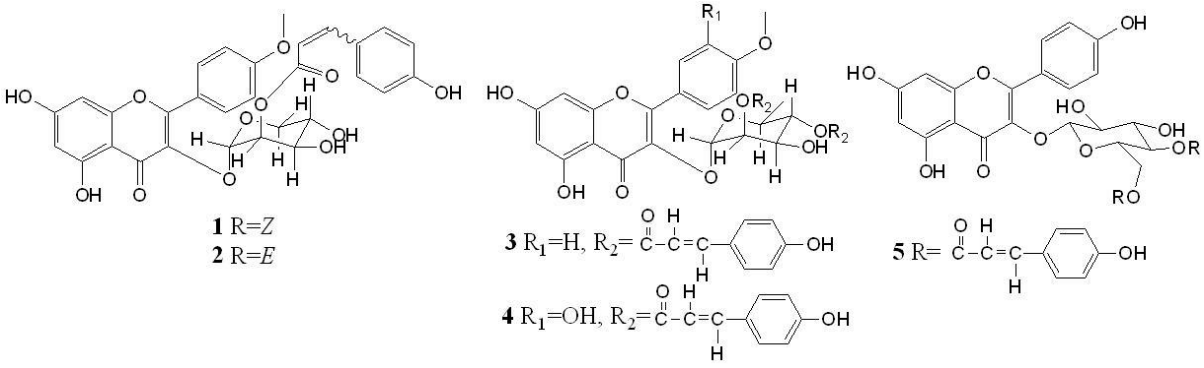
Lindera akoensis (Lauraceae) is an endemic evergreen tree that grows in broad-leaved forests in lowlands throughout Taiwan. Aporphines, alkaloids, sesquiterpenoids, butanolides, furanoids, chalconoids, and phenolic compounds were widely distributed in the plants of the genus Lindera. Some isolates exhibit biological activies, including anti-mycobacterial, anti-inflammatory, anti-human lung cancer (SBC-3), osteoclast differentiation inhibitory, human slowing down of the pregession of diabetic nephropathy in mice, anti-nociceptive, and LDL anti-oxidation effect et al.. In this study, we have isolated five new flavonoids, 4'-*O*-methyl-2"-Z-p-coumaroylafzelin (**1**), 4'-*O*-methyl-2"-*E*-*p*-coumaroylafzelin (**2**), 4'-*O*-methyl-2",4"-di-*E*-*p*-coumaroylafzelin (**3**), 4'-*O*-methyl-2",4"-di-*E*-*p*-coumaroyltamarixetin (**4**), kaemferol-3-*O*-α-*L*-4",6"-di-*E*-*p*-coumaroylglucoside (**5**). The structures of these new compounds were determined by the analysis their spectroscopic date. The compounds **1**, **2**, **3**, **4** and **5** exhibited anti-inflammatory activity decrease the LPS-stimulated product of nitrite in RAW 264.7 cells with IC_{50} values of 14.9, 11.3, 6.9, 25.5 and 15.6 μg/ml, respectively. (Indomethacin was used as positive control with IC_{50} value of 182.9μM)



PM-53

New sesquiterpenes from *Neurolaena lobata*

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The Maya ethnopharmacological plant *Neurolaena lobata* (L.) R.Br. ex Cass (Asteraceae) has been widely applied in traditional medicine in Central and South America for the treatment of different types of cancer, ulcers, inflammatory skin disorders and diabetes. The main constituents of the plant were identified as sesquiterpene lactones, which are responsible for its different pharmacological effects. Our previous studies revealed the presence of 13 sesquiterpenes of the germacranolide, eudesmanolide, eudesmane and furanoheliangolide types and some of them were reported as active compounds against cancer and inflammation *in vitro* and *in vivo*. In continuation of our studies on this field, the aim of the present work was the isolation and identification of new bioactive sesquiterpenes from *N. lobata*.

The aerial parts of the plant were extracted with methanol. After evaporation it was subjected to solvent–solvent partition with *n*-hexane and CH_2Cl_2. The CH_2Cl_2 fraction was chromatographed by a combination of different methods, including CC, VLC and preparative TLC. The structure determinations of the isolated compounds were carried out by means of MS and NMR (1D- and 2D-NMR) spectroscopy.

The results allowed the identification of four new sesquiterpenes, all esterified with isovaleric acid at C-8 or C-9. The new compounds are lobatin E, 8β-hydroxy-9α-isovaleroyloxy-calyculatolide, an unsaturated 1,10-epoxy-germacranolide ester, and an unprecedented non-lactone type 6,10-epoxy-germacranolide ester. Furthermore, one known furanoheliangolide lobatin C, and one known eudesmane type sesquiterpene volenol were also isolated from the plant. In the case of lobatin C, the previously published spectral data were supplemented with complete ^{1}H- and ^{13}C-NMR shift assignments.

Acknowledgement: This work was supported by the Hungarian Scientific Research Fund (OTKA K109846) and a János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

PM-54

High-resolution α-glucosidase and radical scavenging profiling combined with HPLC-HRMS-SPE-NMR for identification of bioactive constituents in crude extract of *Pueraria lobata*

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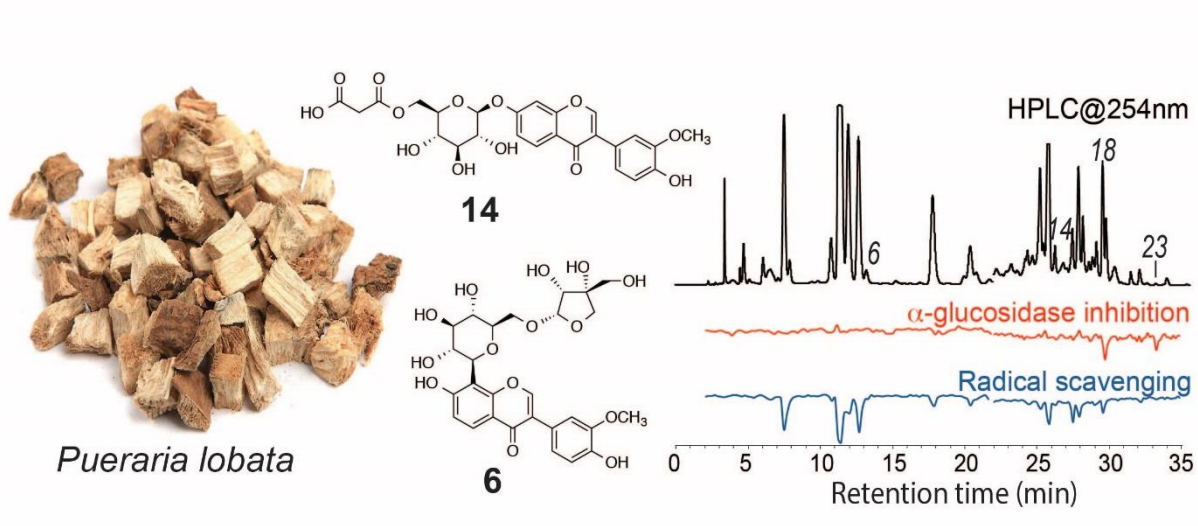
Pueraria lobata is a perennial leguminous vine, which is widely distributed in China where it is used as a dietary supplement and herbal medicine due to its profound pharmacological functions. *P. lobata* extract has previously

shown antioxidant activity - one of the most important effects of functional food, dietary supplements and anticancer natural products - as well as α-glucosidase inhibitory activity - an important effect for managing blood glucose for type 2 diabetics. This work describes the identification of active constituents in *P. lobata* root extract by dual high-resolution α-glucosidase inhibition and radical scavenging profiling combined with HPLC-HRMS-SPE-NMR [1,2]. The resulting HR-bioassay/HPLC-HRMS-SPE-NMR analytical platform enabled pinpointing of bioactive constituents in HPLC chromatograms directly from crude extracts. Bioactive constituents were cumulatively trapped on SPE cartridges and the structures identified and elucidated by spectral data obtained in the HPLC-HRMS-SPE-NMR mode. A total of 24 compounds were identified, including the new compounds **6** and **14** (Figure 1). Several of these showed radical scavenging activity, while compunds **18** and **23** (Figure 1) showed α-glucosidase inhibitory activity.



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PM-55

In vitro cytotoxic potential of novel semi-synthesised clusianone and cardamonin analogues

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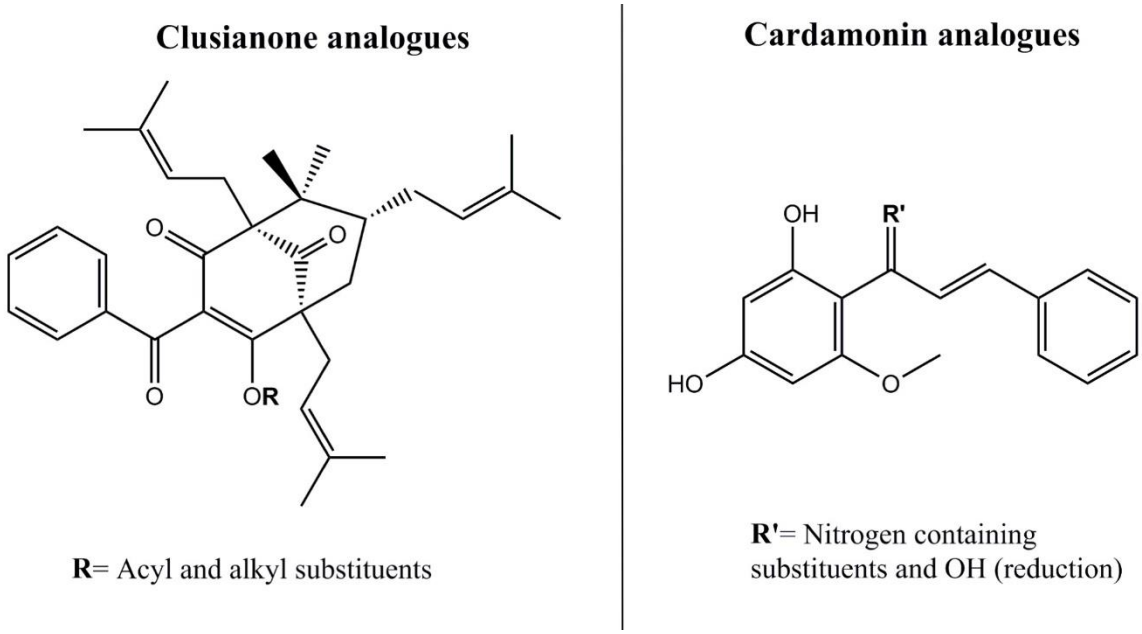
Secondary metabolites derived from natural products are extremely diverse with wide-ranging biomedical implications. In an effort to establish new candidates with improved cytotoxic activity, two naturally occurring compounds, namely clusianone and cardamonin, have been investigated due to their potent cytotoxic activities [1]. Clusianone extracted from the leaves

of *Garcinia parvifolia* and commercially available cardamonin were used as starting materials for the semi-synthesis of 7 and 15 analogues respectively. Synthesised clusianone analogues mainly involved alterations to the hydroxyl group via acylation and alkylation reactions while cardamonin analogues involved modifications of carbonyl group via reduction and condensation reactions. Most compounds were characterised via melting point measurements, IR and mass spectrometry while further analysis has been performed via ^{1}H NMR. All of the synthesised compounds were subjected to *in vitro*cytotoxic evaluation against squamous nasopharynx cells (HK1) and lung adenocarcinoma cells (A549) in the concentration range of 6.25-100 μM. Results revealed the importance of the hydroxyl group for clusianone's cytotoxicity, whereby its modification resulted in a loss of activity. On the contrary, modification of the carbonyl group of cardamonin enhanced cytotoxicity. Metal complexes of both natural products exhibited improved bioactivity as evident from copper complexes. The overall data obtained revealed significant cytotoxicity against both cell lines, with IC_{50} below 10 μM after 72h treatment for some of the analogues tested. The above results warrant further research into elucidating the mode of action of these promising lead compounds as well as establishing a concise SAR study.



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PM-56

Prenylated acylphloroglucinols from *Hypericum annulatum*

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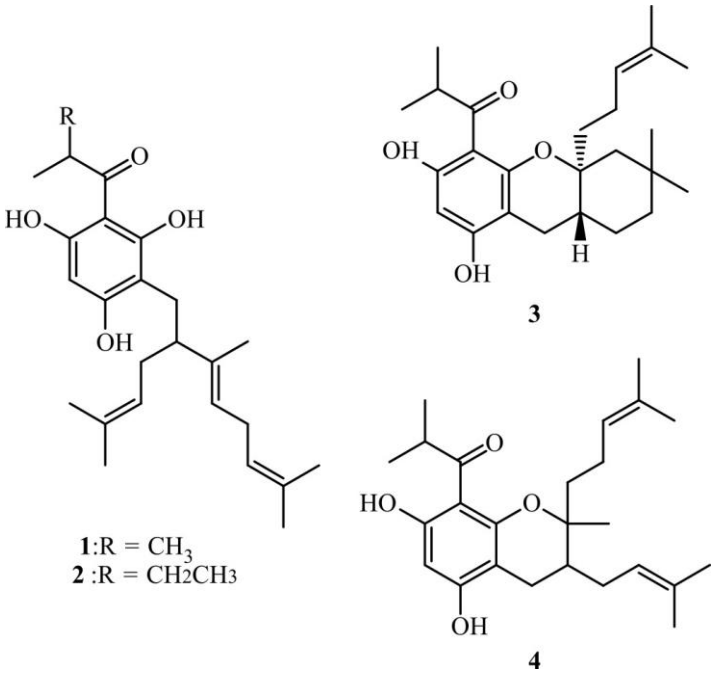
The genus *Hypericum* L. (Hypericaceae) includes more than 480 species that are found in every continent of the world, except Antarctica [1]. *Hypericum annulatum* Moris is a perennial

herb distributed in Sardinia, Balkans, East Africa and Saudi Arabia [2]. A phytochemical investigation of the hexane extract of the aerial parts of the titled species led to the isolation of three new (1-3) and a known (**4**) prenylated acylphloroglucinol derivatives (Fig. 1). The new compounds were identified as 1-{3-[(3E)-3,7-dimethyl-2-(3-methylbut-2-en-1-yl)octa-3,6-dien-1-yl]-2,4,6-trihydroxyphenyl}-2-methylpropan-1-one (**1**), 1-{3-[(3E)-3,7-dimethyl-2-(3-methylbut-2-en-1-yl)octa-3,6-dien-1-yl]-2,4,6-trihydroxyphenyl}-2-methylbutan-1-one (**2**) and 1-((4aS,9aR)-6,8-dihydroxy-3,3-dimethyl-4a-(4-methylpent-3-en-1-yl)-2,3,4,4a,9,9a-hexahydro-1H-xanthen-5-yl)-2-methylpropan-1-one (**3**) by means of spectral methods (MS, NMR, IR, UV). The known compound has been identified as hypercalyxone A (**4**) by comparing its spectral data with that reported in the literature [3]. The cytotoxicity of isolated compounds were established on a panel of tumor cell lines (EJ, HL-60, HL-60/DOX, MDA-MB, SKW-3, BV-173 and K-562) was determined using MTT based assays. Copounds **1** and **2** were the most cytotoxic with IC_{50} values ranging from 0.61 to 4.63 μg/mL.



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PM-57

Bioguided isolation of cytotoxic compounds against melanoma cells from *Carissa spinarum* L.

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Malignant melanoma is the most aggressive form of skin cancer. As natural products are an important source of new anti-cancer lead compounds, the aim of this study is to screen selected Saudi medicinal plants for anti- melanoma activity by evaluating their effects on cell proliferation, cell cycle profile and apoptosis induction, as well as to characterize the active principle compounds.

Based on our preliminary results [1], several extracts of selected Saudi medicinal plants were active against melanoma cell lines. Among them *Carissa spinarum* L. (synonym *Carissa edulis* (Forssk.) Vahl) was one of the most promising plants. The active extract of this plant was subjected to VLC, preparative TLC and the isolated compounds were characterised by NMR and MS. The cytotoxic effects on A375 melanoma cells of the extracts were determined by Sulforhodamine B staining assay. Further mechanistic studies were performed namely caspase 3/7 activity and cell cycle analysis.

The hexane extract retained most of the cytotoxicity (IC_{50}≈40µg/ml), followed by the chloroform (IC_{50}≈47µg/ml) and methanolic (IC_{50} >100µg/ml) extracts.

Cell cycle analysis showed that the hexane extract was able to induce an arrest at S phase and this was accompanied by the induction of caspase 3/7 activities. Its fractionation using VLC (normal phase, Silica gel) afforded 11 fractions. All fractions were screened for cytotoxicity and fraction no. 11 (eluted with 100% ethyl acetate) was one of the most active ones. Preparative TLC (Silica gel 60 using hexane:ethyl acetate as solvent system) was used to purify the fraction, which afforded 2 compounds (IC50≈20µg/ml). Their spectral and spectroscopic data are consistent with ursolic acid and a closely related compound.

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PM-58

Chemical constituents of *Artemisia asiatica* roots

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[1] *Department of Pharmacognosy, University of Szeged, Szeged, Hungary*
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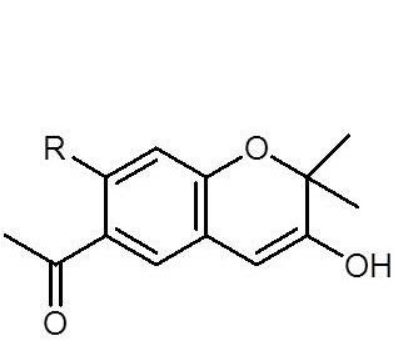
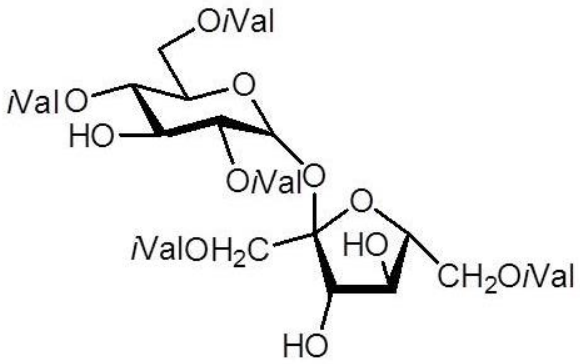
Characteristic secondary metabolites of the genus *Artemisia* are terpenoids, flavonoids, coumarins, caffeoylquinic acids and acetylenes. Some *Artemisia* species possess anti-inflammatory, antimicrobial and anti-cancer properties. In earlier studies cytoprotective,

antioxidative, xanthine oxidase inhibitory and antiproliferative activities of *A. asiatica* have been reported [1-4]. In contrast to the promising pharmacological properties, the chemistry of the plant, especially the roots, has not been studied thoroughly, therefore the aim of our study was the investigation of the chemical constituents of the roots.





R

1 OH

2 H

3

Dried roots were extracted with MeOH, and this extract was partitioned between *n*-hexane, $CHCl_3$ and H_2O. The *n*-hexane and $CHCl_3$ phases were separated by CC, VLC, CPC and PLC, affording 6-acetyl-3,7-dihydroxy-2,2-dimethyl-chromene (**1**), 6-acetyl-3-hydroxy-2,2-dimethyl-chromene (**2**), 1-(5-acetyl-2,4-dihydroxy-phenyl)-3-methyl-butan-1-one, (-)-6-hydroxytremetone, (*E*)-coniferyl aldehyde, and 6α-D-2-[(2,4,6-triisovaleryl)-glucosyl]-β-D-(1,6-diisovaleryl)-fructose (**3**). The compounds, including the relative stereochemistry, were identified by ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, NOESY, HSQC and HMBC experiments.

All isolated compounds were described for the first time from *A. asiatica*, and **1**, **2** and **3** are new natural products, On comparison of the chemical constituents of the aerial parts [3, 4] and roots it can be stated that the composition of the plant organs is different. In the aerial parts flavonoids, sesquiterpenes are the typical compounds, but in case of the root prenylated acetophenones, (*E*)-coniferyl aldehyde and a sucrose ester predominated.

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PM-59

Oligomeric procyanidins as inductors of cellular differentiation of epidermal keratinocytes

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Procyanidins are natural healers of wounds through their anti-inflammatory and antioxidant actions. On skin cells, procyanidins induce proliferation, differentiation and stimulate cellular viability. Differentiation of epidermal fibroblasts and keratinocytes are important in the wound healing process and in remedying skin conditions such as psoriasis and atopic dermatitis [1,2,3]. In this study, one flavan-3-ol and oligomeric procyanidins; epicatechin, procyanidin B2 (epicatechin(4β→8)epicatechin), procyanidin B5 (epicatechin(4β→6)epicatechin), procyanidin C1 (epicatechin(4β→8)epicatechin (4β→8) epicatechin and procyanidin D1 (epicatechin(4β→8)epicatechin(4β→8)epicatechin)(4β→8)epicatechin) were investigated for their ability to induce cellular differentiation of primary normal human epidermal keratinocytes(pNHEK) by immunoflourescence staining and SDS-PAGE Western blot methods for involucrin and cytokeratin 10 expressions at concentrations of 0.1 to 100 μM. Procyanidin B2 stimulated pNHEK to undergo cellular differentiation at 1 and 10 μM whilst its (4β→6) connected dimer, procyanidin B5 did not induce pNHEK to cellular differentiation. Epicatechin, procyanidins C1 and D1 did not induced cellular differentiation. Induction of keratinocytes to cellular differentiation was restricted to procyanidin, B2 which could be due to stimulation of specific cellular mechanisms due to interactions with specific receptors.

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PM-60

Celastrol, an active constituent of the TCM plant *Tripterygium wilfordii*, inhibits prostate cancer bone metastasis

Kenny Kuchta[1], Yucheng Xiang[2], Shuai Huang[2], Yubo Tang[2], Xinsheng Peng[2], Xi Wang[2], Yuexing Zhu[2], Jiukun Li[2], Jing Xu[2],Zhenhua Lin[2], Tao Pan[2]

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Prostate cancer (PCa) is one of the most common malignant tumours and a leading cause of cancer deaths. Treatment failure of PCa is often due to bone metastasis. Celastrol, an active

constituent of the roots of *Tripterygium wilfordii* Hook.f., has shown anti-tumour effects in previous studies in accordance with its traditional use in China [1]. Here we report for the first time an in-depth study of the effects of celastrol on PCa bone metastasis and its mechanism of action. Using a PC-3 cell model, *in vitro* assays were performed to evaluate the effects of celastrol on proliferation, migration (wound healing assay), invasion of healthy tissues (Transwell-Matrigel penetration assay), and secretion of Vascular Endothelial Growth Factor (VEGF) (ELISA assay). An intra-tibia injection mouse model was used to assess the effect of celastrol treatment on PCa bone metastasis *in vivo*. Pre-treatment with celastrol significantly reduced proliferation of PC-3 cells in a dose-dependent manner and cell migration was much slower than in untreated controls. In the penetration assay, significantly fewer cells penetrated through the gel-membrane after celastrol administration and their skeletal invasive ability was also significantly reduced in a dose-dependent manner. Correspondingly, a significant, dose dependent decrease in VEGF secretion was observed. In the *in vivo* mouse model, pre-treatment with celastrol (8 μmol/L) inhibited the tumourigenicity of PC-3 cells so that almost no bone invasion occurred as compared to control injections. Histological examinations using H&E-staining showed that tibiae injected with celastrol pre-treated PC-3 cells retained their natural bone structure. Our results suggest that celastrol may have major preventive potential against PCa bone metastasis.

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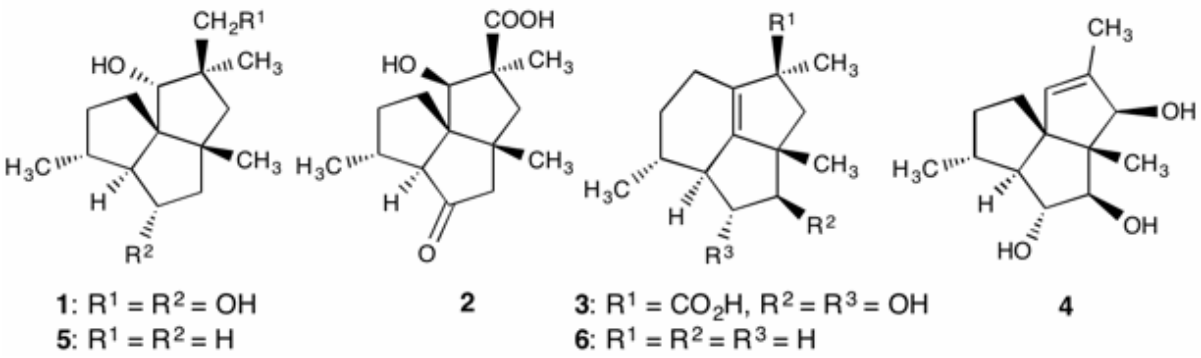
PM-61

Sesquiterpene derivatives from cultured lichen mycobionts of *Diorygma* sp.

Takao Tanahashi, Yukiko Takenaka

Kobe Pharmaceutical University, Kobe, Japan

Lichens are distinctive organisms resulting from a symbiosis between algae and fungi. Lichens are well known for their production of unique lichen substances, some of which are potentially useful and biologically active compounds. Although the cooperative mechanisms for the biosyntheses of lichen substances are not fully understood, they are thought to be produced by the fungal portion (mycobiont) of the symbiotic system. However, our previous studies demonstrated that isolated lichen mycobionts are, in some cases, capable of producing new compounds, which are not detected in the natural lichen thalli [1,2]. These findings suggested that laboratory cultures of isolated lichen mycobionts could provide a potential source of novel secondary metabolites. In continuing our chemical studies on the cultured lichen mycobionts [3], we have cultivated the spore-derived mycobionts of Diorygma sp. collected in Vietnam on conventional malt-yeast extract medium supplemented with 10% sucrose at 18 ˚C in the dark. Purification of their metabolites afforded four new sesquiterpene derivatives **1**-**4** related to cameroonanol (**5**) and presilphiperfol-7-ene (**6**) [4]. Their structures were elucidated by spectroscopic and chemical means. This is the first instance of isolation of this type of metabolites from cultured mycobionts of lichen.



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PM-62

Separation and analysis of bioactive flavonoids in *Tanacetum parthenium* supercritical fluid extracts

Krisztina Végh[1], Ágnes Alberti[1], Eszter Riethmüller[1, 2], Anita Tóth[1], Szabolcs Béni[1], Ágnes Kéry[1]

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[2] *Compound Profiling Laboratory, Gedeon Richter Plc., budapest, Hungary*

Feverfew (*Tanacetum parthenium* L., Asteraceae) is a perennial medicinal plant which has been used to alleviate the symptoms of migraine, headache, rheumatoid arthritis and has many pharmacological properties. The herb contains various potentially active constituents such as sesquiterpene-γ-lactones, flavonoids and volatile oil. The main sesquiterpene-lactone in feverfew is parthenolide which is considered to be responsible for the therapeutical effects. The herb also contains lipophilic flavonoids which has been reported to posses notable anti-inflammatory activity. It has been proved, that sesquiterpene lactones and flavonoids as a result of pharmacokinetic interaction are improving the pharmacological potency when they are present in the same product. Presumably the lipophilic flavonoids of feverfew herb could also escalate the effect of the sesquiterpene lactones.

The aim of our work was to prepare a supercritical fluid extract containing the two significant groups of constituents: sesquiterpene lactones and methylated flavonoids. Supercritical carbon dioxide with 7 % ethanol was used at 22 MPa and 64 °C based on our previous studies. The sample was studied by thin layer chromatography (TLC), preparative high performance liquid chromatography (Prep-HPLC) and high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-DAD-ESI-MS/MS). TLC was used for quick comparison of the various product samples. Fifty-two fractions were collected by prep-HPLC. The flavonoids, parthenolide and minor sesquiterpene-lactones in the fractions of the SFE extract were analysed with LC-MS/MS. Two non-conjugated flavones: apigenin and luteolin were

identified besides parthenolide and some minor sesquiterpene lactones. Additionally, eleven mono-, di-, tri- and tetramethoxylated flavone and flavonol aglycones were characterized. Flavonoid profiles have potential as chemical fingerprints beside parthenolide in quality control of feverfew extracts.

PM-63

Antioxidant activity of the root extract of *Combretum dolichopetalum* and the isolated constituents

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Natural antioxidants from plants such as ascorbic acid, tocopherol, carotenes, phenolic acids and phytoestrogens have been recognized as having the potential to reduce the risk for diabetes and other chronic diseases. *Combretum dolichopetalum*Engl. and Diels (Combretaceae) aqueous root extract is used traditionally in management of diabetes and in stomach disorders. The aim of the present research is to investigate the antioxidant activities of *C. dolichopetalum* root extract and to isolate the antioxidant constituents.

On successive solvent-solvent partitioning, the methanol extract (ME) of *C. dolichopetalum* root afforded the ethyl acetate (EAF) and butanol (BuF) fractions. Through various chromatographic separations, arjunolic acid (**1**) and dihydrophaseic acid (**2**) were isolated from EAF, while ellagic acid (**3**) and 3,4,3'-tri-*O*-methylellagic acid (**4**) were obtained from BuF. The compounds were identified based on their ^{1}H- and ^{13}C-NMR, mass spectra and comparison of the data with literature reports. The in vitro antioxidant activity of the extract, fractions and isolated compounds was assessed using the 2,2- diphenyl-1-picrylhydrazinyl (DPPH) and compared to the standard drug ascorbic acid.

Results showed that the ME, EAF, BuF and compound **3** possessed antioxidant activity (IC_{50}=540.00±6.34, 538.02±2.78, 165.43±10.10, 30.00±1.25 µg/ml respectively) which were lower than that of ascorbic acid (IC_{50}=16.20±0.69 µg/ml). Compounds **1**, **2** and **4** showed no inhibition of DPPH.

It is concluded that *C. dolichopetalum* root possesses antioxidant activity and ellagic acid is identified as the main antioxidant principle of the plant.

PM-64

Searching for antimicrobial and antiviral xanthones: a molecular docking study coupled to multivariate analysis

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Xanthones are naturally occurring compounds present almost exclusively in Gentianaceae, Guttiferae, Moraceae, Clusiaceae, and Polygalaceae plant species [1], considering them as chemomarkers. These compounds have demonstrated important biological activities, including DNA-polymerase inhibitory, anti-inflammatory [1] and anti-infective [2,3]. Hence, as part of our current research on virtual screening-aided drug discovery, an in silico study was conducted to evaluate the potential of xanthones as inhibitors of enzymes that play important roles in microorganism metabolic pathways. In depth, molecular docking to more than 200 compounds was carried out employing 10 different receptors of fungal and viral pathogens. Several xanthones exhibited affinity values greater than those for co-crystallized or natural inhibitors. For strongest xanthone – enzyme complexes, detailed analysis of structural interactions and their relevance was accomplished. Molecular docking showed interaction of xanthones with 3 to 10 residues per enzyme. Moreover, affinity values were correlated by means of multivariate statistical analysis. It let to demonstrate partial classification of xanthones and a relationship between affinity and xanthone-type was therefore found. This approach let us to propose at least four hit xanthone structures for anti-infective drugs.

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PM-65

Cancer-chemopreventive actvity of secondary metabolites isolated from *Xanthoparmelia conspersa* lichen

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Secondary metabolites are substances produced primarily by mycobionts and deposited on the lichen hyphae. They are derived from various metabolic pathways such as: mevalonic, shikimic and acetyl-polymalonyl acid pathways. Four metabolites and their parent drugs were isolated by using solvent extraction (Soxhlet extraction method) and repeated chromatographic methods (HPLC) and next determined by spectroscopic methods including nuclear magnetic resonance (NMR) and spectroscopy correlation of NMR. One new compound for *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale was identified and confirmed one more time by our team, as atraric acid (2,4-dihydroxy-3,6-dimethylobenzoate) [1]. Furthermore, the activity using a battery of 13 luciferase cancer-related reporter gene assays for the parent drug and some of its metabolites showed that atraric acid and usnic acid exhibited a significant cancer-chemopreventive activity. However, little or weak anticancer activities were observed for stictic and norstictic acids. Our present study is important for further investigates of cancer-chemopreventive activity of the secondary metabolites isolated from lichens, their use in the traditional medicine and to understand their metabolic fate or deposition in human organism.

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PM-66

Greek *Iris* species as sources of agents potentially effective in bone metabolism

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Osteoporosis is a potentially crippling disease and a major cause of morbidity in the elderly, women in particular. Treatment of osteoporosis mainly includes hormone replacement therapy (HRT) with estrogen and selective estrogen receptor modulators (SERMs, raloxifene). Since, HRT is characterized by high breast and uterine cancer risk, with other therapies being less effective, the discovery of new remedies remains a goal. Even the beneficial effects of plants in bone health hade been known since the ancient Greek physicians (e.g. Dioscorides) and Greece is one of the richest floral diversity regions, there are scarcely any reports connecting Greek flora herbs with prevention of osteoporosis. Aim of our study is to identify and characterize plant derived extracts and isolated compounds that are potentially capable of

safely preventing postmenopausal osteoporosis. Based on traditional medicine sources and current literature, 65 plant species were selected and 130 extracts were prepared using several techniques (ASE, MAE, SFE). Phytochemical profiling provided 80 extracts for further biological evaluation and preliminary results revealed that the extracts from three different species of genus *Iris*(*I. germanica, I. attica* and *I. unguicularis ssp. cretensis*) are capable to induce MC3T3-E1 differentiation to osteoblasts, with *I. germanica* extracts being the most promising one. Since differentiation of MC3T3-E1 is promoted beyond others by estrogens, the estrogenic activity of extracts was determined using MCF7 and Ishikawa cell line and all extracts, except EtOAc extract of *I. unguicularis ssp. cretensis*, lacking estrogenic activity. Furthermore, the isolation of the major compounds from *I. germanica* was performed and the structure elucidation was implemented mainly by NMR spectroscopy. Among the isolated compounds, isoflavone derivatives (irigenin, irisflorentine), benzophenones (iriflophenone), acetophenones and iridales derivatives were identified.

PM-67

Sesquiterpenes from the rhizome of *Cyperus rotundus* with cytotoxicity on human cancer cells *in vitro*

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The rhizome of *Cyperus rotundus* L. (Cyperaceae) have been used in traditional Chinese medicine. In this study, two new sesquiterpenes, cyperusol A_3 (**1**) and 3-hydroxycyperenoic acid (**2**), along with twelve known compounds, britanlin E (**3**), 1*β*,4*β*-dihydroxyeudesma-11-ene (**4**), and 10*β*-eudesm-4-en-3-one-11,12-diol (**5**), pinellic acid (**6**), fulgidic acid (**7**), scirpusin A (**8**), scirpusin B (**9**), 6'-acetyl-3,6-diferuloylsucrose (**10**), 4',6'-diacetyl-3,6-diferuloylsucrose (**11**), *p*-coumaric acid (**12**), ferulic acid (**13**) and luteolin (**14**) were isolated from an EtOAc-soluble fraction of the rhizome of *Cyperus rotundus* L. The structures of compounds **1** and **2** were elucidated by physical and spectroscopic data (^{1}H-NMR, ^{13}C-NMR, 2D NMR, and MS) interpretation. All the isolates **1**-**14** were evaluated for their cytotoxicity against human ovarian cancer cells (A2780) and endometrial adenocarcinoma cells (Ishikawa) using MTT assays, with the most active compound being 10*β*-eudesm-4-en-3-one-11,12-diol (**5**).

PM-68

Chemical constituents of *Digitalis viridiflora*

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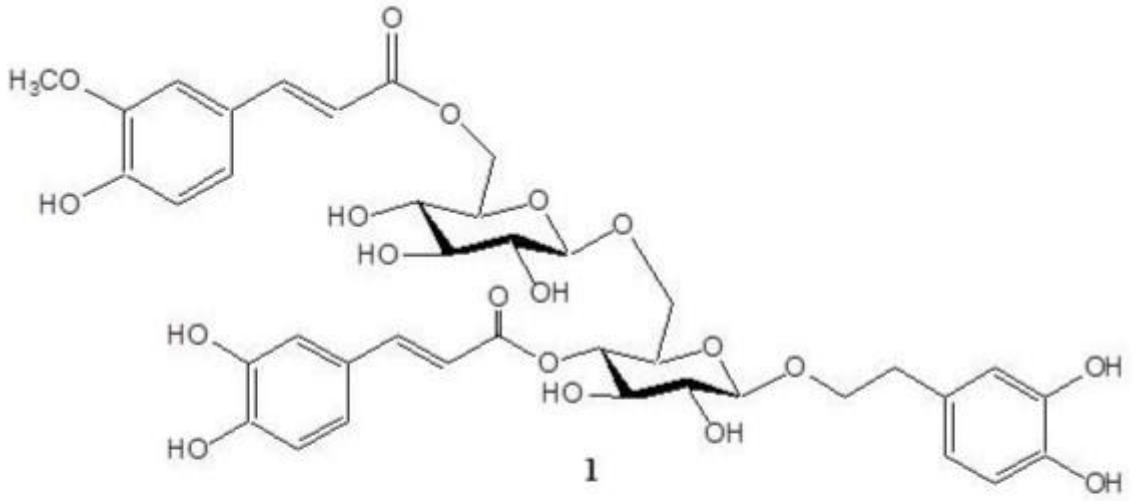
The genus *Digitalis* (Plantaginaceae) contains biennial or perennial species. It is represented by nine species in the flora of Turkey including *D. viridiflora* Lindley [1]. Phenylethanoid glycosides, cardiac glycosides, steroidal saponins and pregnane glycosides constitute the main

group of secondary metabolites of the genus *Digitalis* [2, 3]. In continuation of our systematic survey on the chemical composition of *Digitalis* species growing in Turkey, we recently reported five phenylethanoid glycosides from *D. viridiflora* as the preliminary phytochemical research [4]. Further detailed chromatographic studies on the chemical constituents of the leaves of *D. viridiflora* led to the isolation of a new phenylethanoid glycoside named as digiviridifloroside (**1**) along with a known phenylethanoid glycoside (calceolarioside A), two flavonoids (scutellarein 7-*O*-β-D-glucopyranoside and hispidulin 7-*O*-β-D-glucopyranoside), two cleroindicins (cleroindicins B and F), a nucleoside (adenosine), as well as a mixture of β-D-glucopyranosyl-(1→6)-4-*O*-caffeoyl-α/β-glucopyranose and 3,4-dihydroxyphenylethanol which could be an artefact formed during isolation procedure. The structure of the new compound was established as 3,4-dihydroxy-β-phenylethoxy-6-*O*-(*E*)-feruloyl-β-D-glucopyranosyl-(1→6)-4-*O*-(*E*)-caffeoyl-β-D-glucopyranoside (**1**) based on extensive 1D- and 2D-NMR spectroscopy as well as MS. Digiviridifloroside (**1**) is the third example of rare phenylethanoid glycosides obtained from the genus *Digitalis* bearing two aromatic acyl units; the first two ones were reported from *D. lanata* [5].



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PM-69

Identification of corilagin, punicalagin and ellagic acid derivatives from antibacterial and antioxidative extracts of African savanna woodland tree *Terminalia laxiflora*

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Terminalia laxiflora Engl & Diels. is used as decoctions against bacterial infections and their symptoms such as cough and diarrhea [1]. Stem wood fumigations are used against malaria parasite, venereal diseases and skin disorders [2,3,4]. Previous research demonstrated in vitro anti-acne properties of stem wood of *T. laxiflora* and agrees with the traditional use of the stem wood against skin ailments [4]. This study aimed on identification of antioxidative ellagitannins and ellagic acid derivatives in the roots of *T. laxiflora*.

Air dried root (100 g) was sequentially extracted using solvents of increasing polarities. 20 µl of the ethyl acetate extract (50 mg/ml), showing good antimicrobial activity, was applied on RP-18 TLC plates and development was performed using a mobile phase of methanol and water. The plates were dried and sprayed with DPPH reagent to detect antioxidative compounds. Gallic acid and catechin were used as standards.

Our TLC results show that ellagitannins and gallic acid contributed significantly to the antioxidative potential of the ethyl acetate extract of *T. brownii*. Ellagitannins are presumably also related to the good antibacterial effects of this extract. UHPLC/MS-QTOF and HPLC-DAD analysis led to the identification of nineteen ellagitannins among which corilagin and its derivative as well as punicalagin were characterized for the first time from the root of *T. laxiflora*. Ellagic acid xyloside and 3-O-methyl ellagic acid xyloside are likewise presented for the first time in the roots of *T. laxiflora*.

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PM-70

Miconidin acetate, a natural 5-lipoxygenase (5-LOX) inhibitor from *Eugenia hiemalis* Camb. (Myrtaceae)

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Eugenia hiemalis grows as a tree in South America [1]. Previous studies showed primin is present in its leaves [2] and inhibits both COX-2 and 5-LOX [3]. In this study, a primin-related metabolite, miconidin acetate (MA), was isolated from CH_2Cl_2 extract of *E. hiemalis* collected in Southern Brazil. In the search for protein targets mediating a possible anti-inflammatory effect of MA, a similarity ensemble approach (SEA) [4] was pursued using the publicly available SEA search tool [5]. Among the predicted targets, 5-LOX is linked to inflammation. MA was evaluated for 5-LOX inhibition in vitro using a cell-based assay [3] and detecting LTB4 with an ELISA kit. MA (20 μM) inhibited LTB4 formation to an extent of 59±12%; the positive control zileuton (10 μM) inhibited 5-LOX 69±12%. MA was docked into an X-ray crystal structure (PDB entry 3o8y) [6] using GOLD 5.2 (CCDC, GB). The resulting poses place MA close to the catalytic iron and the OH group is predicted to form H bond with the terminal Ile676. The pentyl moiety occupies the hydrophobic substrate channel (Fig. 1).

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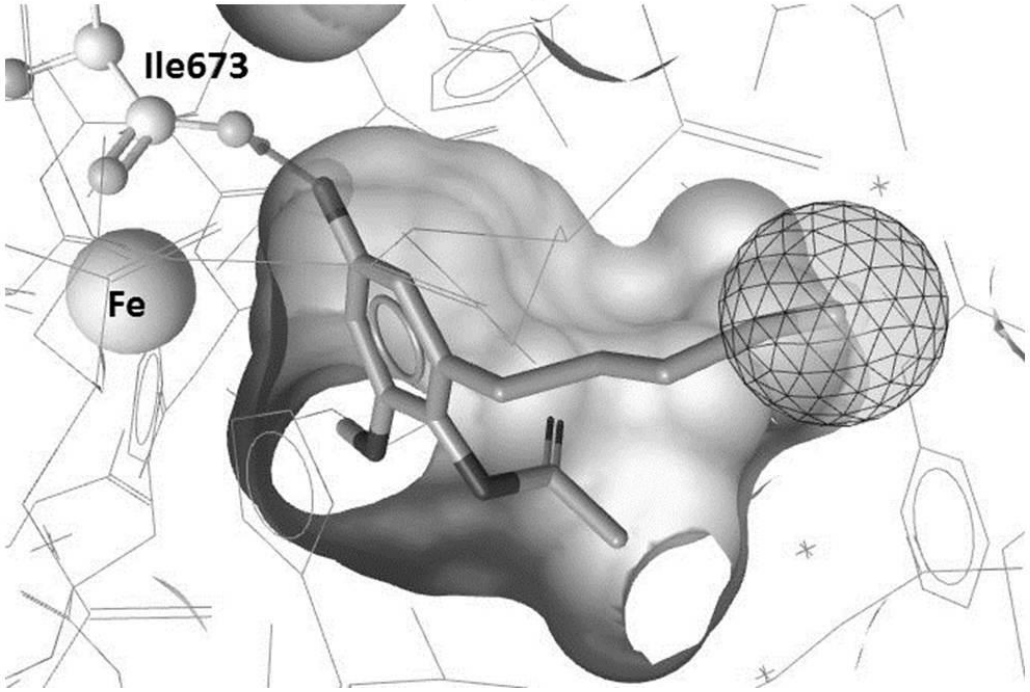
Figure 1. Predicted protein-ligand interactions of miconidin acetate and its target 5-LOX. Chemical interactions are depicted: sphere – hydrophobic contact; arrow – hydrogen bond donor.



PM-71

First isolation and identification of polyphenols from the halophyte *Armeria maritima*

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While there is a rich bibliography concerning the chemical composition of some genus belonging to Plumbaginaceae family [1-2], secondary metabolites of *Armeria* genus have slightly been investigated. This phytochemical study concerns the halophyte *Armeria maritima* Willd., with a specific focus on phenolic compounds whose presence have already been reported in this species but whose structures have never been accurately identified [3].

A hydro-alcoholic extract prepared from dried aerial parts of *A. maritima* was first partitioned with water and cyclohexane, dichloromethane, ethyl acetate and then n-butanol. Ethyl acetate and n-butanol parts, very rich in phenolic compounds according their TLC profiles, were then fractioned by RP-VLC and some complex fractions were also submitted to size exclusion chromatography (Sephadex LH20). The polyphenolic enriched fractions were then submitted to semi-preparative RP-HPLC. This approach and combination of LC-HR-ESI-TOF-MS and 1D and 2D NMR experiments led us to the isolation and identification of many phenolic compounds, such as phenolic acids (gallic, protocatechuic, *p*-hydroxybenzoic and caffeic

acids) and also flavonoid glycosides: myricetin-3- *O*-α-L-rhamnopyranoside, quercetin-3- *O*-(4''- *O*-acetyl)-α-L-rhamnopyranoside, kaempferol-3- *O*-(6''-*O*-*p*-*trans*-coumaroyl)-β-D-glucopyranoside, myricetin-3- *O*-[α-L-rhamnopyranosyl—(1->6)- *O*-β-D-glucopyranoside]. All of them are firstly identified in this halophyte.

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PM-72

In vitro antibacterial and antiproliferative screening of Hungarian bryophytes

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The bryophytes comprising of liverworts (Marchantiophyta), mosses (Bryophyta) and hornworts (Anthocerotophyta), can be found everywhere in the world except in the sea. In the Hungarian flora, more than 600 species are present, with the predominance of mosses. Although not applied in human nutrition, a number of bryophytes have been widely used as medicinal plants, especially in China for various illnesses, including diseases of bacterial origin (cystitis, pharyngitis, tuberculosis, pneumonia, erysipelas) or as local antiseptics [1].

The most intensively studied activities of bryophytes are the antimicrobial and antiproliferative effects. However, compared to the higher plants, bryophytes have been less extensively studied. Taking into consideration the permanent need for medicines with improved antibacterial and antitumour effects, the phytochemical and pharmacological analysis of this taxon seems promising.

The aim of our study was the investigation of antibacterial and antiproliferative effects of some Hungarian bryophytes in vitro. 20 species were collected for our screening assay. In vitro antibacterial testing of extracts of different polarity of 20 species (4 extracts each) revealed significant antimicrobial activity of 9 species (*B. rutabulum, C. cuspidata, C. dendroides, O. hians, P. longifolium, P. cuspidatum, P. undulatum, P. purum, T. muralis*) on MRSA (ATCC43300 and 64326) or *S. aureus* (ATCC29213) with mild to strong efficacy. Antiproliferative assays on 3 human cancer cell lines (Hela, A2780 and T47D) confirmed >50% activity in 6 of the tested 10 species (*S. ruralis, C. dendroides, P. purum, R. squarrosus, A. abietina* and *P. cuspidatum*, 10 or 30 μg/ml). From these, 4 were active on at least 2 cell lines, and *C. dendroides, P. purum* were active in the tested lower concentration (10 μg/ml).

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PM-73

Antimicrobial and antioxidant activities of methanolic leaf extract of *Argemone mexicana* Linn (Papaveraceae)

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Natural products play an important role in drug discovery programs of many research organizations. An important reason for the use of natural products as a source of lead compounds is the tremendous variety of species found in nature and the resulting molecular diversity of the isolated compounds [1] .Over the years, World Health Organization (WHO) advocated traditional medicines as safe remedies for ailments of both microbial and non-microbial origins.[2]

The present study aimed at investigating the antimicrobial and antioxidant activities of the methanolic leaf extract of *A. mexicana.* An antimicrobial assay was carried out against four bacterial and three fungi strains using the agar well diffusion method. The formation of a clear zone of inhibition was observed and compared with that of Levofloxacin standard. Antioxidant assay was carried out.

The result indicates that extract of *A. mexicana* was able to inhibit the growth of micro-organisms such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* by the formation of a clear zone of inhibition.Antioxidant assay indicates that the extract has a high percentage inhibition as compared with the standard. The table below shows the result of the antibacterial assay;

Organism	Zones of inhibition of Extract (mm)	Zones of inhibition of Standard (mm)
P. aeruginosa	10.5	19.5
E.coli	0.5	20.5
S. typhi	0.2	24
S. aureus	11.5	20

Extract is at 200 mg/ml and standard is at 20 mcg/ml

The methanolic leaf extract of *A. mexicana* is effective in the inhibiting the growth of certain micro-organisms and can be used to prevent infectious diseases. Also, it possesses antioxidant activities useful in fighting or free radicals which can cause cell damage.

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PM-74

In vivo immunomodulatory effect of wild carrot oil and its fractions

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Daucus carota L. ssp. carota (wild carrot) oil extract (DCOE) and fractions (F1, F2, F3 and F4) were recently shown to exhibit *in vitro* and *in vivo*anticancer activity [1,2]. The aim of this study is to examine the *in vivo* immunomodulatory effect of DCOE and its fractions on the levels of IFN–γ, TNF-α, IL-10, IL-17, and VEGF in spleen tissues of BALB/c mice. Animals were injected with 200 mg/Kg of DCOE or fractions and spleens were collected at 6 and 24 hrs post treatment. Whereas, DCOE and F1 did not induce a significant increase in IFN–γ levels at either interval, F2, F3, F4 and LPS caused 6.7, 4.6, 3.5 and 2.3 fold increases, respectively. The level of TNF-α displayed 2.4, 2.8, 6.2, 3.8, 2.5 and 3.1 fold increase compared to control 24 hrs post treatment with DCOE, F1, F2, F3, F4 and LPS, respectively. DCOE, F1 and F4 induced a 1.3 fold increase and F2 and F3 induced a 1.4 fold increase in the IL-10 levels, 6 hrs post treatment. However, no significant elevation of IL-10 was observed 24 hrs post injection of F2, F3 and F4 fractions. While 1.7, 1.6, and 1.75 fold increases in IL-17 were observed 6 hrs post treatment with F2, F3 and F4 respectively, these increments did not reach significance after 24 hrs. In addition, F1 and F2 were shown to inhibit the production levels of spleen VEGF. In conclusion, the *in vivo* antitumor activity of DCOE and fractions may be attributed to the enhancement of IFN-γ, TNF-α and IL-17 production and down regulation in the level of VEGF.

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PM-75

New Limonoid from the Seed of Swietenia macrophylla with Inhibitory Activity on Neutrophil Pro-Inflammatory Responses

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Swietenia macrophylla King (Meliaceae) is a tropical timber tree, natively distributed throughout tropical regions of the Americas, mainly in Mexico, Bolivia and Central America. Limonoids, steroids, and their derivatives are widely distributed in plants of the genus Swietenia. Many of these compounds exhibit anti-inflammatory, antimalarial, and antifungal activities. In our studies of Formosan plants for in vitro anti-inflammatory activity, *S. macrophylla* was found to be an active species.

In the present study, to isolate anti-inflammatory constituents from the EtOAc-soluble part of the MeOH extract of the seed of *S. macrophylla*, they were purified by repeated silica gel column chromatography, MPLC, and preparative TLC to give a new limonoid, **1**, along with five known compounds, **2**–**6**. Swietemacrophin (**1**), humilinolide F (**2**), 3,6-*O*,*O*-diacetylswietenolide (**3**), 3-*O*-tigloylswietenolide (**4**), and swietemahonin E (**5**) exhibited inhibition ($IC_{50} \leq 45.44$ μM) of superoxide anion generation by human neutrophils in response to fMLP.

PM-76

In vitro protection and toxicity assessment of Chios Mastic Gum extracts and isolated triterpenic acids using human hepatocarcinoma cells.

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Chios mastic gum (CMG), is the resin obtained from *Pistacia lentiscus* (L.) var. *chia* (Duham). The plant is cultivated in Chios Island, Greece, and it is known since ancient times for its pharmacological activities.

In the current work, total mastic extract (a) was fractionated giving the neutral (b) and the acidic fraction (c). The latest was used for the purification of several characteristic mastic triterpenic acids at a Supercritical Fluid Chromatography (SFC) system.

The cytotoxic and potential genotoxic effects of (a), (b), (c) and of the isolated triterpenic acids masticadienonic (d) and isomasticadienonic (e) were investigated in vitro using human hepatocarcinoma (HepG2) cells. Cytotoxicity was determined using the MTT assay at

concentrations 0.5 to 200 μg/mL for the extracts (a, b, c) and 10-12 to 10-5 M for the pure compounds (d, e) after 24-hr incubation in HepG2 cells. The results showed significant cytotoxic activity of all the extracts at 100 μg/mL. Isolated compounds exhibited no cytotoxic activity at the concentrations tested. For the determination of the genotoxic potential, the single cell gel electrophoresis (Comet assay) was used. Pure compounds were tested at concentrations 10-8 to 10-6 M and extracts at concentrations 0.5, 1, 5, 10 and 25 μg/mL for 24 hrs in HepG2 cells. Isolated compounds showed no genotoxic potential for the concentrations tested whilst only total mastic extract with polymer exhibited genotoxic activity (DNA damage) at the highest concentration tested. The protective activity of CMG against hydrogen peroxide-induced DNA damage in HepG2 cells has also been tested with the Comet assay and results obtained show a protective activity of the acidic fraction at concentrations of 0.5 and 1 μg/mL. Genotoxicity and cytotoxicity are also currently tested with the γ-H2AX – In Cell Western assay using the Odyssey CLx Infrared system to corroborate the results of the Comet assay.

PM-77

Mechanism based approach for screening of decoction and infusion of *Swertia chirata* for antimalarial activity

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Malaria has been affecting the populations residing in the tropical and subtropical region. Medicinal and aromatic plants have been very successfully used in treatment of malaria. Quinine from *Cinchona* species and artemisinin from *Artemisia annua* have been used globally in the treatment of malaria. In traditional medicine, *S. chirata* decoction and infusion but no mechanistic approach it is reported to show antimalarial activity. So we propose to explore the potential of decoction and infusion of *S.Chirata* as an antimalarial through various mechanistic antimalarial assays

Decoction and infusion of *S. chirata* were prepared, standardized and mechanistic antimalarial assay were carried out using β-hematin [1], PfHRPII [2], Falcipain2 [3]. Antiplasmodial analysis using SYBR Green I (3D7 *P. falciparum*) [4]. Cytotoxicity assay was carried using MTT assay and brine shrimp toxicity [5,6].

Decoction and infusion of *S. chirata* were found to be non-cytotoxic on mammalian cell lines up to 100 μg/mL and Brine shrimp 600 μg/mL.

Assay Method	Decoction IC_{50} Value (μg/mL)	Infusion IC_{50} Value (μg/mL)
β-hematin	50	35
PfHRPII	25	10
Falcipain2	100	12.5
SYBR Green I (3D7 *P. falciparum*)	75	8.5

The results indicated infusion of *S. chirata* has good *in vitro* antimalarial activity as compared to decoction. Decoction and infusion are found to be nontoxic. It upholds the traditional use as antimalarial and it may be given alone or in combination with antimalarial drugs. The studies are in progress to understand the synergism of the antimalarial drugs and infusion and whether it can lead to additive, synergism or toxic effects.

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PM-78

Two new jatrophane diterpenes from *Euphorbia guyoniana*

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Euphorbia guyoniana Boiss. and Reut. is endemic to the northern Sahara in Algeria, Tunisia, Libya and Morocco. In Algeria, this species is used in the folk medicine as a wart remover, and against venomous bites of scorpion [1]. In Saharan region of Tunisia, *E. guyoniana* is an antitussive and analgesic remedy in the ethnomedicine [2]. Diterpene content of *E. guyoniana* of Algerian origin was investigated earlier, and *ent*-abietane, atisane, jatrophane and tigliane type diterpenes have been isolated from the roots [3-4], and the jatrophanes guyonianin A–F were isolated from the aerial parts [1, 5-6].

We report herein the isolation and structure determination of two jatrophane diterpenes from *E. guyoniana* collected in Tunisia. The chloroform extract of the dried aerial parts was fractionated by column chromatography on polyamide, then by vacuum liquid chromatography on silica gel. Selected fractions from these separations were further purified by HPLC to yield two pure compounds. The structure elucidation was carried out by HRMS and extensive NMR studies (^{1}H NMR, JMOD, ^{1}H-^{1}H COSY, HSQC and HMBC). The isolated compounds were identified as 14-oxojatropha-6(17),11-diene pentaester derivatives acylated with acetic, isobutanoic and benzoic acids. Both compounds are new natural products. Interestingly the main diterpene constituents of *E. guyoniana* in our experiment were not identical with the previously reported compounds of this species, indicating the high chemical diversity of this taxon. Isolated compounds are structurally similar to multidrug resistance reversing diterpenes, therefore they are worthy for anti-MDR studies.

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PM-79

In vitro antifungal activity of various Persian cultivars of *Punica granatum* extracts against *Candida*species

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Resistance of *Candida* species to antifungal agents has potentially serious implications for management of infections. *Candida* species are now the fourth most common organisms isolated from hospitalized patients. In the past decade numerous reports of treatment failures were reported. Prevention and control of these infections will require new effective antimicrobial agents. Plant-derived antifungal compounds have always been a source of novel therapeutics. The aim of this study was to investigate the antifungal effect of methanolic extracts of pomegranate peel and pulp against *Candida*species.

Samples from eight cultivars of *Punica granatum* L. were collected from the Saveh Agricultural Investigation Center in Iran. Both pomegranate pulp and peel were dried and powdered separately. The dried powders were extracted by using a Soxhlet extractor. The antifungal effects (minimum inhibitory concentration, MIC) of methanolic extracts of pomegranate peel and pulp were determined *in vitro* against five standard species of *Candida*, namely *C. albicans* (ATCC 10231), *C. parapsilosis* (ATCC 22019), *C. tropicalis*(ATCC 750), *C. glabrata* (PTCC 5297), and *C. kroseii* (PTCC 5295).

The most potential antifungal inhibition was observed in the case of peel extracts deriving from sour malas, sour white peel, and sour summer cultivars, respectively. The antifungal activity of pulp extracts against *Candida* species was negative. *Punica granatum* peel extract has been shown to posses antifungal activities, which suggests a potential applivcation in the treatment and prevention of candidiasis.

PM-80

A subchronic oral toxicity study on medical herb extract, KIOM CRC#BP10A, in male and female mice

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Our recent study demonstrated that KIOM CRC#BP10A (BP10A), a 1:1 mixture of two medicinal herb extracts,*Peucedanum praeruptorum* DUNN and *Descurainia sophia* (L.) Webb ex Prantl, exerts a potent anti-tumor effects in diverse animal xenograft cancer models. But there is limit information concerning its toxicities in vivo. The present study is aimed at conducting histopathological and biochemical studies in a 28-day subchronic toxicity studies using 4 weeks old male and female ICR mice.

Ethanolic extracts of *Peucedanum praeruptorum* DUNN and *Descurainia sophia* (L.) Webb ex Prantl were prepared separately and mixed at 1:1 ratio and named as BP10A. BP10A was administered at dosages of 0 (vehicle control), 500, 1000 and 2000 mg/kg by gastric lavage. Histopathological studies of major organs and blood chemistry analyses were performed after euthanization.

There were no statistically significant differences in body weights between the treatment groups and the control group in each week. But there were some sporadic, statistically significant changes in some hematology and serum biochemical parameters. Differences between two groups in all absolute organ weights and relative organ weights (organ-to-body weight ratios) seem not to be related with drug treatment for male and females. Also, no macroscopic pathological findings were observed in liver and kidney of all animals.

In conclusion, BP10A is considered to be non-toxic when orally administered to ICR mice during 4 weeks. Our present study demonstrates that the NOAEL (no observed adverse effect level) for BP10A was 2000 mg/kg bw/day, the highest dose tested, in male and female mice.

PM-81

Potent antimicrobial prenylated isoflavonoids from *Maclura aurantiaca* fruits

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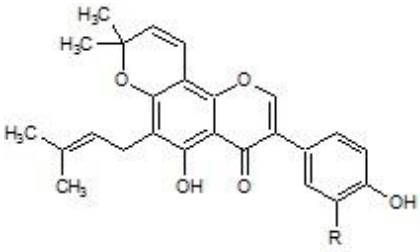
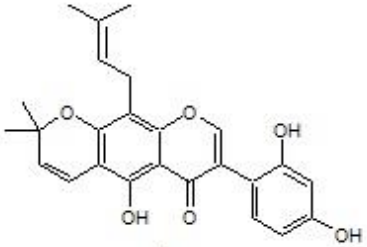
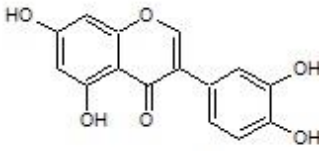
Bioassay guided fractionation of the ethanolic extract of *Maclura aurantiaca* L. led to the isolation of 4 prenylated isoflavonoids identified as osajin (**1**) [1], pomiferin (**2**) [2], auriculatin (**3**) [3] and orobol (**4**) [4]. Compound **1** showed antibacterial activities against Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin-sensitive *Staphylococcus aureus*

(MSSA) and *E. coli*with IC $_{50}$ values all less than 0.8 ug/ml. Osajin (**1**) also showed antibacterial activities against *Pseudomonas aeruginosa* with an IC $_{50}$ value of 9.9 μg/ml and against *Mycobacterium intracellulare* with an IC $_{50}$ value of 2.3 μg/ml. Compound **2** showed antifungal activities against *Cryptococcus neoformans* with an IC_{50} value of less than 0.8 μg/ml and against both of *Candida glabrata* and *Candida krusei* with IC_{50} values of 5.7 μg/ml and 5.0 μg/ml, respectively. Compound **2** also showed antibacterial activities against (MRSA) and (MSSA) with IC $_{50}$ values of 2.6 μg/ml and 6.9 μg/ml, respectively. Compound **3** showed an antibacterial activity against MRSA with an IC $_{50}$ value of 11.0 μg/ml and an antifungal activity against*Cryptococcus neofomans* with an IC $_{50}$ value of 3.0 μg/ml. Compound **4** showed an antibacterial activity against (MRSA) with an IC $_{50}$ value of 4.7 μg/ml .







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PM-82

Chemical constituents from the leaves of *Clausena lansium* and their anti-inflammatory activity

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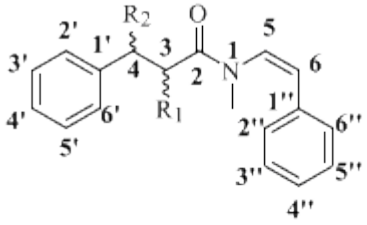
Five new acyclic amides clausenalansamides C–G (**1**–**5**) together with 39 known compounds were characterized from the leaves of *Clausena lansium* (Lour.) Skeels (Rutaceae). Their structures were established by spectroscopic methods, and the absolute configurations were determined by electronic circular dichroism and single-crystal X-ray diffraction analyses with

Cu Kα radiation. Most of the isolated compounds were evaluated for their potential anti-inflammatory activity. Among the test compounds, imperatorin (11) and wampetin (12) displayed the most significant inhibition of fMLP/CB induced superoxide anion generation with IC_{50} values of 1.7±0.3 and 6.8±1.1 μM, respectively. Moreover, the neuroprotective effect of the isolated compounds on Aβ25–35 neurotoxicity in vitro was also investigated. Among all the compounds, 6-*O*-methyl-epi-neoclausenamide showed protective activity of Aβ 25–35 cortical neuron cells death with 22.4% at 50 μM.



	R_1	R_2	C3	C4
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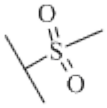
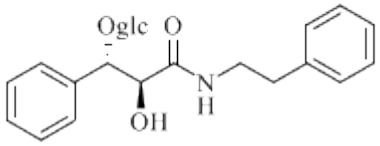
2	Cl	OH	*S*	*S*
3	OH	Cl	*S*	*R*
4	OH		*S*	*R*



5

PM-83

Assessment of the effects of a betulinic acid nanoformulation on ear inflammation

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Betulinic acid is a compound of natural origin, member of the pentacyclic triterpenes family, intensively studied in the last two decades based on its multiple biological activities, like as: anticancer, antiangiogenic, anti-inflammatory, immunomodulatory, anti-HIV and hepatoprotective effects. This compound possesses a very low solubility in aqueous solutions and finding a proper formulation to improve its solubility became a real challenge for the researchers.

The present study was purported to evaluate the effect of a betulinic acid nanoformulation on ear inflammation experimental model induced to SKH1 mice.

The ear inflammation model was obtained by topical application of a 2μg TPA (tetradecanoyl phorbol-13-acetate)/20 μl acetone solution on the mouse ears. The test solutions (betulinic acid nanoformulation and the nanoformulation blank) were administered intraperitoneally at 30 minutes post-application of TPA solution. After 4h the mice were sacrificed and the ears were

measured, weighted and kept in formalin for histological analysis. The hydration of the stratum corneum was also measured by the means of non-invasive techniques.

Our results indicate that administration of betulinic acid nanoformulation (2 mg/ml/body weight) reduced the ear inflammation by 70% as compared to the group that received only TPA. The group of mice that received the solution of the nanoformulation blank presented similar values concerning the weight of the ears with the ones obtained in the TPA-treated group. The hydration status of the treated group with betulinic acid was significantly higher as compared with the untreated groups. Moreover, the histopathological analysis confirmed the anti-inflammatory effect of betulinic acid.

These data show that betulinic acid nanoformulation is a potent anti-inflammatory agent after intraperitoneal administration.

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PM-84

Metabolomic variation in *Senecio graveolens* (Asteraceae) in altitudinal populations

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S. graveolens Wedd (Chachacoma; Asteraceae) is an altiplanic plant that grows above 3500 masl in Chile, Peru, Argentina and Bolivia, and is highly used by the Aimaras to ameliorate high-altitude sickness. Although the genus Senecio is reported as rich in pyrrolizidine alkaloids, exhibiting several activities, they are not present in *S. graveolens.*

We have reported the cytotoxic properties of the *S. graveolens* ethanolic extract and the identification of 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone with antibacterial activity. Here, we used a metabolomic approach to investigate potential chemical differences based on altitude. We also isolated and characterized the activity of a new *S. graveolens* compound.

Samples were collected from 4116-4611 masl (XV region, Chile). Metabolomic profiling was conducted using 1H NMR spectroscopy with principal component analysis (PCA) and high performance thin layer chromatography (HPTLC). Furthermore, the new *S. graveolens*

compound was isolated by high-speed countercurrent chromatography and its structure elucidated by NMR and mass spectrometry. Compound's cytotoxic activity was evaluated by MTT in MCF7 cells and the antibacterial activities by the minimum inhibitory concentration.

The PCA analysis of the 1H NMR spectroscopy fingerprints of *S. graveolens* clustered samples from the same altitude, establishing a relationship between altitude and principal component 1. In concordance, HPTLC analysis revealed a change in the metabolomic pattern with altitude. The new compound isolated from *S. graveolens* was identified as 2-hydroxy-5-(3-methylbut-2-enyl)acetophenone, an isomer of the acetophenone previously described by us, with cytotoxic activity on MCF-7 cells (IC50 139 μM) but no antibacterial properties. This compound has been described to exert a wide range of biological activities and this is the first time that it is isolated from a natural source.

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PM-85

The flavone apigenin blocks SREBP-2 activation in hepatic cells

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SREBP-2 is a pivotal transcriptional factor in cholesterol metabolism. Factors interfering with the proper functioning of SREBP-2 potentially alter plasma lipid concentrations. Consuming fruits and vegetables is associated with beneficial plasma lipid profile. The mechanism by which plant foods induce desirable lipid changes remains unclear. Apigenin, a common plant food flavonoid, was shown to prevent the nuclear translocation of SREBP-2 in the hepatic cells WRL and HepG2 in the current study. The processing of SREBP-2 protein occurred after translation, and apigenin blocked this activation route. Further study indicated that AMPK was activated by the flavone and co-administrating the AMPK-specific inhibitor compound C could release the blockage. Reporter gene assay revealed that the transactivation of SRE-containing HMGCR promoter was suppressed by the flavone. Similarly, EMSA result also demonstrated a reduced DNA-binding activity on the SRE domain under the same treatment. The reduced transactivity and DNA-binding activity could be attributed to a decreased amount of SREBP-2 translocating from cytosol to nucleus as depicted by confocal microscopy. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay demonstrated that the transcription of HMGCR followed the same pattern of SREBP-2 translocation. In summary, the present study showed that apigenin modulated SREBP-2 translocation and reduced the downstream gene HMGCR transcription.

PM-86

Evaluation of protein kinase C-activating effect of different type of *Euphorbia* diterpene phorbol esters in human platelets

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Phorbol esters are the tetracyclic diterpenoids derived from Croton tiglium and from other plants of the family Euphorbiaceae. Phorbol esters, such as phorbol 12-myristate 13-acetate, are known as tumor promoters and many biological activities of the compounds are mediated through their direct activation of protein kinase C (PKC). On the other hand, some kinds of phorbol Euphorbia diterpene esters, such as ingenol 3-angelate and prostratin, can activate PKC without tumor-promoting activities. Moreover, ingenol 3-angelate is able to induce primary necrosis in dysplastic keratinocytes, an effect that may be mediated by activation of a non-classic type of PKC- PKCδ. Recently, ingenol 3-angelate has been approved for the topical treatment of actinic keratosis. Therefore, finding novel phorbol diterpene esters from natural sources seems to be an important task. Human platelets contain 5 isoforms of PKC, including α, β, δ, θ, and ζ, and activation of PKC results in platelet aggregation. Therefore, in the present study, platelets were used to evaluate PKC-activating activities of five phorbol diterpene esters, which were isolated from European Euphorbia species. We found that 20-deoxyingenol 3-angelate (1) and 12-deoxy-16-hydroxyphorbol-20-acetate-6-angelate-13-isobutyrate (2) induced significant platelet aggregation accompanied by induction of phosphorylation of PKC substrates in platelets. In contrast, two jatrophanes (3, 4), and a myrsinol-type diterpene (5) neither induced platelet aggregation nor PKC activation. PKD, a substrate of PKCδ, was phosphorylated in response to 1 and 2, indicating that these two compounds are able to activate PKCδ. Our results suggest that 1 and 2 are PKC activator, but their selectivity to different isoforms of PKC remains to be determined.

PM-87

Antibiotic activity of crude terpenoid extract of *Lantana camara* on *Plutella xylostella* (Lepidoptera: Plutellidae)

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The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most destructive insect pest of Brassicaceae worldwide [1]. Broad-spectrum insecticides can induce resistance in insect population and are harmful to non target organisms [2]. Lantana camara L. (Verbenaceae) is one of these plants that has insecticidal activity on insect pests of agricultural crops. Crude terpenoids extrect (2, 5, 10 and 20% W/V) from aerial parts of *L. camara* were tested on DBM. The third instar larvae were fed by treated leaves. The extract was toxic to insect and reduced fecundity of females in parent and offspring generations. Also the extract changed the biological parameters such as net reproductive rate (R0) (Control=69.77±4.03 a, 2%=19.30±3.02 b, 5%=16.65±2.60 b, 10%=8.92±1.86 b,

20%=10.35±1.93 b), intrinsic rate of increase (rm) (Control= 0.193±0.003 a , 2%=0.157±0.011 ab, 5%=0.137±0.010 bc, 10%=0.116±0.013 bc, 20%=0.107±0.013 c) and finite rate of increase (λ) (Control=1.231±0.004 a, 2%=1.170±0.012 b, 5%=1.147±0.011 bc, 10%=1.123±0.015 c, 20%=1.113±0.015 c). In F1 generation. It seems the presence of phytojuvenile hormones in crude terpenoid extract is responsible for antibiotic activity against DBM. Therefore *L. camara* could be a good choice for control of DBM. However, further studies are necessary to demonstrate the active ingredients of the extract with insecticidal activity.

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PM-88

Antioxidant activity and polyphenol content of endemic plant species *Centaurea ragusina* L.

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The genus of *Centaurea* (Asteraceae) represents an attractive source of bioactive substances [1]. The goal of this work is to determine polyphenol content and antioxidant activity in endemic Croatian plant species *Centaurea ragusina* L. cultivated *in vitro* (½MS 2.9 μM GA_3+0.5 μM BA and ½MS 2.5 μM IBA) [2] and collected at natural habitats (Katalinić brig - K and Sustipan - S). To clarify biological activity of *C. ragusina*, interactions of extracts with double stranded polynucleotides (poly A – poly U and ctDNA) were studied. The highest level of total phenols, flavonoids and flavonols was determined in calli ethanol/aqueous extracts cultivated *in vitro* (2.9 μM GA_3 + 0.5 μM BA) while the highest value of hydroxycinnamic acids and proanthocyanidins was detected in ethanol/aqueous extracts of leaves after acclimatization from culture media (½MS 2.5 μM IBA). Significant antioxidant activity measured by DPPH and ABTS methods was observed in almost all extracts with respect to gallic acid as a standard. Extracts of leaves after acclimatization showed the highest stabilization on poly A - poly U (18.36) and ctDNA (5.75) evaluated by changing of melting temperature of the polynucleotide (ΔTm). Results of circular dichroism (CD spectroscopy) indicate significant impact of all tested extracts on the conformation of both polynucleotides. The results obtained suggest that the plant species *C. ragusina* grown *in vitro* can be efficiently used as a potential source of polyphenols and antioxidants in food, pharmacological and cosmetics industry as it is equally rich with phytochemicals as its wild grown counterparts.

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PM-89

Determination of alpha-glucosidase inhibitory effects of anthraquinone aglycons by molecular docking and *in vitro* studies

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Rumex acetosella L. (Polygonaceae) has been commonly used for diabetes in traditional medicine [1]. According to World Health Organization, the global prevalence of diabetes was estimated to be 9% among adults in 2014 [2] and 90% of which was type 2 diabetes [3].

Alpha glucosidase inhibitors delaying breakdown of complex carbonhydrates in small intestine and slowing glucose absorbtion are significant to prevent development of hyperglycemia [4].

In this study, anthraquinone aglycons were investigated by molecular docking studies and in vitro α glucosidase inhibition assay to determine whether the effect depends on the compounds or not. According to the docking studies simulated by the docking program AutoDock Vina 4.0, emodin, chrysophanol, physcion, aloe-emodin and rhein had moderate binding energies ranging from -6.8 to -7.5 kcal/mol. Because anthraquinone aglycons showed similar and inconclusive docking results, we in vitro measured the α glucosidase inhibition for each. In comparison to the standard compound acarbose (IC_{50}=1.75 mg/ml) their activity was low except for chrysophanol (IC_{50}=0.25 mg/ml). In our further studies, antidiabetic potential of glycosidic 1,8-dihydroxy anthraquinone derivatives will be investigated.

Acknowledgments: This study was supported by grants from Hacettepe University Scientific Research Projects (Project No: 1216).

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PM-90

Characterization of antibacterial flavonoids and stilbenes of the root extract of *Anogeissus leiocarpus* by UHPLC-MS-QTOF

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Anogeissus leiocarpus occurs in savannas in tropical and subtropical regions of Africa and is used among traditional medicinal practitioners for treatment of various diseases, among them bacterial infections [1]. Eight flavonoids [2], methyl ellagic acid, ellagic acid [4, 5] and the ellagitannins castalagin and flagallonic acid [6] have been reported from the leaves and bark of *A. leiocarpus*. There are just a few investigations on the phytochemistry of the roots [1], however. This study has aimed on in depth investigations on the phytochemistry of the root part.

Ten microliters of 50 mg/ml methanol extract of the root of A. leiocarpus was applied on RP18 TLC reversed phase. Methanol, water and orthophosphoric acid were used as mobile phase. DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent was used for detecting antioxidative compounds. In order to identify these compounds HPLC-DAD and UHPLC/MS/QTOF methods [3] were used.

Aromadendrin, taxifolin, methyltaxifolin, ampelopsin and eriodictyol as well as pinosylvin and methylpinosylvin and 3,3´-Di-*O*-methyl ellagic acid glucoside were identified for the first time in the roots of *A. leiocarpus*. In addition pentagalloyglucose and digalloylglucose were found in the root. We have demonstrated that a methanolic root extract of *A. leiocarpus* gives promising antibacterial effects. These effects might be connected to antioxidative flavonoids and ellagic acid derivatives in this extract.

Acknowledgements: This study has been supported by Ella and Georg Ehrnrooth Foundation in Finland. This abstract is dedicated to the memory of Professor Raimo Hiltunen (1944-2014).

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PM-91

Bioactivity based phytochemical studies on *Scutellaria salviifolia* Benth.

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Genus *Scutellaria* (Lamiaceae), is widespread in all over the world, has 25 taxa in Turkey. Among them 14 taxa are endemic [1,2]. Scutellaria species and their active principles possess antitumor, anti-angiogenesis, hepatoprotective, antioxidant, anticonvulsant, antibacterial, and antiviral activities [1]. In this study, endemic *S. salviifolia* aqueous extract is evaluated for its liver X receptors (LXRs) ligand activity using a LXRα luciferase reporter assay, antioxidant activity on DPPH, NO, SO radicals and cytotoxic activity against HEp-2 cell line. LXRs play role in de novo synthesis of cholesterol, excretion and detoxification of bile acids, or lipids, glucose homeostasis, neurological functions and inflammation. Recent discoveries found LXRs could regulate tumor growth in a variety of cancer cell lines [3]. Since the extract showed potent LXRα agonistic activity, it was subjected to polyamide column for fractionation of active compounds. The extract and fractions were tested for their antioxidant and cytotoxic activities. Flavonoid-rich fractions (Frs. D-E) were found to be the most active fractions in the both test systems. IC_{50} values of Frs. D-E were determined as 116 and 33 µg/mL for cytotoxic and 20-202 µg/mL for antioxidant activities, respectively. The active fractions were applied to serial column chromatographies to give compounds 1 (apigenin), 2 (luteolin-7-*O*-β-glucopyranoside) and 3 (apigenin-7-*O*-β-glucopyranoside) in pure form. Studies on active constituents are still in progress.

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PM-92

Inhibition of angiogenic key features *in vitro*: the alkaloid narciclasine blocks proliferation, migration, and tube formation of primary human endothelial cells

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Plants of the Amaryllidaceae family have been used in traditional medicine for the treatment of cancer for a long time. Narciclasine, an isocarbostyril alkaloid from *Narcissus* and *Haemanthus* species, was reported to exhibit pro-apoptotic effects selectively for cancer cells.

Although narciclasine is an interesting compound for cancer therapy, its effects on endothelial cells in the context of tumor angiogenesis have been neglected so far.

We aimed to elucidate the action of narciclasine on *in vitro* key features of angiogenesis, *i.e.* on the proliferation, migration, and tube formation of cultured endothelial cells (ECs). Treatment of ECs with narciclasine up to a concentration of 300 nM for 24 or 48 h did not affect the metabolic activity, while cell proliferation was concentration-dependently reduced with an IC50 value of 61 nM. The compound lowered the number of endothelial cells in the S- as well as G2/M-phase and increased the cell number in the G1/G0-phase of the cell cycle (flow cytometric analysis). Interestingly, narciclasine (300 nM) did neither inhibit the activation of Erk1/2 and Akt (Western blot analysis), nor interfere with endothelial nitric oxide (NO) production (DAF-2 and arginine/citrulline conversion assay). Moreover, narciclasine (300 nM) reduced the undirected migration of ECs (scratch assay) by 54 % and the chemotactic migration (Boyden chamber assay) by 77 %. Microscopical analysis of the cytoskeleton revealed that narciclasine increased the formation of F-actin stress fibers. Most importantly, the alkaloid inhibited the formation of endothelial tube-like structures on Matrigel, since it reduced the number of junctions and tubules as well as the total length of tubules.

Taken together, we could demonstrate that the isocarbostyril alkaloid narciclasine effectively decreases proliferation, migration, and tube formation of human endothelial cells and, therefore, will be further evaluated for its anti-angiogenic properties.

PM-93

Flavonoid constituents and biological studies of *Dobera glabra* leaves.

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The first phytochemical investigation of *Dobera glabra* (Forssk.) Poir. (Salvadoraceae) led to the isolation of seven flavonoids, namely isorhamnetin-3-*O*-β-glucopyranoside-7-*O*-α-rhamnopyranoside (1), isorhamnetin-3-*O*-α-rhamnopyranoside-7-*O*- β-glucopyranoside (2), kaempferol-3,7-di-*O*-α-rhamnopyranoside (3), isorhamnetin-3-*O*-β-glucopyranoside (4), kaempferol-3-*O*-β-glucopyranoside (5), isorhamnetin (6) and kaempferol (7).Their structure elucidation was performed by chromatographic, chemical and spectroscopic methods. Antioxidant and cytotoxic activities were also determined for five successive extracts of the plant. The ethyl acetate, chloroform, butanol, methanol and aqueous plant extracts exhibited moderate antioxidant effects (DPPH assay). Their cytotoxic activity was carried out against four tumor cell lines (MTT assay). The ethyl acetate and butanol extracts expressed the greatest antiproliferative activity against colon cancer cells (HCT116) with IC_{50} (8.7 and 5.3 mg/mL), respectively. In addition *D. glabra* is a highly valued plant species with diverse importance such as drought food and source of feed, a special mineral source feed, a tool for forecasting the droughts [1]. The crude protein content of *D. glabra* is also high to support animals' requirement and the laboratory analysis on the nutritive value of the edible part of *D. glabra* also revealed that this plant has nutritive value nearly comparable to the most common wild food fruits [2].

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PM-94

Anticancer effect of *Alnus japonica* extracts through the caspase-dependent pathway

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In this study, *Alnus japonica* extracts were evaluated for their *in vitro* antioxidant potential and anticancer effects in AGS human gastric carcinoma cell lines. The antioxidant properties of *A. japonica* extracts were evaluated by several biochemical assays, including FRAP (ferric reducing antioxidant power) assay, ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), alkyl and hydroxyl radical scavenging activities. These results show that ethanol extracts of *A. japonica* (AJE) have greater antioxidant activity than water extracts of *A. japonica* (AJW). AJE extracts inhibited cell growth and induced cell death by increasing reactive oxygen species (ROS) production in AGS cells. Moreover, AJE extracts specifically triggered apoptosis when caspase-8, 7, 3, and poly ADP ribose polymerase (PARP) were activated. These results suggest that treatment with AJE extracts could be a new promising strategy for clinical chemotherapy.

PM-95

LC/PDA/ESI-MS/MS polyphenols profiling of the bioactive fractions of *Croton zambesicus* fruits against*Madurella mycetomatis*

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Croton zambesicus Müll. Arg. locally known as Umgelagla belongs to the Euphorbiaceae family. It has a wide application in African traditional medicine. Ethnobotanically, the leaf decoction is used as an antimicrobial to treat various infections and for treating fever associated with malaria [1]. Different types of diterpenes including phorbol esters, clerodane, labdane, kaurane, trachylobane, and pimarane types have been isolated from this genus.

Air dried ground fruits of *C. zambesicus* were extracted using 70% methanol. The concentrated methanolic extract was sequentially fractionated with petroleum ether, chloroform and ethyl acetate. The crude extracts together with respective fractions and its seeds oil were tested

against *Madurella mycetomatis* employing a microtitre plate-based antimycetomal assay incorporating resazurin as an indicator of cell growth [2].

The ethyl acetate fraction and seed oil exhibited a significant activity against *M. mycetomatis* with MICs of 39 and 78 μg/mL, respectively.

Reverse phase HPLC-DAD coupled with ESI tandem mass spectrometry employing CID experiments at an alternating mode led to the identification of four methoxylated derivatives of kaempferol and isorhamnetin together with the C-glycosides, vitexin and isoorienten in the ethyl acetate fraction of *C. zambesicus* fruits. We assume that the identified polyphenols, at least to a certain extent, accounts for the activity of *C. zambesicus* against *M. mycetomatis* and this result may validate its traditional use as an antimicrobial.

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PM-96

Seperation and identification of bioactive compounds in *Lepidium meyenii* (Maca) based on the combination of medium-pressure liquid chromatography and preparative HPLC

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Lepidium meyenii (Maca) has health benefits for relieving menopausal symptoms and improving sexual function [1]. The main bioactive components of Maca were unsaturated fatty acids and macamides [2]. The isolation of these bioactive components from Maca has been a challenge. In this work, the preparative separation of bioactive components was effective process with two steps: the preparation of fractions using medium-pressure liquid chromatography and the purification of components by the preparative HPLC. The 8 compounds in the Maca were obtained with the amount of 76 to 836 mg and of high purity (98%, HPLC) from the 200 g ethanol extracts. The chemical structures of these compounds were identified by HR-ESI-MS, 1H and 13C NMR. To the best of our knowledge, ethyl linolenate and ethyl linoleate were separated from Maca for the first time. Besides, the HPLC method has also been developed to determine the contents of eight main bioactive components. Fig 1 is the typical HPLC chromatogram of ethanol extract of *Maca*. The HPLC conditions were: the column was Thermo ODS hypersil C18 (4.6 mm×250 mm, 5 μm). The mobile phase was water-phosphoric acid-acetonitrile (200:1:800, *v/v*). The column temperature was 40 °C. The flow rate was 1.0 mL/min. The detection wavelength was 220 nm. The results showed that the total eight bioactive components was about 1500 mg/100 g ethanol extracts and about 80 mg/100 g dried Maca roots. The preparation and analysis method proposed in the work could be applied for the quality control of Maca or Maca extracts from different origins.

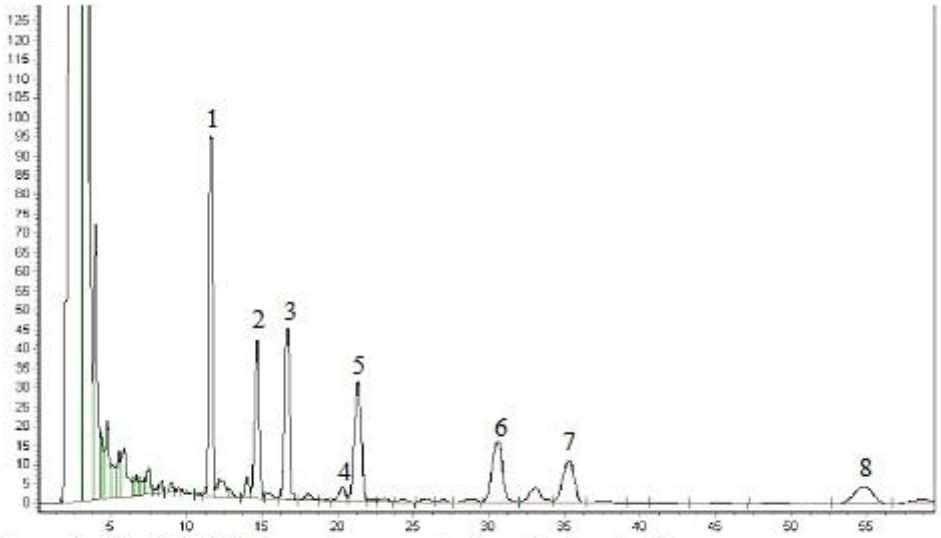

Figure 1. The HPLC chromatogram of ethanol extract of maca.
1. Linolenic acid. 2. N-benzyl-(9Z, 12Z, 15Z)-octadecatrienamide. 3. Linoleic acid. 4. N-(3-methoxybenzyl)-(9Z, 12Z)-octadecadienamide. 5. N-benzyl-(9Z, 12Z)-octadecadienamide. 6. N-benzylhexadecanamide. 7. Ethyl linolenate. 8. Ethyl linoleate

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PM-97

Synthesis and biological evaluation of 1,3,6-trisubstituted β-carboline derivatives for cytotoxic and anti-leishmanial potential

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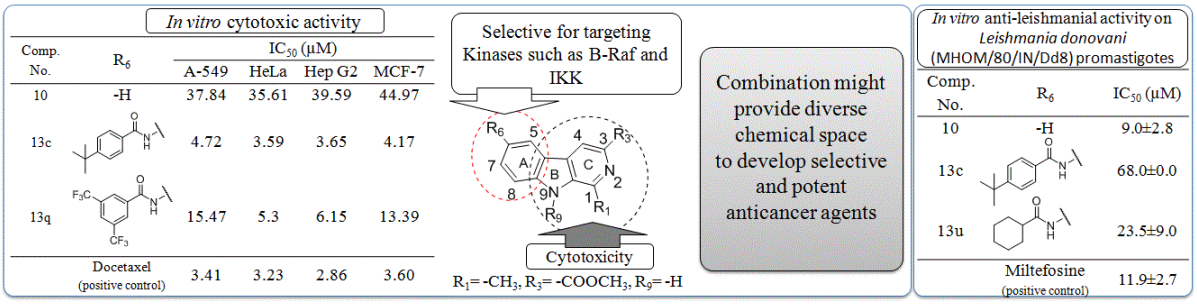
β-carbolines have been a privileged natural product scaffold for developing potent anti-cancer and anti-leishmanial compounds. [1] β-carbolines modified at C_1, C_3, N_2 & N_9 have been reported with increased cytotoxicity towards cancer cells. [2] Recent reports suggest that substitution on A-ring improves cytotoxicity towards cancer cells and inhibition of kinases.

[2-4] With this background, 23 β-carbolines were synthesized and screened for cytotoxic and anti-leishmanial potential. 1-methyl & 3-methoxycarbonyl substitutions were selected based on literature [5], while C_6-substitution was kept variable. Compounds **13c** & **13q** showed potent cytotoxicity as compared to docetaxel in four human cancer cell lines and induced apoptosis in A-549 and MCF-7. SAR suggests that the bulky substitution on benzamido group favors the cytotoxicity. However, increased IC_{50} in *in vitro* anti-leishmanial assay suggests that C_6-substitution was found to be unfavorable. These lead compounds need to be further optimized for more potent molecules.



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PM-98

Isolation and structure elucidation of acylphloroglucinols from *Hypericum triquetrifolium*

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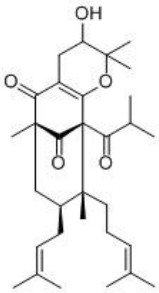
Several species of the genus *Hypericum* (Hypericaceae) accumulated prenylated acylphloroglucinols as prominent secondary metabolites. *Hypericum triquetrifolium* Turra

(Hypericaceae) mainly distributed in the Mediterranean region has been intensively investigated regarding its essential oil composition [1]. Additional studies contain investigations on the hypericins and other phenolics like I3-II8 biapigenin [2, 3]. Nevertheless, a systematic study on the occurrence of acylphloroglucinols is lacking. Thus, a petroleum ether raw extract from the aerial parts of *H. triquetrifolium*, collected on Golan Heights, Israel in July 2012 was separated using silica gel flash chromatography, centrifugal partition chromatography, RP-18 column chromatography, as well as semi-preparative RP-18 HPLC techniques. Two new acylphloroglucinols triquetrireboudin (**1**) and triquetriborin (**2**) were isolated and their structures were confirmed with 1H-, 2H-NMR and MS experiments.



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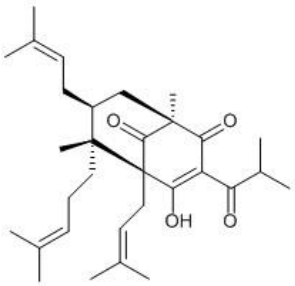

2

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PM-99

Alkaloid profiling in *Galanthus gracilis* from Western Aegean by GC/MS

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Amaryllidaceae is a family consisting of mostly tropical or subtropical plants with 85 genera and 1100 species. Among the Amaryllidaceae genera growing in Turkey, *Galanthus* L. possess Amaryllidaceae alkaloids with interesting chemical structures and a range of biological activities. A widespread and amply investigated alkaloid, galanthamine, is a marketed drug for the treatment of Alzheimer's disease. Another widely distributed alkaloid, lycorine has been proven to have prominent biological activities. *Galanthus gracilis* Celak. is distributed in Western Aegean in Turkey. In the present study, we investigated the chemical constituents of *G. gracilis* collected from western Aegean (Alankıyı/Bayındır) by using GC/MS. The GC/MS system was operated in the electron impact mode (EI, 70 ev). Helium gas was used as the carrier gas at a constant flow rate 0.8 mL/min. The temperature conditions followed the program: 80 °C for 1 min, 80-250 °C at 10 °C/min, 2 min hold at 250 °C, 250-300 °C at 10

°C/min and a 10 min hold at 300 °C. İnjector temperature was 250 °C. As a result, 11 alkaloids were detected by GC-MS, including graciline, demethylhomolycorine and tazettine as the major ones.

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PM-100

Antimicrobial and antinematode activity of *Hypericum androsaemum*

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Hypericum androsaemum L. (HA) grows wild in shadowy sites, namely in the northern region of Portugal, where it is widely used as a medicinal herb. This species is used in popular medicinal preparations as a cholagogue, hepatoprotector, and diuretic and in kidney failure [1]. The HA water extracts were assayed against the microorganisms *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae* and Candida albicans, and the plant parasitic nematode *Meloidogyne javanica*. Either the minimum inhibitory concentration or the minimum lethal concentration were higher than 8 mg/ml when the infusion was tested against *E. coli*, *C. albicans*, and *S. cerevisiae*, showing their low sensibility to HA. However, when tested against *B. subtilis*, the minimum inhibitory concentration was lower than 0.5 mg/ml and the minimum lethal concentration was lower than 2.0 mg/ml. Cumulative eclosion of *M. javanica* juveniles of second stage (J2) was calculated by counting, every 24 h, the juveniles ecloded from samples of 20 eggs exposed to 4, 6, 8, and 10 mg/ml of lyophilized extract. For mortality studies egg masses of M. javanica were placed on a small square piece of muslin with 30 µm diameter openings supported as a small sieve in a glass block (2 ml cap.); about 1 ml tap water was added, until the muslin was just submerged. Juveniles collected during the first 24 h were discarded and the subsequent J2 collected were used. Twenty J2 were exposed to 2 ml of each concentration of the lyophilized extract: 4, 6, 8 and 10 mg/ml. A relation between the concentration of the extract and hatching of *M. javanica* was found. However, apparently there was no eclosion in the first 24 h. The mortality of J2 of *M. javanica* was also directly dependent on the concentration of the extract being observed in the first 24 hours for 6, 8 and 10 mg/ml but only after 72 h at the concentration of 4 mg/ml.

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PM-101

Anti-HIV activity of *Hyptis* Jacq. (Lamiaceae)

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Hyptis species are known to be used in folk medicine to treat various diseases such as flu and constipation (*H. fruticosa*), respiratory diseases (*H. macrostachys*), stomach and intestinal disorders (*H. martiusii*), colic and liver disease (*H. pectinata*), nasal and ear disorders (*H. umbrosa*) and fever (*H. suaveolens*) [1]. It is a genus of Lamiaceae, comprising of approximately 144 species which are distributed in tropical and subtropical regions. *Hyptis radicans* (Pohl) Harley & J.F.B. Pastore, *H. lappulacea* Mart. ex Benth, *H. multibracteata* Benth. and *H. comaroides* (Briq.) Harley & J.F.B. Pastore, are species from the Atlantic Forest easily found in Paranapiacaba, SP, Brazil, except *H. comaroides* which is found in Southern Brazil, in a field area. Currently there is no literature describing the pharmacological potential about those species. Therefore the anti-HIV1 potential of hydroethanolic extracts was investigated. Plant material was collected, dried at 40°C for a week, powdered and subjected to maceration in ethanol 70% for 7 days at room temperature in the dark. Extracts were lyophilized and all samples were dissolved in DMSO 10% to achieve concentrations of 0.25, 0.50, 0.75 and 1 mg/mL for an anti-HIV1 assay (Roche®). A Foscarnet standard (0.025-1 μg/mL) was used as a positive control. The crude extract that showed the lowest EC_{50} was from *H. comaroides* (EC_{50} 33.9 μg/mL) followed by *H. radicans* (EC_{50} 158.7 μg/mL), *H. lappulacea* (EC_{50} 865.0 μg/mL) and *H. multibracteata* (EC_{50} 1096.0 μg/mL). In comparison to the stardard Foscarnet (EC_{50} 0.51 μg/mL) *H. comaroides* and *H. radicans*, both from *Peltodon* section, are promising species to search for substances with anti-HIV1 activity.

Acknowledgements: FAPESP for financial support (2013/24841-4; 2014/21233-6), CAPES for scholarship of MDSP and KPS, and CNPq.

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PM-102

Bioassay guided isolation and identification of anti-inflammatory active components from *Ficus microcarpa*

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Ficus microcarpa L. fil. belongs to the genus *Ficus* of family Moraceae, one of the largest species of flowering plants with about 800 genera of deciduous trees. The aerial root, bark and dried leaves were traditional use for the treatment of chronic bronchitis, alleviating fever, pain-relieving, ulcers, and diabetes.

The anti-inflammatory constituent was isolated from the methanol extracts using bioassay guided fractionation evaluated by estimating the levels of nitric oxide (NO) after 24 h of LPS

induction (1 μg/ml). Asiaticoside, a triterpene from *Centella asiatica*, was used as control. The extract was partitioned with hexane, ethyl acetate, *n*-butanol and water, and the active partition was further fractionated using flash chromatography system. Fractions isolated from column chromatography were assayed for anti-inflammatory and cytotoxicity activities. The isolated components from the active fraction, oleanolic acid and β-sitosterol, were confirmed by NMR and LC-MS analysis.

PM-103

Ten new 9,11 secosterols isolated from the formosan corgonian coral *Pinnigorgia* sp.

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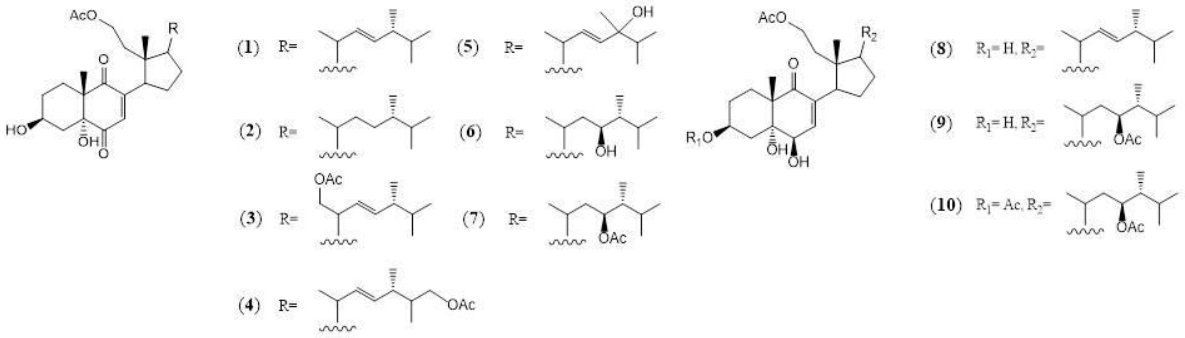
Induction of hepatic stellate cells (HSC) apoptosis by natural products is considered an effective strategy for treating liver fibrosis. The marine environment may be explored as a rich source for novel drugs. Multiple studies have been carried out to characterize marine bioactive compounds with an aim to cure human diseases and conditions; the focus on liver diseases is very limited. Using our experience searching for new bioactive compounds from soft corals and gorgonian corals, we carried out the first chemical investigation on the gorgonian coral *Pinnigorgia* sp. with the aim of discovering interesting new natural products and ten new 9,11-secosterols **1**–**10** were isolated. Structures of sterols **1**–**10** were elucidated by spectroscopic methods, particularly with 2D NMR experiments. In bioactivity test, cytotoxicity of sterols **1**–**10** against the proliferation of HSCs was evaluated. The results showed that sterol **1** inhibited cell viability of HSC-T6 with an IC_{50} value of 6.21±0.14 μM. Thus, Steroid **1** could be a promising bioactive agent and may warrant further biomedical investigation.



Pinnigorgia sp.

PM-104

Caatinga plants inhibit DNA replication and quorum sensing of *Staphylococcus aureus.*

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Caatinga plants have been described as excellent weapons against *Staphylococcus aureus* and this work evaluated the ability of 30 extracts from 22 Caatinga plants to (i) inhibit *S. aureus*, (ii) prevent the dimerization of DnaN, (iii) inhibit quorum sensing (QS). The anti-*S. aureus* action was evaluated and DnaN-DnaN interaction was performed using a Bacterial two-hybrid system. The effects of each extract on quorum sensing was performed using *spa::lacZ* or *hla::lacZ* report strains. The initial screening revealed 8 extracts were able to inhibit *S. aureus* growth (table 1), but only 3 extracts blocked the *in vivo* interaction of DnaN-DnaN: methanolic and ethyl acetate extracts from *Buchenavia tetraphylla* leaves and aqueous extract from *Libidibia ferrea* fruits. This result was confirmed using *S. aureus* strain overexpressing DnaN which resulted in resistance towards these plant extracts. The antimicrobial activity of these three plants has been reported by our group. On the other hand, the extract from *Manilkara rufula* increased the expression of *recA* involved in SOS response (evaluated using a *recA::lacZ* reporter strain). Finally, four aqueous extracts were able to inhibit QS (upregulate *spa* and down-regulate*hla* expression): *Cnidoscolus quercifolius, Harpochilus neesianus,*

Senna splendida, *Sida galheirensis*. *S. aureus* biofilm formation was also strongly inhibited by *H. neesianus* and *S. splendida* extracts. This study (i) confirms the antimicrobial potential of *L. ferrea* and *B. tetraphylla*, and shows that inhibition of DNA replication is one target of their action; (ii) reports by the first time the antimicrobial activity of *M. rufula, Senna lechriosperma and Trischidium molle*; (iii) shows that *M. rufula* extract can active SOS response; (iv) reveals *C. quercifolius*, *H. neesianus*, *S. splendida*, *S. galheirensis*extracts as QS Inhibitors. The purification and structural characterization of active compounds from all these plants are the next step of our research.

Table 1; Caatinga plants inhibit DNA replication and quorum sensing of *Staphylococcus aureus*

Plant			
Anadenanthera colubrina	*Fruits*	*Ethyl acetate*	*Anti-S. aureus action*
Buchenavia tetraphylla	*Leaves*	*Methanolic*	*Anti-S. aureus action, inhibition of DnaN dimerization*
Buchenavia tetraphylla	*Leaves*	*Ethyl acetate*	*Anti-S. aureus action, inhibition of DnaN dimerization*
Cnidoscolus quercifolius	*Branches*	*Aqueous*	*Quorum sensing inhibition*
Harpochilus neesianus	*Branches*	*Aqueous*	*Quorum sensing inhibition*
Libidibia ferrea	*Fruits*	*Aqueous*	*Anti-S. aureus action, inhibition of DnaN dimerization*
Manilkara rufula	*Branches*	*Aqueous*	*Anti-S. aureus action, activation of SOS response*
Senna lechriosperma	*Branches*	*Aqueous*	*Anti-S. aureus action*
Senna splendida	*Branches*	*Aqueous*	*Quorum sensing inhibition*
Sida galheirensis	*Branches*	*Aqueous*	*Quorum sensing inhibition*
Stryphnodendron pulcherrimum	*Branches*	*Aqueous*	*Anti-S. aureus action*
Trischidium molle	Branches	Aqueous	Anti-S. aureus action

PM-105

Macrocylic diterpenes as modulators of *Candida albicans* multidrug transporters

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Fungal infections constitute a serious global health concern. The most prevalent fungal pathogens belong to the *Candida*genus namely *Candida albicans*. *Candida* infections have been treated mainly with azoles. However, the widespread and prolonged use of antifungals, mainly due to the use of antifungal prophylaxis in immunocompromised individuals, has resulted in the development of multidrug-resistant strains. Resistance to azole antifungal agents in *Candida* species is a multifactorial phenomenon, being one of the most significant mechanisms the overexpression of ABC and major facilitator superfamily (MFS) membrane transporters. Thus, a promising approach for overcoming drug resistance is the development of inhibitors of these efflux-pump proteins.

Euphorbia species are characterized by an unusual diversity of chemical constituents, including a wide range of macrocyclic diterpenes that were found to strongly modulate the transport activity of the human P-glycoprotein, considered of major importance in the MDR phenomenon in cancer. Aiming to find new reversers of antifungal resistance mediated by efflux pumps, a set of macrocyclic diterpenes of the jatrophane and lathyrane-type was evaluated for their ability to inhibit drug-efflux activity of CaCdr1p and CaMdr1p multidrug transporters of *C. albicans* overexpressed in a *Saccaromyces cerevisiae*strain. Their inhibitory potential was assessed through a functional assay, monitoring Nile Red (NR) accumulation by flow cytometry. A chemosensitivity assay, using the checkerboard method, was also performed with some the most active compounds in order to evaluate the type of interaction with fluconazole. In the transport assay most of the compounds were found to be dual inhibitors. Two jatrophanes were selective for CaMdr1p or CaCdr1p. Moreover, three jatrophanes were able to strongly sensitize yeast growth to the antifungal activity of fluconazole.

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PM-106

GC-MS Investigation of Amaryllidaceae Alkaloids in *Galanthus cilicicus*

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The genus *Galanthus* L. belongs to the Amaryllidaceae family and is distributed throughout Europe, Asia Minor and the Near East. *Galanthus* is represented by 14 species (15 taxa) in Turkey. Among these species, *Galanthus cilicicus* Baker, an endemic species of the genus *Galanthus*, is distributed in southern Turkey mainly in the province of Içel [1].

*Galanthus*species are among the most important native flowerbulbs exported from Turkey, therefore they have an economical importance [2]. They are known to possess Amaryllidaceae alkaloids with interesting chemical structures and biological activities [3]. Among the Amaryllidaceae alkaloids, galanthamine is used for the treatment of Alzheimer's disease [4]. In this study, the alkaloidal content of *G. cilicicus*, collected from Yenikoy (Icel) during the flowering season, was investigated by means of gas chromatography/mass spectrometry (GC/MS). The crude alkaloidal extracts were obtained from the aerial parts and the bulbs. As a result, the GC/MS analysis of the prepared extracts afforded twenty alkaloids belonging to different skeletal types of Amaryllidaceae alkaloids. In the aerial parts, among the detected alkaloids haemanthamine and tazettine were the main alkaloids whereas in the bulbs galanthamine and tazettine were the major alkaloids.

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PM-107

Rhamnosyl gallates and proanthocyanidins produced by *Inga* species and their antioxidant and antitumoral potential

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Fabaceae species are widespread in tropical/subtropical regions as shrubs and trees [1] and used as medicinal plants in Brazil to treat inflammation. The presence of flavonoids and phenolics in *Inga* extracts has been reported previously and might be associated to their popular uses [1,2]. Preliminary analysis of EtOAc fraction from crude extracts of *Inga laurina* (S.W.) Willd, *I. edulis* Martius, and *I. marginata* Willd. indicated phenolics as major compounds by HPLC-DAD-UV. Such fractions were submitted to antiradicalar evaluation in the DPPH assay and liposome model which indicated their antioxidant potential. In addition, the cytotoxic activity was evaluated by the MTT assay using HT-29 cancer cells (human colon) and best results for cytotoxicity were shown by the EtOH extract from *I. laurina* leaves (ED_{50} 7.20 µg/mL), which led to its selection for further work. RP-HPLC of this extract afforded gallic acid rhamnosyl derivatives whereas the fruits extract afforded proanthocyanidins. Their structures were determined by NMR and MS analysis as 2-rhamnopyranosyl-3,5-dihydroxyphenyl gallate, 2-rhamnopyranosyl-4,6-dihydroxyphenyl gallate, and

proanthocyanidin A2. Literature data highlight the prevalence of phenolics in leaves extracts of *Inga* species and their antioxidant activity associated to the chemopreventive potential which corroborates our data [3]. Cytotoxicity evaluation of the isolated compounds indicated low activity towards HT-29 cell line whereas their strong free radical scavenging activities (IC_{50} 22.6 μM, 19.9 μM and 25.2 μM, respectively) were confirmed by the DPPH assay and compared to quercetin (IC_{50} 18.9 μM), evidencing the antiradicalar potential of *Inga laurina* and the importance of bioprospecting studies in the search of novel bioactive compounds.

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PM-108

Aciculatin inhibit RANKL-induced osteoclastogenesis in RAW 264.7 cells

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Chrysopogon aciculatis (Retz.) Trin. (Poaceae) is a perennial herb and widely distributed in lower altitudes in Taiwan. It has been used to treat fever and common cold as a traditional Chinese medicine. In the current study, the whole grass of *C. aciculatis* was heated under reflux with 95% ethanol, and then partitioned with a sequence of ethyl acetate (EtOAc), n-butanol and water. Aciculatin, 8-(2,6-dideoxy-beta-ribo- hexopyranosyl)-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4H-1-benzopyran-4-one, was obtained and identified from EtOAc extract. We investigate the effects of aciculatin on osteoclastogenesis. Receptor activator of nuclear factor kappa-B ligand (RANKL)-induced osteoclastogenesis in RAW 264.7 cells was used as a platform for osteoclast assessment. Cell viability was examined using thiazolyl blue tetrazolium bromide (MTT) assay. Tartrate resistant acid phosphatase (TRAP) activity and staining were determined in multinucleated osteoclasts. The protein and mRNA expression was analyzed by Western blotting and real-time PCR. The results showed that aciculatin can inhibit osteoclast differentiation in RANKL-induced RAW 264.7 cells. Aciculatin regulated expression of nuclear factor of activated T cells (NFATc1), which plays an important role in osteoclast differentiation, and nuclear factor kappa B (NFκB) signaling pathway. The present *in vitro* results suggest that aciculatin may be useful in treating or preventing osteoporosis *in vivo*.

PM-109

A novel HILIC-method for the analysis of Mycosporine-like amino acids and their biological relevance on anti-inflammatory targets and collagenase

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Depletion in stratospheric ozone and subsequent increases in solar radiation promoted research on UV absorbing compounds from different organisms. Mycosporine-like amino acids (MAAs), a group of small secondary metabolites predominantly found in algae, cyanobacteria, lichen and fungi came in the focus because of their pronounced photo protective and anti-oxidative activities [1]. In literature the HPLC analysis of MAAs has always been described using reversed phase material [2]. In our study, a fully validated HILIC method is presented for the first time, which offers many advantages like a precise quantification of the analytes, enhanced retention and the option of coupling to MS. Excellent linear correlation coefficients (R2>0.9998) were observed for selected compounds with LOD values from 0.10-0.16 µg/mL. Additionally, the same MAAs were investigated for collagenase inhibition and anti-inflammatory properties. Collagenase activity was measured in a previously validated fluorogenic assay indicating a dose dependent inhibition of the enzyme by all three derivatives, with IC_{50} values in the range of 37.73 µg/mL (porphyra) to 34.42 µg/mL (shinorine). Their effect on the inflammatory pathway was investigated in a cellular context (NFkB activity on THP-1 blue cells). Interestingly, shinorine and porphyra were able to induce and not inhibit NF-kB activity, which might suggest pro-inflammatory or immune stimulating properties. Especially unstimulated cells were significantly activated by the two MAAs.

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PM-110

Pseudobulbs of the Columbian orchid *Cyrtopodium paniculatum* Garay, a source of multiple stilbenoid derivatives

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The genus *Cyrtopodium* is constituted of about 50 orchids located in South America. Some species are used in traditional medicine for their antioxidant activity due to either their polysaccharides [1] or their stilbenoids [2] content. As part of our continuing studies on

tropical orchids [3], we herein report the first phytochemical investigation of a Colombian orchid*Cyrtopodium paniculatum* Ruiz & Pav. A classical phytochemical approach of a dichloromethane extract by multi-step fractionation allowed the isolation of 17 stilbenoids. Their structures were elucidated by UV, HRMS and NMR spectral analysis. Among the obtained compounds, three are newly described:

- **9-Hydroxyerianthridin** or 3,4 dimethoxy-9,10-dihydrophenanthrene-2,7,9-triol
- **Cyrtopodin** or 1,3,5,6-tetramethoxyphenanthrene-2,7-diol
- **Cyrtopodinol** or 3,5,6-tetramethoxyphenanthrene-1,2,7-triol

Both chromatographic and spectrometric data from each isolated compound were collected and used for building a rapid RP-HPLC-DAD/UV-MS/MS dereplication method. Therefore, the stilbenoid content of *C. paniculatum* leaves and roots will be detected online by means of this dereplication strategy.

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PM-111

New cytotoxic and antifungal amides from the fruit of *Piper retrofractum*

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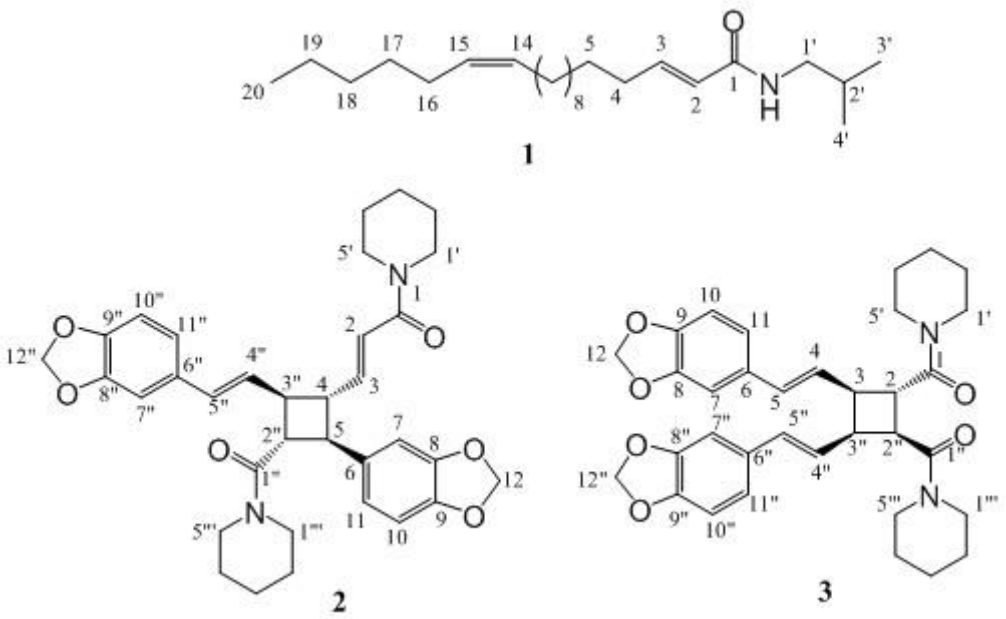
Piper retrofractum is an economically and medicinally important species in the genus *Piper* (Piperaceae). The fruit extract has been reported to possess various bioactivities such as antifungal, insecticidal, antibacterial, anticancer, antileishmanial, antidiabetic and antiobesity activity [1,2]. In search of new natural products with respect to pharmaceutical lead discovery of antifungal and anticancer agents, bioassay-guided fractionation and chemical investigation of the methanol extract of the dried fruit of *Piper retrofractum* led to the isolation of three new amides, namely (2*E*,14*Z*)-N-isobutyleicosa-2,14-dienamide (**1**), dipiperamides F and G (**2** and **3**), together with 30 known compounds. Their structures were elucidated by extensive spectroscopic analyses including 1D and 2D NMR as well as MS, and by comparison with the literature. All isolated compounds were screened for their antifungal and cytotoxic activities. Piperanine (**9**) showed potent activity against the plant pathogenic fungus, *Cladosporium cladosporioides*, compared to nystatin (positive control). Dipiperamides F and G (**2** and **3**),

chabamide (**30**), nigramide R (**31**), dehydropipernonaline (**24**), pipernonaline (**25**), guineensine (**22**), brachystamide B (**23**), retrofractamide C (**20**), pellitorine (**13**) and pipericine (**14**) exhibited cytotoxicity against L5178Y mouse lymphoma cells with IC_{50} values of 10.0, 13.9, 11.6, 9.3, 8.9, 17.0, 17.0, 16.4, 13.4, 28.3 and 24.2 μM, respectively. The presence of a methylenedioxyphenyl moiety in the amides structure tends to increase the cytotoxicity.



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PM-112

Novel, bioactive homoisoflavonoids from a Madagascan *Rhodocodon* species (Hyacinthaceae, *sensu* APG II)

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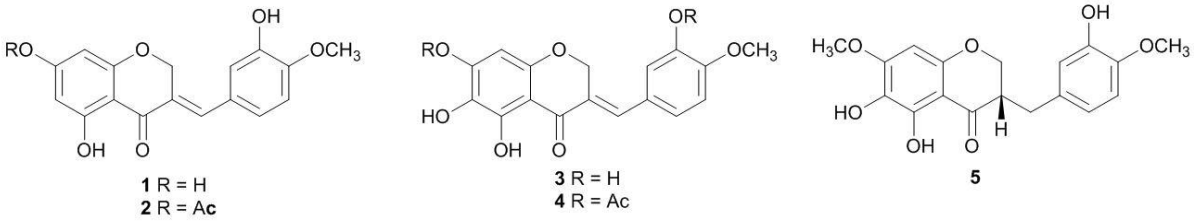
Rhodocodon species are endemic to Madagascar and their circumscription has been a topic of debate. Speta [1] treated*Rhodocodon* as synonymous with *Rhadamanthus* and Manning [2]

included the species in *Drimia*. Knirsch *et al.* [3] have recently presented evidence for a separate genus, *Rhodocodon*. Three novel homoisoflavonoids (Compounds **1**, **3** and **5**) were isolated from the dichloromethane extract of the bulbs of *Rhodocodon* (Hyacinthaceae, sensu APGII). The structures of these compounds together with the acetates (compounds **2** and **4**) were determined by spectroscopic techniques. The absolute stereochemistry at C-3 of compound **5** was determined by circular dichroism and found to be *S*, unusual for 3-benzyl homoisoflavonoids from the Hyacinthaceae, which are typically *R*. The effect of compounds **2-5** on cyclooxygenase-2 (COX-2) expression in the HCA7 colorectal cancer (CRC) cell line was investigated with compound **3** giving the best result (12% reduction compared to the control). Compounds **2**, **3** and **4** were tested for antiangiogenic activity on human retinal microvascular endothelial cells (HREC) and gave GI50's of 17.6 μM, 17.1 μM and 15.8 μM respectively. Compounds inhibiting angiogenesis are of interest to treat eye diseases characterized by neovascularization of the retina, as well as being of interest as novel anticancer therapies.



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PM-113

Alkaloids can synergistically enhance the activity of trypanocidal drugs against *Trypanosoma brucei brucei in vitro*

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Trypanosoma brucei is a parasitic flagellate, which currently endangers 70 million people in Africa by causing the deadly sleeping sickness [1]. Only four drugs are registered against African trypanosomiasis, which exhibit severe side effects and many strains have become resistant to them [2]. Therefore, new strategies and drugs are urgently needed. In this study, we investigated a combination of bioactive alkaloids with common trypanocidal drugs in order to discover synergistic interactions. Eleven alkaloids were selected, which differ in their mode

of action: intercalation of DNA (berberine, chelerythrine, emetine, harmine, quinine, sanguinarine), inhibition of protein biosynthesis (homoharringtonine), assembly of microtubules (vinblastine, colchicine, noscapine) and induction of apoptosis (piperine). We investigated their trypanocidal activity against *Trypanosoma b. brucei in vitro* using the MTT cytotoxicity assay [3]. In addition, alkaloids were combined in a non-toxic concentration with the trypanocidal drugs suramin, diminazene and pentamidine in order to evaluate potential synergistic or additive effects. Homoharringtonine had the strongest trypanocidal effect among the alkaloids and trypanocidal drugs, while berberine affected the activity of all three trypanocidal drugs in a synergistic fashion. Our experiments clearly show that combinations of bioactive alkaloids with trypanocidal drugs can enhance their efficacy, often in a synergistic fashion.

Table 1. Summary of combinations of alkaloids with trypanocidal drugs

Summary of synergistic or additive effects of alkaloid combinations with trypanocidal drugs suramin, diminazene and pentamidine. CI values are interpreted as follows: <0.1 very strong synergism (+++++), 0.1–0.3 strong synergism (++++), 0.3–0.7 synergism (+++), 0.7–0.85 moderate synergism (++), 0.85–0.90 slight synergism (+), 0.90–1.10 nearly additive (±), 1.10–1.20 slight antagonism (−), 1.2-1.45 moderate antagonism (−−), 1.45-3.3 antagonism (−−−), 3.3-10 strong antagonism (−−−−), > 10 very strong antagonism (−−−−−) [4].

Alkaloid added in the combination	IC_{50} (suramin; nM)	CI	CI (+/-)	IC_{50} (diminazene; nM)	CI	CI (+/-)	IC_{50} (pentamidine; nM)	CI	CI (+/-)
Trypanocidal drug alone	130 ± 9.00	/	/	260 ± 30	/	/	40 ± 7.00	/	/
Berberine	64.4 ± 8.6	0.78	++	137.37 ± 17.91	0.81	++	18.4 ± 0.26	0.70	+++
Chelerythrine	69.3 ± 1.21	0.73	++	110.40 ± 37.40	0.64	+++	34.1 ± 3.68	1.01	±
Colchicine	65.5 ± 10.89	0.88	+	278.20 ± 2.20	1.47	--	72.1 ± 2.10	2.08	---
Emetine	95.97 ± 22.6	0.87	+	264.20 ± 34.86	1.40	---	67.97 ± 10.13	1.97	---
Harmine	73.10 ± 2.25	0.71	++	284.83 ± 15.66	1.51	---	72.00 ± 2.86	2.09	---
Homoharingtonine	72.7 ± 1.49	0.80	++	257.33 ± 17.86	1.25	--	71.40 ± 7.04	1.93	---
Noscapine	124.2 ± 4.15	1.34	--	290.67 ± 15.66	1.54	---	61.4 ± 2.30	1.84	---
Piperine	79.33 ± 17.17	1.00	±	292.77 ± 10.77	1.53	---	132.87 ± 1.18	3.51	----
Quinine	133.8 ± 8.9	1.44	--	131.67 ± 26.17	0.78	++	72.67 ± 4.34	2.09	---
Sanquinarine	70.1 ± 4.98	0.69	+++	131.60 ± 16.48	0.93	±	44.27 ± 10.11	1.46	--
Vinblastine	72.83 ± 1.19	0.69	+++	130.87 ± 25.11	0.80	++	68.5 ± 10.67	1.65	---

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PM-114

Inhibition of LPS-induced inflammatory responses by caffeoyl glucosides from *Nandina domestica*

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Endothelial dysfunction is a critical pathological feature of many vascular inflammatory diseases including sepsis and atherosclerosis. In the present study, a new calleryanin derivative (**1**), along with a new (**2**) and a known (**3**) caffeoyl glucoside were isolated from the fruits of *Nandina domestica*. The compounds were investigated for their effects against lipopolysaccharide (LPS)-mediated endothelial inflammatory responses in the Human Umbilical Vein Endothelial cells (HUVECs). As compared to the control, compounds **2** and **3** (at 20 μM) reduced LPS-induced hyperpermeability in the HUVECs. Pretreatment with LPS increased the adherence of leukocytes to HUVECs to around 86%. Compounds **2** and **3**, at 20 μM, reduced the adherence to 30.0 and 33.1%, respectively in comparison to 20% in control, and significantly suppressed their migration across the HUVECs monolayer. Taken together, these results suggest that caffeoyl glucosides could be a potential scaffold for the development of drug leads against vascular inflammatory diseases.

PM-115

Chemical constituents and biological properties of liverworts from South Africa

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Liverworts (Marchantiophyta) are considered to be the oldest terrestrial plants. At present more than 1500 terpenoids and 350 aromatic compounds (excluding flavonoids) have been isolated from or detected in the Marchantiophyta [1]. Several of these constituents are unique to liverworts and exhibit interesting biological activities, including antibacterial and antifungal activities [2]. Although 1200 species occur in southern Africa, a Scopus search revealed that no studies related to the chemistry of these liverworts have been done. Liverwort specimens, 48 specimens representing nine species, were collected from several localities in South Africa. Volatile compounds, extracted using solvent-based extraction, were identified by gas chromatography-mass spectrometry (GC-MS). Solvent extracts (MeOH: $CHCl_3$) were evaluated for their antimicrobial activities against *Candida albicans* (ATCC 10240), *Cryptococcus neoformans* (ATCC 1416), *Enterococcus faecalis*(ATCC 29212), *Escherichia coli* (ATCC 8740), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus*(ATCC 25923). The most active extract was that from *Plagiochasma rupestre* against *P. aeruginosa* and *E. faecalis*, with minimum inhibitory concentrations of 0.5 mg/mL and 1.8 mg/mL respectively. Reversed phase preparative high performance liquid chromatography purification of the extract from *Dumortiera hirsute* yielded a compound, identified as the dumortane-type derivative 1,1,1α,6-tetramethyl-3-

methylidenedecahydrocyclopropa[e]indene, using GC-MS, and one and two dimensional nuclear magnetic resonance spectroscopy. This is first report on the volatile profiles of *Fossombronia swaziensis*, *Pallavicinia lyellii* and *Marchantia pappeana*.

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PM-116

4,15–isoatriplicolide-esters: new inhibitors of trypanothione reductase

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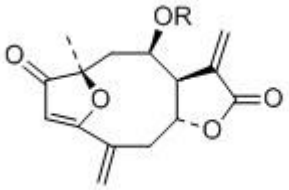
The sesquiterpene lactone 4,15-isoatriplicolide tiglate **1** was recently discovered as an extremely potent trypanocidal agent with an in vitro IC_{50} of only 15 nM against *Trypanosoma brucei rhodesiense*, causative pathogen of East African Human Trypanosomiasis (HAT) [1]. Trypanosomatids possess a unique mechanism to maintain their redox state and defend themselves against oxidative stress, in which the glutathione/glutathione reductase system, serving this purpose in other Eukaryotes, is replaced by Trypanothione/Trypanothione reductase (TR). TR is therefore a potential target for new leads and drugs against HAT and related diseases [2]. In an attempt to identify potential molecular targets of the isoatriplicolide ester in trypanosomes, we have investigated the possibility that it inhibits the parasites' most crucial redox enzyme.



	R
1	tiglate
2	methacrylate
3	isobutyrate

Fig. 1: Structures of 4,15-isoatriplicolide esters **1-3**

In vitro studies with *T. cruzi* (*Tc*) and *T. brucei* (*Tb*) TR have clearly shown that the enzyme is inhibited to variable extent by the tiglate **1** as well as the corresponding methacrylate **2** and isobutyrate **3**; Under the chosen assay conditions [2], e.g. 20, 40 and 100 µM of **1** inhibited the *Tc* enzyme enzyme by 52, 71 and 89 % after only 15 min pre-incubation. Time dependent inhibition experiments with the *Tb* enzyme furthermore indicate that the enzyme is inhibited in an irreversible manner. This observation may point towards a covalent modification in the enzymes' active site which contains two cysteine residues with essential function in the

catalytic mechanism [3]. Further studies aiming at a full characterization of the inhibition mechanism are in progress.

Acknowledgments: This work is part of the activities of ResNetNPND (http://www. ResNetNPND.org/) and was performed as a cooperation within COST action CM1307.

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PM-117

Anti-inflammatory effect of streptochlorin via TRIF-dependent signaling pathways in cellular and mouse models

Do-Wan Shim[1], Hee Jae Shin[2], Ji-Won Han[1], Woo-Young Shin[1], Xiao Sun[1], Eun-Jeong Sim[1], Tack-Joong Kim[3], Tae-Bong Kang[1], Kwang-Ho Lee[1]

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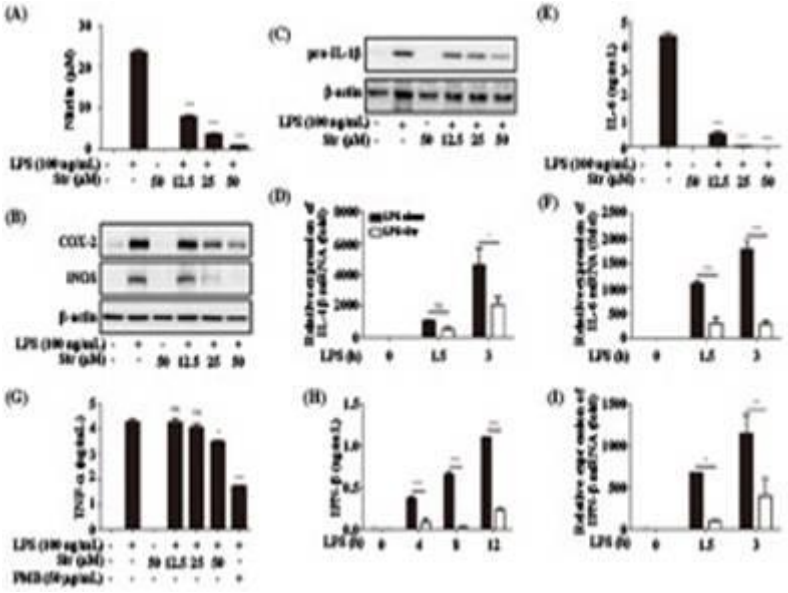
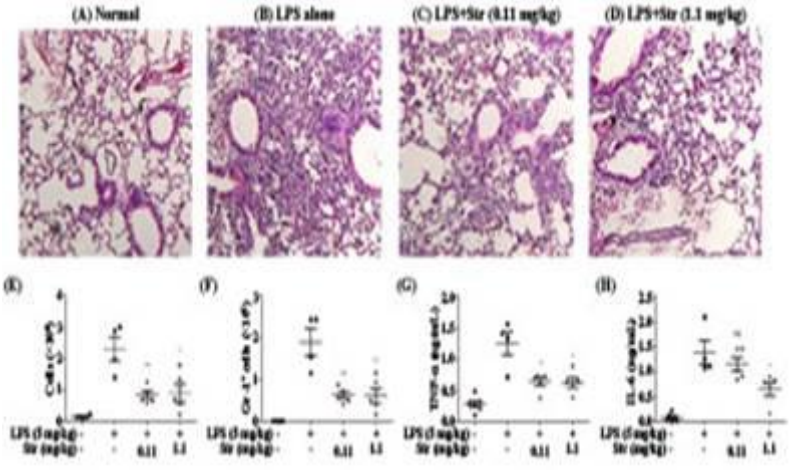
Streptochlorin, a small compound derived from marine actinomycete, has been shown to have anti-angiogenic, anti-tumor, and anti-allergic activities. However, the anti-inflammatory effects and underlying mechanisms have not yet been reported. In the present study, we investigated the effect of streptochlorin on lipopolysaccharide (LPS)-induced inflammatory responses *in vitro* and *in vivo*. As shown in Fig. 1, streptochlorin attenuated the production of proinflammatory mediators such as nitric oxide, cyclooxygenase-2, pro-interleukin (IL)-1β, and IL-6 in LPS-stimulated RAW264.7 cells through inhibition of the Toll/interleukin-1 receptor (TIR)-domain-containing adapter-inducing interferon-β (TRIF)-dependent signaling pathway. IC50 to inhibit those inflammatory mediators is 25μM.

Fig.1



To verify the *in vivo* efficacy of streptochlorin, we injected 0.11~1.1 mg/kg of streptochlorin by intra peritoneal injection in mouse (Fig. 2). Streptochlorin suppressed the infiltration of immune cells such as neutrophils into the lung and proinflammatory cytokine production such as IL-6 and TNF-α in broncho-alveolar lavage fluid (BALF) in the LPS-induced acute lung injury (ALI) mouse model.

Fig. 2



Taken together, streptochlorin has potent anti-inflammatory effects through regulating TRIF-dependent signaling pathways, suggesting that streptochlorin may provide a valuable therapeutic strategy in treating various inflammatory diseases.

PM-118

Antileukemic lanostanoids from *Poria cocos*

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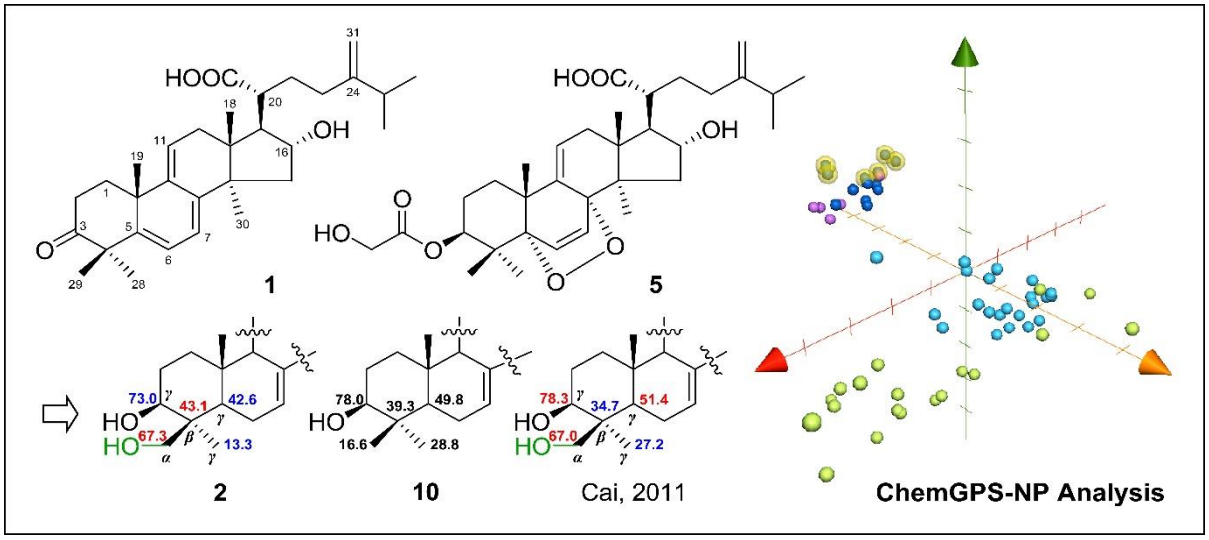
Seven new lanostanoids, isolated from the sclerotia of *Poria cocos*, were elucidated to be (20*ξ*)-16*α*-hydroxy-3-oxo-24-methyllanosta-5,7,9(11),24(31)-tetraen-21-oic acid (**1**), (20*ξ*)-3*β*,16*α*,29-trihydroxy-24-methyllanosta-7,9(11),24(31)-trien-21-oic acid (**2**), (20*ξ*)-3*β*,16*α*,30-trihydroxy-24-methyllanosta-7,9(11),24(31)-trien-21-oic acid (**3**), (20*ξ*)-3*β*-acetyloxy-16*α*,24*α*-dihydroxy-lanosta-7,9(11),25-trien-21-oic acid (**4**), (20*ξ*)-5*α*,8*α*-epidioxy-3-*O*-hydroxyacetoxy-3*β*,16*α*-dihydroxy-24-methyllanosta-6,9(11),24(31)-trien-21-oic acid (**5**), (20*ξ*)-3*β*,16*α*-dihydroxy-7-oxo-24-methyllanosta-8,24(31)-dien-21-oic acid (**6**) and (20*ξ*)-3*α*,16*α*-dihydroxy-7-oxo-24-methyllanosta-8,24(31)-dien-21-oic acid (**7**), based on the extensive spectroscopic analyses. The antileukemic activity of the new compounds (except **3** and **4**), along with the fifteen known lanostane-type triterpenoids, was evaluated against four leukemic cell lines (Molt 4, CCRF-CEM, HL 60 and K562). Dehydropachymic acid (**9**), dehydroeburicoic acid (**12**), pachymic acid (**14**) and lanosta-7,9(11),24-trien-21-oic acid (**20**) exhibited cytotoxic effect on CCRF-CEM cancer cell line with IC_{50} values of 1.43, 2.96, 2.61 and 5.96 μg/mL, respectively. Both dehydropachymic acid (**9**) and dehydroeburicoic acid (**12**) showed cytotoxicity against Molt 4 (IC_{50} 7.26 and 6.67 μg/mL) and HL 60 (IC_{50} 3.84 and 2.79 μg/mL) leukemic cell lines. ChemGPS-NP analysis on the active lanostanoids from *P. cocos* suggested that targets other than topoisomerases may be involved in the cytotoxic effect.



PM-119

Cytotoxicity and antimicrobial activities of secondary metabolites produced by endophytic fungi isolated from *Gongronema latifolia* and *Loranthus micrantus*

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This study was carried out to evaluate the cytotoxicity and antimicrobial properties of secondary metabolites produced by endophytic fungi isolated from *Gongronema latifolia* and *Loranthus micranthus*. Isolation of endophytic fungi from plant leaves was carried out using a previously described method. Solid state fermentation was carried out in rice medium for 30 days at 25-27 °C and the secondary metabolites were extracted using ethyl acetate. Cytotoxic effects of the extracts were tested on mouse lymphoma cell line (L5178Y) using MTT assay. The antimicrobial properties of the extracts were evaluated using agar well diffusion assay method. HPLC-DAD analysis was carried out to identify the compounds that may be responsible for the recorded activities. Six endophytic molds were isolated from *G. latifolia* and four from *L. micranthus*. The results of the cytotoxicity test revealed that the extracts of three fungi from *G. latifolia*, ACA-L1-2.2, ACA-L-1, and ACD-S2-1.1 showed significant inhibition of the growth of mouse lymphoma cell line with percentage inhibitions of 57.9, 96.8 and 98.7 respectively. Extracts from two fungi from *L. micranthus*, ACC-S2-1.1 and ACD-S2-1.1 showed moderate inhibition of 32.9 and 45.9 % respectively, others showed poor activity. At 1mg/mL, some extracts showed antibacterial activity against all test bacteria. At same concentration, none of the extracts recorded antifungal activity. Extracts from ACC-S2-1.1 and ACC-S2-2.1 specifically showed good antimicrobial activity against the test bacteria with inhibition zone diameter in the range of 2-10 mm. HPLC-DAD analysis of the active extracts revealed the presence of xanthones, depsidones, cinnamic acid, protochatechic acid and indole acetic derivatives. The present study may serve as the baseline for the isolation and

characterization of the active molecules which may be used as anticancer or antimicrobial compounds; or as lead compounds for further development into novel therapeutic molecules.

PM-120

Tri- and diterpenoids from some species of Euphorbiaceae. Evaluation of their antiinflammatory and cytotoxic properties.

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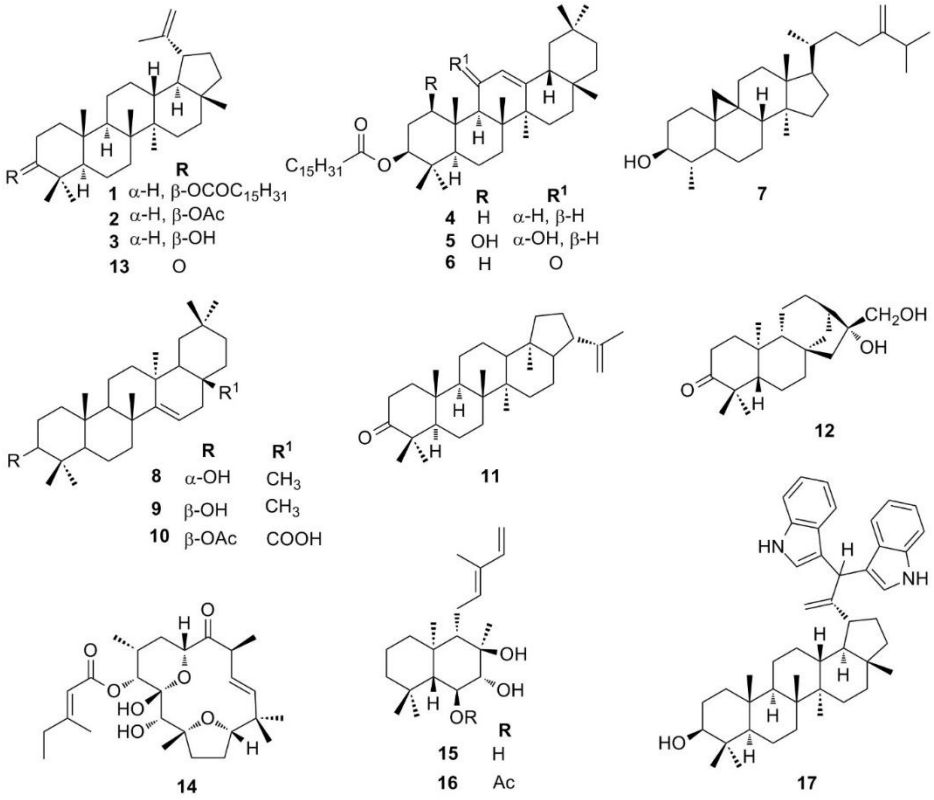
The Euphorbiaceae family is one of the largest in the plant kingdom; it is widespread throughout the world and biosynthesizes a variety of bioactive metabolites [1, 2]. The aim of present study is the characterization and bioevaluation of the secondary metabolites from four Euphorbiaceae species, *Sapium nitidum*, *Sebastiania longicuspis*, *Sapium macrocarpum*and *Croton glabellus*.

From the aerial parts of *S. nitidum* were characterized lupeyl palmitate (**1**), lupeyl acetate (**2**), 19βH-lupeol (**3**), β-amyrinyl palmitate (**4**), 1β,3β,11α-trihydroxy-olean-12-enyl-3-palmitate (**5**), 3β-hydroxy-11-oxo-olean-12-enyl-3-palmitate (**6**), cycloeucalenol (**7**), β-sitosterol, stigmasterol, β-sitosteryl-3-*O*-β-D-glucopyranoside and β-sitosteryl-3-*O*-β-D-glucopyranoside-6'-*O*-palmitate. From *S. longicuspis* were isolated 3-epi-taraxerol (**8**), 3β-taraxerol (**9**), 3-acetyl-aleuritolic acid (**10**), 3-oxo-21αH-hop-22(29)-ene (**11**), ent-kaurane-3-oxo-16α,17-diol (**12**) and **3**. Lupenone (**13**), sitostenone, β-sitosterol, tonantzitlolone (**14**) and **3** were isolated from the aerial parts of *S. macrocarpum*. From the leaves of *C. glabellus*were isolated austroinulin (**15**), 6-*O*-acetylaustroinulin (**16**) and phytosterols. Preliminary biological evaluation of some natural compounds indicated moderate activity. In order to explore the bioactivity, some semisynthetic compounds were prepared. Diindolylmethane derivative (**17**) was obtained from 30-oxo-lupeol, which was prepared from the oxidation of **3**with SeO_2. Compound **17** displayed good selectivity against human leukemia-cancer cell lines.



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PM-121

Isolation and identification of chemical constituents from the roots of *Peucedanum praeruptorum*

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The dried roots of *Peucedanum praeruptorum* Dunn, Baihuaqianhu, is well known traditional Chinese medicine. It has been used as anti-asthma, antitussive and as a remedy for angina. Recent pharmacological studies showed that the extract of *P.praeruptorum* has various beneficial pharmacological activities. Chromatographic separation of a 70% EtOH extract of roots of *P. praeruptorum* led to the isolation of a new angular-type pyranocoumarin, along with fifteen known compounds including twelve pyranocoumarins, two furanocoumarins, and a polyacetylene. The structures of all compounds were determined on the basis of 1D and 2D NMR techniques (COSY, NOESY, HSQC, HMBC) in association with HR-ESI-MS data analysis. Their stereochemistry were determined by optical rotation and circular dichroism studies. All of the isolates were evaluated for their protective activity against chemotherapy-

induced myelosuppression using *ex vivo* hematopoietic CFC assay system. Four compounds isolated from *P. praeruptorum* showed protective effects. In this study, we report the isolation and structural elucidation, and biological activities of these compounds from the roots of *P. praeruptorum*.

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PM-122

Evaluation of the antibacterial activity of some African medicinal plants

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Aiming to find new antibacterial compounds from African medicinal plants, forty extracts of different polarity from eight plant species were screened against Gram positive and Gram negative bacteria. The selected plants, *Cleistochlamys kirkii, Colophospermum mopane, Cladostemon kirkii, Chrysophyllum viridifolium, Gardenia ternifolia, Grewia hexamita, Xeroderris stuhlmannii* and *Albizia adianthifolia*, are used in Mozambique traditional medicine to treat infectious diseases. The best results were obtained for both apolar and polar extracts of *C. kirkii* root bark (Annonaceae) with minimum inhibitory concentration (MIC) of 7.5-62 μg/ml against Gram positive strains. Bioassay-guided fractionation of the methanol extract of *C. kirkii* allowed the isolation of several known compounds with different scaffolds, namely three flavanones, two α,β-unsaturated lactones, one mono-tetrahydrofuran derivative, one triterpene and one alkaloid. Their structures were assigned based on spectroscopic methods namely 1D-NMR and 2D-NMR experiments.

The evaluation of the antibacterial activity was performed by the microdilution method, against Gram positive*Staphylococcus aureus* (sensitive and resistant strains), vancomycin-resistant *Enterococcus faecalis, Bacillus subtilis* and Gram negative *Salmonella typhimurium* and *Pseudomonas aeruginosa.* MIC and MBC (minimum bactericidal concentration) values were determined for all compounds. The best results were obtained for some of the flavanones that displayed MIC values lower than 4 μg/mL against all the Gram positive strains tested. Furthermore, using the checkerboard method, all the compounds, excepting the alkaloid, were also evaluated for synergistic effects in combination with the β-lactam antibiotics amoxicillin and oxacillin against *Staphylococcus aureus* (methicillin-resistant and sensitive strains). The fractional inhibitory concentration index (FICI) values indicated synergistic activities for two of the compounds.

PM-123

Exploring epoxylathyrane derivatives to overcome ABCB1-mediated multidrug resistance in human colon adenocarcinoma cells

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Multidrug resistance (MDR) in cancer is nowadays regarded as the leading problem in chemotherapy. The different mechanisms of MDR have been intensively studied. One of the most important is related with the overexpression of the energy-dependent membrane efflux proteins known as ATP-binding cassette (ABC) transporters, which include, among others, the ABCB1 transporter (P-glycoprotein, P-gp). ABCB1 confers significant resistance to a wide range of drugs, including taxanes, Vinca alkaloids and anthracyclines. For this reason, the development of molecules that are able to modulate the ABCB1 mediated efflux has been one of the most promising strategies to overcome MDR. In the last decade, macrocyclic diterpenes with the lathyrane and jatrophane scaffold isolated from *Euphorbia* species have been shown to be potent inhibitors of ABCB1-related efflux activity. However, despite of the already existing work, there is still a long way ahead to be followed for optimizing macrocyclic diterpenes as ABCB1 modulators.

Aiming to obtain a set of homologous bioactive compounds in order to develop further structure-activity relationship studies, an epoxylathyrane diterpene, isolated from *Euphorbia* sp., was submitted to several chemical transformations, including hydrolysis, reduction and acylation reactions. Overall, twenty three novel derivatives were prepared, and fully characterized using spectroscopic methods. Their anti-MDR potential was evaluated by flow cytometry in MDR human colon adenocarcinoma (Colo 320) cells. Most epoxylathyrane derivatives, in particular those with aromatic moities showed a remarkable upgrade in their MDR-modifying activity enabling the establishment of structure activity relationships. Moreover, ATPase inhibition by three representative MDR-modifying compounds was investigated and revealed that they all act as ABCB1 slowly transported substrates.

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PM-124

In silico pharmacological profiling of *Ganoderma* secondary metabolites to unravel the polypharmacological nature of Reishi

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The species complex around the so-called mushroom of immortality (Reishi), *Ganoderma lucidum* Karst. (Ganodermataceae), is known for a plethora of bioactive secondary metabolites, mainly lanostane-type triterpenes. This remarkable amount of structural information in combination with limited compound availability makes a rationalization of the individual *Ganoderma* constituents to biological actions on a molecular level extremely challenging. Taking these issues into account, a database was generated containing 279 chemical structures of so far known *Ganoderma* constituents. This was followed by subjecting this 3D multi-conformational molecular dataset to *in silico* parallel screening against an in-house collection of validated structure- and ligand-based pharmacophore models [1]. Influenced by traditional application fields of *Ganoderma* sp. the selection focused on representative druggable targets in the field of viral infections (5) and diseases related to the metabolic syndrome (22). In sum, 89 and 197 *Ganoderma* compounds were predicted as ligands of at least one of the selected pharmacological targets in the antiviral and the metabolic syndrome screening, respectively. Among them only a minority of individual compounds (around 10%) has ever been investigated on the hit targets or for the associated biological activity. By disclosing putative ligand-target interactions this study guided us towards assaying for inhibition of the influenza virus and human rhinovirus (e.g. see Fig.), and further serves as a basis to access yet undiscovered biological actions of *Ganoderma* secondary metabolites on a molecular level.

Acknowledgement: Supported by the Austrian Science Fund (FWF: P24587) & the European Social Fund (ESF & TMWAT Project 2011 FGR 0137).

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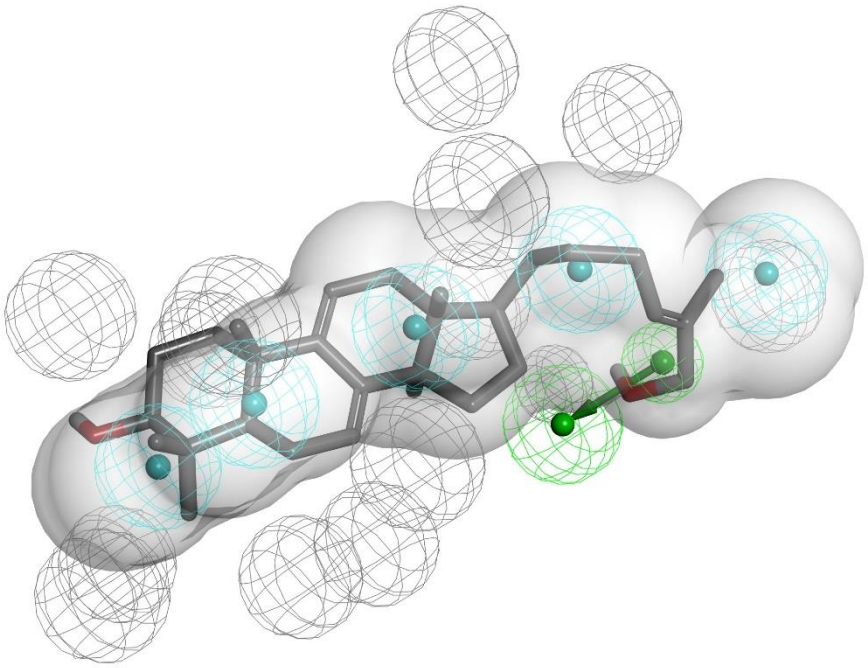

PM-125

Isolation, structures, and structure-anti-inflammatory activity relationships of cembranoids from two cultured soft corals *Sinularia sandensis* and *Sinularia flexibilis*

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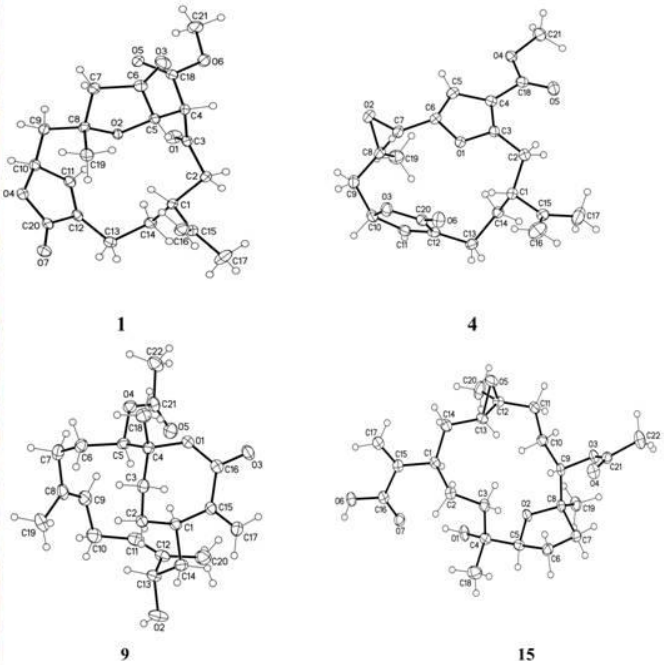
Five new cembranoids, sandenlides A (**1**) and B (**2**), 3-epi-diepoxycembrene A (**6**), and flexibinoids A (**8**) and B (**9**), along with eleven known related metabolites **3**–**5**, **7** and **10**–**16** have been isolated from the two cultured soft corals *Sinularia sandensis* and *Sinularia flexibilis*. The structures were elucidated by means of IR, MS, and NMR techniques, and the absolute configurations of **1**, **4**, **9** and **15** were further confirmed in conjunction with a single-crystal X-ray diffraction analysis. In the *in vitro* anti-inflammatory effects test, compounds **9**–**14** were found to significantly inhibit the accumulation of the pro-inflammatory iNOS and COX-2 proteins of the LPS-stimulated RAW264.7 macrophage cells. Structure-activity relationships analysis indicated that cembrane-type with one seven-membered lactone moiety at C-1 are potential anti-inflammatory agents. Furthermore, to the best of our knowledge, this is the first farming system for *Sinularia sandensis* in the world.



PM-126

Cytotoxic effect of wild carrot oil fractions on human epidermal keratinocytes

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Wild carrot has been used in traditional medicine in Lebanon to treat several diseases. Recently, *Daucus carota* oil extract and its fractions, F1 (pentane; 100%), F2 (pentane-diethyl ether; 50:50), F3 (diethyl ether; 100%) and F4 (chloroform-methanol; 93:7), were shown to possess anticancer and antioxidant activities.1,2 The present study aims to complete spectrum of the anticancer activity of the 4 fractions against three types of human epidermal keratinocytes (HaCaT cells) cell lines HaCaT-II4 (non-invasive), HaCatT-A5 (invasive) and HaCaT (immortalized). Cells were treated with 10, 25, 50 and 100 µg/ml of the fractions for 48 h and cell survival was determined using WST-1 assay. The 4 fractions exhibited a dose-dependent inhibition of cell viability, with F1 and F2 being more potent ($p<0.05$) than F3 and F4. The immortalized HaCat cell line was more resistant to cytotoxicity compared with the two malignant counterparts ($p<0.05$). The IC_{50} values of F1, F2, F3, and F4 fractions in the immortalized HaCat cells were respectively 34, 37, 50 and 45 µg/ml, which were significantly ($p<0.05$) higher than that of HaCat-A5 cells with IC_{50} values of 26, 32, 38 and 36 µg/ml

respectively and of HaCat-II4 cells with IC_{50} values of 26, 24, 39 and 34 µg/ml. In conclusion, the results show that F1 and F2 are more toxic to invasive and non-invasive HaCaT than the immortalized cells. Further studies are needed to isolate and characterize the biologically active compounds in F1 and F2.

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PM-127

Mechanism of cardanol isolated from Thai propolis on apoptosis of BT474 breast cancer cells

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Cardanol was isolated from *Apis mellifera* propolis in Nan province, Thailand as previously described. Due to MTT assay, the IC_{50} against BT474 was 15.57±1.73 µg/ml. The chemical structure of cardanol was analysed by mass spectroscopy. Cardanol inhibited BT-474 cells with time- and dose-dependent manner. Morphology change of cardanol treated BT-474 cells such as cell shrinkage, cell detachment from substratum which, later, caused the cell death could be observed. By annexin V and propidium iodide (PI) staining, BT-474 cells were dead via late apoptosis. At 72 h of treatment, the death of cardanol treated cells was by both late apoptosis (27.20 ± 1.13%) and necrosis (25.40±1.41%) while the death of cells treated with doxorubicin, a chemotherapeutic drug, was different with the lower percentage in late apoptosis (4.30±0.42%) but the higher percentage in necrosis (35.80±13.01%). By PI staining, cell cycle was arrested at G1 subphase. Moreover, change in expression of genes involving in cell division was observed. The obtained data from qRT-PCR and western blot was coincided in term that cardanol increased the expression level of ERK, JNK, and p38. Also, it up-regulated the p21 expression but decreased the expression of cyclin D1 and CDK4 at G1 subphase. Also, it decreased the expression of cyclin E and CDK2 which were important for the cell cycle to continue to the S subphase. Hence, within cells, no DNA replication and cell division occurred. That led to the death of BT-474 cells.

PM-128

LC-MS-based phytochemical characterization of an antiproliferative *Daphne altaica* stem bark extract

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Daphne altaica Pall. (Thymelaeaceae) is a deciduous shrub native to central Asia. The plant has long been used in traditional Kazakh Medicine to treat different types of cancer and ailments like rheumatism, common cold and sore throat. In a previous study, *D. altaica* stem bark extracts have been shown to possess antiproliferative activity in human esophageal squamous cell carcinoma, gastric carcinoma, hepatoma and cervical carcinoma cells [1]. In the current investigation, *D. altaica* stem bark was submitted to sequential extraction with *n*-hexane, dichloromethane (CH_2Cl_2) and methanol, and the extracts were tested for antiproliferative activity in human CCRF-CEM leukemia and MDA-MB-231 breast cancer cells. The most active CH_2Cl_2 extract was analyzed by LC-DAD-MS^n and by LC-DAD-HRESIMS in positive mode. This allowed the unambiguous identification of the four diterpene orthoesters daphnetoxin,1,2-dihydrodaphnetoxin, gnidicin and excoecariatoxin, of the bis-coumarin daphnoretin (Fig. 1), and the tentative identification of 9 further diterpene orthoesters. All compounds were identified in *D. altaica* for the first time.Daphnane-type diterpene orthoesters are characteristic of the Thymelaceae family. For daphnetoxin, gnidicin, excoecariatoxin and some of the tentatively identified diterpene orthoesters, anticancer activity has been described [2, 3]. We found that 1,2-dihydrodaphnetoxin was moderately active in CCRF-CEM and MDA-MB-231 cells. Also, daphnoretin is known to possess anticancer effects *in vitro* [4, 5]. Therefore, it can be assumed that the identified constituents are of high relevance for the antiproliferative activity of the *D. altaica* CH_2Cl_2extract.

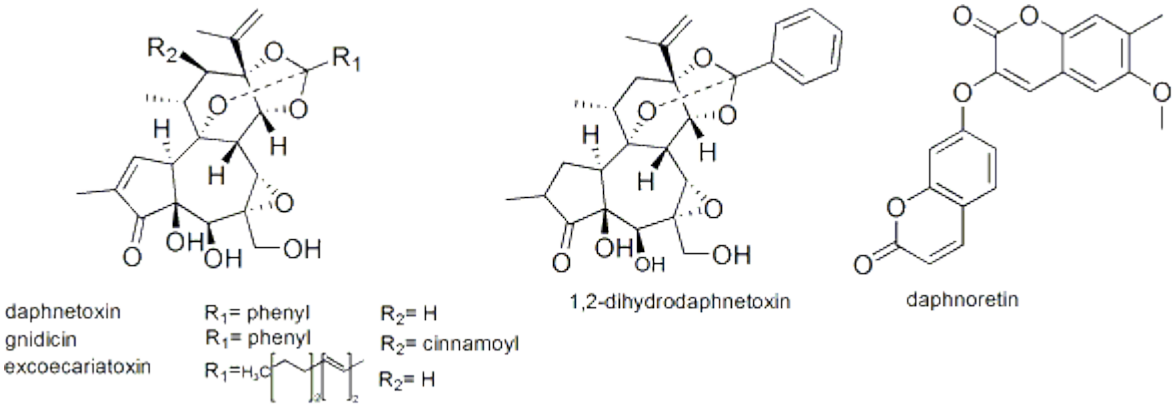

Fig. 1: Constituents unambiguously identified from D. altaica CH_2Cl_2 extract

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PM-129

The effect of diterpene alkaloids on the GIRK channels

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The GIRK *(G protein-activated inwardly rectifying potassium channel)* channel is a potential target in the treatment of atrial fibrillation. These channels are selectively expressed in the atrium and they are not present in the ventricle thus the electrical remodelling of atrial heart muscle might be achieved by their inhibition. Diterpene alkaloids isolated from *Aconitum* and*Consolida* species exert activity on Na^+ and K^+ channels and most of them are highly toxic. Depending on skeletal type and substitution pattern the binding affinity thereby the physiological activity may be diverse. Compounds with selective inhibitory activity on GIRK channels might be useful in the treatment of chronic atrial fibrillation. The aim of this work was the investigation of diterpene alkaloids on the GIRK channel.

Diterpene alkaloids were isolated from Ranunculaceae species. From *A. anthora* compounds 10-hydrox-8-*O*-methyltalatizamine (**1**), hetisinone (**2**), isothalatisidine (**3**), from *A. moldavicum* lycoctonine (**4**), gigactonine (**5**), ajacine (**6**), swatinine (**7**), from *A. vulparia* finetiadine (**8**), acovulparine (**9**), septentriodine (**10**), delcosine (**11**) and from *C. orientalis*takaosamine (**12**) were isolated. The electrophysiological effects of involved bisnor- (**12**), nor- (**1**, **3**-**11**) and diterpene alkaloids (**2**) were investigated on stable transfected HEK-

GIRK1/4 (Kir3.1 and Kir3.4) cell lines using automated patch clamp equipment (Patchliner, Nanion, 1 μM and 10 μM).

Compounds exerted low (-5-27%) and moderate (30-45%) inhibition. Norditerpene alkaloids with aromatic moiety exerted higher activity. The highest activity was exerted by delcosine (**11**) (45±1%) at 10 μM. These results may contribute to the understanding of the structure-activity relation of diterpene alkaloids on the GIRK channel.

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PM-130

Two new steroidal glycoalkaloids from *Solanum pseudoquina* berries

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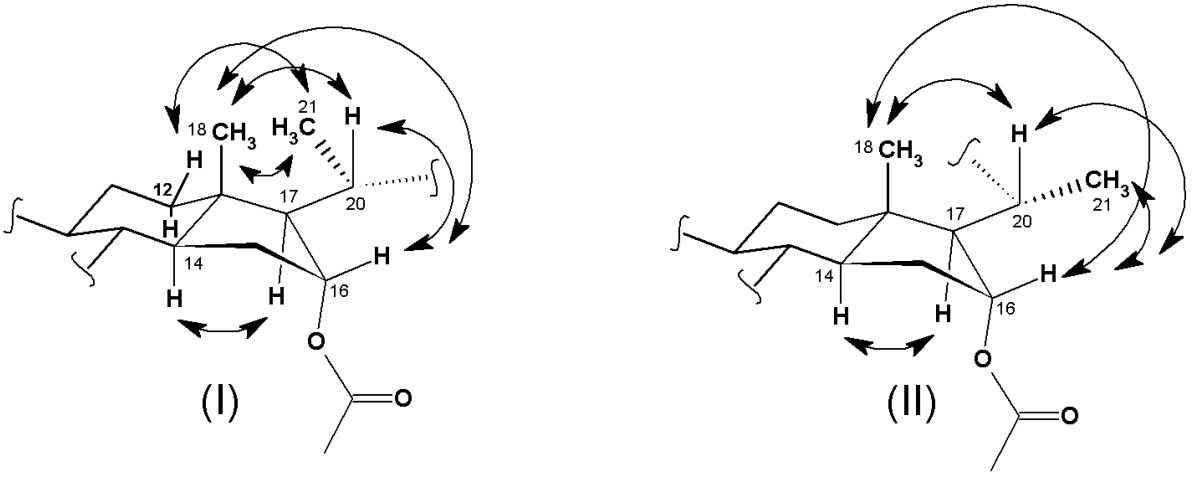
Solanum pseudoquina St.- Hil. is an endemic plant from Brazil with traditional use as tonic and febrifuge in the Brazilian southern region. Previous studies on the convulsive action of the isolated steroidal alkaloid (25S)-isosolafloridine were reported. The present report communicate the isolation of two new steroidal glycoalkaloid (SGA) isomers from the green berries of *S. pseudoquina* St.- Hil. The isolated SGA are: (**1**) 3-*O*-(b-D-glucopyranosyl) (20S,25ξ)-23,26 – epimino-16a-acetyl-3b-hydroxycholesta-5,23(*N*) - dien- 22- one and its 20R isomer (**2**). Green berries of *S. pseudoquina* St. Hil. were homogeneized with 5% aqueous acetic in a laboratory blender and then sonicated for 30 min. in an ultrasonic bath. The homogenate was successively passed through Celite and then XAD-2. The resin bed was washed with water and the alkaloid mixture was eluted with methanol. The methanol fraction from XAD-2 was subjected to several medium pressure liquid chromatography procedures to get enriched SGA fractions. These fractions were subjected to semi-preparative HPLC leading to the isolation of the two new SGA from *S. pseudoquina* St.-Hil. The compounds structures were elucidated by 1D and 2D NMR experiments, ESI/HRMS, APCI/MS and by comparison with literature data. MS data of positive ESI/HRMS for compound (1) and (2) showed the same formulae: $C_{35}H_{54}NO_9$ (calc. m/z 632.3793 $[M+H]^+$) and errors of 0.5 ppm for (1) and -0.8 ppm for (2). Relative configurations of the isomers were confirmed by 2D ROESY NMR as follows: H-16 showed correlations with CH3-18 , H-20, bH-15 and bH-12 in (**1**) and, in (**2**), H-16 ROE correlations were observed with CH3-21, bH-15 , CH3-18 and aH-17.

Acknowledgements: CNPq, FAPERJ and CAPES.



PM-131

Isolation and structure determination of novel jatrophane diterpenes from *Euphorbia dulcis* and their GIRK-channel-inhibitory activity

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GIRK channels (G protein-activated inwardly-rectifying potassium channels) play an important role in the regulation of action potentials in the heart. These membrane proteines are selectively expressed in the atria. Under physiological circumstances GIRK channels facilitate the hyperpolarization and prolong the refractory phase in the myocytes, thus decrease the heart rate. The malfunctions of GIRK channels are considered to be involved in the development of atrial fibrillation (AF). Selective blockade of GIRK channels may offer an alternative way in the treatment of AF [1].

The methanol extract of aerial parts of *Euphorbia dulcis* L. was subjected to liquid-liquid partition. Multistep chromatographic purification (CC, VLC, PLC, RP-HPLC) yielded five pure compounds. The structure determination was carried out by means of HRMS, together with 1D (^{1}H, JMOD) and 2D NMR-methods (HSQC, HMBC, ^{1}H-^{1}H COSY, NOESY). Electrophysiological effects of diterpenes were investigated on stable transfected HEK-GIRK 1/4 cell lines using automated patch clamp equipment.

As a result, four novel and one known [2] jatrophane diterpenes were obtained from the plant. All of the compounds contain a rare olefin bond between C5-C6 and are acylated with acetic, benzoic and angelic acids. The isolated diterpenes were tested on HEK-GIRK 1/4 cell lines and exerted substantial inhibition. IC_{50} values of the three most active compounds are 1.33±0.16 μM, 1.63±0.19 μM and2.65±0.48 μM, respectively. Moreover the GIRK-inhibitory effect of jatrophane diterpenes is reported here for the first time.

[1] Dobrev D, Friedrich A, Voigt N, Jost N, Wettwer E, Christ T, Knaut M, Ravens U. The G protein-gated potassium current $I_{K,ACh}$ is constitutively active in patients with chronic atrial fibrillation. Circulation 2005; 112:3697–706.

[2] Yamamura S, Shizuri Y, Kosemura S, Ohtsuka J, Tayama T, Ohba S, Ito M, Saito Y, Terada Y. Diterpenes from*Euphorbia helioscopia*. Phytochemistry 1989; 28: 3421–3436.

PM-132

Isolation, characterisation and chemotaxonomic significance of secondary metabolites from *Polygonum persicaria* L.

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One of the largest genera of Polygonaceae is *Polygonum*, comprising about 300 species, primarily grows in northern temperate regions of the world. It is well known to produce a variety of secondary metabolites, such as terpenoids, flavonoids, anthraquinones, coumarins, phenylpropanoids, stilbenoids and tannins. *Polygonum persicara* L. is a morphologically extremely variable perennial plant, naturalized in various parts of the world. Our previous phytochemical studies revealed the presence of a new highly methoxylated flavon and three new flavonols in this species. The present work is a continuation this project with the aim of isolation and identification of secondary metabolites from this plant.

The plant materials were extracted with methanol. The extract was concentrated and then solvent–solvent partition was performed with *n*-hexane, and $CHCl_3$. The $CHCl_3$ extract was subjected to a multiple chromatographic purification (RP-MPLC, VLC, TLC, gel filtration on Sephadex LH-20 and RP-HPLC). Structure determinations were carried out by means of MS and NMR spectroscopy.

The results allowed the identification of flavanones, chalcones, an ionon-glucoside and a carboxystilbene, persilbene. 5-Hydroxy-7,8-dimethoxyflavanone, onysilin, 6-hydroxy-5,7-dimethoxyflavanone, 2’-hydroxy-3’,4’,6’-trimethoxychalcone, pinostrobin-chalcone and (6*R*,9*S*)-3-oxo-α-ionon-*β*-D-glucopyranoside, were obtained for the first time from this species. Pinostrobin and pinostrobin-chalcone, and 5-hydroxy-7,8-dimethoxyflavanone and pashanone are biogenetically related flavanones and chalcones. These compounds are chemotaxonomic markers in genus *Polygonum*, since the literature data suggest that, the synthesis and accumulation of flavone derivatives are not typical of other genera of Polygonaceae.

Acknowledgement: This work was supported by the Hungarian Scientific Research Fund (OTKA K109846) and a János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

PM-133

Anti-inflammatory action of an antimicrobial model peptide that suppresses the TRIF-dependent signaling pathway via inhibition of toll-like receptor 4 endocytosis in lipopolysaccharide-stimulated macrophages

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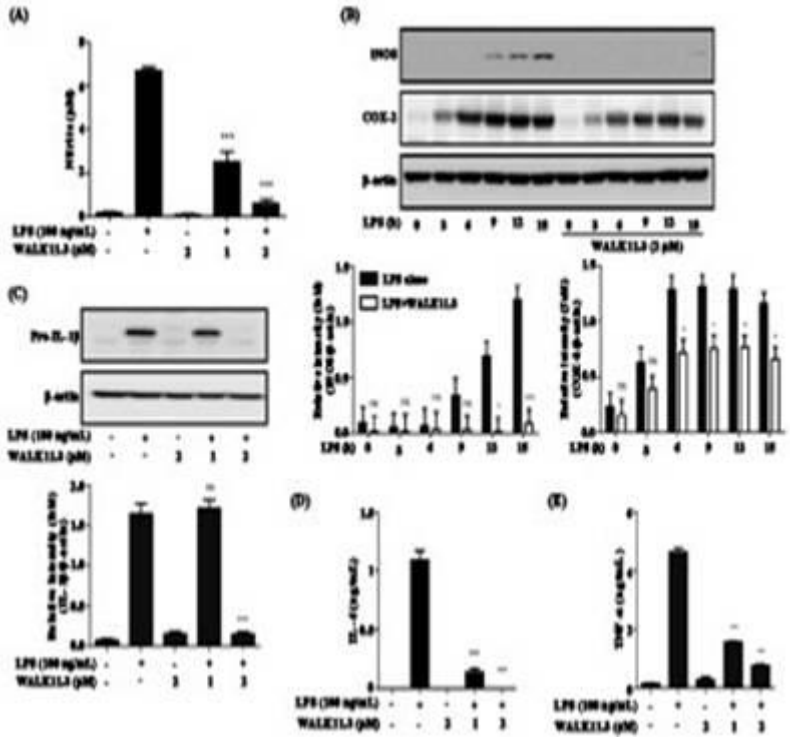
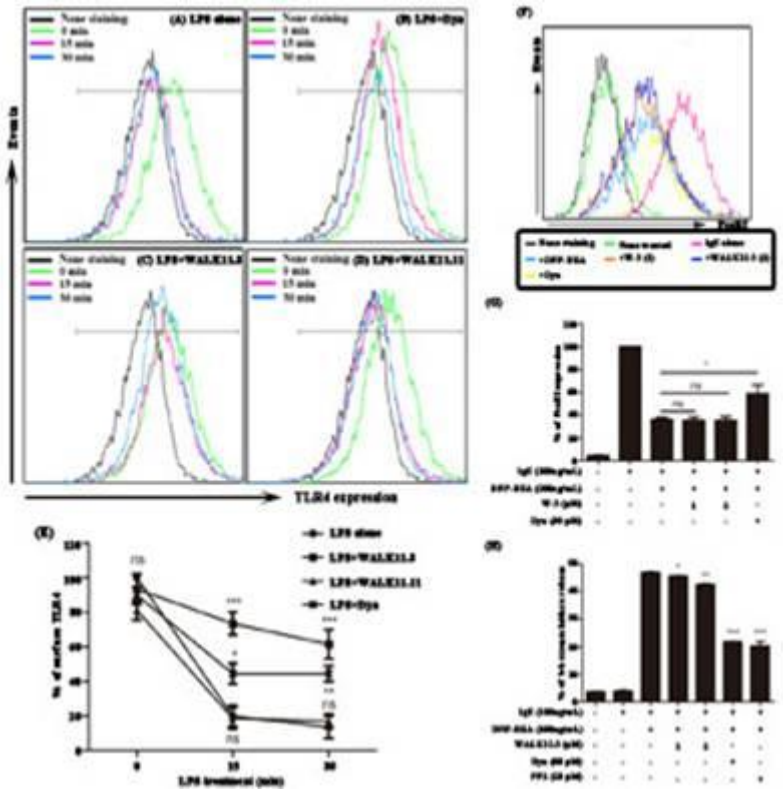
Antimicrobial peptides (AMPs), also called host defense peptides, particularly those with amphipathic helical structures, are emerging as target molecules for therapeutic development due to their immunomodulatory properties. Although the antimicrobial activity of AMPs is known to be exerted primarily by permeation of the bacterial membrane, the mechanism underlying its anti-inflammatory activity remains to be elucidated. We report potent anti-inflammatory activity of WALK11.3, an antimicrobial model peptide with an amphipathic helical conformation, in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. This peptide inhibited the expression of inflammatory mediators, including nitric oxide, COX-2, IL-1b, IL-6, INF-b, and TNF-a. IC_{50} to inhibit inflammatory reaction was 1μg/ml (Fig. 1).

Fig. 1.



Although WALK11.3 did not exert a major effect on all downstream signaling in the MyD88-dependent pathway, toll-like receptor 4 (TLR4)- mediated pro-inflammatory signals were markedly attenuated in the TRIF-dependent pathway due to inhibition of the phosphorylation of STAT1 by attenuation of IRF3 phosphorylation. WALK11.3 specifically inhibited the endocytosis of TLR4, which is essential for triggering TRIF-mediated signaling in macrophage cells (Fig. 2).

Fig. 2.



Hence, we suggest that specific interference with TLR4 endocytosis could be one of the major modes of the anti-inflammatory action of AMPs. Our designed WALK11 peptides, which possess both antimicrobial and anti-inflammatory activities, may be promising molecules for the development of therapies for infectious inflammation.

PM-134

Antiviral activity of the *Echinacea purpurea* extract Echinaforce® against the highly pathogenic avian influenza virus (H7N9)

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The Echinacea purpurea extract Echinaforce® has shown in recent years in vitro its strong inhibitory activity against respiratory viruses. The influenza A virus subtype H7N9 is a novel avian influenza virus first reported to have infected humans in 2013 in China. So far, 486 laboratory confirmed cases for this virus have been reported with 185 deaths [1]. These are the first experiments of a herbal extract to show antiviral activity against the H7N9 virus.

An ethanol extract (65% V/V) from fresh Echinacea purpurea roots (5%) and herba (95%) (Echinaforce®), was tested on its ability to inhibit A/Anhui/1/2013 (H7N9) influenza virus via Focus Assay by using MDCK-II-cells. The test preparation was tested as a newly produced tincture, a concentrated spissum extract and an 8 year old tincture.

The newly produced tincture inhibited the viruses with an IC_{50} of 7.01µg/mL, the spissum extract with a IC_{50} of 27.31 µg/mL, and the 8 year old tincture was still highly active with an IC_{50} of 9.37µg/mL.

The influenza A virus subtype H7N9 is a highly pathogenic virus for which no standard treatments are available either for treatment or for prevention. Here a standardized echinacea preparation showed good effects against the virus. As also an aged preparation showed similar results to a newly produced one, the antivirally active substances are obviously stable. Further research should elucidate the actives by analytically comparing these two preparations and also test their bioavailability.

[1] WHO. Influenza at the human-animal interface. Summary and assessment as of 26 January 2015

PM-135

Cytotoxic, antioxidant, iNOS, AChE, BChE inhibitory and antimicrobial activities of ethanol extract of Cyprus Endemic plant *Salvia veneris*

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Salvia veneris Hedge (Lamiaceae) is an endemic species of Cyprus. This species finds use as an herbal tea in the region by the misidentification instead of *S. officinalis*. There is no information on the biological activity of *S. veneris* in the literature. Here we report the biological activities of ethanol extract of this species. The cytotoxicity of the extract was evaluated against PC3, HeLa, CaCo-2, MCF-7, U87MG, HEK293, mPanc-96 cell lines. Highest activity was observed against PC3, mPanc-96 and HeLa cell lines 6.158, 8.704 and 9.907 µg/mL (IC_{50}) respectively. The extract produced 11.00 µg/mL iNOS inhibitory activity which is higher concentration than the positive control parthenolide 0.6 µg/mL (IC_{50}). Low AChE and BChE enzyme inhibitory activity was observed at 10 mg/mL concentration which produced 26.54±0.58 % and 35.87±0.53 respectively (*n=3*). Additionally the extract produced highest antimicrobial activity against *C. albicans*, *S. epidermis*, and *E. faecium* microorganisms with 15.6, 31.25, 31.25 µg/mL MIC values respectively. Highest DPPH scavenging activity was observed at 10 mg/mL concentration 90.83±0.0 % (n=3) which is lower than positive control (α-tocopherol at 10 mg/mL; 92.38±0.01 % (n=3)). The PRAP activity was 0.92±0.04 AU at 10 mg/mL which corresponds to the activity observed for 2.1 mg/mL *α*-tocopherol (y=0.2251x+0.4354; R^2=0.9932; *n=3*). The FRAP activity of the 10 mg/mL extract corresponds to activity observed for 5.70±0.10 mg/mL *α*-tocopherol concentration (*n=3*;*y=0.1268x+0.0548*; R^2=0.9922). The extract showed high potential for most of the activity tests studied. According to the presented activity results identification of the active compounds is currently under investigation.

PM-136

Secondary metabolites from *Pulsatilla patens* and *Pulsatilla vulgaris* and their biological activity

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[2] *Department of Forestry in Hajnówka, Bialystok University of Technology, Hajnowka, Poland*
[3] *Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, Oxford, United States*
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The aim of this research was the identification of biologically active secondary metabolites isolated from rare plant species*Pulsatilla patens* (L.) Mill. and cultivated *Pulastilla vulgaris* Mill. (Ranunculaceae). Chromatographic fractionation of the ethanolic extract of the roots of *P. patens* resulted in the isolation of two known oleanane-type glycosides identified as hederagenin 3-*O*-β-D-glucopyranoside and hederagenin 3-*O*-β-D-galactopyranosyl-(1→2)-β-D-glucopyranoside [1]. The structures of the isolated compounds were determined by ^{1}H and ^{13}C-NMR spectroscopy. Chromatographic analysis using GC-MS of the silylated methanolic extract of the leaves of *P. vulgaris* revealed the presence of carboxylic acids, such as benzoic, caffeic, malic, and succinic acids [2]. Relative composition (%) of the isolated compounds in total ion current (TIC) was calculated on the basis of peak area in the chromatogram. The extracts of *Pulsatilla* species were evaluated for their antifungal, antimicrobial, antileishmanial and antimalarial activities. Both *P. patens* and *P. vulgaris* were active against the fungus *Candida glabrata* with IC_{50} values of 9.37 μg/mL [1] and 11 μg/mL.

[1] Sharma VK, Łaska G, Radhakrishnan SVS, Jacob MR, Zjawiony JK. Phytochemical investigation and pharmacological evaluation of *Pulsatilla patens* var. *patens*. Planta Med. 2014; 10: 777

[2] Łaska G, Sienkiewicz A, Stocki M. Secondary metabolites from *Pulsatilla* species and their role in pharmacology. Planta Med. 2014; 10: 835

PM-137

Effect of the aqueous leaves extract of *Clerodendron splendens* on physiology of HaCaT keratinocytes

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[3] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

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Clerodendron splendens G. Don (Verbenaceae) has been used for centuries ethnopharmacologically to treat a variety of medicinal problems in Africa, including wounds, blisters and bruises [1].Thou several investigations have been conduct on the biological activities of the extracts, none so far determines the effect of the extract on skin cells.This research aims at investigating the *in vitro* physiological effect of the aqueous leaves extract of *C. splendens* on HaCaT keratinocytes. Keratinocytes are the main cells of the epidermis and any agent used for the treatment of skin conditions will firstly come in contact with these cells. To determine the effect of the aqueous leaves extract on keratinocytes, the functional activities of the extract were investigated on HaCaT cells by determining effect on viability, proliferation and necrotic cytotoxicity by the MTT, BrdU and LDH assays respectively. Positive controls respectively were 5% and 1% FCS and 10 % triton-X 100. For quality control, the HPLC fingerprint chromatogram was also developed. Results from this study showed a significant increase in metabolic activity of HaCaT cells at low concentrations of 0.1 and 1 µg/mL (***$p<0.001$) with cellular viability of 113±5 % and 113±6 % respectively followed by a decrease in viability at 10 and 100 µg/mL (**$p<0.01$). There was a significant increase in proliferation also at 0.1 and 1 µg/mL (***$p<0.001$ and *$p<0.05$) with a stimulation of 108±3 % and 105±7 % respectively. In the LDH assay, the extract showed a possible cytoprotective effect over the concentrations tested by a significant decrease in LDH leakage in comparison to the untreated control (***$p<0.001$). The positive effect on viability and proliferation at 0.1 and 1 µg/mL suggests the absence of toxicity, but a decrease in metabolic activity and proliferation of the cells at higher concentrations could indicate a dose dependent cytotoxic effect.

[1] Koffi K. et al (2013) BMC Complement Altern Med, 13:149.

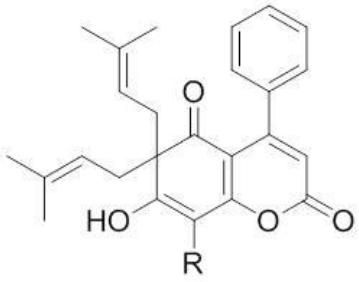
PM-138

Lepidotols and lepidotins: new phenylcoumarins from Malaysian *Mesua* species

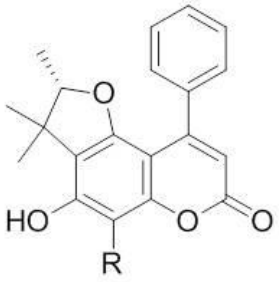
Caroline Rouger[1], Séverine Derbré[1], Thomas Cauchy[2], Marc Litaudon[3], Khalijah Awang[4], Pascal Richomme[1]

[1] *EA921 SONAS/SFR4207 QUASAV, Université d'Angers, Angers, France*
[2] *Laboratoire MOLTECH-Anjou, CNRS UMR6200, Université d'Angers, Angers, France*
[3] *ICSN, CNRS, Gif-sur-Yvette, France*
[4] *University of Malaya, Department of Chemistry, Faculty of Science, Kuala Lumpur, Malaysia*

Clusiaceae and *Calophyllaceae* are pantropical plants well known to biosynthesize polyprenylated polyphenols [1]. Among them, several xanthones, coumarins and benzophenones have shown anti-inflammatory or immunomodulatory properties [2-3]. In order to identify natural products exhibiting potential anti-inflammatory and immunosuppressive activities, a dereplication analysis was conducted through HPLC-PDA-MSn on the DCM and MeOH extracts (bark, leaves and occasionally fruits) obtained from nine *Calophyllum* and three *Mesua* species (Calophyllaceae) native to Malaysia. It appeared that the fruits of *Mesua lepidota* T. Anderson are a rich source of original phenylcoumarins named as lepidotols and lepidotins. A biosynthetic hypothesis accompanied with experimental and calculated specific rotations were used to determine the stereochemistry of these compounds. The lepidotol series was also detected in the bark and in another *Mesua*species.



Lepidotols



Lepidotins

[1] Cechinel Filho V. et al. (2009) Chem Biodivers 6: 313-327.

[2] Anantachoke N. et al. (2012) Pharm Biol 50: 78-91.

[3] Fu Y. et al. (2014) J Agric Food Chem 62: 4127-4134.

PM-139

Antiadhesive effect of leaves extracts from *Orthosiphon stamineus* extract against uropathogenic *E. coli*

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Uropathogenic *Escherichia coli* (UPEC) colonize host cells and tissue of the urogenital tract after specific attachment to cell surface structures by mainly type 1 and P-fimbriae. Specific inhibition of bacterial adhesion is an alternative approach for specific prevention. The

following study investigated the in vitro antiadhesive potential of extracts from leaves of*Orthosiphon stamineus* B. against UPEC to T24 bladder cells.

Dried plant material was extracted with water (OWE, drug-extract ratio 100:20) and acetone (OAE, drug-extract ratio 100:4.5). Both extracts did not influence cell viability of T24 cells (MTT assay) or exhibited cytotoxicity against UPEC (agar diffusion assay). OAE significantly decreased bacterial adhesion at 100 µg/mL (Fig. 1) while OWE increased the adhesion due to bacterial agglomeration as shown by flow cytometry and Giemsa staining. Tannin-like compounds were removed from OWE by treatment of the extract with polyvinylpyrrolidone and skin powder, resulting in a tannin-free extract OWE^{øTannin} which did not cause any more bacterial aggregation, but did also not influence the bacterial adhesion significantly.

Interestingly preincubation of bacteria with pooled urine and OWE led to 40% reduction of bacterial adhesion. This means that OWE in combination with human urine is capable to exert significant antiadhesive effects, a phenomenon which cannot be explained at the moment.

Beside antiadhesive effects of the extracts the influence of OWE and OAE on UPEC-induced biofilm formation was also monitored (crystal violet assay). Additionally the effect of the extracts against UPEC invasion into T24 bladder cells was studied by gentamicin protection assay.

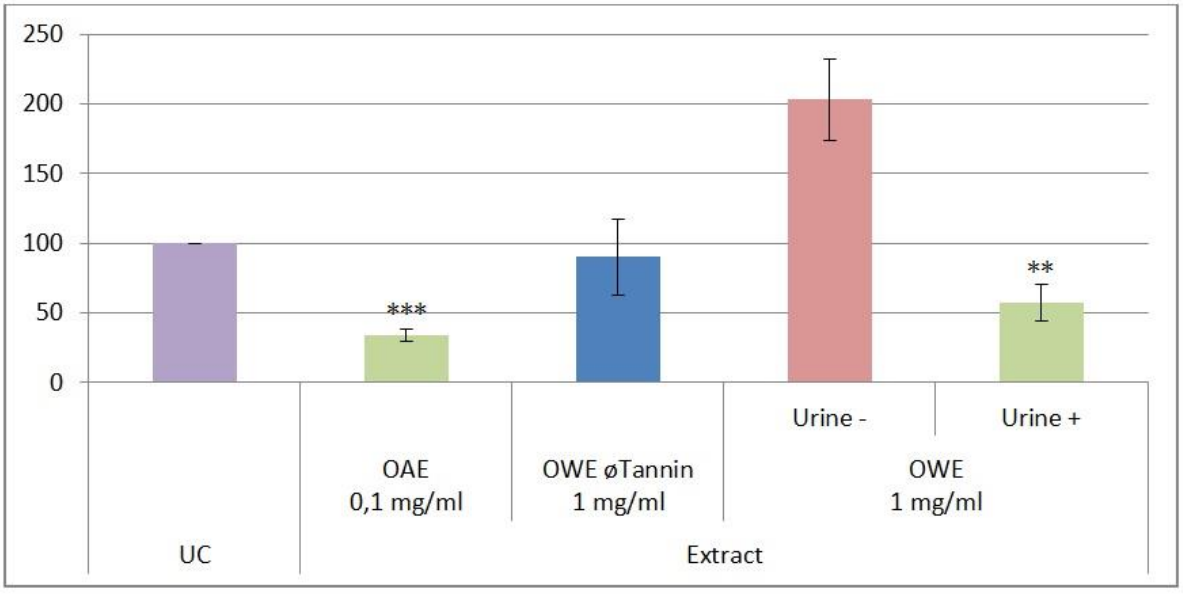

Fig. 1
Influence of OAE, OWE and OWE^{øTannin} on the adhesion of FITC labelled UPEC (strain NU14) to T24 cells (FACS assay) after 2 h of incubation.
UC: untreated control.

PM-140

Constituents of the fruits of *Vaccinium uliginosum* (bog bilberry)

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[2] *Department of Life and Nanopharmaceutical Science, Kyung Hee University, Seoul, Korea, Republic of (South)*

Vaccinium uliginosum L. (also known as bog bilberry) is a low-growing deciduous shrub classified in the Ericaceae family of plants, which includes numerous *Vaccinium* berries, blueberries, and cranberries. *V. uliginosum* have been reported to contain an abundance of flavonoids, anthocyanins, and flavonols. Flavonoids have been extensively studied in many kinds of berries (grape, cranberry, black currant, etc.), and have been shown to have potential health promoting benefits including antioxidant, anti-inflammatory and anticancer properties.

As a part of our ongoing project to search novel secondary metabolites from medicinal plants, we chose the fruits of *V. uliginosum* for detailed phytochemical study. Repeated chromatography of freeze-dried extract of berries led to isolation of eleven compounds, comprising an anthocyanin, six flavonoids, two phenyl propanoids, and two iridoids. The isolates were identified as cyanidin-3-*O*-*β*-D-glucopyranoside (**1**), quercetin (**2**), hyperoside (quercetin-3-*O*-*β*-D-galactopyranoside) (**3**), quercetin-3-*O*-*α*-L-arabinopyranoside (**4**), myricetin (**5**), myricetin-3-*O*-*β*-D-galactopyranoside (**6**), syringetin-3-*O*-*β*-D-galactopyranoside (**7**), methylchlorogenate (**8**), chlorogenic acid (**9**), loganic acid (**10**), and 6,7-dihydromonotropein methyl ester (splendoside) (**11**) by physical (mp, $[\alpha]_D$) and spectroscopic data (^{1}H NMR, ^{13}C NMR, 2D NMR, and MS) measurement and by comparison with published values. Although loganic acid (**10**) and 6,7-dihydromonotropein methyl ester (splendoside) (**11**) were isolated from other *Vaccinium* species, they have not been reported from *V. uliginosum* to date.

PM-141

Euphrasia spp. as a natural stabilizer of hyaluronic acid - A step closer to physiological human tear fluids?

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Natural tears contain water, sodium chloride and various other components like proteins, lipids, mucins, antioxidants and buffer substances. Tear substitutes which are used for therapy of the dry eye syndrome mostly lack natural tear components and ingredients with antioxidative potential. Thus, the aim of our study was to identify substances with protective function against ultraviolet (UV) light and ozone, which could be used in artificial tear substitutes. Viscosity measurements were perfomed using a 0.25 % hyaluronic acid solution with a viscosity of 2.12 centi Stokes (cSt) [range 2.09–2.15], as well as extracts and substances with possible antioxidative effects after irradiation with ultraviolet light and ozone. UV light and ozone lead to a depolymerisation of hyaluronic acid associated with an decrease of viscosity to 1.76 cSt [range 1.56–1.87] and 1.46 cSt [range 1.41–1.63], respectively. Eleven substances with possible hyaluronic acid stabilizing effects were investigated. Eyebright

(Euphrasia spp.) and mannitol could significantly protect hyaluronic acid from a degradation ($p<0.01$). The viscosity was 2.05 cSt [range 2.04–2.05] and 2.07 cSt [range 2.05–2.08] through influence of UV light and ozone, respectively, with the protective components. Uric acid (1.76 cSt) and melatonine (1.75 cSt) were able to achieve a significant stabilizing effect of hyaluronic acid against ozone ($p<0.01$), but not against UV light. Arginine, curcumin, fructose, urea, lysine, spermidine and taurine did not stabilize hyaluronic acid after UV and ozone stress.

For maintaining the antioxidative function of tears euphrasia spp. extracts and mannitol could be added to artificial tears. Both substances are capable of stabilizing hyaluronic acid in tears as well as in artificial tears by preventing UV light and ozone induced degradation of hyaluronic acid. This stabilizing effect extends the retention time and lubricating effect on the ocular surface.

PM-142

Inscecticidal plant extracts from the Greek biodiversity: Biological activity and phytochemical characterization.

Aikaterini Termentzi[1], Vivek Ahluwalia[2], Nikolaos Tsafantakis[2], Panagiotis Milonas[3], Antonios Michaelakis[3], Dimitrios Papachristos[3], Helen Karasali[1], Nektarios Aligiannis[2], Nikolas Fokialakis[2], Alexios-Leandros Skaltsounis[2]

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Plant-derived extracts and compounds have been suggested as potential alternative insecticides. The large biodiversity, followed by incredible chemodiversity, even for species of the same family, creates a pool of bioactive ingredients, very little explored by now. Opposite to the synthetic ones, biopesticides are generally considered safer, posing fewer risks to the environment, with minimal impacts on animal and human health. Furthemore, natural compounds often act at multiple target sites, reducing the potential for resistance.

In the present study, 25 extracts from plants of the Greek biodiversity, belonging to 15 different families were tested concerning their insecticidal activity against representative species of coleoptera, diptera and lepidoptera. The selection of these plant extracts was based on bibliographic search concerning potential active inscecticidal secondary metabolites: plants with known content of possible bioactive ingredients, as well as relative species were selected for this biological screening. Contact and feeding bioassays on selected insect species were performed in the laboratory at constant temperatures. Both positive and negative controls were included in the experimental design for the bioassays.

Results showed that certain plants from Thymelaceae family (e.g. *Daphne* sp. & *Thymelaea hirsuta*) have an important insecticidal potency. Thus, a detailed phytochemical analysis was performed for selected extracts and their content was characterized by HPLC-HR-MS/MS. Also, specific compounds were isolated and their structure was elucidated by 1&2D NMR experiments. The phytochemical investigation revealed the presence of coumarins, coumarin glycosides, lignans and daphnane diterpenoids. Also flavonoids, bisflavonoids and other

phenolic derivatives were detected. These findings enhance the possibility that specific natural compounds could serve as lead potentials for the development of naturally-derived insecticides.

PM-143

Studies on the chemical constituent and bioactivities of *Pandanus amaryllifolius*

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Pandanus amaryllifolius Roxb. (Pandanaceae) is a tropical shrub that distributed in Southeast Asia, and was introduced to Taiwan as a cultivated plant from Vietnam in 1980s. In Vietnam, Thailand, Indonesia and Malaysia, the plant is popular as a kind of spices and folk medicine for the treatment of hyperglycemia, hypertension and cancer.

In a previous study, the ethanolic extract of *P. amaryllifolius* aerial part showed potential antioxidant, anti-biofilm and anti-inflammatory activities. This extract was separated by an acid-base extraction method to give an alkaloid-containing fraction. Three new compounds designated pandamarine B (**1**) and pandalizines C and D (**2** and **3**) were isolated from the alkaloid-containing fraction. All of the structures were elucidated by spectroscopic analysis, and were classified into two skeletons, pandamarilactones (**1**), indolizinones (**2** and **3**).

In literature reports, the alkaloid-rich fraction from *P. amaryllifolius* showed antioxidant activity. However, all alkaloids in our investigation were inactive in anti-biofilm, anti-inflammatory, anti-platelet aggregation and cytotoxic assays.

PM-144

Study of Chung-Pae as a potential therapeutic agent against chronic obstructive pulmonary disease

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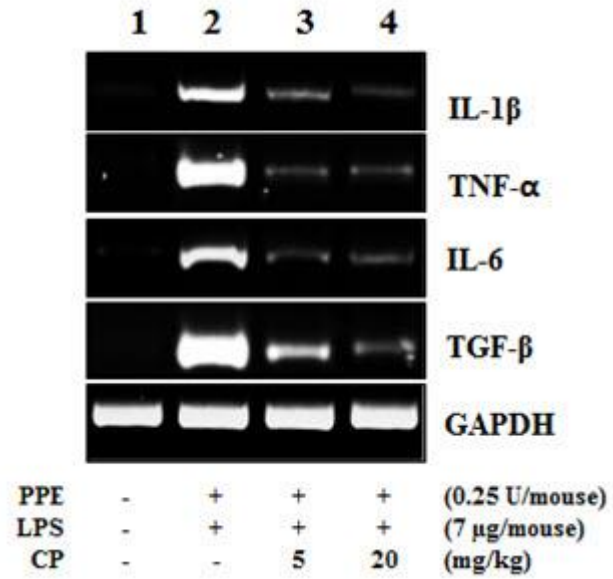
Chung-pae (清肺, CP), an empirical traditional Korean medicine formula, composed of Ephedrae Herba (麻黃) Caryophylli Flos (丁香) Pogostemonis (Agastachis) Herba (藿香) Zingiberis Rhizoma Crudus (生薑), is being prescribed as an inhalant for the treatment of respiratory symptoms, such as dyspnea and cough, at Kyung Hee University Korean Medicine Hospital. The aim of this study is to investigate the safety and efficacy of this formula as a therapeutics against COPD.

As CP is given to patients as an inhalant, mice received CP in aerosol. For the study of therapeutic effects of CP on COPD, a COPD mouse model were established by administering an i.t. spraying of 0.25 Unit of porcine pancreatic elastase on day 1 and 7 μg of lipopolysaccaride (LPS) on day 4 in a week for 3 consecutive weeks. For the test of therapeutic effects of CP, mice received either an i.t. CP (5mg/kg or 20mg/kg) or PBS 2h after each i.t. spraying of LPS. 3 days after the final i.t. LPS administration, mice were euthanized for the analysis of lung. The therapeutic effect of CP was assessed by lung histology and semi-quantitative RT-PCR analysis of COPD-associated proinflammatory cytokines(IL-1β, TNF-α, IL-6, and TGF-β) in lung tissue. Adverse effect of CP was determined by measuring changes of weights of body and internal organs and biomarkers for the integrity of liver and kidney.



In COPD mice, i.t. CP relieved the manifestations of COPD by suppressing inflammation and vacuolization in the lung. CP did not significantly affect the whole body and internal organ weights and biomarkers in blood for liver and kidney. CP delivered in aerosol is safe and effective in remitting COPD in a COPD mouse model. Based on the results, we suggest that CP can be developed as a therapeutics against COPD.

PM-145

An approved herbal medicinal preparation of *Salvia officinalis* as potential source of new agents against*Trypanosoma brucei rhodesiense*

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Sleeping sickness, Chagas disease and Leishmaniases, are infectious diseases caused by unicellular eukaryotic parasites ("protozoans"). They are classified as Neglected Tropical Diseases by World Health Organization. These life-threatening diseases, due to the lack of vaccines as well as safe medicines and in view of the lack of industrial interest, urgently require development of new therapies.

The aim of our ongoing study is to investigate the potential of legally approved and marketed herbal medicinal products (HMPs) as antiprotozoal agents.

Up to now, 66 extracts from 53 HMPs have been assayed by a Multiple-Target-Screening (MTS) against protozoan parasites of the genera *Trypanosoma*, *Leishmania* and *Plasmodium*. 19 HMPs showed in vitro activity against at least one of the pathogens (IC_{50}<10µg/ml). In particular, a preparation of *Salvia officinalis* exhibited promising activity against *T.b. rhodesiense* (East African Sleeping Sickness) with 100% of growth inhibition (GI) at 10µg/ml, IC_{50}=1.86 µg/ml and SI=17. Separation on Sephadex LH-20 afforded fractions that were assayed *in vitro* against the parasite and analyzed by UPLC/ESI-QqTOF-MS to generate the analytical fingerprints. Fractions 18, 19, 20, 22 and 24 exhibited significant trypanocidal activity ranging from 75.6 to 100% of GI at 10 µg/ml. Partial Least Squares regression models (PLS) were constructed to correlate the activity of the fractions and their LC-MS profiles in order to localize those compounds most likely responsible for the biological activity. The obtained PLS models explained about 98% of the variance in the biological data with 2-3 latent variables. Targeted isolation of the most important compounds highlighted by the PLS models is in progress.

Acknowledgments: NLM is grateful for a fellowship from Heinrich-Böll-Stiftung. Financial support by Apothekerstiftung Westfalen-Lippe is gratefully acknowledged. We thank R. Brun and M. Kaiser, Swiss TPH, Basle, for performing the bioassays.

PM-146

New alkaloids from Taiwanese Zoanthid *Zoanthus kuroshio*

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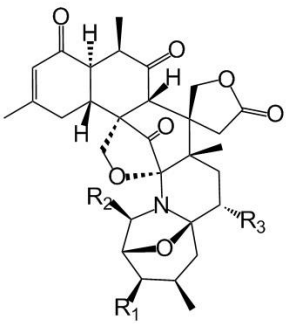
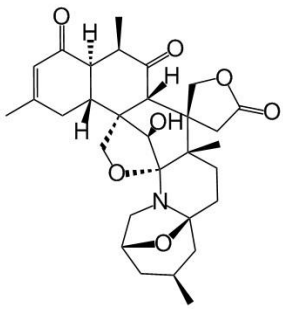
Fractionation of an ethanolic extract from *Zoanthus kuroshio* has resulted in the isolation of four new alkaloids, kuroshines C-F (**1**-**4**), together with three known compounds, kuroshines

A and B (**5** and **6**) and zoanthenamide (**7**). Kuroshine C (**1**) may be categorized as a new oxidative derivative of kuroshine A (**5**) with a ketone group at C-11. All of these marine alkaloids possess a densely functionalized octacyclic ring system on the basis of the zoanthamine framework. The structures of these compounds were elucidated through the interpretation of spectroscopic methods, especially 2D NMR technologies (COSY, HMQC, HMBC, and NOESY). The cytotoxic activities of the isolates against A-549, Hep-G2, and MDA-MB-231 cancer cell lines were evaluated.



1 R_1 = H, R_2 = H, R_3 = H
2 R_1 = OH, R_2 = H, R_3 = H
3 R_1 = H, R_2 = OH, R_3 = H
4 R_1 = H, R_2 = H, R_3 = OH



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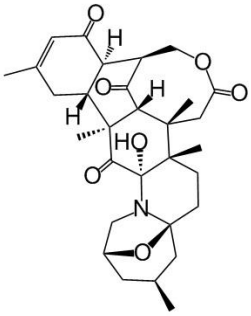

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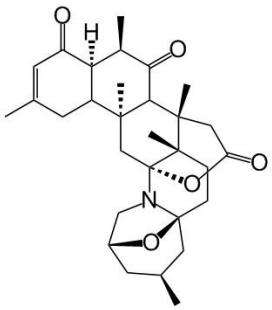

7

PM-147

Chemical constituents from *Xylocarpus rumphii* (Meliaceae)

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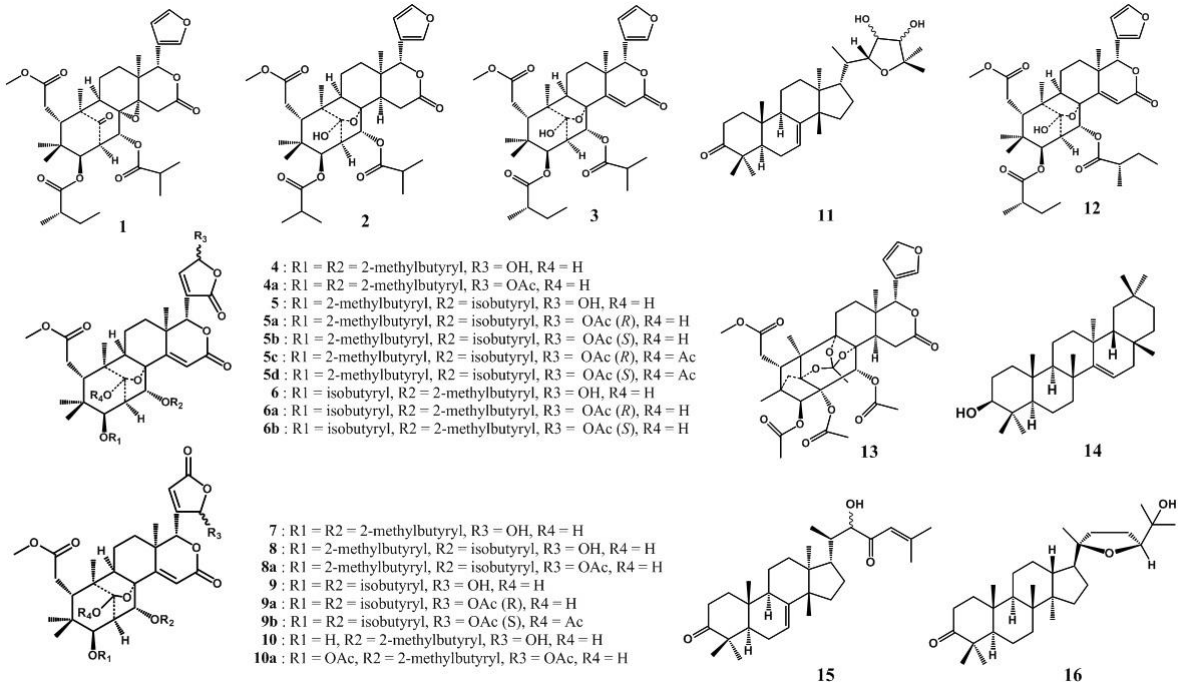
Eleven compounds were isolated from the heartwood of *X. rumphii* and were identified as xylorumphins A and C (**1** and **2**), xylorumphiin C (**3**), xylorumpholides A-G (**4-10**) and odoratone (**11**). Compounds **4-10** have a hemiacetal group which opens and closes in solution making it impossible to purify. Acetylation enabled separation of the α and β forms. Column chromatographic separation of the acetylated fraction of impure **4-10** led to the isolation of one acetylated derivative of **4**(**4a**), four acetylated derivatives of **5** (**5a-d**), two acetylated derivatives of **6** (**6a** and **6b**), one acetylated derivative of **8** (**8a**), two acetylated derivatives of **9** (**9a** and **9b**) and one acetylated derivative of **10** (**10a**). In addition, five compounds were isolated from the bark of the same plant and were identified as xylorumphin B (**12**), xyloccensin E (**13**), taraxer-14-en-3β-ol (**14**), 22Ɛ-hydroxytirucalla-7,24-dien-3,23-dione (**15**) and 3-oxo-(20S,24S)-epoxydammaran-25-ol (**16**). Compounds **1**, **2**, **4-10** and **12** have not been described previously. Chemical constituents from the *Xylocarpus* genus are reported to exhibit several biological activities such as antidiarrhoeal [1], antimalarial[2], anti-inflammatory[3] and insect antifeedant [4], and the aqueous extract of *X. granatum* is reported to express significant antifilarial activity [5]. Eight compounds, **1-6**, **4a** and**13** were tested at one concentration, 1 x 10-5 M, against several leukemia, non-small cell lung, colon, CNS,

melanoma, ovarian, renal, prostate and breast cancer cell lines. The compounds did not meet activity criteria in the one-dose NCI59 cell test for further testing.



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PM-148

Pharmacological activities of Lichens from Russia

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Lichens are supposed to have nearly 600 unique chemicals. Usnic acid, one of them, is reported to possess a broad spectrum of pharmacological activities, related to its antioxidative properties. We characterized and screened different lichen extracts/fractions for their antioxidative, antimicrobial, and cytotoxic activity.

The lichens *Cladonia arbuscula* (L.) Hoffm., *Evernia prunastri* (L.) Ac. and *Usnea barbata* (L.) Wigg. were collected in Mari El Republic of Russia (June 2011). Chloroform ($CHCl_3$)-,

hexane (Hex)-, dichloromethane (DCM) and acetonitrile (ACN) extracts were prepared and analysed by HPLC. As reference (+)-usnic acid was used. The total antioxidant activity was evaluated with phosphomolybdenum method. The total content of phenols was determined by Folin-Ciocalteau method. The antimicrobial activity was estimated by double microdilution method. Toxicity was evaluated (24hrs) in human skin fibroblasts (HSKF) and in U-87 glioblastoma cells by resazurin assay. Phosphorylation of ERK1/2 was measured in U-87 cells by Western blot.

The usnic acid content of the crude extracts (%) of *C. arbuscula*, *E. prunastri* and *U. barbata* were 8.91±1.18, 5.68±0.47, 74.49±8.64 respectively. The tested antimicrobiobal activities (*Staphylococcus aureus, Candida albicans, Escherichia coli, Pseudomonas aeruginosa, Mycobacterium tuberculosis*) and the antioxidative capacity differed for the 3 extracts/fractions and were unrelated to the usnic acid content. In HSKFs the IC_{50} were 29.3 µg/ml, 78.6 µg/ml, 44.2 µg/ml and 15.5 µg/ml for the extracts of *E.prunastri* (DCM, ACN) and *C.arbuscola* (Hex, DCM) respectively. In U87 cells IC_{50} values ranged from 10.8 µg to 98.1 µg/ml. Phosphorylation of ERK1/2 was concentration dependently reduced.

The tested pharmacological activities did not relate to the usnic acid content. The identification of further secondary metabolites with in depth investigations of different modes of action is required for drug development.

Acknowledgement: A. Shcherbakova was supported by the DAAD.

PM-149

The evaluation of wound healing potential of rosmarinic acid isolated from *Arnebia purpurea*

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The genus *Arnebia* (Boraginaceae) is represented by 5 species in Turkey. *Arnebia* species and their secondary metabolites have antimicrobial, anti-inflammatory, cytotoxic and wound healing effects [1]. Rosmarinic acid is known with its significant medicinal usage such as antioxidant, anti-inflammatory, astringent, antimutagen, antibacterial and antiviral activity [2-4]. The present study reports that wound healing activity of rosmarinic acid, the major component isolated from the methanol extract of the aerial parts of *A. purpurea* S. Erik & H. Sumbul, which is an endemic species in Turkey. Excisional wound model was used on Wistar albino female rats. The three basic phases of wound healing (inflammation, proliferation, remodeling) was evaluated [5]. Histopathological evaluation was performed with lymphocyte density, vascular proliferation, edema formation and fibrosis and scoring was made with between groups. We observed that topical rosmarinic acid (120 mg/kg, 0.1%) administered group showed a statistically significant difference compared to the other groups. Further

investigations for explain the mechanisms of the antioxidant properties of rosmarinic acid are needed.

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PM-150

Changes in the anti-inflammatory activity of aurone and chalcone class flavonoids from *Cotinus coggygria*extracts after complexation with cyclodextrins

Diana S. Antal[1], Iulia Pinzaru[1], Dorina Coricovac[1], Codruta Soica[1], Stefan Schwaiger[2], Hermann Stuppner[2], Evelyne Ollivier[3],Cristina A Dehelean[1]

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Aurones and chalcones are less studied flavonoid classes with emerging therapeutical potential. Using the heartwood of European smoke tree (*Cotinus coggygria* Scop. syn *Rhus cotinus* L.) which contains aurones (sulfuretin, S) and chalcones (butein, B) [1], the aim of the present research was to obtain cyclodextrin (CDX) derivatives with enhanced water solubility and improved biological activity. Following steps were performed: 1) A flavonoid-enriched extract was prepared and standardized to its content in S, B, fustin, 2,3-dihydroquercetagetin, and quercetin using an established HPLC method [1]; 2) *C. coggygria* extract, S and B were each complexed through kneading with hydroxypropyl-beta cyclodextrin (HPBCD) and randomely methylated beta-cyclodextrin (RAMEB). A molar ratio of CDX: flavonoid = 2:1 was employed; 3) Formation of CDX complexes was confirmed by DSC, FT-IR and Karl Fisher titration techniques; 4) Anti-inflammatory effects of *C. coggygria* extract, S, B, and their CDX complexes were assessed in the model of the mouse ear edema (SKH1 male mice). Corneometry was used to measure the hydration level of the stratum corneum, this level being inversely correlated with skin irritation. Results showed that the noncomplexed extract, B, and S reduced inflammation with 50%, 33% and 50 %, respectively. They are as well capable to lessen (with 26%, 97% and 30%, respectively) the skin dehydration induced by TPA. Complexation with CDXs enhances the anti-inflammatory effect of B (maximal for HPBCD) and S (maximal for RAMEB), but reduces it in case of the extract. These findings encourage further development of S- and B-CDX derivatives with improved biological effects.

Acknowledgement: This work was supported by grant UEFISCDI, PN II, CT-397/30.06.2014, contract nr. 789/30.06.2014.

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PM-151

Anti-apoptotic effect of *Phyllanthus emblica* extract prevents contrast-induced acute kidney injury in rats

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Contrast-induced acute kidney injury (CI-AKI) occurs after the administration of intravenous iodinated contrast agents. Apoptosis has been proposed as one of the most important mechanisms in the pathogenesis of CI-AKI. The objective of this study was to investigate the anti-apoptosis effect of the extract from *Phyllanthus emblica* L. (PE) in preventing CI-AKI.

Male Sprague Dawley rats were given water (control) or PE extracts (500 mg/kg/day) for 5 days before the induction of CI-AKI. Blood and renal tissues were collected to investigate renal function and pathohistological examination. The expression levels of Bax, and Bcl-2 in kidney were also determined to indicate anti-apoptotic effect using realtime PCR and western blotting.

In the CI-AKI group, an increase in blood urea nitrogen and serum creatinine was demonstrated which correlated with severity of tubular necrosis, peritubular capillary congestion and interstitial edema. In contrast, CI-AKI-induced rats administrated with PE extract significantly improved the renal function and ameliorated the renal injury. Realtime PCR and western blot analysis showed that the expression of Bax was up-regulated in the CI-AKI group, whereas that of Bcl-2 was down-regulated. However, PE treatment increased the Bcl-2 expression.

These findings suggest that pretreatment with PE extract provides the anti-apoptotic activity against CI-AKI in rat model.

PM-152

Synergistic effect of KIOM-CRC#BP10A, an ethanol extract of two medicinal herbs, with cisplatin in A549 lung cancer xenograft model

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Lung cancer is the most frequent malignancy and also one of the leading causes of mortality in both men and women worldwide. Despite current advances in diagnostics and medicines, the incidence of lung cancer is increasing and effective treatment is very challenging.

Using medicinal plants to fight against various human diseases including cancer have been widely practiced all around the world, especially in China, India as well as Korea. It is well recognized that the traditional medicine has long history of human use without or little harmful effect, so it is expected to have little malicious side effect.

In this study, we tested the anticancer activity of KIOM-CRC#BP10A (BP10A), along with its effect on the chemotherapeutic activity of cisplatin in mouse tumor xenograft model using A549 human lung cancer cell line. BP10A is an ethanol extract of 1:1 mixture of two medicinal herbs, *Peucedanum praeruptorum* Dunn and *Descurainia sophia* (L.) Webb ex Prantl.

The oral administration of BP10A attenuated tumor growth in A549 xenograft model compared to vehicle (0.5% carboxymethyl cellulose). Although the antitumor effect of BP10A was lower than that of cisplatin, mice treated with BP10A showed no sign of body weight loss and organotoxicity which were typical adverse effects of cisplatin. Additionally, BP10A enhanced the anticancer efficacy of cisplatin, so only half dose of cisplatin was required for the same anticancer effects in this animal model.

Our data suggest that herbal medicines and phytochemicals such as BP10A can be a good candidate for adjuvant therapy for lung cancer which effectively enhance the efficacy and decrease side effects of conventional chemotherapeutic drugs.

PM-153

Polyphenols from *Cyclopia genistoides* and their xanthine oxidase inhibitory activity

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The genus *Cyclopia* (Fabaceae), comprising four species, *Cyclopia intermedia, genistoides, subternata*, and *sessiliflora* has significant commercial value as the source of the popular caffeine free herbal tea, honeybush. Several studies investigated the chemical composition of *Cyclopia*, most of which are directed to the analysis of their polyphenolic substances [1]. The bioactivity of different species was also recorded, such as antimutagenic and estrogen-like effects, but anti-gout activity (xanthine oxidase inhibition) has not yet been investigated.

Bioactivity-guided isolation was undertaken with the methanolic extracts of the fermented and non-fermented herb of *C. genistoides* in order to investigate its xanthine-oxidase activity, by the means of HPLC, VLC, MPLC, RPC, OCC and preparative TLC. Fourteen compounds - comprising isoflavones, flavanones, flavones, benzophenons and other phenolic compounds - were isolated, from which five are first reported in this species, and two in the genus *Cyclopia*. From the fourteen constituents four acted as inhibitors of the enzyme xanthine oxidase. The most active *in vitro* xanthine-oxidase inhibitors were luteolin and diosmetin (IC_{50}=1.76±0.037 μM, 0.54±0.011 μM). The inhibitory activity of both compounds significantly exceeded that of allopurinol (IC_{50}=7.49 ± 0.29 μM) which was used as a positive control.

Acknowledgement: This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program'. Financial support from the Hungarian Scientific Research Fund (OTKA K109846) is gratefully acknowledged.

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PM-154

Acetylcholinesterase inhibition and antioxidant activity of isolated compounds from *Galenia africana* and*Combretum apiculatum*

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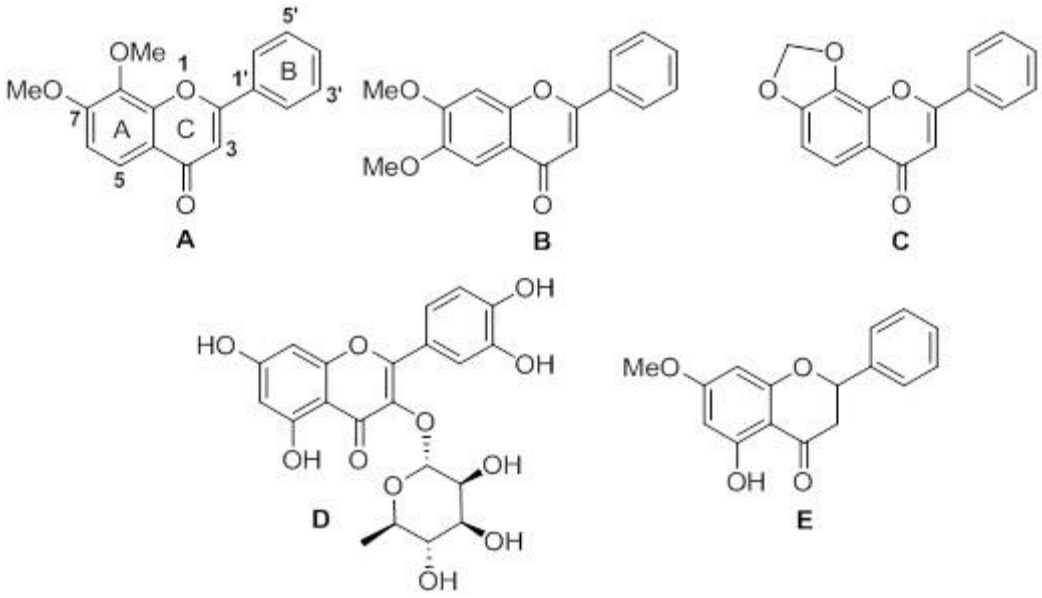
Alzheimer's disease (AD) is a neurodegenerative disorder which is characterized by loss of memory, cognitive decline, and severe behavioural abnormalities and ultimately death [1]. AD is the most common form of dementia in our aging society, which affects more than 37 million people worldwide. Inhibition of acetylcholinesterase (AChE), the key enzyme in the breaking down of acetylcholine thus serves as a promising strategy for the treatment of neurological disorders such as AD.Oxidative stress has been considered a mechanism involved in the pathogenesis of AD, and it has also played a major role in the aging process [2]. Oxidative damage by free radicals has been well investigated within the context of oxidant/antioxidant balance.

Two south African plant species *Galenia africana* and *Combretum apiculatum* were investigated for their potential to inhibit acetylcholinesterase. The DCM leaf extarct of *G. africana* let to the isolation of 7,8-dimethoxy-2-phenyl-4H-chromen-4-one **A**, 6,7-dimethoxy-2-phenyl-4H-chromen-4-one **B**, 8-phenyl-6H-[1,3]dioxolo[4,5-h]chromen-6-one **C** and the MeOH leaf extract of *C. apiculatum* led to the isolation of quercetrin **D** and pinostrobin **E**. The isolated compounds were screened for AChE inhibition and radical scavenging activities using a TLC bioautographic method. Compound **C** showed acetylcholinesterase inhibitory activity and compound **D** and **E** showed radical scavenging activity.



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PM-155

Isolation of two diterpenoids from mangrove plant *Rhizophora mangle* by countercurrent chromatography

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Mangrove plants are potential sources of biologically active compounds what is revealed by their numerous traditional and medicinal uses [1]. Of Brazil's 7408 km coastline, 6786 km contain mangrove forests, covering 25.000 km^2. *Rhizophora mangle* (Rhiphoraceae), known as red mangrove, is a Brazilian native tree and occurs in all Brazilian mangrove areas [2]. The plant is used for the extration of tannins, which make 15-36% of the dry bark [3]. Phytochemical studies on the species reported the isolation of flavonoids, tanins and triterpenes from the leaves [4]. In this work two labdane diterpenes, manool and jhanol (Figure 1), were isolated from the hexane extract of aerial roots by countercurrent chromatography using a biphasic non-aqueous solvent system composed of hexane-acetonitrile-methanol 1:1:0.5 (v/v/v).

The literature reports the presence of kaurane, labdane, pimarane and beyerane diterpenes in Rhizophoraceae family [4], but not manool and jhanol specifically. Labdane diterpenes have several biological activities such as antibacterial, antifungal and antiprotozoal properties [3]. These structures will serve as starting point for semi-synthesis of pharmacologically active compounds.

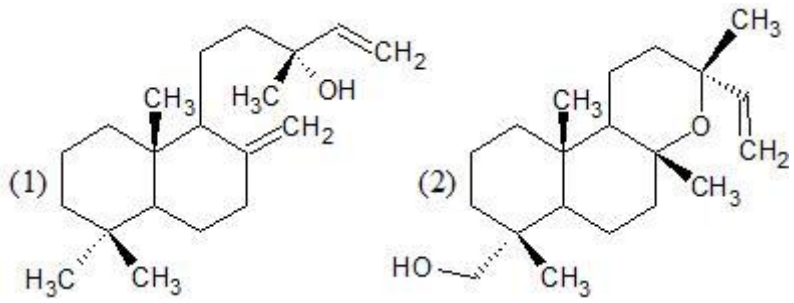

Figure 1. Isolated diterpenes (1) manool and (2) jhanol

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PM-156

A polyphenol enriched fraction of rose oil distillation water inhibits proliferation in HaCaT cells and induces apoptosis.

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Water steam distillation of rose flowers (*Rosa damascena*) separates the essential oil from the polyphenol containing rose oil distillation waste water (RODW). While the essential oil represents the desired liquid for the cosmetic industry, the polyphenol containing RODW is in the center of our interest. Recently, a strategy was developed to separate RODW into a polyphenol depleted water fraction and a polyphenol enriched fraction [RF20-(SP-207)]. Polyphenols are known to have a wide spectrum of biochemical and pharmacological effects. In the present study, we investigated possible anti-proliferative effects of RF20-(SP-207) and fractions thereof F(I)-(IV) in immortalized human keratinocytes (HaCaT). The BrdU cell proliferation assay was used to measure cell proliferation. Cell migration was elucidated by time lapse microscopy. The data demonstrated that from all tested fractions only F(IV) revealed a concentration dependent anti-proliferative effect which is comparable to RF20-(SP-207) (IC_{50} of approx. 10 µg/mL). This effect is similar to both positive controls LY294002 (PI3K-inhibitor, 30% inhibition) and NVP-BEZ235 (dual PI3K/mTOR-inhibitor, 30 % inhibition) and clearly exceeds the anti-proliferative action of quercetin (approx. 20% inhibition). Time lapse microscopy revealed that cell migration was dramatically decreased under influence of RF20-(SP-207) and F(IV). This effect was comparable to LY294002 and NVP-BEZ235. Fluorescence microscopy images confirm the qualitative increase of apoptosis under influence of RF20-(SP-207) and (IV).

PM-157

Discovery of natural products potentially active against myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is a genetically inherited muscle disorder that is characterised by progressive muscle wasting and weakening, cataracts, and cardiac conduction defects. At present there is no cure or effective treatment for this disabling disease. In this context, a collection of 70 pure compounds and 2100 extracts from different plants and fungal strains were screened with a novel DM1-based biochemical assay for their ability to inhibit the formation of the pathogenic complex formed between (CUG)n-RNA and the splicing-factor muscleblind-like 1 (MBNL1). As a result, eight extracts from different plant species were found to be active (≥50% inhibition at 100 µg/ml). Active constituents were tracked using HPLC-based activity profiling, an approach which combines bioactivity data, structural

information from online HPLC-UV-MS and offline microprobe NMR analyses, and database searches. Methylenetanshinquinone and 1,2-dihydrotanshinquinone were found to be the most active compounds in *Salvia miltiorrhiza*. The β-carboline alkaloid harmine was responsible for the activity of *Peganum harmala*, and the iridoid-glycoside auroside was identified as the active constituent in *Lamium album*. The HPLC profiles suggested the presence of tannins in the remaining five active extracts. Retesting of these extracts after tannin removal by filtration over polyamide confirmed the nonspecific interaction of the original extracts with the protein-based screen. In addition, the protoberberine alkaloid berberine was identified as a potent hit from the library of pure compounds. Overall, this study identified several small molecules of natural origin which are promising hit compounds in (CUG)n-MBNL1 complex inhibition. In a secondary cellular assay some of the identified small molecules partially reversed the splicing defects associated with DM1. Detailed secondary *in vitro* and *in vivo* investigations on these compounds are ongoing.

PM-158

Evaluation of antibacterial activity of combinations of *Platostoma africanum* and *Psidium guajava* on multidrog-resistant bacteria

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This study was undertaken to evaluate the possible synergistic antimicrobial activity of combinations of ethanol leaf extracts of *Platostoma africanum* and *Psidium guajava* on multidrug-resistant bacteria. Three (3) strains of extended spectrum beta lactamase (ESBL)-producing *Escherichia coli*, 2 strains of methicillin resistant *Staphylococcus aureus* (MRSA) and one susceptible strain each of *E. coli* and *S. aureus* as control organisms were used in this study. Preliminary antimicrobial screening of the ethanol extracts of the two plants was carried out on the test isolates using the agar well diffusion method. The minimum inhibitory concentrations (MICs) of the plant extracts were determined against the test isolates using the agar dilution method. Synergistic interactions of the plant combinations against the test isolates were evaluated by the checkerboard method and their fractional inhibitory concentrations (FICs) indices were used as indicators of synergistic activities of the plants combinations. In the antimicrobial screening of the ethanol leaf extracts of the plants, *P. guajava*showed good antimicrobial activity against both groups of resistant organisms. *P. africanum* showed lower antimicrobial activity against the Gram-negative organisms compared to the Gram-positive strains. The plant combinations showed more synergistic, than indifferent or antagonistic effects, against the test isolates. The 8:2 combination of the two plants recorded best synergistic activities against all the test isolates (both resistant and susceptible Gram-negative and Gram-positive isolates) with FIC indices ranging from 0.106 to 0.825. This study has shown the combinations of ethanol leaf extracts of*P. africanum* and *P. guajava* to possess synergistic antibacterial activity against multidrug resistant bacteria and thus provide the initial steps for further isolation and identification of antibacterial agents from these plants.

PM-159

Antimicrobial activity of endophytic fungi isolated from *Catharanthus roseus* and *Euphorbia hirta*

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Fungal endophytes of medicinal plants are important sources of secondary metabolites of pharmaceutical importance. The aim of this research was to investigate the endophytic fungi of *Catharanthus roseus* (L.) G. Don (Apocyanaceae) and*Euphorbia hirta* L. (Euphorbiaceae) for antimicrobial activity. A total of 10 fungi endophytes; 6 from C. roseus and 4 from E. hirta were isolated and subjected to solid state fermentation on rice media. The metabolites were extracted using ethyl acetate and the dried extracts tested for antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus subtilis, Aspergillus fumigatus* and *Candida albicans* using the agar well diffusion technique. The minimum inhibitory concentrations (MICs) of the extracts were also determined using the agar dilution method. The bioactive compounds of the extracts were detected using HPLC-DAD. At the concentrations evaluated (1−0.0625 mg/mL), antimicrobial activity was recorded by the extracts with inhibition zone diameters (IZDs) ranging from 0−16.33 mm (antibacterial) and 0−7.30 mm (antifungal). The MICs of the extract against the test organisms ranged from 0.125−0.5 mg/mL. Best antimicrobial activity was recorded by MR1B and MRB.2 with MIC of 0.125 mg/mL recorded against all test organisms. HPLC-DAD analysis of the extracts revealed the presence of some compounds with known antimicrobial property such as citreoisocoumarin, paxilline, nigricinol, fatty acid, sceptrin, cladosporin, desmethyldichlorodiaportin, desmethyldiaportinol and *N*-5(3-(5-Isopropyl-3,6-dioxo-pipperazin-2-yl)-propyl)-2-phenyl- acetamide. It can be concluded that these endophytic fungi could be a promising source of novel bioactive compounds, thus necessitating further studies.

PM-160

Synergistic effects of Helichrysi flosextract in combination with ciprofloxacin against lower respiratory tract pathogens

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Combination of antibiotics with plant extracts is an efficient approach to overcome microbial resistance. The aim of this study was to evaluate the antibacterial activity of Helichrysi flos (*Helichrysum arenarium* (L.) Moench subsp. *arenarium*, Asteraceae) methanolic extract and ciprofloxacin, alone and in combination, against methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae* isolates from patients with lower respiratory tract infections. The effects against standard strains were also assessed. The phenolic content and profile of the extract were studied by Folin-Ciocalteu assay and RP-HPLC-DAD-ESI-MS. The antibacterial activity was evaluated by the agar diffusion, broth microdilution and checkerboard assays [1]. According to the MIC values, the clinical isolates

showed a decreased susceptibility to Helichrysi flos extract and ciprofloxacin than the standard strains. Checkerboard assay enabled the identification of Helichrysi flos extract-ciprofloxacin combinations showing partially synergistic effects (FIC index=0.5-0.75) on both the clinical isolates and standard strains.

Standard strain/ Clinical isolate	MIC*$_E$	MIC*$_{Cip}$	MIC*$_{E\ in\ combination}$	MIC*$_{Cipin\ combination}$	FIC_E	FIC_{Cip}	FIC index
S. aureus ATCC 25923	0.62	$1x10^{-3}$	0.31	$0.25x10^{-3}$	0.5	0.25	0.75
S. aureus CI 1	2.5	$4x10^{-3}$	1.25	$0.5x10^{-3}$	0.5	0.12	0.62
S. aureus CI 2	2.5	$4x10^{-3}$	1.25	$0.5x10^{-3}$	0.5	0.12	0.62
S. pneumoniae ATCC 49619	1.25	$0.25x10^{-3}$	0.31	$0.12x10^{-3}$	0.25	0.48	0.73
S. pneumoniae CI 1	2.5	$2x10^{-3}$	0.62	$0.5x10^{-3}$	0.25	0.25	0.5
S. pneumoniae CI 2	2.5	$2x10^{-3}$	0.62	$0.5x10^{-3}$	0.25	0.25	0.5

* mg/mL, E= *Helichrysi flos* extract, Cip=ciprofloxacin, CI=clinical isolate

This study reveals a potential use of Helichrysi flos extract and ciprofloxacin in the combination therapy of lower respiratory tract infections.

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PM-161

Medicinal plant constituents interact with membrane-acting local anesthetics

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Specific constituents in medicinal plants have been suggested to act on lipid bilayers and modify the physicochemical properties of biomembranes as well as amphiphilic membrane-active drugs. The interactions between selected phytochemicals and local anesthetics were studied at a membrane lipid level to verify the possibility that their concomitant use may influence local anesthesia. Biomimetic lipid bilayer membranes were prepared as unilamellar vesicle suspensions by the injection method using different phospholipids and cholesterol to mimic the lipid composition of neuronal membranes. They were treated at 37 ºC for 30 min

with apple flavonoid phloretin, chili pepper capsaicinoid capsaicin, harmala alkaloid tetrahydroharman and local anesthetic lidocaine separately or in combination, followed by measuring fluorescence polarization to determine their induced membrane fluidity changes. Lidocaine increased the fluidity of biomimetic membranes at clinically-relevant concentrations. In contrast, phloretin (10-50 μM) and tetrahydroharman (10-500 nM) decreased the membrane fluidity, but capsaicin (50-100 μM) increased. Phloretin (25 μM) and tetrahydroharman (~15 nM) inhibited or counteracted the membrane-fluidizing effects of lidocaine (0.05-1 mg/mL), whereas capsaicin (50 μM) potentiated. Such membrane interactions were also found in bupivacaine. Local anesthetics mechanistically act on neuronal membranes besides voltage-gated sodium channels. The antagonistic or synergistic membrane interactions with medicinal plant constituents suggest the potentially beneficial or adverse effects that the counteraction by phloretin would successfully discontinue anesthesia after the treatment and the cooperation with capsaicin should prolong the duration of nerve block, while the inhibition by tetrahydroharman might reduce the anesthetic efficacy in certain patients. The membrane interactivity will be also an experimental clue to discover the drug leads for anesthetic adjuncts.

PM-162

A Brazilian sample of a New *Laurencia* sp. (Ceramiales, Rhodophyta), yields sphingosines new to the marine environment and their antioxidant activity

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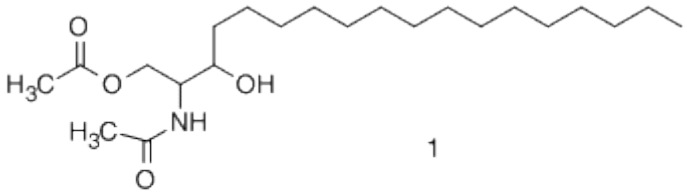
Marine algae belonging to the genus *Laurencia* are the most prolific producers of secondary metabolites in comparison to other marine algae. The secondary metabolites produced by these plants are typically halogenated and have a wide variety of biological properties. The most common structure classes are sesquiterpenes, diterpenes, triterpenes, sterols, C15 acetogenins and long-chain hydrocarbons. Sphingosines, are well known amino-alcohol-lipids constituents of animal nerve tissue [1], that are uncommon constituents of plants, particularly algae. In this presentation we report the isolation from an algal sample belonging to a new species of the genus *Laurencia* collected in southeastern Brazil of two sphingosines (**1** and**2**) new to the marine environment. (2S,3S)-2-acetamido-3-hydroxyoctadecyl acetate (**1**) was recently reported from edible mushrooms and demonstrated inhibition of osteoclast formation without cytotoxicity [2] while *N*-(1-hydroxy-3-oxooctadecan-2-yl)acetamide (**2**) is a new chemical entity. In our antioxidant assays at 50 μg/mL, **1** and **2** quenched 18.5% and 13.0%, respectively, of free radicals in a 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay and exhibited antioxidant activities of 4.92 μM and 4.63 μM $FeSO_4$, respectively, in a Ferric Reducing Antioxidant Power (FRAP) assay, showing them both to be very weak antioxidants. Currently, both of these compounds are being further investigated for other potential biological activities.



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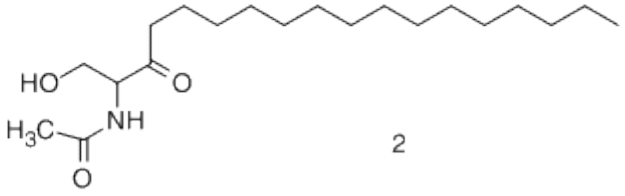

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Acknowledgement: University of Hawaii at Hilo, DKI-College of Pharmacy; Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and Faculdade de Ciências Farmacêuticas da Universidade de São Paulo (FCF-USP) for financial and infrastructure support.

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PM-163

Bioactive polypeptides from marine-derived fungi, *Trichoderma* spp. by LC-MS/MS and molecular networking analysis

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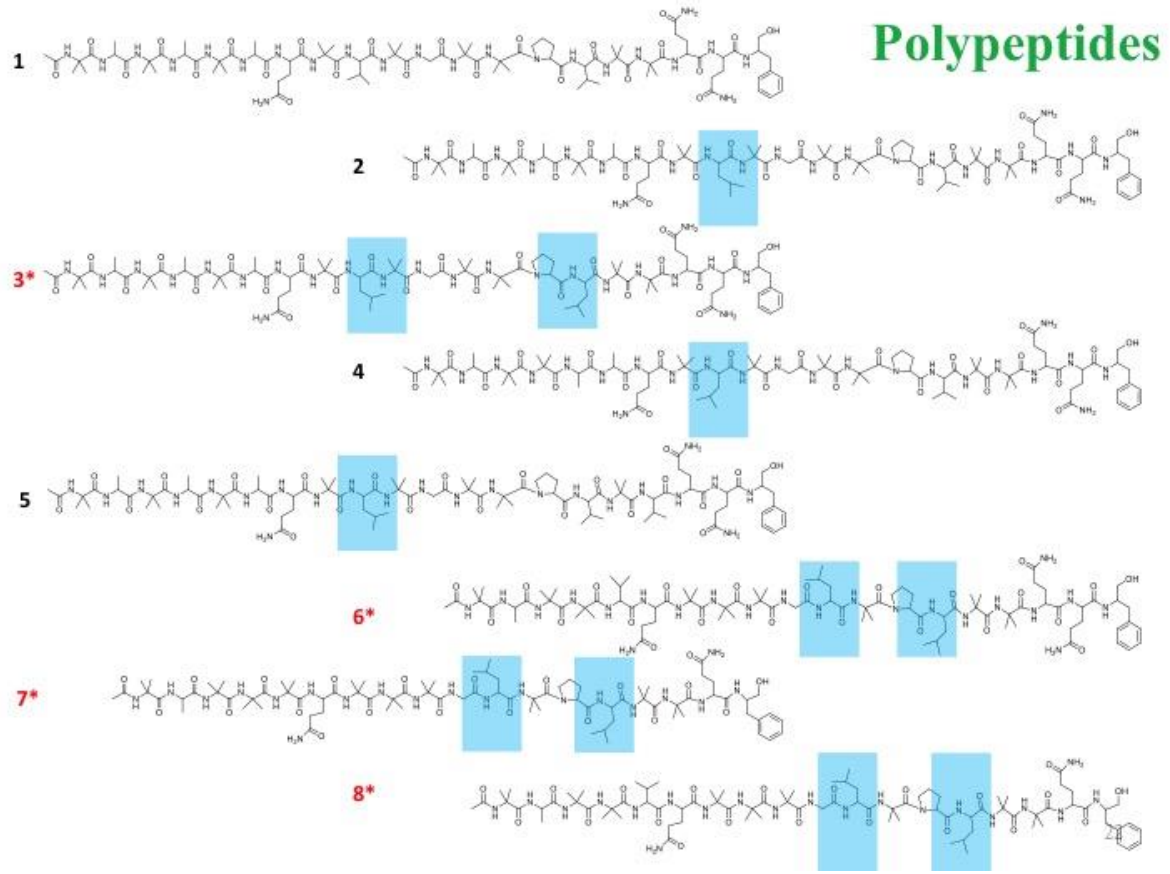
Oceans cover nearly three-quarters of the earth surface. The unique extreme environments in ocean, such as deep-sea volcano, estuaries, intertidal zones, and other special circumstances, harbor the abundant biodiversity of marine organisms, which generate a series of completely different secondary metabolism from those of terrestrial organisms. On the other hand, trace metals in ocean are present at picomolar levels but essential to marine organisms. Marine microbes develop organic ligand systems to seize these trace metals for surviving in the different extreme environments of the oceans. Siderophore (iron-chelating)-like organic ligands are one of the specialized biological mechanisms that help marine microorganisms harvest the trace metal, iron. In preliminary screenings, we found that two marine fungi, *Trichoderma reesi*(MR13-TR1) and *T. atroviride* (MR13-TA1), isolated from a sponge, *Niphates* sp., collected in Wan-Li Tong, Pingtung County, showed clearly iron-chelating effect

by chrome azurol S (CAS) medium and also exhibited clear inhibitory effect against *Acinetobacter baumanii*.

By LC-PDA biofunction-guided fractionation isolation, HPLC purification and LC-MS/MS molecular networking analysis, five non-ribosomal polypeptides (**1**-**5**) were isolated from the ethyl acetate extract of *T. reesi* MR13-TR1, and three non-ribosomal polypeptides (**6**-**8**) from those of *T. atroviride* MR13-TA1. Among them, **3** and **6**-**8** are new compounds. All of these polypeptides in 100 ppm showed clear inhibitory effects against *A. baumanii* by the punching-plate assay. Compounds **5**, **6** and **8** in 10 ppm exhibited good effect to chelate iron. More detailed mechanism of these actions of these organic ligands will be further under the investigation.



PM-164

Flavonoids from *Deguelia duckeana* inhibit the eukaryotic elongation factor 2 (eEF2) in SK-N-SH neuronal cells

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Deguelia duckeana A.M.G. Azevedo (Fabaceae) is known as timbó and used by indigenous people for killing fish. Recently the phytochemical analysis showed flavonoids [1]. Reinvestigation of *D. duckeana* afforded further flavonoids identified by means of MS and ^{1}H and ^{13}C NMR as 3,5,4'-trimethoxy-4-prenylstilbene (1), 4-methoxyderricidine (2), lonchocarpin (3), 4-hydroxylonchocarpin (4), 4-methoxylonchocarpin (5), 5-hydroxy-4',7-dimethoxy-6-prenylflavanone (6), 4'-hydroxyisolonchocarpin (7), 5-hydroxy-4'-methoxyisolonchocarpin (8), 3',4',7-trimethoxyflavone (9), 3',4'-methylenedioxy-7-methoxyflavone (10), 2",2"-dimethypyrano-5,4'-dihydroxy-5'-methoxyflavone (11). Except of 1, 3, and 4 all these flavonoids have been described for the first time in *D. duckeana*.

Chalcones are known for their cytotoxic activity [2], therefore compounds 2, 3, 4, 7, 9, and 10 were studied for their cytotoxicity in the neuronal cell line SK-N-SH using the LDH assay. The chalcone 4 and the flavanone 7 showed significant cytotoxicity and also activation of caspase-3. The other compounds exhibited neglectible cell death. To elucidate whether cell death can be connected to reduced protein synthesis, all these compounds were studied on the eukaryotic elongation factor 2 (eEF2). In mammalian cells, peptide-chain elongation requires eEF2. Its phosphorylation impairs interaction with ribosome and thus the protein synthesis [3]. Interestingly, all studied flavonoids activated eEF2 whether they induce cell death or not. Further studies are needed to elucidate the importance of this effect, because activation of eEF2 can promote cell survival, reduce hypoxic injury and regulate autophagy in response to nutrient deprivation [3].

Acknowledgements: CT-Agro/CNPq, Pro-amazônia/CAPES, Programa Ciência Sem Fronteiras/CNPq, PPBio/CNPq, INCT/CENBAM/CNPq.

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PM-165

Phytochemical and pharmacological studies of *Chelidonium majus* L. (Papaveraceae)

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[2] *Corvinus University of Budapest, Faculty of Food Science, Department of Microbiology and Biotechnology, Budapest, Hungary*

The interest for the *Chelidonium majus* L. species is still high, but in Romania the species is poorly valorized. The plant material was collected from Tirgu Mures, in 2014. Our own collection have been qualified according to pharmacopoeia The preliminary analyses were performed by TLC, foreign matter, loss on drying, alkaloid content expressed in chelidonine. The total content in alkaloids was in normal limits with content recommended by the Pharmacopoea. Analgesic activity of the celandine ethanolic extract was tested using hot-plate test on mice [1]. Basal reaction time has been determined, and two doses of the extract (100 mg/kg and 200 mg/kg body weight) were administrated to two groups. Extracts with Chelidonii herba in a dose of 100 mg/kg in every 60' or 90' showed a lower analgesic effect than that of aspirin (reference) at a dose of 200 mg/kg, but the extracts in dose of 200 mg/kg showed similar effects to aspirin and after 90 ' was greater than aspirin at a dose of 200 mg/kg.

Antibacterial and antifungal activities of Chelidoniii herba extract have been screened against 5 pathogenic bacterial and 3 opportunistic pathogenic *Candida* species using the well diffusion test. *Pseudomonas aeruginosa, Enterococcus faecalis, Listeria monocytogenes* and *Staphylococcus aureus* proved to be sensitive for up to 20-times dilution of the herbal extract, while *Candida glabrata* was only partially inhibited by this dilution rate. Minimal bactericidal and fungicidal concentrations of the extract for the sensitive species were determined in microplate cultures.

The existence of antinociceptive and antimicrobial effects of Chelidonii herba extracts could lay the scientific basis of future clinical trials and open a new clinical perspectives.

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PM-166

Antibacterial activity of 19 species from the Juncaceae family, and bioactivity guided fractionation of the most active species *Juncus inflexus*

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The increasing prevalence of multidrug resistant pathogens encourages the development of new antimicrobial agents. Plants of the Juncaceae family accumulate a high diversity of

phenanthrene type compounds, which have attracted great interest from phytochemical and pharmacological points of view. Previously the cytotoxic, antimicrobial, antiviral and anti-inflammatory activities of the phenanthrenes were reported.

The main object of this work was to investigate the antibacterial activity of 96 extracts (*n*-hexane, CH_2Cl_2, EtOAc and remaining aqueos MeOH) of 19 species from the Juncaceae family against methicillin-resistant *S. aureus*, extended-spectrum β-lactamase (ESBL)-producing *C. freundii, E. coli, E. cloacae, K. pneumoniae,* multiresistant *A. baumannii* and *P. aeruginosa*. Antibacterial susceptibilities were screened for inhibitory zones and MICs determined by the microdilution method.

16 Extracts (CH_2Cl_2) from *Juncus* species and 3 extracts (CH_2Cl_2) from *Luzula* species showed mild to strong (inhibition zones=6.7–14.6 mm) inhibitory activities against MRSA strains. Among them the CH_2Cl_2-soluble fraction of the roots of *J. inflexus* showed the highest activity (MIC=9.75 μg/mL). Therefore this extract was subjected to bioactivity guided fractionation. The extract was chromatographed by CC on polyamide, and then on silica gel vacuum CC, MPLC, preparative TLC and finally by RP-HPLC. Structure determinations were carried out by means of MS and NMR spectroscopy and comparison of the spectral data with literature values. The results allowed to the identification of 5 phenantherenes, substituted with vinyl, hydroxyl, methyl and aldehyde groups. These compounds were evaluated for their antibacterial activity, and juncusol showed significant activity (inhibition zone=12 mm) against MRSA strains.

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PM-167

Identification of plasmodial enoyl-ACP reductase inhibitors of *Acacia nilotica* stem bark and their molecular docking with special reference to DPPH radical scavenging activity

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Plasmodium falciparum is the most serious health threat in Sub-Saharan Africa [1]. Due to the arising resistance, new targets and active compounds are urgently needed. Targeting enoyl-ACP reductase (PfENR), which catalyses the rate limiting step in each elongation cycle in type II fatty acid synthesis pathway, has been validated as an important target [2]. This study aims to discover new antiplasmodial molecules based on PfENR inhibition using in vitro and in silico tools. The study correlates between the antiplasmodial and antioxidant activity of the investigated plant extracts/fractions, to point out molecules which interfere with the redox metabolism of plasmodium.

Selection of the plants studied was based on interviewing traditional healers on the use of locally available antimalarial and/or antipyretic medicinal plants. Accordingly 10 plants were subjected to preliminary screening for their in vitro inhibition of PfENR. Among them five plants were subjected to bioactivity guided fractionation.

The ethyl acetate fraction of *Acacia nilotica* stem bark revealed a significant PfENR inhibition (IC50 0.87 µg/ml) and reasonable diphenylpicrylhydrazyl scavenging activity (12.5 µg/ml). Therefore, it was further analysed by LC/MS/MS which eventually resulted in the identification of four prominent antiplasmodial compounds. The four compounds and some of their diastereomers were subjected to in silico evaluation by docking against PfENR using SYBYL-X1.1 package and they exhibited a binding affinity of -12.00, -10.66, -9.18, -9.18, -4.7, -5.98 and -9.25 Kcal/mol for (-)catechin,(+)catechin, (+)epicatechin,(-)epicatechin, catechin-7-*O*-gallate, chrysoeriol and naringenin chalcone, respectively (Figure1). Remarkable correlations between the antiplasmodial and antioxidant activity were discerned.

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PM-168

The effects of *Panax ginseng* on experimental rat model of benign prostatic hyperplasia

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Benign prostatic hyperplasia (BPH) is one of the most common disease among elderly men. BPH can be treated with 5α-reductase inhibitor (Finasteride) that reduces serum dihydrotestosterone (DHT) in internal mediacal method. In this study, we investigated the therapeutic effects and action mechanism of *Panax ginseng C. A. Mey* with a BPH induced by castration and testosterone treatment. Sprague-Dawley rats were treated with testosterone after castration for induction of experimental BPH, which is similar to human BPH in histopathological profiles. *P. ginseng* as an experimental specimen, and Finasteride as a positive control, were administered orally. The prostates were evaluated by histopathological changes, testosterone levels. Rats administered *P. ginseng* extracts (200mg/kg) for 4 weeks had significantly higher serum testosterone levels (16 ng/dL) than castrated rats (9 ng/dL). The serum acid phosphatase in castrated rats treated with testosterone and administered*P. ginseng* extracts was significantly lower than the BPH model control group (all $P<0.001$). While prostates of control rats revealed severe acinar gland atrophy and stromal proliferation, the rats treated with *P. ginseng* showed a diminished range of the tissue damage. These findings suggest that *P. ginseng* may protect the glandular epithelial cells and also inhibit stromal proliferation in association with the suppression of 5α-reductase. From theses results, we suggest that *P. ginseng*could be a useful remedy agents for treating the BPH.

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PM-169

Inhibition of COX-2 expression in PMA differentiated THP-1 macrophages by extracts of *Epipremnum pinnatum*

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COX-2 is a mediator of inflammation and highly expressed in diseased conditions like rheumatoid arthritis, asthma and several autoimmune diseases. Since many anti-inflammatory agents on the market treating these diseases cause severe side effects, natural sources offer a great pool for discovering novel lead compounds targeting COX-2 [1].

Epipremnum pinnatum (L.) Engl. (Araceae) is traditionally used as an analgesic and anti-inflammatory agent in various areas of Asia [2]. Little is known about the mode of action and the chemical composition of this plant. With a PMA differentiated, LPS stimulated THP-1 inflammation model [3], we evaluated the influence of leaf extracts (hexan, dichlormethan, methanol) on COX-2 gene expression. At the concentration of 20µg/ml, the methanol extract showed potent COX-2 mRNA inhibition (54.3% +/- 9.2). Extracts prepared by two different extraction methods (ASE, Soxhlet) were compared and showed similar inhibition of COX-2 gene expression.

We could demonstrate for the first time that the methanol extract of *Epipremnum pinnatum* exerts strong COX-2 gene expression inhibition in vitro. The observed effect may help us to understand the traditional use of *Epipremnum pinnatum*and possibly lead to identification and isolation of novel COX-2 gene expression inhibitors.

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PM-170

Oleuropein inhibited Th17 response and reduced intestinal IL-17 and IFN-γ release in dextran sulfate sodium (DSS)-induced acute colitis in C57BL/6 mice

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Numerous studies on intestinal inflammation have established a critical role for the recently discovered Th17 cells, since an amount of this subtype of infiltrating leukocytes and their related cytokines are found in inflamed mucosa of colitic mice and in ulcerative colitis patients, as well. Once we demonstrated that oleuropein acts as an anti-inflammatory agent in such pathological condition, the present study attempts to determine if oleuropein exerts any effect on Th17 cells in a DSS-induced colitis model. Acute colitis was induced to C57BL/6 mice through oral administration of 3% DSS (w/v) in water for 7 days. Animals were randomly assigned to four groups: blank, control, oleuropein (100 mg/kg) and dexamethasone (2.5 mg/kg). After mice were sacrificed at day 8 by cervical dislocation, a piece of 0.5 cm of the distal part of the colon was cut and submitted to cytokine determination by ELISA. Mononuclear cells of lamina propria were isolated and stimulated with ionomycin (500 ng/mL) and phorbol myristate acetate (5 ng/mL). After 4 h, cells were collected, incubated with antibodies against CD4, CD3, IL-17A, IFN-γ, and Rorγt, and analysed by FACS.

The percentage of CD4+ Rorγt+ cells and IL-17+IFN-γ+ expressing CD4+ Rorγt+ cells was higher in C group than in B group. Both subpopulations were inhibited in mice treated with oleuropein (25% and 48%, respectively) and with dexamethasone (40% and 30%, respectively). IL-17A and IFN-γ levels in colon samples were reduced by oleuropein (95% and 66% inhibition, respectively) and by dexamethasone (98% and 87% inhibition, respectively) respect to the control group.

In conclusion, oleuropein is able to reduce the Th17 response in DSS-induced acute colitis.

PM-171

Comparison of the phenolic profile of six *Lysimachia* species

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The genus *Lysimachia* comprises about 200 species, wild and cultivated, that are native to throughout temperate and subtropical regions of the Northern Hemisphere.

Lysimachia species have been used in folk medicine and have been the subject of numerous pharmacological studies. Previous phytochemical investigations of plants from this genus showed that the most typical chemical constituents are flavonoids and triterpenoid saponins [1]. However many of these works are incomplete.

The aim of our investigations was to develop a chemical screening method for analysis of six *Lysimachia* species.*Lysimachia vulgaris* L., *L. nummularia* L., and *L. punctata* L., *L. christinae* Hance, *L. ciliat*a L. *var. Firecracker*, and *L. clethroides* Duby. Samples were

analyzed by RP-LC-DAD-ESI-MS/MS method to identify or characterize their phenolic compounds.

The results demonstrate that there is significant variation in the phenolic composition of these six *Lysimachia* species. The qualitative LC-MS/MS analyses resulted in identification of more than 50 various and distinctive phenolic components.

In the extracts caffeic acid derivatives, chlorogenic acid, free flavonol aglycones, as kaempferol and quercetin, and various flavonoid glycosides were identified. In *L. vulgaris*, *L. nummulari*a and *L. punctata* mainly flavonol-O-glycosides (quercetin-, kaempferol- and myricetin – O -mono- di- and triglycosides) were detected. In *L. christinae* beside O-glycosides C-glycosides, as vitexin- and isoorientin- derivatives and various methylated flavonoids were characterized. The results also confirmed that *L. ciliata* and *L. clethroides* native in North-America and Asia, respectively, show higher similarity to *L. vulgaris*, *L. nummularia* and *L. punctata* (native in Europe) than to *L. christinae* (native in China).

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PM-172

Metabolic profiling of the Chinese herbal mixture Huang Qi Jian Zhong Tang and its immunomodulatory effects in U937 cells

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Huang Qi Jian Zhong Tang (HQJZT), a classical TCM formulation, has been used traditionally for chronic gastritis, peptic ulcers, inflammatory bowel disease, autonomic dystonia, chronic hepatitis and chronic nephritis. The formula is a mixture of six herbs consisting of 9 g Astragali radix (Huang Qi), 18 g Paeoniae radix (Shao Yao), 9 g Cinnamomi ramulus (Gui Zhi), 8 g Jujubae fructus (Da Zao), 6 g Glycyrrhizae praeparatae radix (Zhi Gan Cao) and 9 g Zingiberis rhizoma recens (Sheng Jiang). TLC- and HPLC-methods, including LC-DAD-MS/MS, have been developed for the analysis of the metabolic profiles of the single herbs and of the mixture. Decoctions of the single herbs and of the mixture have been fractionated with n-hexane, dichloromethane, ethyl acetate and butanol, and have been analysed by TLC and HPLC in order to trace the herbs in the mixture. As a result, five constituents of Huang Qi, eight constituents of Shao Yao, two constituents of Gui Zhi, twenty constituents of Zhi Gan Cao, six constituents of Sheng Jiang, and no known constituent of Da Zao have been assigned in the chemical profiles of the formula, which now allows a standardization of the mixture. The results of the pharmacological testing showed that the DCM, EtOAc and butanol fractions of the mixture decoction of HQJZT significantly inhibited the expression of TNF-α, IFN-γ, IL-1β and IL-4 in U937 cells. The water fraction of the mixture decoction also inhibited the expression of TNF-α, IFN-γ and IL-1β in U937 cells, but stimulated the expression of IL-4.

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PM-173

Antioxidant and immune-enhancing potentials of leaf extract and active constituents of Millettia aboensis.

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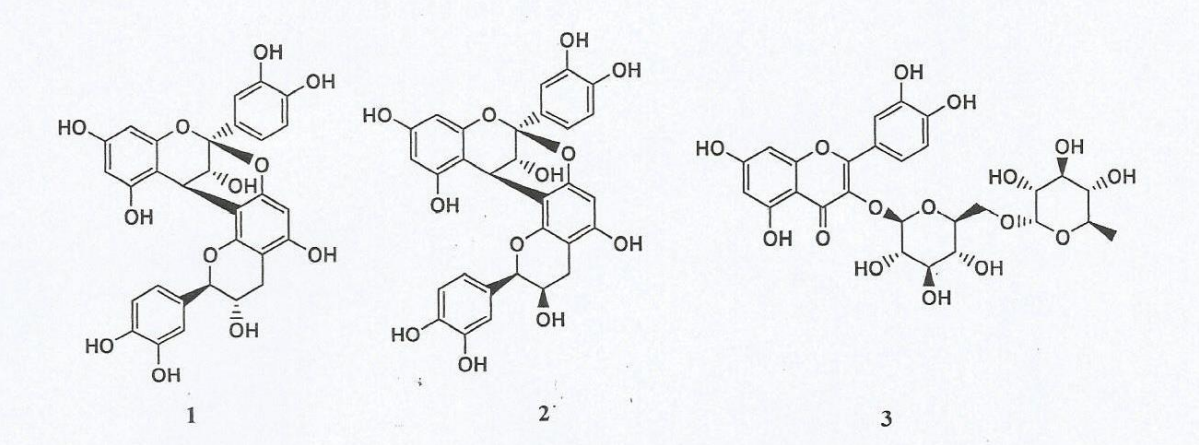
Antioxidant and immune-enhancing potentials of ethanolic leaf extract of *M. aboensis*, fractions and isolated compounds were determined using in vitro and in vivo models. In vitro antioxidant activities were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and hydrogen peroxide scavenging activity tests; while in vivo protection against oxidative damages was assessed by carbon tetrachloride (CCl_4) induced liver damage and streptozotocin (STZ) induced systemic oxidative stress models. In vivo immune enhancing properties were monitored using primary and secondary immune responses to tetanus toxoid. Bioassay guided separations led to the isolation of compounds 1, 2 and 3. Their structures were elucidated by a combination of 1D and 2D NMR and mass spectrometry. In vitro inhibition of liver microsome lipid peroxidation was used to evaluate the antioxidant activity of compounds 1 and 2 while stimulation of specific T-lymphocytes was used for evaluating immune enhancing activity of compound 3. The extract exhibited both antioxidant and immune-enhancing properties however, antioxidant activity was prominent in the ethyl acetate fraction, while butanol fraction expressed more immune-enhancing activity. Structural elucidation revealed compounds as epicathechin-(2β→O→7, 4β→8)-cathechin (procyanidine A1) (**1**), compound epicathechin-(2β→O→7, 4β→8)-epicathechin (procyanidine A2) (**2**) and quercetin-3O-rutinoside (rutin) (**3**). Compounds **1** and **2** demonstrated strong inhibition of liver microsome lipid peroxidation, with an EC50 of 46 and 55 μg/ml respectively. Compound **3** showed up-regulation of specific $CD4^+$ lymphocytes with Up to 38% stimulatory effect of IFNγ at 6.25 μg/ml compared to the baseline effect in DMSO control group. The extract, fractions and isolated compounds from *M. aboensis* expressed strong antioxidant and immune-enhancing properties which may be responsible for its ethnopharmacological use for general healing



PM-174

Active ingredients and anticancer potential in *Eupatorium clematideum*

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Certain species of the genus *Eupatorium* (Asteraceae) have been used as antimalarial, antibacterial, antifungal, anti-inflammatory, hepatoprotective, and immunostimulant agents for decades. *Eupatorium clematideum* (Wall. ex DC.) Sch. Bip. var. *gracillimum* (Hayata) C.-I Peng & S. W. Chung is an endemic plant in Taiwan. However, the pharmacological activites of this species have not been studied so far. The present research focuses on the analysis of active ingredients of *Eupatorium clematideum* and their antiproliferaive properties.

Extract from the whole plant of *Eupatorium clematideum* was purified by chromatographic methods and active ingredients as well as anticancer potential were investigated. Two thymol derivatives, 8-methoxy-9-*O*-angeloylthymol and 8,10-epoxy-9-acetoxy thymol angelate were identified from the EtOAc-soluble fraction. Crude extracts and the two thymol derivatives were treated on four different cancer cell lines, i.e. KB (human nasopharyngeal carcinoma), CAL-27 (human oral tongue cancer), Colon (human colon cancer) and A549 (human lung epidermoid) to realize the anticancer potential. Preliminary results showed that both crude extracts and 8,10-epoxy-9-acetoxy thymol angelate exhibited significant cytotoxicity activity against the four cancer cell lines. Further studies on the secondary metabolites from this plant shall be carried out to reveal the mechanisms of anticancer abilities.

PM-175

Nephroprotective effect of dropwort (*Filipendula hexapetala*) on cisplatin-induced toxicity in rats

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One of the most widely used anticancer drugs is the inorganic complex cisplatin (CP) but with many undesirable side effects and toxicity. *Filipendula hexapetala* Gilib. (Rosaceae) use in traditional medicine is based on the plants´s diuretic, astringent, antirheumatic and anti-inflammatory properties [1]. Our work aimed at investigating the nephroprotective effect of *F. hexapetala* aerial part (FHA) and root (FHR) methanolic extracts on CP-induced toxicity in Wistar rats. The investigated extracts were phytochemically characterized by LC-DAD-MS^n analysis. Rats were treated with three doses of FHA and FHR extracts (100, 200 and 400 mg/kg body weight, respectively), for 10 days. CP-toxicity was induced with a single injection of CP (7.5 mg/kg, *i.p.*) on 5^{th} day of treatment. Negative and positive control (only CP) groups were also evaluated. The results of serum parameters showed that extracts significantly reduced ($p < 0.05$) the levels of ALT, AST, ALP, γGT, uric acid and urea and increased the levels of total proteins, as compared to the positive control. The extracts treatment significantly increased the activities of CAT and SOD in kidney tissue. GSH levels were slightly, but not significantly higher in groups treated with the extracts. Significant reduction in the formation of MDA was observed. Using LC-DAD-MS^n analysis, flavonoid glycosides like spiraeoside and hyperoside and hydrolysable tannins (di- and trigalloyl-hexahydroxydiphenol-glucoses) were identified as major constituents of FHA, and catechin and epicatechin were identified in FHR. This study demonstrates that extracts of*F. hexapetala* are able to markedly attenuate the cisplatin-induced toxicity in kidney and to ameliorate the observed change of serum parameters.

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PM-176

Application of Zebrafish embryos toxicity test to evaluate the alkaloid fraction of *Psychotria deflexa*

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The *Psychotria* L. genus is chemically characterized by the presence of indole alkaloids. Our research group has already demonstrated that these alkaloids are able to inhibit enzymes *in vitro*, such as cholinesterases and monoamine oxidases. The fish embryo acute toxicity (FET) test is an important dose-screening tool: it is a rapid, simple and effective method. This screening established by the OECD 236 (Organization for Economic Co-operation and Development) was used to define the best dose that can be use in the future studies to evaluate the biochemical parameters in the nervous system of adults Zebrafish. In addition, a preliminary chemical evaluation of enriched alkaloids fraction (EFA) obtained from *P. deflexa*leaves is also objective of this study. The leaves of *P. deflexa* were collected in the Rain Forest at Blumenau (SC-Brazil). The air-dried material was powdered and macerated with EtOH. After solvent evaporation, the crude extract was submitted to acid-base partition to obtain the EAF. This fraction was analyzed by HPLC-DAD and High-resolution Mass Spectrometry (HRMS) to determine their chemical profiles. In addition, the FET evaluation of the EAF (1; 5; 10; 20; 30; 40; 50; 75 e 100 μg/mL) was performed. The following parameters were evaluated: coagulation of fertilized eggs; lack of somite formation; lack of detachment of the tail-bud from the yolk sac; and lack of heartbeat. The HPLC-DAD analysis showed the presence of several monoterpene indole alkaloids in the EAF. HRMS analysis identified the major product having a molecular ion *m/z* = 517.2188 ($C_{26}H_{32}N_2O_9$) as strictosidinic acid. The FET test demonstrated that the EAF (1-50 ug/ml) did not produce morphological changes after 96 hpf. However, higher concentrations resulted in coagulation of embryos at the period of 48 hpf. From these results it was possible to establish an appropriate dose for the study of biochemical parameters in zebrafish central nervous system such as cholinergic activity.

PM-177

Sesquiterpenoids from Taiwanese *Vernonia cinerea*

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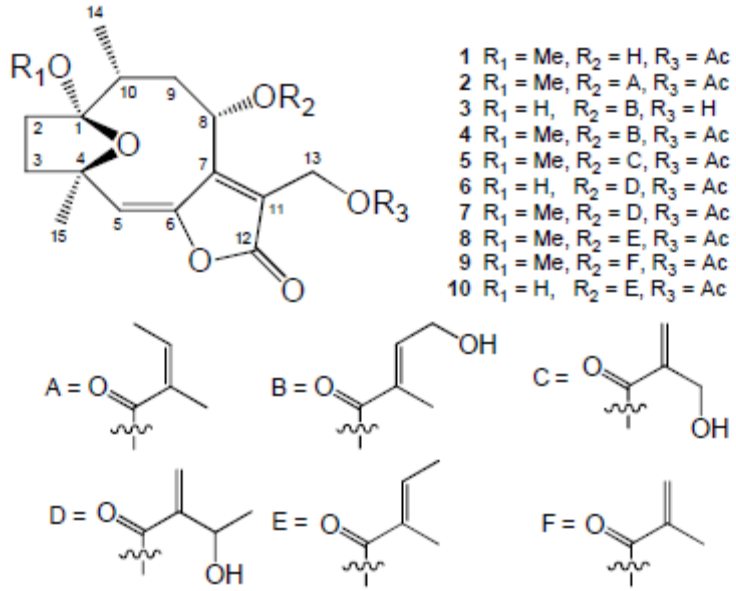
A phytochemical investigation of the ethanol extracts of Taiwanese *Vernonia cinerea* L. resulted in the isolation of ten hirsutinolide-type sesquiterpenoids, including seven new ones, named vercinolides A~G (1~7). All structures were elucidated by a combination of detailed spectroscopic analyses (NMR and MS) and comparison with reported data. Furthermore, in *in vitro* anti-inflammatory assays, compounds 4, 5, and 8~10 exhibited strong inhibitory activities of NO production by LPS-induced RAW264.7 macrophages, IC_{50} values of 1.16, 0.91, 0.75, 0.55, and 0.28 μM, without affecting cellular viability at 40 μM. Structure-activity relationship studies implied that the functional groups at C-1, C-8, and C-13 may enhance inhibition of NO production, especially the ether group at C-8.



PM-178

Myrsinane and related diterpenes from *Euphorbia falcata* with selective potassium ion channel activity

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Plants belong to the genus *Euphorbia* are promising sources of biologically active compounds. Over 650 diterpenoids have been isolated from the members of this genus and a wide range of therapeutically relevant activities (e.g. antitumor, cytotoxic, multidrug resistance-reversing, antiviral and anti-inflammatory activity) have been reported.

Previously twenty diterpenes were isolated from *E. falcata* by our group. The isolated compounds contain myrsinane, premyrsinane and cyclomyrsinane skeletons, and are esterified with acetic, propanoic, isobutanoic, methylbutanoic, benzoic and nicotinic acids. All but one are new natural products.

In the present study, the effects of these compounds on the G protein-activated inwardly rectifying K^+ (GIRK) channel and on the human Ether-à-go-go-Related Gene (hERG) channel were investigated. The GIRK channels regulate the electrical activity of cardiac atrial myocytes among others. Compounds that inhibit GIRK channel are promising tools in the treatment of atrial fibrillation. Compounds with hERG blocking activity may modify the action potential of the heart muscle which can lead to prolongation of the action potential and an increase risk of severe ventricular arrhythmias. The aim of our work was to find compounds which inhibit GIRK channel but do not influence the functions of hERG channel.

Thirteen of the tested compounds possessed significant blocking activity on GIRK channel and 5 of these exerted low activity on hERG channel. Compounds with a myrsinane skeleton with a keto function at C-7 were proved to be highly active. In addition, esters of 2-deoxy-cyclomyrsinane with aliphatic ester group at C-8 were also found to have remarkable selective ion channel activity.

Acknowledgement: This work was supported by the Hungarian Scientific Research Fund (OTKA K109846) and a János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

PM-179

Quantitative determination of flavonoids in *Centaurea kurdica*

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The Asteraceae family is one of the richest vascular plant families in the world with 1600-1700 genera and 24000-30000 species. *Centaurea* genus is represented with 192 taxa in Turkey, 114 of which are endemic. Many species of the genus*Centaurea* have traditionally

been used for their antibacterial, antirheumatic, diuretic, stomachic, astringent, choleretic, cytotoxic, antipyretic and tonic properties [1,2].

In this study, *Centaurea kurdica* was collected from Elazig, Turkey. It was identified by Ugur Cakilcioglu, Department of Plant and Animal Production, Tunceli University. The herbarium of the plant is deposited in Ege University Faculty of Pharmacy, Department of Pharmacognosy (Herbarium no:1457). The chloroform, methanol:water (1:1) and n-hexane extracts of the aerial parts were prepared. According to the previous studies, the chloroform extract has more terpenoids and flavonoids than the other extracts [3,4]. The presence of hesperidin, naringin, eupatorin, quercetin dihydrate and chrysin was analyzed using LC-MS method. In the present study, the amount of the eupatorin was found as 1.4 % in the chloroform extract. The amount of eupatorin was calculated by using the equation y=20635.1+9985.84*x (R^2=0.9998). This compound was detected for the first time from this plant by LC-MS.

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PM-180

In vitro biomass of *Salvia corrugata*: chemical analysis and evaluation of antimicrobial activity

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The major diterpenoids of the exudate of the fresh aerial parts of *Salvia corrugata* Vahl., demethylfruticuline A (1) and fruticuline A (2), showed significant antibacterial activity [1] and inhibited in vitro the synthesis of biofilm of multiresistant strains of *Staphylococcus* and *Enterococcus* [2]. In order to identify efficient *in vitro* methods of production of these compounds, a protocol for micropropagation and for induction of adventitious shoots from the leaves of *S. corrugata* was developed. The percentage of nodal explants producing shoots was

high in MS medium containing BA 1.5 μM or TDZ 3.0 μM, while the leaf explants showed a poorly suited source of explants for the induction of adventitious shoots. For the quantitative determination of 1 and 2 in the methanolic extracts of the two types of biomass an HPLC method was set up and validated. Regenerated shoots showed the presence of both 1 and 2, while micropropagated plants contained only 2. The yield of 2 was higher in regenerated shoots than in the exudate. In addition to 1 and 2, the two methanolic extracts afforded three new diterpenoids, an icetaxane (3) and two abietanes (4 and 5) with a royleanone skeleton, identified by UV, IR, 1D and 2D-NMR, and HR-MS analysis. 4 and 5 were active on multidrug resistant clinical strains of *Staphilococcus aureus, S. epidermidis, Enterococcus faecalis* and *E. faecium* displaying MIC values of 64 μg/mL.

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PM-181

Marine guanidine derivatives affect the redox biology of *Leishmania infantum* and downregulate cytokines of macrophages

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Marine guanidine compounds have been shown promising antimicrobial and antiparasitic activities [1,2]. Considering the need for novel drugs for neglected protozoan diseases as Visceral Leishmaniasis, we evaluated the in vitro antileishmanial activity of a series of fifteen synthetic guanidines and investigated the lethal action and the immunomodulatory potential of two most selective compounds. Six synthetic guanidines displayed selective antiparasitic activity against *Leishmania* (L.) infantum intracellular amastigotes, with IC_{50} values in the range between 2.2 μM and 18.8 μM. The mammalian cytotoxicity demonstrated CC_{50} values in the range between 42.2 μM and > 150 μM. The lethal action studies demonstrated that two synthetic guanidines induced alteration of reactive oxygen species (ROS) levels in *Leishmania* parasites, resulting in the depolarization of mitochondrial membrane potential. The immunomodulatory assays using flow cytometry, suggested a NO-independent effect on macrophages. An anti-inflammatory effect was observed in *Leishmania*-infected macrophages co-cultived with lymphocytes, reducing the production of the cytokines MCP-1 and INF-$_\gamma$ without modulation of TNF, IL-6 and IL-10. By affecting the redox balance of *Leishmania* and attenuating the cellular immune response of macrophages, the two synthetic guanidines selectively eliminated parasites and may be explored as hit compounds for VL.

Acknowledgement: FAPESP 2013/50228-8

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PM-182

Antioxidant and antimicrobial activities of different parts of *Pancratium maritimum*

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Pancratium maritimum L. (Amaryllidaceae) is grown in the Mediterranean regions shows a significant medicinal interest.This study was conducted to evaluate the antioxidant and antimicrobial activity of the extracts of *Pancratium maritimum* parts (root, bulb, leaves, flowers and seeds) .The antioxidant activity was estimated by different methods. All the methanolic extracts of *Pancratium maritimum* parts exhibited good antioxidant activity. Flowers and leaves extracts recorded the highest DPPH radical scavenging activity (85.22%±1.23 and 81.34%±0.74) for flowers and leaves methanolic extract respectively. The highest Reducing power was found in flowers extract (0.844±0.005 while the lowest (0.465±0.004) was recorded with bulbs methanolic extract. Flowers extract exhibited the highest radical scavenging activity using ABTS assay (72.31%±0.928). On the other hand bulbs extract had the superiority in metal chelating activity it recorded 76.92%±1.33. Leaves extract contains the highest total phenolic and flavonoid contents (5.36±0.082 mg gallic/g DW and 1.17±0.03 mg quercetin/g DW respectively). Chloroform extract of all parts exhibited a strong fungicidal action against the selected multi-drug resistant *Candida albicans* [1]. Also the extract of roots showed strong activity against Gram-negative bacteria by *Klebsiella pneumoniae* (32 mm), *Staphylococcus aureus* (28 mm) and *Salmonella Typhi* (23 mm), while the extracts of bulbs and seeds displayed strong activity against *Salmonella Typhi*, *Staphylococcus aureus*(40mm). Compound (**1**) was isolated from the leaves of *Pancratium maritimum* using different spectroscopic analysis (ESI-MS, 1D and 2D HNMR) and identified as *N*-cyanomethylnorboldine. Present results revealed that some parts of *Pancratium maritimum* have remarkable antioxidant and antimicrobial activity.

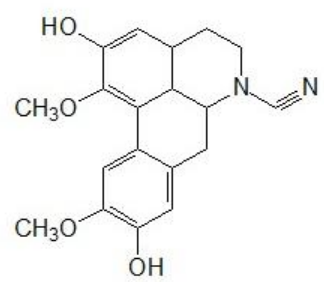

Fig. 1.Chemical structure of compound (1).

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PM-183

Secondary metabolites from *Scorzonera latifolia* roots

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Scorzonera latifolia (Fisch. & Mey.) DC. which belongs to the Asteraceae family, is widely distributed in the Middle and East part of Anatolia as well as Caucasia and Iran [1]. Roots of this plant have been used as analgesic and antihelmintic internally and against women infertility externally in Turkish folk medicine [2]. Analgesic, antiinflammatory, wound healing and antioxidant activities of *S. latifolia* have been reported previously [3]. In current study total methanolic extract of the roots were prepared and partitioned by liquid-liquid extraction succesively by petroleum ether, chloroform and ethyl acetate. Ethylacetate extract was subjected to silicagel column to obtain several subfractions. Further separation procedures on subfractions allowed us to obtain nine phenolic compounds. Structures of the compounds were elucidated by spectroscopic techniques (^{1}H, ^{13}C and 2D-NMR, MS) and identified as chlorogenic acid, chlorogenic acid methyl ester, 1,5-dicaffeoyl quinic acid, 3,5-dicaffeoyl quinic acid, methylester of 3,5-dicaffeoyl quinic acid, hydrangenol-8-*O*-glucoside, hydrangenol-4´-*O*-glucoside, scorzotomentosin-4´-*O*-glucoside and a new isocoumarine derivative named dihydromalicine-4´-*O*-glucoside.

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PM-184

Bioactivity guided isolation studies on *Hypericum microcalycinum*

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The genus *Hypericum* L. (Hypericaceae) is represented by 100 taxa grouped under 19 sections 45 of which are endemic in Turkey *Hypericum* species are used as antispasmodic, sedative and antihelmintic internally; antiseptic and for wound healing externally in Anatolia [1,2]. They contain mainly hyperforins, naphthodianthrones, flavonoids, tannins, xanthones, essential oils [3]. In the present study, bioguided isolation were performed on the aqueous extract of *Hypericum microcalycinum*Boiss. & Heldr. on the basis of 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) and superoxide (SO) radical scavenging activities. The aqueous extract was subjected to polyamide column chromatography to afford eight main fractions. Aqueous extract and polyamide column fractions were tested for their radical scavenging activities and fraction E was found the most active one. Repeated column chromatographies of the fraction E resulted in the isolation of two catechins and three flavonoid glycosides. The structures of the isolated compounds were identified as catechin and epicatechin [1], apigenin-6-C-2''-*O*-acetyl glucopyranoside [2], quercetin-3-*O*-glucopyranoside [3] and quercetin-3-*O*-arabinopyranoside [4] on the basis of spectroscopic (1D/2D NMR and FAB-MS) data. Radical scavenging activities of compounds were also tested. While compound 1, a mixture of catechin and epicatechin, was found the most effective compound, their activity was found comparable to that of known antioxidant, ascorbic acid.

Acknowledgement: US Harput has been supported by TUBA-GEBIP (USH/2013) Award program.

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PM-185

Mucus-normalizing activity of Bronchipret® film-coated tablets in bronchoalveolitis: reduction of LPS-induced goblet cell metaplasia and mucin production *in vivo*

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Increased secretion of viscous mucus is a hallmark symptom of acute bronchitis. Using an *in vivo* model of bronchoalveolitis we aimed to investigate whether Bronchipret® film-coated tablets (BRO) possess the ability to positively regulate determinants of mucus secretion and

consistency. BRO is a herbal medicinal product containing the fixed combination of thyme herb and primula root extract and is clinically used for the treatment of acute bronchitis.

Bronchoalveolitis in male Wistar rats was induced by intratracheal LPS instillation (100 µg/animal). BRO was given daily at 1-10-fold equivalents of the human daily dose (68-680 mg/kg) for up to three days. Animals were sacrificed at 24h intervals up to 72h post LPS challenge to analyze epithelial goblet cell numbers as well as MUC5AC protein expression in lung tissue homogenate.

LPS strongly increased both the density of bronchial epithelial goblet cells as well as MUC5AC levels in a time-dependent fashion with the highest levels reached at 72h post LPS administration. Treatment with BRO at all dose levels led to significant reduction in MUC5AC at 48h and 72h as well as goblet cell numbers at 72h post LPS. The magnitude of these effects was comparable to that of the positive control dexamethasone (5mg/kg).

The herbal medicinal product Bronchipret® (a fixed combination of thyme and primula dry extract) normalizes pathologically altered determinants of viscous mucus secretion typically accompanying inflammation of the lower respiratory tract. These effects most likely contribute to the clinical efficacy of Bronchipret® in acute bronchitis.

PM-186

Evaluation of main anthraquinones as novel Matrix Metalloproteinase-1 (MMP-1) inhibitors

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Matrix Metalloproteinases are responsible for breaking down extracellular matrix proteins [1]. MMP-1 is known as interstitial collagenase and associated with breast [2], lung [3], and prostate [4] cancer. Recent studies have shown that high levels of MMP-1 in such cancers that inhibition of MMP-1 can prevent the growth and spread of tumours.

We selected natural compounds, emodin, aloe-emodin, rhein, physcion and chrysophanol in order to discover their new biological effects. In this research, inhibitory effects of these anthraquinones were investigated on MMP-1 enzyme by using spectrophotometric method. Ac-PLG-(2-mercapto-4-methyl-pentanoyl)-LG-OC2H5 was used as substrate and NNGH (N-Isobutyl-N-(4-methoxyphenylsulfonyl) glycyl hydroxamic acid) was used as MMP-1 inhibitor. Concentrations of anthraquinones were selected as 100 µg/ml, 400 µg/ml, 800 µg/ml respectively. Inhibitor activities of all antraquinones were measured at 412 nm. Physcion showed the highest inhibitor activity with 65.18% at 100 µg/ml on MMP-1 while NNGH showed 90.80% inhibition. This study shows that anthraquinone molecules may be potential scaffolds for the discovery of novel MMP-1 inhibitors.

Acknowledgments: This study was supported by grants from Hacettepe University Scientific Research Projects (Project No: 1216).

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PM-187

Two Saponins from *Harpullia pendula*

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Harpullia pendula Planch. (Sapindaceae) is a large tree found in coastal regions in Australia from Bellingen in New South Wales to Cairns in Queens land [1]. Genus *Harpullia* is represented in Egypt by two species *Harpullia pendula* and *H. cupanoides*. Saponins were proved to be one of the main phytoconstituents of genus *Harpullia* [2, 3]. From the seeds of *H. pendula*, we report here the isolation of two new saponins characterized as 22-*O*-angeloyl-A1-barrigenol 3-*O*-β-D-glucopyranosyl(1→2)[α-L-arabinofuranosyl(1→3)]β-D-glucuronopyranoside and 22-*O*-angeloyl-A1-barrigenol 3-*O*-β-D-galactopyranosyl(1→2)[α-L-arabinofuranosyl(1→3)]β-D-glucuronopyranoside. The structures of saponins were elucidated by chemical hydrolysis, 1D and 2D NMR spectra as well as mass spectra. The triterpene part of the saponins was identified from the close similarity of its NMR data to those of A1-barrigenol except the low field positions of C-3 due to glycosylation and C-22 due to acylation with angelate moiety. The sugar units were recognized after acidic hydrolysis and complete assignments of resonances of each sugar unit using 2D NMR analysis. The observed glycosylation shift of carbon resonances and HMBC correlations at the sites of sugar connectivity, determined the interglycosidic linkages and sequences.

The two saponins were tested *in vitro* for their cytotoxic activity on a number of human tumor cell lines. The results showed that the two saponins were active with IC_{50} values between 5-8 μM.

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PM-188

The effect of ontogenetic factor on active agent content of *Achillea collina*

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Wild yarrow (*Achillea collina* Becker) is popular medicinal plant which is applied in phytotherapy for its antiinflammatory, antispasmodic, digestive and cholagog effect. However, the quality of the drug is often variable. The effect of harvesting time on the essential oil and chamazulene content of yarrow was investigated by several authors, the results are contradictious while experiments focusing on other active compounds, such as flavonoids and phenolics are only sporadic.

In our trial the effect of plant development on essential oil, proazulene, total flavonoid and phenolic content of *A. collina* was investigated. The experiment was installed in Budapest during 2013-2014 using the cultivar 'Proa'. The flowering tops of the plants were harvested in five ontogenetical stages from early budding till late flowering. The essential oil, chamazulene and total flavonoid content was determined in the air-dried samples according to Ph. Eur. VII. [1]. The total phenolic content was measured by the modified method of Singleton and Rossi [2].

The results are summarised in the table below (mean of two years). These results shows that the main active compounds follow different dynamics during ontogenesis of yarrow, therefore harvesting should be optimised for reaching the desired quality according to market/processors' needs.

Plant development stage	Green bud	White bud	Beginning flowering	Full flowering	End of flowering
BBCH scale	55	59	61	65	69
Essential oil content (ml/100 g)	0.180	0.252	0.243	0.220	0.156
Proazulene content (%)	0.073	0.114	0.083	0.085	0.049
Total flavonoid content (%)	1.69	2.04	2.45	2.15	1.99
Total phenoloid content (mg GAE/g)	222.65	207.42	179.93	159.13	152.92

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PM-189

Caffeic acid derivates in spent coffee ground as potential crude material for drug discovery

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The aim of this work is to evaluate the methodology to obtain an extract rich in caffeic acid (CAD) derivatives in spent coffee ground (SCG) and evaluate its free radical scavenging activity. Eighteen SCG samples and coffee beverage were collected from different brands and coffee shops. The SCG extracts were prepared by three methods: A) ethanol 96o according Campos et al [1]; B) extracts prepared in A were hydrolysed with HCl 37% (1:10), according Markham [2]; C) water extraction. All samples were screened by HPLC/DAD for phenolic profile characterization and structural identification and quantification of CAD [1]. Among caffeine the majority of the compounds found in the coffee beverage were CAD and in the SCG the compounds remaining the same. The hydrolysed extracts confirmed its existence, possible as glycosides (Fig 1). The matrix resulting from the hydrolysis of the SCG can also be used to explore further bioactivities for therapeutic consideration once the released aglycones are in general more active. Method A was the most efficient (4.305 mg CAD/g SCG). Nevertheless the results were different according to the products. The caffeine ratio between SCG and Expresso coffee was 17 % and the ratio of CAD in both samples was 8 %. A significant free radical scavenging potential (activity of DPPH) was founded yet in SCG (EC_{50}=1.857µg/mL) comparing with the coffee beverage (EC_{50}=0.172 µg/mL) around 10 % lower. The results show that the SCG has potential for future biological screening showing a stable compound profile and constitutes a much simpler matrix than the beverage, simplifying its analysis and further studies for potential bioactivities.

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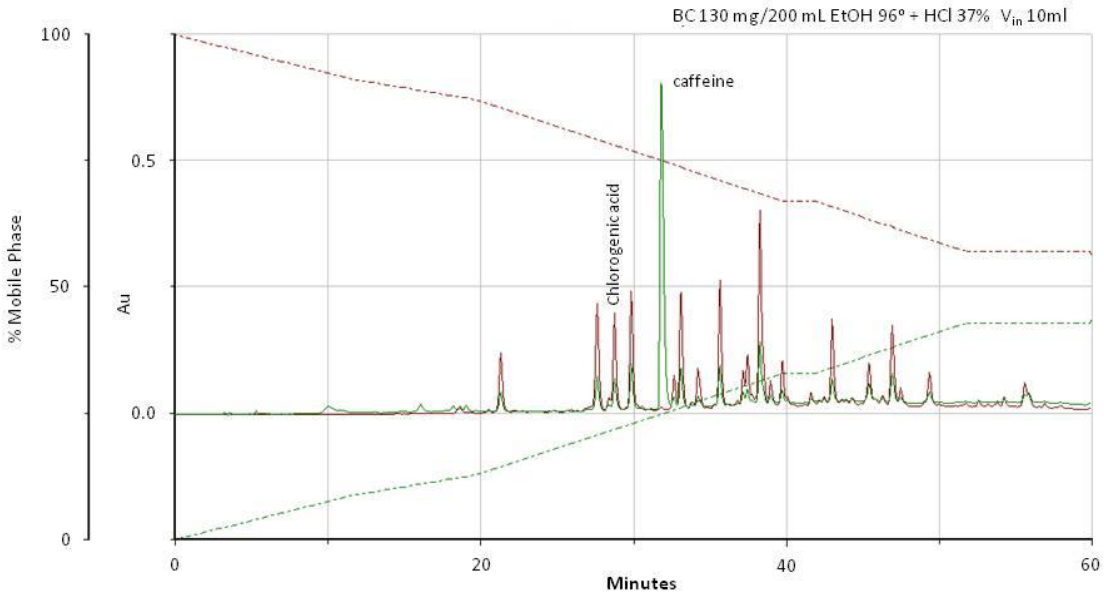

Figure 1. HPLC/DAD profile of acidic hydrolyse of ethanolic extract recorded at λ_{max} 260 (for caffeine)(green line) and 340 nm (for caffeic acid derivatives)(red line).

PM-190

Protective effect and mechanism of triterpenoids isolated from *Cornus walteri* against anticancer drug-induced nephrotoxicity in LLC-PK1 cells

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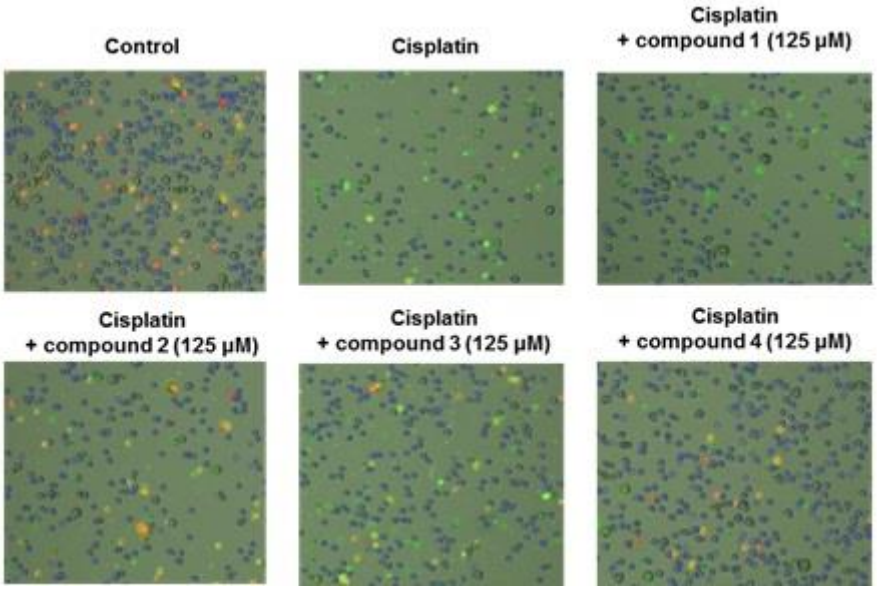
Cornus walteri Wanger (Cornaceae) is a deciduous shrub distributed in valley areas of Asia, and its fruits and leaves have been used in Chinese folk medicine for treatment of inflammation of the skin or boils caused by lacquer poison [1]. In our search for bioactive constituents from Korean medicinal plants, a phytochemical investigation of the MeOH extract of the stems and stem bark of *C. walteri* resulted in identifying twelve major triterpenoids (**1–12**). Compounds **1-4** ameliorated cisplatin-induced nephrotoxicity to 80% of the control value at 125 μM. Phosphorylation of mitogen-activated protein kinases (MAPKs) was decreased following cisplatin treatment after treatment with compounds **1-4**. These results show that blocking the MAPKs signaling cascade plays a critical role in mediating the renoprotective effect of the isolated compounds**1-4** from the stems and stem bark of *C. walteri*.



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PM-191

Perspective sesquiterpene lactones from endemic plant species of Kazakhstan

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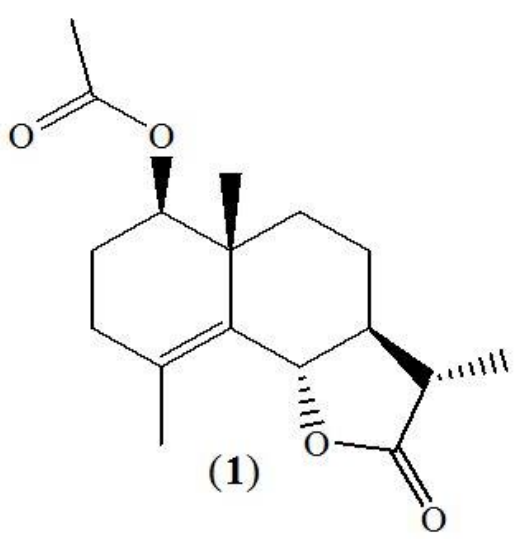
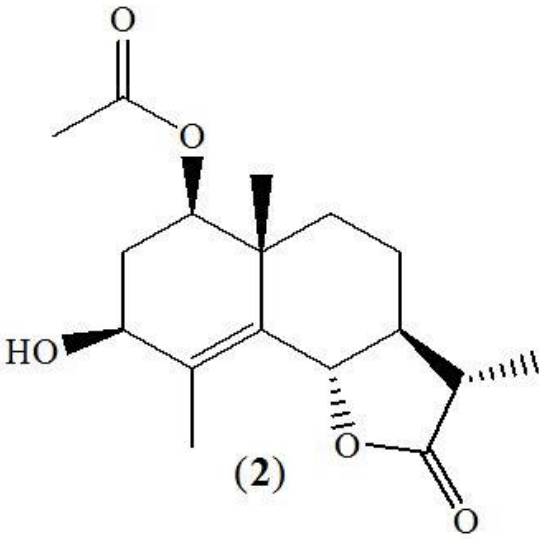
There are more than 6000 plants species native to Republic of Kazakhstan, 667 of which are endemic and can be considered as potential sources of new, previously unexplored natural compounds. With the aim of searching for new sesquiterpene lactones we have studied endemic plants of Kazakhstan's flora. Based on results of screening for perspective sources of sesquiterpene lactones for detailed research we have chosen the following *Artemisia* species: *A. aralensis* Krasch., *A. filatovae* A.Kuprijanov sp.*nova*, *A. glabella* Kar. et Kir., *A. gracilescens* Krasch., *A. halophila* Krasch., *A. hippolyti* A. But. sp. *nova*, *A.karatavica* Krasch., *A. leucodes* Schrenk, *A. radicans* A.Kuprijanov sp. *nova*, *A. semiarida* (Krasch. et Lavr.) Filat., *A. succulenta* Ldb., *A. tournefortiana* Rchb. Furthermore *A. halophila* was identified as a rich source of new eudesmanolids: 1b-acetoxy-7a,6,11b(H)-eudesm-4(5)-en-6,12-olid (**1**) and 1β-acetoxy-3β-hydroxy-6,11β,7α(H)-eudesm-4(5)-en-6,12-olid (**2**). From *A. filatovae* ludartin, isoepoxyestafiatin, arglabin and hanfillin were isolated and identified, from *A. aralensis* and *A.gracilescens* argracin was isolated. In *A. hippolyti*ludartin, arglabin, isoepoxyestafiatin, hanfillin and artefin were detected, in *A. leucodes* achillin, austricin, leucomisin, grossmisin were identified, in *A. radicans* argolid, 8-desoxicumambrin, achillin, grossmisin and austricin were discovered, in *A. semiarida* taurin, 8α-acetoxitaurin, in *A. succulenta* α-santonin, in *A. tournefortiana* tourneforin was identified.

Some isolated sesquiterpene lactones showed remarkable biological activity. From the endemic species *A. glabella* Kar. et Kir. a sesquiterpene lactone from the guaianic group, arglabin was isolated, which is the active component of the original anitumor drug «Arglabin»

and on the basis of the guaianolide leucomisin from *A. leucodes* Schrenk. the new hypolipidemic agent «Aterolid» was developed.





PM-192

Susceptibility of *Listeria monocytogenes* to South African medicinal plants

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Thirteen South African medicinal plants were screened for activity against the pathogenic bacterium, *Listeria monocytogenes*. The plants were selected based on information on their traditional medicine use for treating symptoms of listeriosis (diseases caused by *L. monocytogenes* infection). Ethyl acetate and chloroform extracts of each plant were tested using the disc diffusion and microtitre methods against *L. monocytogenes*. Out of the thirteen plants screened for activity and it was found that *Acacia karroo* Hayne (family *Fabaceae)* and *Plectranthus ecklonii* Benth. (family *Lamiaceae)* had a high antilisterial activity as compared to other plants investigated. Through the bioassay guided fractionation of the ethyl acetate extract of *A. karroo* two bioactive compounds were isolated and identified. The MICs and the MBCs were determined by the micro titre plate method. The MIC of *A. karroo* ethyl acetate crude extract against *L. monocytogenes* was 3.125 mg/ml and the MBC 3.125 mg/ml. The two isolated compounds isolated from A. *karroo* that were found to be active against *L. monocytogenes*. These were β-sitosterol and epigallocatechin. Their MICs were 31.25 µg/ml and 62.5 µg/ml against *L. monocytogenes*. Compound 2(β-sitosterol) had IC_{50} of 63.62±1.614 (SD) µg/ml and compound 4 (epigallocatechin) was more active (IC_{50} 28.91±1.525 (SD) µg/ml). The ethyl acetate crude extract of *A.karroo* had IC_{50} of 45.49±7.86 (SD). *P. ecklonii* ethyl acetate extract led to the isolation of two known compounds, parvifloron D and parvifloron F. Parvifloron D and F exhibited a minimum inhibitory concentration (MIC) of 15.6 and 31.25 µg/ml, respectively against *L. monocytogenes*. The two active compounds isolated from *A. karroo* and the two from *P. ecklonii* have potential to play a role in finding the most effective novel drugs from plants against *L. monocytogenes*, this could reduce the risk of multidrug resistant species and reduce the treatment costs.

PM-193

The effect of the acetone extract of *Arctotis arctotoides* (Asteraceae) on the growth and ultrastructure of some opportunistic fungi associated with HIV/AIDS

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In this study, the effect of the acetone extract of *Arctotis arctotoides* (L.f.) O. Hoffm. (Asteraceae) on the growth and ultrastructure of some opportunistic fungi associated with HIV/AIDS was analyzed by means of scanning electron microscope (SEM). Remarkable morphological alterations in the fungal mycelia which were attributed to the loss of cell wall strength ranged from loss of turgidity and uniformity, collapse of entire hyphae to evident destruction of the hyphae. The elements responsible for giving the fungi their characteristic virulence were detected and quantified by energy dispersive X-ray microanalysis techniques. X-ray microanalysis showed the specific spectra of sodium, potassium and sulfur as the principal intersection of the four pathogenic fungi studied. Since these ions have the potential of fostering fungal invasion by altering the permeability of hosts' membranes, their presence was considered inherent to the pathogenicity of the opportunistic fungi. Hence, these findings indicate the potential of the crude extract of *A. arctotoides* in preventing fungal invasion and subsequent infection of host's membranes.

PM-194

"Ligand fishing" in chemical space reveals new potential leishmanicidals

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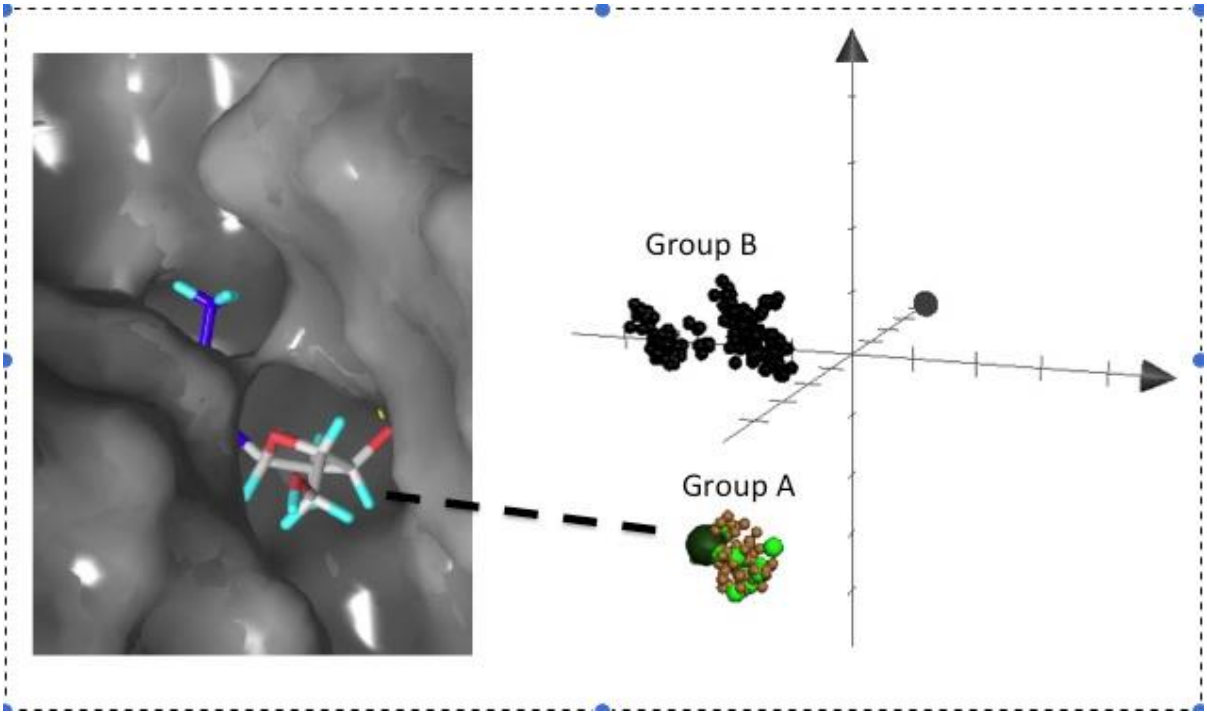
Pteridine reductase 1 (PTR1) is suggested to be a potential drug target in *Leishmania* parasites, because it is predicted to be essential for the pathogen's survival and it appears to lack human homologues [1]. The aim of this study is to elucidate if "ligand fishing" in chemical space using ChemGPS-NP can be used to find new potential PTR1-inhibitors of natural origin. PTR1 in complex with 7,8-dihydrobiopterin (DHB), was obtained from the Protein Data Bank. Two sets of compounds, A and B, of natural origin were retrieved using ChemGPS-NP. ChemGPS-NP positions compounds in chemical space according to their physical-chemical properties [2,3]. Group A included natural compounds that are positioned near DHB. Group B included natural compounds positioned far from all ligands in all crystalized structures of PTR1. The inhibitory effects of the compounds in group A and B, on PTR1 were assessed by predicting their affinity towards the enzyme using molecular docking. Thirteen of the 78 compounds in Group A were predicted to bind with a higher affinity than DHB to PTR1, and nine of these, interacted with the binding pocket of PTR1 in other ways than known ligands. None of the 191 compounds in Group B, were predicted to bind to PTR1 with the same or higher affinity than DHB. Hence, "ligand fishing" in chemical space using DHB as bait can be a successful path for finding new potential PTR1 inhibitors of natural origin.



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PM-195

Studies on angiogenic potential of *Rubia cordifolia*, *Mimosa pudica* and *Hemidesmus indicus* by chick embryo chorioallantoic membrane as a model system.

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In wound healing process, angiogenesis plays an important physiological role by restoring blood flow to tissues after injury.

The chorioallantoic membrane (CAM) is a vascular extra-embryonic membrane found in eggs of some amniotes, such as birds, and is formed on day 4 of incubation. It is formed by the

fusion of the allantois and chorion. The CAM model has been widely used to screen and evaluate the wound healing potential of various compounds. *Rubia cordifolia* (RC), *Mimosa pudica* (MP) and *Hemidesmus indicus* (HI) are reported for their wound healing property. But till date no reports were available for their mechanistic role in wound healing via angiogenesis.

The aqueous extracts and fractions of RC, MP and HI were tested for angiogenesis using chick embryo chorioallantoic membrane as a model system.

Result:

RC: The percentage increase in number of blood vessels observed were 48.81% with 0.1 µg/ml, 32.65% with 1 µg/ml. The concentration 10µg/ml was found to be toxic to CAM.

HI: The percentage increase in number of blood vessels observed were 30.25% with 1µg/ml, 41.45% with 10 µg/ml and 61.37% with 100 µg/ml.

MP: The percentage increase in number of blood vessels observed were 48.21% with 1µg/ml, 68.32% with 10 µg/ml and 70.43% with 100 µg/ml.

Rubia cordifolia, *Mimosa pudica* and *Hemidesmus indicus* promoted angiogenesis in CAM model. The angiogenesis potential of the plants extracts could be contributed to the alkaloids and flavonoids which are reported to be responsible for wound healing. Hence, we propose "angiogenesis" as a probable mechanism towards wound healing properties of these extracts. Angiogenesis,the increased blood vessel growth can promote both the extent and direction of fibroplasia. Neo vascularization, therefore, would be contributing significantly to wound healing activity of Rubia cordifolia, Mimosa pudica and Hemidesmus indicus.They may serve as a good source for isolating lead compounds for wound healing property.

PM-196

In vitro assessment of antioxidant activity of essential oils and methanol extract of *Pelargonium graveolens*

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This work aimed at evaluating the antioxidant activity of essential oils and methanol extracts of *Pelargonium graveolens* L. which is widely spreading in Algeria. The extraction of essential oils from the dried plant accomplished by steam and hydro-distillation gave 1.25 and 0.5% yields, respectively.

Antioxidant assessment of the methanol crude extract and essential oils was carried out using three methods such as TLC bioautography, DPPH test and ferric reducing ability of plasma (FRAP) assay [1,2]. In the first TLC technique, yellow spots were observed after spraying the plates with DPPH solution indicating the antioxidant power of the extracts under study. In the second method, the methanol extract and essential oils showed a pronounced DPPH scavenging potency with IC_{50} values of 0.237 and 1.12 mg/ml, respectively. It was also noted that our extracts exhibited higher reducing potential of ferric ions to ferrous ones which depends upon extract concentration. Furthermore, the kinetic behaviour of DPPH radical

scavenging activity of extracts under investigation allowed us to determine some kinetic parameters such as $t_{1/2}$, time reaction (t) and the remaining DPPH·percent.

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PM-197

Evaluation of androgenic potential of *Pedalium murex* in male albino rats

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Pedalium murex L. is extensively used as traditional remedy for several diseases and male infertility. The present study evaluated the androgenic activity of *P. murex* fruit extract. Male rats were orally treated with sulphasalazine (SSZ) 100 mg/ kg b. wt. for induction of infertility and further treated with *P. murex* extract at doses of 50, 100 and 200 mg/kg b.wt. for 60 days. Significant increase was observed in body weight and weights of reproductive organs i.e. testis, cauda epididymis, seminal vesicle, ventral prostate and vas deferens. Sperm motility and density were significantly elevated. Biochemical analysis in treated rats revealed significant increase in protein, sialic acid, seminal vesicular fructose, ascorbic acid in the adrenal gland and testicular glycogen was also increased. Cholesterol content in the testis, cauda epididymis, seminal vesicle, and ventral prostate decreased in significant manner. LH and testosterone levels showed significant increase. Histopathological examination revealed the stimulated growth of accessory glands, seminiferous tubules and the Leydig cells. Our present study suggests that oral administration of methanolic fruit extract of *Pedalium murex* has promise in fertility enhancement and exerts androgenic activity without showing any adverse effects.

PM-198

Isolation and characterization of essential oils and 7 new ecdysteroids from medicinal plant *Ajuga turkestanica*

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Ajuga turkestanica (Lamiaceae) is an endemic perennial plant and grows in areas at 600-1000 m above sea level in Southern Pamir-Alay mountains on Southwest slopes of the Hissar Mountain (in the territory of Uzbekistan) [1]. This plant has been widely used in folk medicine for enhancement of muscular strength, against heart disease, muscle and stomach aches [2].

The objective of this study was to characterize the chemical constituents (essential oils and ecdysteroids) of *A. turkestanica*.

The hydro-distilled essential oils obtained from the aerial parts of *A. turkestanica* was analysed by gas chromatography-mass spectrometry (GC-MS). Column chromatography, multi-step preparative HPLC (combining RP- and NP-HPLC systems) were used to purify the ecdysteroids present in the roots of *A. turkestanica*. Isolated compounds were identified by high-resolution mass spectrometry, 1D and 2D NMR.

The chemical composition of the essential oils of *A. turkestanica* was investigated for the first time and it was characterized by a broad structural diversity of monoterpenes, hydrocarbons, aliphatic acids and sterols.

A set of 14 ecdysteroids was isolated, among which seven new ecdysteroids (25-hydroxyatrotosterone A, 11-hydroxy-Δ24-capitasterone, 11-hydroxycyasterone, 11-hydroxysidisterone, 22-oxo-turkesterone, turkesterone 22-acetate, turkesterone 20,22-acetonide), all of which bearing a 11a-hydroxy group. *A. turkestanica* ecdysteroids are characterized by the abundance of 11a-hydroxylated compounds, and by the simultaneous presence of 24C, 27C, 28C and 29C ecdysteroids.

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PM-199

Isolation and screening of secondary metabolites from endophytic fungi of *Vernonia amygdalina* and*Carica papaya* for their cytotoxic activity

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Searching for cytotoxic compounds from endophytic fungi is a promising way to meet the public health burden posed by cancer, both in developed and developing countries. This study was carried out to evaluate the potential cytotoxic property of secondary metabolites produced by endophytic fungi isolated from *Vernonia amygdalina* Delile and *Carica papaya*L. Isolation of endophytic fungi from plant leaves was carried out using standard methods. Solid state fermentation was carried out in rice medium for 21 days at 25-27 °C and the secondary metabolites were extracted using ethyl acetate. The extracts were screened for cytotoxicity against L5178Y mouse lymphoma cells using the MTT assay. High-performance liquid chromatography coupled to photodiode array detection (HPLC-DAD) was used to identify the

compounds which may be responsible for the recorded cytotoxic activity. A total of ten endophytic fungi were isolated from the leaves of *V. amygdalina* and *C. papaya*. Of all the extracts produced by the ten fungi, only one (Cp1) from *C. papaya* recorded cytotoxic activity. At 10 µg/mL, Cp1, showed a significant cytotoxicity activity against the cancer cells, having an inhibition of 70.8% while the other extracts had no anticancer effect on the cells. HPLC-DAD analysis of the fungal extracts revealed the presence of cytotoxic compounds (catechin, kahalalide and palitantin) present in Cp1 extract. The result indicates the potential of endophytic fungi from *C. papaya* to produce bioactive compounds with cytotoxic/anticancer properties.

PM-200

Metabolic profiling of *Lupinus* species from Cundinamarca (Colombia)

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Plants belonging to the genus *Lupinus* are widely distributed around the world. They are characterized by attractive flowers, but are also described as invasive. High contents of quinolizidine alkaloids and isoflavonoids are reported for *Lupinus*species [1,2]. Those from Cundinamarca Department in Colombia have almost exclusively blue-colored flowers. In spite of their significantly high distribution in this region, there is a lack of information regarding their chemical composition and metabolic profiling. Therefore, to obtain metabolic profiles of several *Lupinus* taxa, including endemic ones, they were analyzed by means of LC-DAD-ESI-MS and multivariate statistical tools; five species and 39 specimens were examined. Analyses were carried out on different plant parts. Significant inter- and intra-species-dependent differences were observed between the chromatographic profiles, as well as marked chemical variations between the different plant organs. Nevertheless, particular relationships could be established leading to the conclusion that similarities in metabolic pathways are expressed for this set of Colombian *Lupinus* species. Concurrently, representative samples were subjected to alkaloid extraction and analysis by GC-MS. Dissimilarities between species were also found, although to a lesser extent. The results indicated that Colombian *Lupinus* taxa are organisms with dynamic metabolism requiring further phytochemical investigation.

Acknowledgement: The present work is a result of Project IMP-CIAS-1567 financed by Vicerrectoría de Investigaciones at UMNG - Validity 2014.

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PM-201

Chemical and biological investigations of the Egyptian plant *Cleome africana*

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Cleome africana Botsch is a wild medicinal plant growing in Sinai. Chemical investigation of the ethanolic extract of its herb led to isolation of kaempferol-3-*O*-rhamnoside-7-*O*-glucoside and hexadecyl glucosinolate [1,2]. These compounds were isolated using different chromatographic techniques and identified by spectroscopic measurements (UV, MS and NMR). The biological study as an antidiabetic [3] of different fractions [total methanol extract (TM),chloroform (C),ethyl acetate (E), butanol (B) and the total glucosinolate (G)] in streptozotocine induced diabetic rats proved that the fraction B is the most effective (reduction of glucose level from 181 to 96 mg/dl). The anti-inflammatory effect [4] of different fractions was studied in induced diabetic rats, the results showed that, the fractions B, E, TM and G caused a significant reduction in prostaglandin E_2 level than the other fractions. Finally the hepatoprotictive activity [5] of different fractions was studied by measuring of liver enzymes (ALT, AST) in rats of injured liver using CCl_4. It was found that the fraction G normalized both ALT and AST in addition to creatinin and uric acid level.

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PM-202

In vitro antioxidant activity, phenolic content and profile of *Cystoseira barbata* from Romanian Black Sea

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The Romanian Black Sea has abundant seaweed resources, but little effort has been done to screen their biological potential. The aim of the present study was to assess the *in vitro* antioxidant activity, phenolic content and profile of *Cystoseira barbata* (Stackhouse) C. Agardh, Cystoseiraceae, a brown alga inhabiting the costal line of Romanian Black Sea. The 70% acetone extract from *C. barbata* was investigated by HPLC-DAD-ESI-MS^n. Total phenolic content was determined by Folin-Ciocalteu method. The antioxidant activity was assessed using four methods (2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical cation scavenging assay, reducing power and 15-lipoxygenase inhibition assay); gallic acid and caffeic acid were used as positive controls. Several phlorotannins (fucodiphloroethol, fucophloroethols with six, seven and eight phloroglucinol units with $[M+H]^+$ at *m/z* 499, 747, 871 and 995, respectively) were tentatively identified on the basis of their characteristic MS fragmentation pattern. A total phenolic content of 236.03±1.20 mg gallic acid equivalents/g extract was determined. In all antioxidant assays, the activity of the extract increased significantly in a concentration dependent manner.The EC_{50} values in the DPPH, ABTS and reducing power assays were 88.5±0.3, 13.9±0.2 and 17.6±0.0 µg/mL, respectively. The extract showed high 15-lipoxygenase inhibition capacity (EC_{50}=47.3±1.5 µg/mL), when compared with positive controls (EC_{50}=39.0±0.7 µg/mL for gallic acid and 25.5 µg/mL for caffeic acid). The remarkable antioxidant activity of *C. barbata*suggests its potential use in the food and pharmaceutical industries.

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PM-203

Melanogenesis inhibitors from *Oenothera laciniata* extracts

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Oenothera laciniata Hill (Onagraceae), a perennial herb, is used to treat various inflammatory diseases such as systemic lupus erythematosus, ankylosing spondylitis, and rheumatoid arthritis. However, the phytochemical and the anti-melanogenic studies of *O. laciniata* are not identified. The aim of this study compared the anti-melanogenic activity of methanolic extract of *O. laciniata* (OLM) and its different soluble fractions, including ethyl acetate (OLEF), *n*-butanol (OLBF), and water (OLWF), by determing the inhibition of cellular melanin content and tyrosinase activity in B16F10 melanoma cell. The melanin synthesis proteins were also

quantified by western blot assay, including tyrosinase, tyrosinase related protein-1 (TRP-1), tyrosinase related protein-2 (TRP-2), microphthalmia-associated transcription factor (MITF), phospho-cAMP-response element binding protein (p-CREB), phospho-extracellular-signal-regulated kinase (ERK), and phospho-c-jun N-terminal kinase (JNK). The results showed that OLM and its fractions decreased melanin production and inhibited tyrosinase activity in a dose-dependent manner in B16F10 melanoma cells. Moreover, OLM and its fractions demonstrated that induced down-regulation of melanogenesis via inhibiting p-CREB and subsequently reducing the expression of MITF, which in turn down-regulated tyrosinase and TRP-2 activities at the protein levels, but not inhibit TRP-1 expression in B16F10 cell. The results also indicated that OLM, OLBF, and OLWF reduced melanogenesis through an increase in ERK phosphorylation, but OLEF inhibited melanogenesis by inducing JNK phosphorylation. These results suggest that *O. laciniata* has great potential for becoming anti-melanogenesis agents in cosmetics.

PM-204

Graptopetalum paraguayense, an affordable herbal crop, can reverse hepatic damage *in vivo*

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Progression of hepatitis is a common and potentially lethal problem in chronic liver disease in Taiwan. Incidence of cirrhosis is growing as a result of the widespread occurrence of chronic hepatitis, as well as the evident lack of an established therapy for hepatitis. Herbal medicines have been used in Chinese population for thousands of years and have been increasingly popular in western countries as alternative medicines for a wide variety of diseases. The whole plant of *Graptopetalum paragnyense*, a popular herbal medicine, has been considered to have hepatoprotective effects and its freeze-dried powder has been demonstrated in our previous studies to have anti-inflammation, hepatoprotective and antifibrotic effects if fed to rats with dimethylnitrosamine-induced liver injuries at 1.4 g/kg/day. In this study, we applied microarray technology to uncover the underlying molecular mechanisms. By comparing expression profiles among healthy livers, untreated damaged livers and damaged livers treated for 6 weeks, we have identified the 156 damage-related genes whose abnormal expression levels are effectively restored by *G. paragnyense*. Using liquid chromatography–mass spectrometry, we have verified that *G. paragnyense* extracted from 5 different harvests exhibit consistent chemical fingerprints (0.7G. paragnyense does not change expression levels of ALT, AST, bilirubin, AKP, AFP, BUN, PT, and PLT in healthy livers. The above results suggest that *G. paragnyense* makes a potential therapeutic medicine for treating hepatitis.

PM-205

New xanthone and sesquiterpene derivatives from *Scopulariopsis* sp., a marine-derived fungus isolated from the Red Sea hard coral *Stylophora* sp.

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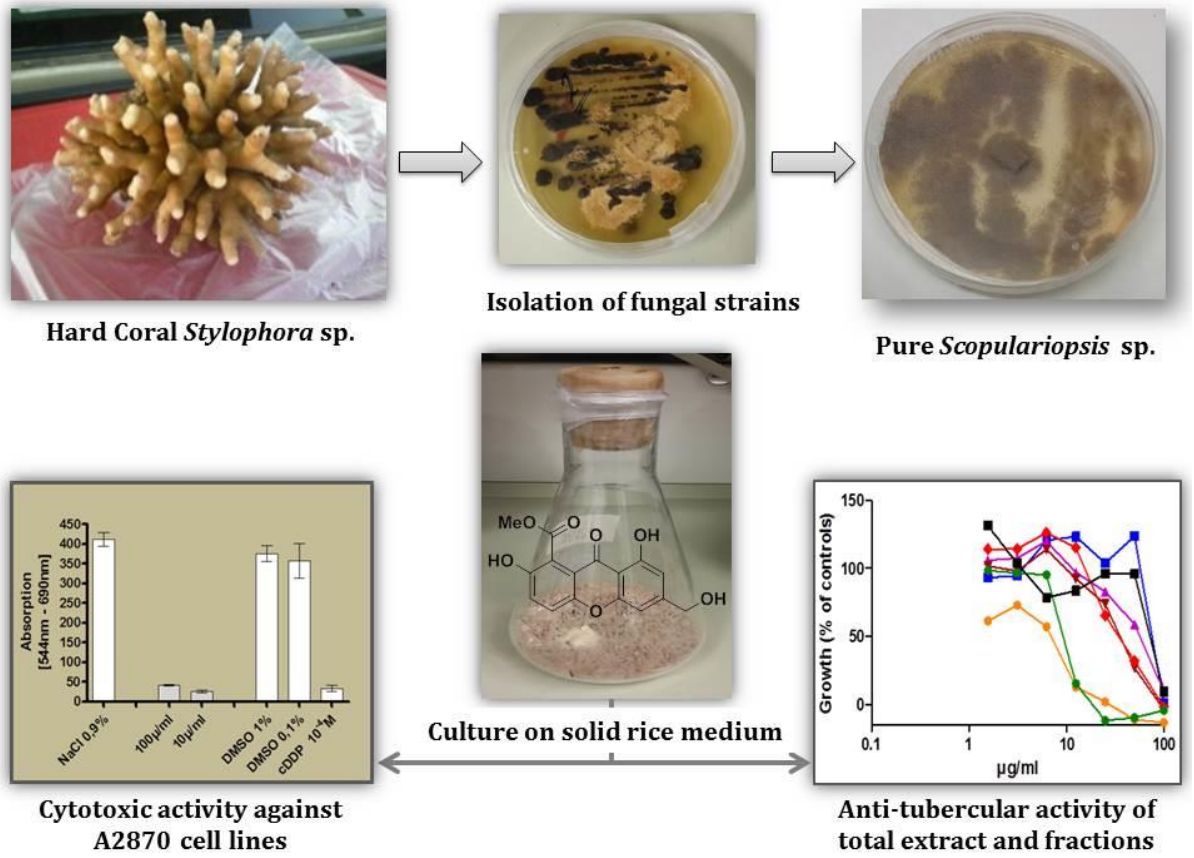
Marine natural products with their diverse unique bioactivities represent a capacious gold mine for the discovery of lead compounds that could be a useful drug entity for the treatment of various malicious diseases. In the last decade, with an increasing interest in the drug discovery from marine system, emphasis has been done mainly concerning various ecosystems exist in the wide marine environment, including hitherto unexploited and geographically and taxonomically undescribed organisms [1]. Recently, an increasing interest was directed toward marine-derived fungi to be an enormous source of structurally novel and potent bioactive metabolites [2].

The marine-derived fungus *Scopulariopsis* sp. was isolated from the inner tissues of a hard coral *Stylophora* sp. collected from the Red Sea, and grew on a solid rice medium. Chemical investigation of the EtOAc extract which exhibited considerable cytotoxic activity against both L5178Y cell line and A2870 cell line and anti-tubercular activity, led to the isolation of six new secondary metabolites including three xanthone derivatives (**1**-**3**), two phenolic bisabolane-type sesquiterpenoids (**4** and **5**), and one alkaloid (**6**). In addition, fourteen known compounds including six xanthone derivatives (**7**- **12**), three bisabolane-type sesquiterpenoids (**13**-**15**), four bi-phenyl ether derivatives (**16**-**19**) and ergosterol (**20**) were also identified. Structures of the isolated compounds were established through extensive analysis of the 1D, 2D NMR as well as MS data, and by comparison with the literature.



PM-206

In vitro biological and toxicological evaluation of *Lathyrus* sp. Extracts

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Lathyrus L. (Fabaceae) species are well known with their edible uses [1] as well as the notorious "*lathyrism*" diseases associated with the oxalyldiaminopropionic acid [2]. There is very limited information on the biological activities of this genus. The *n*-hexane, $CHCl_3$, ethyl acetate and CH_3OH extracts of *Lathyrus* species including *L. sativus*, *L. aphaca*, *L. cicera*, *L. gorgonei*, *L. saxatalis*, *L. blepharicarpos* var. *cyprius* and *L. ochrus* were tested for their DPPH scavenging, PRAP, phytotoxic, *Vibrio fischerii* toxicity, cytotoxicity against NIH/3T3 cell line, LOX enzyme inhibition and as well as antimicrobial activity against a selection of pathogens. The highest DPPH scavenging activity and PRAP activity was observed for the CH_3OH extract of *L. cicera* 79.48±0.79% (*n=5*) and $CHCl_3$ extract of *L. blepharicarpos* var. *cyprius*678.48±15.48 AU (*n=5*; AU TLC-Densitometry) respectively. Highest phytotoxic

activity was observed for $CHCl_3$ extract of L. sativus at 10 mg/mL application concentration 72.86±21.14 % (*n=3*). The highest *V. fischerii* toxicity was observed in *L. cicera* $CHCl_3$ extract after 60 min of application which produced 5 LU at the same conditions positive control $CuSO_4$produced 22 LU. The highest cytotoxicity against NIH/3T3 cell line was observed in *L. ochrus* ethyl acetate extract LC_{50}137.5±3.5 µg/mL (*n=3*). The highest LOX inhibition was observed in ethyl acetate extract of *L. cicera* which afforded 23.96% at 23 µg/mL concentration. All of the extracts produced growth inhibition against the tested microorganisms except for the CH_3OH extracts that had no effect on *C. albicans*. The active principles in the extracts are currently under investigation.

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PM-207

Additional Constituents from *Heterotheca inuloides*. Absolute configuration of some cadinanes

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H. inuloides is one of the plants most used in the Mexican folk medicine and its applications are mainly related with the treatment of inflammatory processes [1]. Cadinane type sesquiterpenes are considered representative constituents of this species, however, the absolute configuration of some of them has not been defined. In the present investigation triterpenes, flavonoids, sterols and eight cadinanes (**1-8**) were isolated from the aerial parts of *H. inuloides*. Among these, **1-4** have not yet been described in the literature. The structures of these metabolites were elucidated on the basis of 1D and 2D NMR spectroscopic data analysis and confirmed by X-ray crystallographic analyses of the majority of them. The absolute configurations of compounds **2-5** were determined by comparing their experimental CD spectra with those calculated by the SCF-CI-DV MO method, and further checked via refinement of the Flack parameter using anomalous X-ray scattering from the oxygen atoms and chemical correlation methods. The 4R,10S configuration for compound **2** is the opposite to that of (+)-δ-cadinene isolated from *Gossypium hirsutum*. These findings are in agreement with previous investigations [2] and allowed to conclude that *G. hirsutum* and *H. inuloides* biosynthesize cadinanes of opposite enantiomeric series. All sesquiterpene compounds were evaluated for their anti-inflammatory potential applying the TPA-induced mouse ear edema model. The results revealed that some of these compounds display moderated activity.

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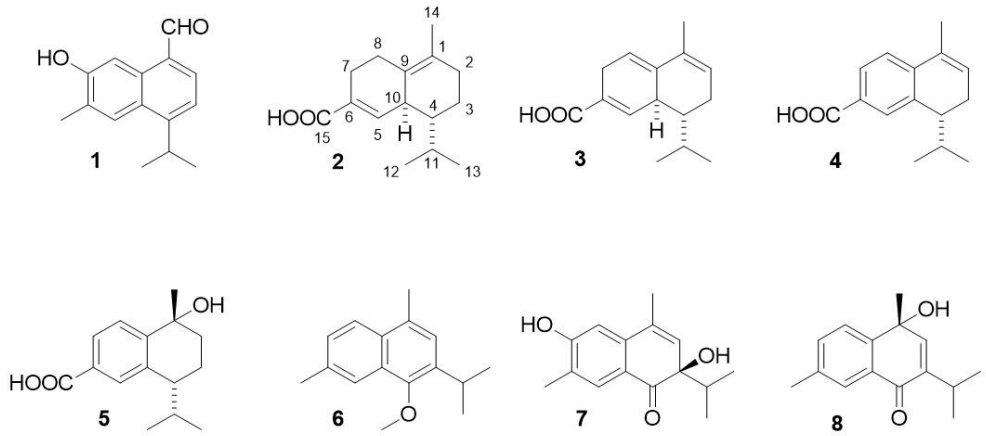

PM-208

Pharmacologically active compounds of *Prosopis africana* and *Parkia biglobossa*

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The air dried pulverized stem bark of *Prosopis africana* and *Parkia biglobossa* were extracted with methanol (MeOH) resulting in crude methanol extracts CMEPA and CMEPB respectively. The quantitative phytochemical analysis and acute toxicity assessment were carried out on the extracts. CMEPA and CMEPB were successively fractionated via column with *n*-hexane, chloroform, ethylacetate and methanol resulting in hexane (HFPA, HFPB), chloroform (CFPA, CFPB), ethylacetate (EFPA, EFPB) and methanol (MFPA, MFPB) fractions respectively. The extracts and fractions were screened for pharmacological activities (anti-ulcer, anti-inflammatory, antioxidant and antimicrobial activities). The most active fractions (EFPB and MFPA) were subjected to vaccum liquid chromatography (VLC) and HPLC. The isolated compounds were further screened for pharmacological activities and subjected to Liquid Chromatography–Mass spectroscopy (LC-MS) and NMR.The extractive yield of CMEPA and CMEPB were 142.56 and 104.43 g respectively. The quantitative phytochemical analysis of CMEPA and CMEPB showed the presence of alkaloids (0.15, 0.19),

glycosides (14.34, 16.54), tannins (6.51, 6.59), saponins (3.09, 4.83), flavonoids (6.67, 10.71), steroids (1.19, 1.62) and terpenoids (0.58, 0.43) mg/g respectively. The LD_{50} of CMEPA was above 5000 mg/kg p.o. and that of CMEPB was 4171.33 mg/kg p.o. EFPB3 (from EFPB) showed significant ($P<0.1$) antiulcer activity, with 92.51% ulcer inhibition in rats. EFPB1 (from EFPB) inhibited rat paw edema by 72.95% in 3h and exhibited anti-oxidant activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) with IC_{50} of 10.55 μM. MFPA2 (from MFPA) showed antimicrobial activities against *E. coli* and *Sal. typhi* (inhibition zone diameter (IZD): 25±0.83 and 20±0.22 mm). The compounds isolated were flavonoids: epigallocatechin-3-*O*-gallate (EFPB3), gallocatechin (EFPB1) and 5,7,dihydroxy -2, 3-dihydroflavone-3-*O*-gallate (from MFPA2).

PM-209

Shikonin induces apoptosis in Caco-2 cells by an increase in caspase-3 and the inhibition of the regulating protein Bcl2

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Shikonin is one of the active principles in the root of *Lithospermum erythrorhizon* Sieb. & Zucc. (Boraginaceae), widely used in traditional Chinese medicine for its anti-inflammatory and wound-healing properties. Recent research highlights shikonin's anticancer properties as well as its preventive ability in acute ulcerative colitis. To evaluate the potential beneficial effects of shikonin on colorectal cancer, a frequent outcome in ulcerative colitis patients, we studied the antiproliferative effect of this naphthoquinone in human colorectal adenocarcinoma cells (Caco-2). Cytotoxicity of shikonin was evaluated using the colorimetric assay described by Mosmann. Flow cytometry was used to study shikonin's proapoptotic activity and to evaluate its effect on cell cycle. Moreover, the study was complemented with the analysis by Western blot of the expression of proteins that play a key role in the apoptotic process.

Our results show that shikonin presents a dose-dependent cytotoxic effect on Caco-2 cells (IC_{50} = 9.84 μM). Cell cycle analysis by flow cytometry demonstrated that this naphthoquinone significantly increased the cell subpopulation in $SubG_0$phase, which is compatible with a proapoptotic mechanism. In addition, it decreased populations located in preparative G_0/G_1 and G_2/M phases, an effect that indicates a metabolic activation of quiescent cells. In agreement with these results, Western blot analyses revealed that shikonin has a marked dose-dependent apoptotic mechanism, evidenced by an increase in caspase 3 (a proapoptotic protein) and the inhibition of the regulating protein Bcl2 (antiapoptotic protein). In conclusion, shikonin shows a promising therapeutic potential as an adjuvant in first-line therapy for colorectal cancer.

PM-210

Screening for anti-inflammatory activity of *Baccharis* species using *in silico* models

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Natural products have always played a vital role in drug discovery. Asteraceae species have showed potent anti-inflammatory activity against cyclooxygenase-1 (COX-1) and 5-lipoxygenase (5-LOX) enzymes [1]. The genus *Baccharis*has more than 400 species and some of them present anti-inflammatory activity. This study aimed at screening extracts of*Baccharis* for their anti-inflammatory potential using UHPLC-HRMS and *in silico* models. EtOH:H2O (7:3 v/v) extracts of 223 *Baccharis* species and 33 other Asteraceae were prepared, their fingerprints were obtained and processed by MZmine. SIMCA-P was used for multivariate analysis and Partial Least Squares Discriminant Analysis (PLS-DA) was used to build prediction models. The model was based on extracts of 14 Asteraceae species that previously presented dual inhibition against COX and LOX and the remaining species presented one or no inhibition. The PLS model was build with two-thirds for training and one-third for testing. As a result, a matrix was obtained with 4,758 variables from the negative mode of ionization. After validation, the PLS-DA model showed statistical significance ($p<0.05$, 95% confidence level) and coefficients of determination (R^2) and prediction (Q^2) of 0.99 and 0.65, respectively. The model was able to sort 50% correctly to *Baccharis* species, where a total of 100 species can present dual inhibition. Among the tested species, *B. boliviensis*, *B. subalata* and *B. incarum* can be cited for their dual inhibition for the first time. Therefore, the purposed model was able to filter the species with anti-inflammatory potential, and this way to avoid waste of time and money.

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PM-211

In vitro antibacterial phenolic extracts from "sugarbag" pot-honeys of Australian stingless bees (*Tetragonula carbonaria*)

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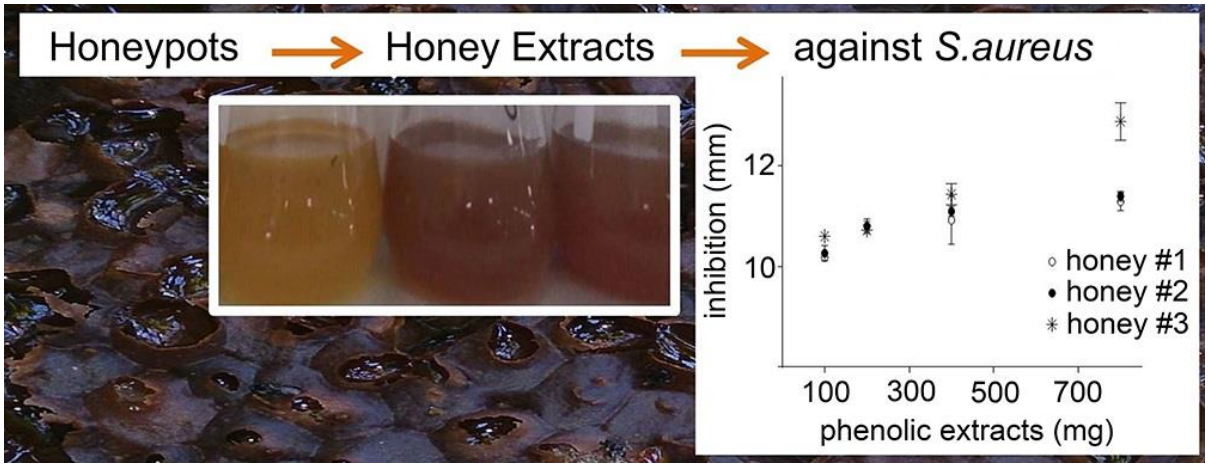
Honey has been used in medicinal preparations since ancient times. Medical research of the antimicrobial effects of honeys can define the potential therapeutic uses [1] further to leads for drug discovery. Medicinal 'manuka' honeys from honeybees (*Apis mellifera*, Apidae) contain the antibacterial methylglyoxal (MGO) [1], and clinical observations showed that honey is effective as a wound dressing [2]. Australian stingless bees *Tetragonula carbonaria* (Meliponini) produce 'sugarbag-pot-honeys' used by Aboriginal people as gastrointestinal cleansers. The antimicrobial properties of sugarbag honeys were reported [1], while their

bioactive factors remained unknown. This study aimed to assess *in vitro* antibacterial effects of phenolic fractions and peroxide contents from *T. carbonaria* honeys.

Methods used *T. carbonaria* honeys harvested in subtropical East Australia. Phenolic extracts were analysed by liquid and gas chromatography mass spectrometry. Antibacterial tests were run against *Staphylococcus aureus* ATCC 25923 by *in vitro* agar diffusion (Fig. 1) and broth dilution assays. Controls were ethanol (negative) and phenolic standard solutions (positive).

Results identified natural products ie. 3-phenyllactic acid, flavonoids and norisoprenoids, while no MGO was found. Total antibacterial activities were partially ascribed to hydrogen peroxide contents as well as phytochemicals [3]: phenolic fractions were bactericidal at 1.2−1.8 mg/mL; hydrogen peroxide content was 155.8 μM while requiring a bactericidal concentration of 760 μM.

In conclusion, *T. carbonaria* honey can source bioactive compounds of antimicrobial interest, and may have a role as a medicinal agent to treat chronic wounds.



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PM-212

Mycochemical study of the mushroom *Tricholoma populinum*

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Tricholoma populinum J.E. Lange also known as cottonwood mushroom is a basidiomycetes species, which belongs to the fairly large genus of *Tricholoma*. It is widespread in Europe and North America, growing on sandy soil under cottonwood trees near a source of water. *T. populinum* is an edible mushroom largely consumed by Salish Indian peoples of British Columbia and also by locals of Sicily. Previous chemical investigations have revealed the presence of ergosterol peroxide, which showed immunosuppressive activity. Now we report on the isolation and structure determination of adenosine type compounds, besides the previously known sterol constituents.

Sporocarps of *T. populinum* were collected in the vicinity of Szeged, southern part of Hungary. The fresh mushroom material was freeze dried to eliminate water. The dried mushroom (310 g) was extracted with methanol at room temperature. The concentrated methanol extract was subjected to solvent-solvent partition first between n-hexane and then chloroform. The chloroform extract was fractionated by repeated rotational planar chromatography. The final chromatographic step was carried out using reversed phase HPLC to obtain pure compounds. The structure elucidation was performed by extensive spectroscopic analyses, including NMR and MS measurements. Based on spectral data the isolated compounds were identified as adenosine, and epimers of 5'-deoxy-5'-methylsulfinyladenosine, which are considered rare fungal metabolites, found previously only in *Ganoderma lucidum*. Pharmacological assays are planned to determine the biological activity of these purine base compounds.

PM-213

Investigation of secondary metabolites of endophytic fungi from *Loranthus micranthus* Linn. and *Citrus jambhiri* Lush. for their antioxidant activity

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Endophytic fungi have proven to be rich sources of novel bioactive compounds with therapeutic potentials. This study was carried out to evaluate the potential antioxidant property of secondary metabolites produced by endophytic fungi isolated from *Loranthus micranthus* Linn and *Citrus jambhiri* lush. Isolation of endophytic fungi from plant leaves was carried out using a previously described method [1]. Solid state fermentation was carried out in rice medium for 21 days at 25-27°C and the secondary metabolites were extracted using ethyl acetate. The extracts were screened for their free radical scavenging properties evaluated using 1, 1 diphenyl-2-picryhydrazyl (DPPH) and ascorbic acid as standard control. HPLC-DAD analysis was carried out to identify the compounds which may be responsible for the recorded antioxidant activities. A total of six (6) endophytic fungi were isolated from *L. micranthus* and five (5) from *C. jambhiri.* DPPH analysis showed that the extracts of endophytic fungi from

C. jambhiri exhibited antioxidant activity with IC_{50} values (17.8, 18.5, 19.3, 19.8 and 106 µg/ml) comparable to that recorded by ascorbic acid (17.5 µg/ml). Extracts of endophytic fungi from *L. micranthus*exhibited low antioxidant activity with IC_{50} values (102,111, 116, 125, 128, and 59.6 µg/ml). HPLC analysis revealed the presence of compounds with established antioxidant properties such as epicatechin, indoly-3-acetic acid, and protocatechuic in the extracts of endophytic fungi from *C. jambhiri*; procyanidin and pinoresinol in the extracts of endophytic fungi from*L. micranthus*. The present study reveals that endophytic fungi are a rich source of bioactive compounds with antioxidant properties.

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PM-214

Investigation of cytotoxic effects of various furanoacridones isolated from *Ruta graveolens*

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Ruta graveolens L. (Rutaceae) is a widely used medicinal plant due to its constituents like furanocoumarins and rutoside. In our study, six furanoacridone alcaloids isolated from *Ruta graveolens* [1] were investigated on human breast cancer cell lines (MCF-7, MDA-MB-361, MDA-MB-231, T47D) for antiproliferative effects and further studies were employed in order to determine the effects on cell cycle and morphology. Breast cancer is by far the most frequent malignant disease among women and accountable for the most cancer-related deaths in the female population worldwide. These data support the urgent need for developing new antiproliferative agents with higher efficacy and better tolerability profile.

The cancer cell lines had been pretreated with the alkaloid components (rutacridone, isogravacridone chlorine (IGC), gravacridonediol monomethyl ether, gravacridonediol, gravacridonetriol, mixture of garvacridonetriol and diol monomethyl ether) and the antiproliferative effects were determined by MTT assay and IC_{50} values were calculated. IGC had the most marked effect on cell proliferation of MDA-MB-231 (IC_{50}=2.27 µM) and MCF-7 (IC_{50}=4.55 µM). Flow cytometric cell cycle analysis had been applied in order to quantify the effect of IGC on the subpopulations of MDA-MB-231 and MCF-7 during cell cycle. It caused cell cycle disturbance decreasing G2/M and G0/G1 and increasing S phase. Hoechst 33258-propidium iodide dual staining was used for the evaluation of morphological changes in MDA-MB-231 and MCF-7 cells pretreated with IGC, resulting in the appearance of nuclear condensation. Caspase-3 activation was additionally determined from IGC-treated MDA-MB-231 cell in order to prove its apoptotic potential.

Based on these *in vitro* findings IGC could be considered for further *in vitro* and *in vivo* studies to characterize the mechanism of its antiproliferative action.

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PM-215

Isolation of the alpha glucosidase inhibitory constituents of *Combretum dolichopetalum* root

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Diabetes mellitus (DM) presents enormous and increasingly important public health issues and its incidence is rapidly increasing globally. Alpha glucosidase enzyme inhibition is an effective way of management of DM. The few α-glucosidase inhibitors that are commercially available are associated with serious gastrointestinal side effects. Therefore, it is necessary to search for alternatives without side effects. *Combretum dolichopetalum* (Combretaceae) root is used traditionally in the management of DM. The aim of the present research is to evaluate and then isolate the alpha glucosidase inhibitory active principles of *C. dolichopetalum* root.

The methanol extract of *C. dolichopetalum* root (ME) was partitioned successively to obtain the ethyl acetate (EAF) and butanol (BuF) fractions. Through various chromatographic separation techniques, arjunolic acid (**1**), cis, trans-dihydrophaseic acid (**2**) and a mixture of two position isomers, echinulin and arestrictin B (**3**) were isolated from EAF while 3,4,3'-tri-*O*-methylellagic acid (**4**) and ellagic acid (**5**) were obtained from BuF. The compounds were identified based on their ^{1}H and ^{13}C NMR as well as mass spectra and comparison of data with literature reports. The α-glucosidase inhibitory activity of the extract, fractions and the isolated compounds was assessed spectrophotometrically at 400 nm using the yeast alpha glucosidase enzyme and the substrate 4-nitrophenyl α-D-glucopyranoside. Acarbose was used as the standard drug.

Our results showed that the ME, EAF, BuF, compounds **1** and **4** exhibited alpha glucosidase inhibitory activity (IC_{50}=1.30±0.05, 0.60±0.16, 1.25±0.20, 12.00±0.39 and 34.08±1.58 µg/ml respectively) better than acarbose (IC_{50}=1305±2.1 µg/ml). Compounds **2**, **3** and **5** did not show the enzyme inhibition. It is concluded that arjunolic acid and 3, 4, 3'-tri-*O*-methylellagic acid contribute to the alpha-glucosidase inhibitory activity of *C. dolichopetalum* root.

PM-216

Bioactivity-guided isolation of compounds with xanthine oxidase inhibitory activity from *Centaurea virgata*

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The genus *Centaurea (Asteraceae)* comprises about 500 species, and has been the object of numerous chemical and pharmacological studies, affording the identification of bioactive acetylenes, sesquiterpene lactones, flavonoids and lignans as the main characteristic secondary metabolites. *Centaurea virgata* Lam, a species native to western Asia, has been used in the

folk medicine for the treatment of allergy and gastric ulcers. The rationale of its traditional application has not been studied previously, therefore the present work aimed at the chemical-pharmacological evaluation of the plant.The aerial parts of the plant material was collected in Elazig (Turkey), and it was extracted with MeOH. The xanthine oxidase (XO) inhibitory activities of the MeOH extract and its subextracts (*n*-hexane, $CHCl_3$ and remaining MeOH-H_2O) were investigated in vitro. XO inhibitory activities were measured by the absorbance of the enzyme induced uric acid production from xanthine at 290 nm, using allopurinol as positive standard (IC_{50} 7.49±0.29 μM). Remarkable activity was exerted by the $CHCl_3$ extract (98.9±15.8 μg/mL), therefore constituents of this extract were analysed. Different purification steps, such as VLC, CPC, PLC and crystallization were used for the isolation, and ESIMS, NMR and LC-MS using authentic standards were applied for identification of the compounds. Sesquiterpenes, cnicin, 8α-hydroxysonchucarpolide and 8α-(3,4-dihydroxy-2-methylene-butanoyloxy)-dehydromelitensine, flavones, apigenin, eupatorin, 3'-methyleupatorin, hispidulin, salvigenin, and the flavonol isokaempferide were identified. The XO inhibitory activity of these compounds was investigated, and found that sesquiterpenes are inactive, and flavonoids containing 7-OMe group have no activity. However, 7-OH flavones, such apigenin and hispidulin exerted significant XO inhibitory effect with IC_{50} values of 0.99±0.33 μM and 4.88±1.21 μM, respectively, therefore these compounds are responsible for the XO inhibitory effect.

PM-217

Primin as antimycoplasmal metabolite from *Eugenia hiemalis*

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Mycoplasmas (mollicutes) are microorganisms without cell wall that have clinical importance for human health and in veterinary sciences, but so far just few antibiotics were found to be active against this class [1]. *Eugenia* species have shown antibacterial activity [2], so in this project bioguided fractionation of *Eugenia hiemalis* extracts in CH_2Cl_2 and methanol was performed. For the growth of bacterial strains, SP4 broth was used for *Mycoplasma capricolum* subsp.*capricolum*, *M. genitalium* and *M. pneumoniae* FH [3], and MLA broth for *M. hominis* [4]. Dichloromethanic extracts of leaves (DEL) and flower buds (DEF) were the most active; extracts of stems (DES and MES) were less active. Successive fractionation of extracts DEL and DEF over silica gel and evaluation of obtained fractions led to isolation of an active compound that was present in both extracts, identified as the benzoquinone primin. Minimal inhibitory concentration (MIC) ranged from 2.60 to 6.94 μg/mL (Table 1). There are several reports of biological activities for primin, including antibacterial activity [5], but this is the first report of activity of primin against bacteria without cell wall.

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4 Velleca WM, Bird BR, Forrester FT. Laboratory diagnosis of mycoplasma infections: course 8226-C. Atlanta: U.S. Department of Health, Education and Welfare; 1979: 120.

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Table 1. Antibacterial activity of extracts, fractions and isolated compound from aerial parts of *Eugenia hiemalis* tested against four species of *Mycoplasma*.

	MIC (µg/mL)			
	M. capricolum* subsp. *capricolum	***M. genitalium***	***M. hominis***	***M. pneumoniae* FH**
Extracts				
DEL	26.04	26.04	6,51	31.25
MEL	333.33	500	104.16	250
DES	166.67	416.67	26.04	104.16
MES	500	416.67	208.3	416.67
DEF	31.25	7.81	0.32	26.04
MEF	83.33	500	104.16	416.67
Active fractions				
L-5	46.87	125	4.55	125
F-7	3.46	6.94	0.40	7.80
Pure compound from L-5 and F-7	Non tested	6.94	2.60	3.47
Levofloxacin	1.56	0.78	25	1.56

PM-218

New triterpenoid saponins with cytotoxic and anti-inflammatory properties from *Weigela stelzneri*.

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Saponins are bioactive compounds occurring in many plant species having a wide range of biological activities such as immunoadjuvant, cytotoxic, analgesic, antimicrobial and anti-inflammatory activities, just to mention a few. The aim of our researches concerns the isolation, structural elucidation and biological investigation of saponins from various plant

species [1]. In this context, the study of the roots and leaves of *Weigela stelzneri* (Caprifoliaceae) led to isolation of four new and two known oleanane-type saponins mainly by medium pressure- and vacuum-liquid chromatography on silica gel (normal and reversed-phase RP-18) [1]. The new compounds were elucidated by a combination of spectroscopic techniques including 1D-, 2D-NMR, and mass spectrometry as glycosides of oleanolic acid, three of them being monodesmosides at C-3 with a branched oligosaccharidic chain made of six sugar units, whereas the known ones were hederagenin derivatives. The six compounds were evaluated for their cytotoxicity against a human cancer cell line (colorectal SW480) and a mouse cancer cell line (mammary EMT6), and for their anti-inflammatory activity. The results show that two new compounds differing only by the first sugar unit of the hexasaccharidic chain at C-3 (arabinopyranosyl instead of xylopyranosyl) exhibited the strongest cytotoxicity on both cell lines (IC_{50} 5.41 μM and 1.54 μM, respectively), which was better than those of the positive controls etoposide and methotrexate. Furthermore, they revealed a significant modulatory effect of the IL-1β production on human lymphocytes stimulated with endotoxins, showing therefore a strong anti-inflammatory potential.

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PM-219

A phenylpropenoic acid glucoside (PPAG) of *Aspalathus linearis* protects H9c2 cardiomyocytes against hyperglycemia-induced cell apoptosis

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Hyperglycemia is responsible for altered myocardial substrate metabolism and subsequent apoptosis in diabetic patients. Adult cardiomyocytes have a limited capacity to regenerate after an injury. Thus, protecting the myocardium against chronic hyperglycemia is crucial for cell survival. Phenylpyruvic acid-2-*O*-β-D-glucoside (PPAG) is one of the major polyphenolic compounds found in *Aspalathus linearis*. PPAG has been shown to protect the pancreatic β-cells from animals with elevated blood glucose levels against cell apoptosis. Therefore, this study investigates the potential anti-apoptotic effect of PPAG against hyperglycemia-induced cardiac injury. H9c2 cardiomyocytes exposed to either normal (5.5mM) or high (33 mM) glucose for 48hrs were treated with PPAG (1 μM) as well as a combination of metformin and PPAG at 1 μM for 12hrs. The efficacy of PPAG to reverse altered cardiac energy metabolism was investigated by measuring the uptake and oxidation of fatty acids. Mitochondrial membrane depolarization was assessed by measuring JC-1 fluorescence while apoptotic cell death was determined by annexin V/propidium iodide staining in addition to caspase 3/7 activity. Results showed that high glucose exposure resulted in an increased fatty acid uptake

and oxidation (35±4%, p<0.0001 and 39±7%, p<0.0001, respectively) when compared to the normal glucose control. In addition, an increase in membrane depolarization (38±4%, p<0.0001), annexin V/propidium iodide (36±2%, p<0.0001/ 37±5%, p<0.0001) and caspase 3/7 activity (32±6%, p<0.001) was observed. PPAG effectively reduced membrane depolarization (10.8±1%, p<0.0003), while treatment with a combination of metformin and PPAG had more effect in reducing annexin V/propidium iodide (14±3%, p<0.0005/ 12±2%, p<0.001) and caspase 3/7 activity (8±1%, p<0.007). Hence, suggesting that a combination of metformin and PPAG might be an effective treatment to protect the myocardium against high glucose-induced cell apoptosis.

PM-220

6-Methoxyflavonol glycosides with *in vitro* hepatoprotective activity from *Chenopodium bonus-henricus* roots

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In Bulgarian folk medicine the roots of *Chenopodium bonus-henricus* L. are known as "chuven" and have been applied externally to treat skin iflammations, wounds and boils. The infusion of the drug has been also used as a mild laxative. In Bulgarian food industry the aqueous extract of the roots has been employed in production of "halva". One new, namely 6-methoxykaempferol 3-*O*-[*β*-apiofuranosyl(1→2)]-*β*-glucopyranosyl(1→6)-*β*-glucopyranoside **2**, and two known flavonoid glycosides, spinacetin 3-*O*-[*β*-apiofuranosyl(1→2)]-*β*-glucopyranosyl(1→6)-*β*-glucopyranoside **1** and spinacetin 3-*O*-gentiobioside **3**, were isolated from the roots of *Chenopodium bonus-henricus* L. Their structures were determined by means of spectroscopic methods (1D, 2D NMR, UV, IR, and HR-ESI-MS). Additionally, **1** and **3** significantly reduced the cellular damage caused by the hepatotoxic agent CCl_4 in rat hepatocytes and preserved cell viability and GSH level, decreased LDH leakage and reduced lipid damage. Effects were similar to those of the positive control silymarin. Control of self-toxic effects were done in a MTT based assay using HepG2 cells and revealed a statistically significant cytotoxic effects only in very high concentrations exceeding mM concentrations and an incubation time of 72 h, making flavonoid glycosides with a 6-methoxykaempferol skeleton a promising and save class of hepatoprotective compounds.

PM-221

Anti-nociceptive and anti-inflammatory effects of the leaf of *Ilex latifolia*

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Ilex latifolia Thunb. (Aquifoliaceae), widely distributed in China, has been used as a functional food and drunk for a long time. Studies have identified the bioactive constituents with antioxidant, antitumor, and anti-inflammatory properties from *I. latifolia*. Previously *I. latifolia* showed neuroprotective effect through anti-oxidative and anti-inflammatory action in stroke and Alzheimer disease models [1, 2]. The purpose of the present study is to investigate anti-nociceptive and anti-inflammatory effects of ethanol extract of the leaf of *I. latifolia*. Writhing responses induced by acetic acid and formalin- and thermal stimuli-induced pain responses for nociception were evaluated in mice. *I. latifolia* (50-200 mg/kg, p.o.) and ibuprofen (100 mg/kg, p.o.), a positive non-steroidal anti-inflammatory drugs (NSAIDs), inhibited the acetic acid-induced writhing response and the second phase response (peripheral inflammatory response) in the formalin test, but they did not protect the thermal nociception in tail flick and hot plate tests and the first phase response (central response) in the formalin test. These results suggest that *I. latifolia* could peripherally, but not centrally, inhibit prostaglandin-induced algesia. *I. latifolia* (50 and 100 μg/ml) and 3,5-di-caffeoyl quinic acid methyl ester (5 μM), isolated from *I. latifolia* as an active compound, significantly inhibited LPS-induced nitric oxide production and mRNA expression of pro-inflammatory mediators, iNOS and COX-2, and pro-inflammatory cytokines, interleukin-1-beta and interleukin 6, in RAW 264.7 cell line. These results suggest that *I. latifolia* could produce anti-nociceptive effect peripherally, but not centrally, via anti-inflammatory activity. Furthermore, these effects of *I. latifolia* may be partly attributable to 3,5-di-caffeoyl quinic acid methyl ester.

[1] Kim et al. (2011) J. Ethnopharmacol. 133. 558-564.

[2] Kim et al. (2012) Arch. Pharm. Res. 35. 1115-1122.

PM-222

Antioxidant and immune- enhancing potentials of leaf extract and active constituents of *Millettia aboensis*

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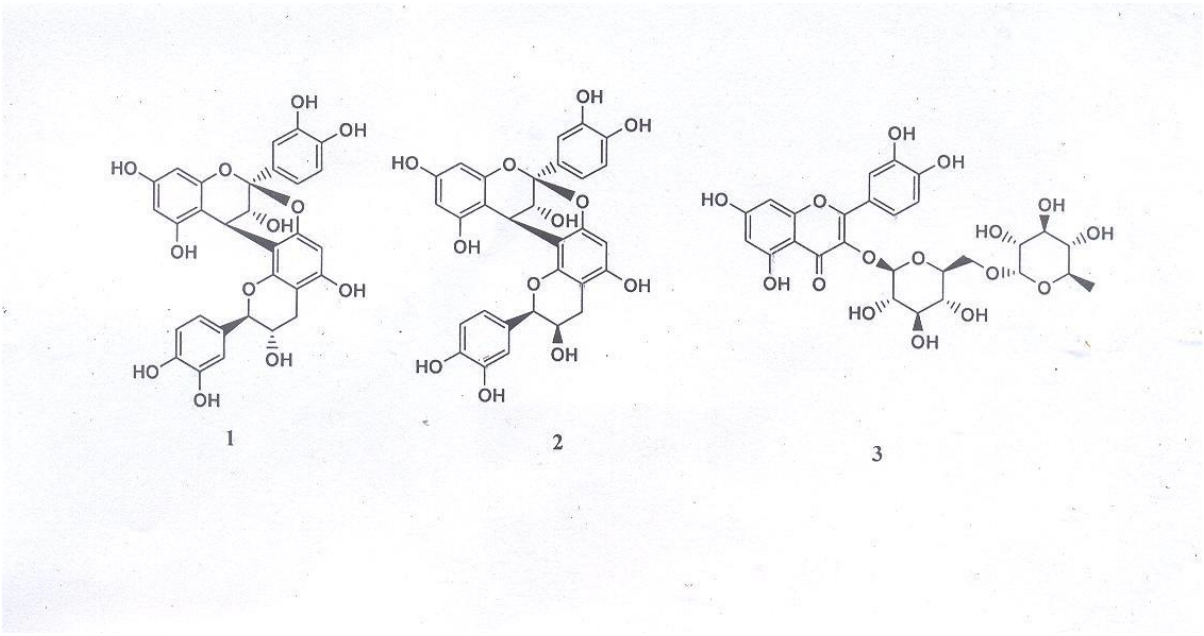
Antioxidant and immune-enhancing potentials of ethanol leaf extract of *Millettia aboensis* (Hook F.) Baker (Leguminosae), fractions and isolated compounds were determined using *in vitro* and *in vivo* models. *In vitro* antioxidant activities were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and hydrogen peroxide scavenging activity tests; while i*n vivo* protection against oxidative damages was assessed by carbon tetrachloride (CCl_4)-induced liver damage and streptozotocin (STZ) induced systemic

oxidative stress models. In vivo immune enhancing properties were monitored using primary and secondary immune responses to tetanus toxoid. Bioassay guided separations led to the isolation of compounds **1**, **2** and **3**. Their structures were elucidated by a combination of 1D and 2D NMR and mass spectrometry. *In vitro* inhibition of liver microsome lipid peroxidation was used to evaluate the antioxidant activity of compounds **1** and **2** while stimulation of specific T-lymphocytes was used for evaluating immune enhancing activity of compound **3**. The extract exhibited both antioxidant and immune-enhancing properties however, antioxidant activity was prominent in the ethyl acetate fraction, while butanol fraction expressed more immune-enhancing activity. Structural elucidation revealed compounds as catechin (procyanidine A1) (**1**), epicatechin (procyanidine A2) (**2**) and quercetin-3-*O*-rutinoside (rutin) (**3**). Compounds **1** and **2** demonstrated strong inhibition of liver microsome lipid peroxidation, with EC50 of 46 and 55 µg/ml respectively against 31 µg/ml of α-tocopherol. Compound **3** showed up-regulation of specific CD4+ lymphocytes with up to 38% stimulatory effect of IFNγ at 6.25 µg/ml compared to the baseline effect in DMSO control group. The extract, fractions and isolated compounds from *M. aboensis* expressed strong antioxidant and immune-enhancing properties which may be responsible for its ethnopharmacological use for general healing.



PM-223

Protective effects of *Nasturtium officinale* extracts against genetic damage induced by cyclophosphamide in mice bone marrow cells

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Chemotherapy drugs are toxic for tissues which have high growth and proliferation. Bone marrow toxicity is one of the major side effects of cyclophosphamide [1]. Brassicaceae vegetables are rich sources of glucosinolates [2] which their anticancer effects have been reported [3]. Watercress (*Nasturtium officinale* W.T. Aiton) is used as an edible vegetable in various parts of the world including Iran. The present study investigated the protective effects of methanolic and aqueous extracts of *N. officinale* against cyclophosphamide-induced genetic damage in mice bone marrow cells using micronucleus assay. The mice were divided into 14 groups with five mice per group. Group 1 (negative control) received 10 ml/kg body weight (bw) of saline solution and Group 2 (positive control) received cyclophosphamide 40 mg/kg bw intra-peritoneally (IP). The other groups were treated IP with the methanolic and aqueous extracts at doses of 20, 50 and 100 mg/kg bw for 15 days (sub acute) and 2h (acute) before injection of cyclophosphamide. The aqueous extract significantly ($P<0.001$) reduced micronucleated polychromatic erythrocytes count at all doses administrated compared to the control which was more effective than the methanolic extract. There was no significant difference between the total phenolic and flavonoid contents in aqueous and methanolic extracts. As a result, it seems glucosinolates in aqueous extract may be responsible for these considerable protective effects. Our results suggest that watercress can be used in the diet of people who receive chemotherapy drugs.

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[3] Park NI, Kim JK, Park WT, Cho JW, Lim YP, Park SU. An efficient protocol for genetic transformation of watercress (Nasturtium officinale) using Agrobacterium rhizogenes. Mol Biol Rep 2011; 38: 4947-4953

PM-224

Protective effects of *Dioscorea membranacea* rhizome ethanolic extract against doxorubicin-induced genotoxicity in human lymphocytes *in vitro*

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Dioscorea membranacea rhizome is commonly used as an ingredient in traditional Thai remedies for inflammatory diseases and cancers. Our previous study reported that the ethanolic extract of *Dioscorea membranacea* rhizome (EEDM) was selectively cytotoxic to lung and breast cancer cell lines [1]. However, the underlying mechanisms of its action are still unclear. One possible mechanism might be their anti-genotoxic activities. To investigate the anti-genotoxic potential of the EEDM, *in vitro* chromosome aberration assay was conducted, testing EEDM against doxorubicin (DXR), a potent genotoxic compound. Genotoxicity indicated by chromatid-type and chromosome-type aberrations and cytotoxicity as shown by mitotic index were analyzed. Human lymphocytes were pretreated with various concentrations of EEDM (0.005-50 ng/ml) followed by 0.1 μg/ml DXR. Plain RPMI and DXR were used as negative and positive controls, respectively. The results demonstrated that EEDM pretreatment at 0.5 ng/ml significantly decreased DXR-induced chromatid-type aberrations by a factor of 4.8. Other EEDM pretreatments at 0.05 and 5 ng/ml also tended to decrease DXR-induced chromatid-type aberrations, although they were not statistically significant. Chromosome-type aberration and cytotoxicity was not detected at all EEDM pretreatments. The outcomes revealed that *in vitro* EEDM pretreatment at a specific dose has anti-genotoxic properties against genotoxic hazards such as DXR in human cells. Therefore, EEDM might be useful not only for anti-cancer treatment but also for cancer prevention. Further *in vivo* anti-genotoxic studies are needed to confirm this *in vitro*.

Acknowledgements: This study was supported by Research Fund, National Research University Project of Thailand. Office of Higher Education Commission and Thammasat University, Thailand.

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PM-225

Simultaneous determination and anti-allergy effects of sesquiterpene lactones in *Aucklandia lappa* Decne

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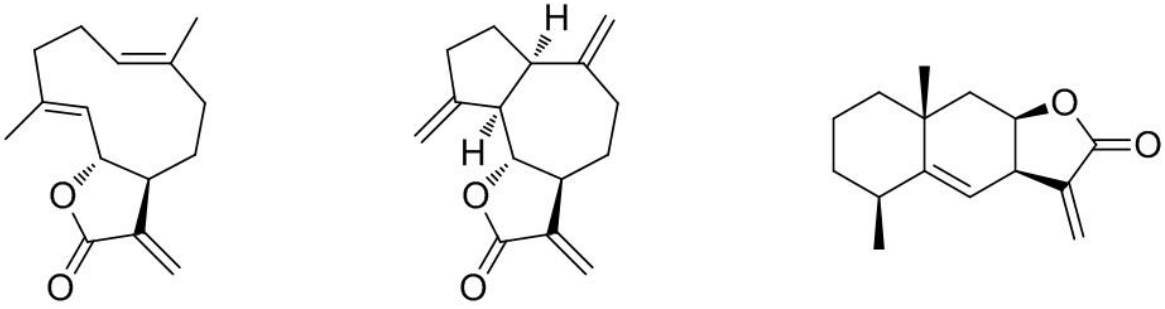
Aucklandia lappa Decne, a well-known traditional herbal medicine, is used for the treatment of asthma, rheumatism, coughs, tuberculosis, and many other diseases. We performed simultaneous analysis of three sesquiterpene lactones, costunolide (1), dehydrocostus lactone (2), and alantolactone (3), obtained from a 70% methanol extract of *A. lappa* using high-performance liquid chromatography–photodiode array techniques. In addition, we determined the inhibitory effects of the three components on the expression of chemokine mRNA in

human keratinocyte HaCaT cells. The established analytical method showed high linearity, with a correlation coefficient ≥ 0.9999. The limit of detection and the limit of quantification of compounds 1–3 were 0.06–0.13 mg/mL and 0.21–0.42 mg/mL, respectively. The recovery of the compounds 1–3 was 97.27–103.00%. The intra- and inter-day relative standard deviations were 0.09–0.97% and 0.09–1.06%, respectively. Treatment with compounds 1–3 caused a significant reduction in the levels of mRNA for a range of chemokines, including thymus and activation-regulated chemokine (TARC/CCL17) and macrophage-derived chemokine (MDC/CCL22), and regulated on activation normal T-cell-expressed and secreted (RANTES/CCL5) chemokine and interleukin-8 in tumor necrosis factor-α and interferon-γ-stimulated HaCaT cells. We suggest that compounds 1–3 may be the active components of *A. lappa* that exhibit anti-atopic effects.



Costunolide (**1**) Dehydrocostuslactone (**2**) Alantolactone (**3**)

PM-226

New 2-(2-phenylethyl)-4*H*-chromen-4-one derivative and bioactive constituents from the stem bark of*Aquilaria sinensis*

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Aquilaria sinensis (Lour.) Gilg. (Thymelaeaceae) is an evergreen tree and endemic to China. It has been used for the treatment of abdominal pain, vomit and asthma in Chinese folk medicine. In our studies on the anti-inflammatory constituents of Formosan plants, many species had been screened for *in vitro* anti-inflammatory activity, and *A. sinensis*was found to be one of the active species. Investigation on active EtOAc-soluble fraction of this plant led to the isolation of a new 2-(2-phenylethyl)-4H-chromen-4-one derivative, aquilarichromone A (**1**), together with 12 known compounds, 5-hydroxy-7,3',4'-trimethoxyflavone (**2**), velutin (**3**), sakuranetin (**4**), apigenin 7,4'-dimethyl ether (**5**), tricin (**6**), 6,7-dimethoxy-2-(2-phenylethyl)chromone (**7**), 2,6-dimethoxy-1,4-benzoquinone (**8**), methyl 3,4-dihydroxybenzoate (**9**), β-sitosteone (**10**), β-sitosterol (**11**), blumenol A (**12**), and α-tocospiro A (**13**). 5-Hydroxy-7,3′,4′-trimethoxyflavone (**2**), velutin (**3**), and sakuranetin (**4**) exhibited potent inhibition against fMLP-induced superoxide ($O_2^{\bullet-}$) production with IC_{50} values of 1.54±0.31, 0.56±0.11, and 0.50±0.05 μg/ml, respectively. The structures of new compound **1** was determined through spectral analyses including extensive 2D NMR data. This meeting describes the structural elucidation of 1 and the anti-inflammatory activities of the isolates.

PM-227

In vitro anticariogenic effects of *Rubus caesius* extracts and their quality evaluation by HPLC-DAD-MS^{3}analysis

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The genus *Rubus* (Rosaceae) comprises around 700 species, naturally occurring in temperate climates. *Rubus* species have been used in traditional medicine for their many medicinal properties [1, 2]. In this study, for the first time, we investigated*in vitro* inhibitory effects of *R. caesius* extracts and their sub-fractions against mutans streptococci. The diethyl ether sub-fraction showed bacteriostatic and bactericidal activities, with minimum inhibitory concentration (MIC) of 1 mg/mL and minimum bactericidal concentration (MBC) in the range of 2-4 mg/mL. Furthermore, the diethyl ether fraction inhibited biofilm formation by Streptococci in dose-dependent manner. However, it was also found that all *R. caesius* preparations exhibited diverse inhibitory effects on *de novo* synthesis of water-insoluble and water-soluble α-D-glucans by glucosyltransferases of mutans group streptococci. The examination of phytochemical profile of investigated samples using HPLC-DAD-MS^3 revealed that all extracts contained polyphenols: tannins or flavonol derivatives as major constituents. Among identified polyphenolic compounds ellagitaninns, especially sanguniin H-6 or kaempferol and quercetin derivatives have been described as dominating. The results demonstrate that *R. caesius* extracts could become useful supplements for pharmaceutical products as a new anticariogenic agent in a wide range of oral care products.

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PM-228

In vitro biological evaluation of polyurethane microstructures genistein based formulation

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Natural products provide a wide range of active agents for an increased number of pathologies. World Health Organization (WHO) reports that a percentage of 60% to 80% of population call for natural medicine in case of primary healthcare needs [1]. The isoflavone genistein (4',5,7-trihydroxyisoflavone) is an example of natural compound included in both preclinical and clinical tests for anti-osteoporotic, cardio-protective, photo-protective, anti-inflammatory, antioxidant and anti-cancer properties [2]. Polyurethane microstructure can offer: (1) protection of the biologically active compound to external agents (UV radiation, strong acidic or alkaline environments, etc., (2) the possibility of amending the lipo- or water-solubility of encapsulated compounds,(3) drug delivery targeted to a specific receptor, (4) retarded activity of the encapsulated drug by the use of transport vehicles having low speed of degradation [3,4]. Since finding such a formulation for genistein could improve its applications, we have conducted a preliminary study regarding the *in vitro* antiproliferative (MCF7, MDA-MB-231 and T47D) and antimicrobial *(Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis* (D), *Bacillus subtilis, Bacillus cereus,* and *Candida albicans*) activity in order to test whether polyurethane microstructures represent a good option for further modulation of genistein's bioavailability. The study concludes that polyurethane microstructures represent a bad in vitro partner for the isoflavone genistein.

Acknowledgement: The study was financed by the UMFT grant -Parteneriate în cercetarea fundamentală inovativă-PIII-C2-PCFI-2015/2016 acronim FLAVOFORM.

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PM-229

Anti-inflammatory and wound healing activity of *Euphorbia characias* and bioassay-guided isolation of some flavonoids

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Euphorbia is the largest genus in the Euphorbiaceae family, which contains at least 2000 species [1]. *Euphorbia* species, containing diterpenoids, flavonoids, volatile compounds and tannins [2], are known to have cytotoxic, antitumor, antibacterial, anti-inflammatory and anti-HIV activity [3], and some of them are used in folk medicine to treat skin diseases and wounds. In this study, *n*-hexane, ethyl acetate and methanol extracts of the aerial parts of *Euphorbia characias* L. were successively prepared and evaluated for their wound-healing and anti-inflammatory properties. Methanol extract of *E. characias* herba, the most potent extract that has been identified, was fractionated by bioassay-guided fractionation, and column chromatography was used for separation. Fractions exhibiting similar chromatographic profiles were combined and tested for their activities. The fractions with the highest activity were subjected to further chromatographic separation to obtain the active compounds quercetin-3-*O*-rhamnoside and quercetin-3-*O*-arabinoside. Chemical structures of these compounds were determined by means of spectroscopy (^{1}H, ^{13}C and 2D-NMR, MS). Isolated compounds were found to be responsible for the activity. Acetic acid-induced increase in capillary permeability test was used for the evaluation of anti-inflammatory activity, and linear incision and circular excision wound models were used for the evaluation of wound-healing activity.

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PM-230

Evaluation of sulphated polysaccharide-protein complexes isolated from marine algae on colon carcinoma by MTT

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Cold and hot extraction methods (CEM and HEM) were used for polysaccharides from *Dictyopetris membraceae, Padina pavonia, Colpomenia sinusa, Ulva fasciata, Enteromorpha intestinalis, Corallina officinalis, Petrocladia capillraceae,Jania rubes* and *Spirulina platensis.* These polysaccharides were tested against a colon carcinoma cell line (HCT115) at various concentrations (100-6.26 µg/ml) using the MTT assay.

The polysaccharides were characterized as sulphated polysaccharide-protein complexes. The hot polysaccharide extract of *J. rubens* and *D. membranacea* exhibited the most potent cytotocicity against HCT115 with an IC_{50} of 24.7 and 38.2 µg/ml, respectively. The other polysaccharides showed various cytotoxic activity against HCT115 with an IC_{50} ranging from 60.8-79.8 µg/ml. Therefore the sulphated polysaccharides isolated from macroalgae might become important sources for drug development for treatment of colon carcinoma.

PM-231

Effects of 25-*O*-acetyl-23,24-dihydro-cucurbitacin F on cell viability, cell cycle distribution and apoptosis induction in human soft tissue sarcoma cells.

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Soft tissue sarcomas represent a rare group of malignant tumors frequently exhibiting increased metastatic potential and chemotherapeutic resistance. Treatment is often unsuccessful or the efficacy limited. Therefore, there is an urgent need for the discovery of new lead compounds. In this study, we investigated the effects of 25-*O*-acetyl-23,24-dihydro-cucurbitacin F (ADCF) on cell viability, cell cycle distribution and apoptosis induction in three different sarcoma cell lines. The compound was previously isolated as most active constituent from *Quisqualis indica* L. (Combretaceae) which gained interest as a result of a systematic bioactivity-based screening of plants used in traditional Chinese medicine [1]. In soft tissue sarcoma cells, ADCF reduced cell viability concentration dependently (IC_{50} values after 48 h: SW-872: 16.2 µM; SW-982: 4.3 µM; TE-671: 1.2 µM). In SW-872 and TE-671 cells, ADCF additionally arrested the cells at the G2/M interphase and led to a significant reduction of cyclin B1, cyclin A, CDK1, CDK2 and survivin. Moreover, it induced apoptosis caspase-3 dependently [2]. However, in SW-982 cells, the cell cycle was only slightly changed and caspase-3 was not activated when treated with the IC_{50}.

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PM-232

The inhibitory effect of peudoshikonin I from Lithospermi radix on matrix-metalloproteinase (MMPs) production and expression in interleukin-1β induced SW1353 chondrosarcoma cells

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A new compound, named as pseudoshikonin I, was isolated from the 70% ethanol extract of Lithospermi radix. The structure was identified as 1-(1,7-dihydroxynaphthalen-3-yl)-4-methylpent-3-enyl-3-methylbut-2-enoate, by means of spectroscopic methods, including HR-FAB/MS, 1D NMR (^{1}H, ^{13}C, DEPT), and 2D NMR (gCOSY, gHSQC, gHMBC, NOESY) spectroscopic analysis. We evaluated the inhibitory effect of pseudoshikonin I (PS) on matrix-metalloproteinase (MMPs) [1] production and expression in interleukin-1β induced SW1353 chondrosarcoma cells. Following treatment with PS (50, 100 μM), active MMP-1, -2, -3, -9, and 13 were quantified in the SW1353 cell culture supernatants using a commercially available ELISA kit. PS treatment effectively inhibited the production of MMPs. The gene expression of MMP-1, -2, -3, 9 and 13, TIMP-2, iNOS and COX-2 in SW1353 cells was investigated by RT-PCR[2]. The MMP-9 (74.2±1.4-fold), MMP-13 (70.2±3.9-fold), iNOS (74.7±2.6-fold) and COX-2 (85.9±3.2-fold) were suppressed by PS treatment in a dose dependent manner. The TIMP-2 mRNA expression was significantly up-regulated by PS (100 μM) treatment compared to the control groups (100-fold). Therefore, pseudoshikonin I from Lithospermi radix might be used to protect cartilage by the inhibitory effect on the production of MMPs as a potential natural ant-inflammatory or anti-osteoarthritis agent.

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PM-233

Determination of Amaryllidaceae Alkaloids in Snowdrop (*Galanthus elwesii*) from Taurus Mountains

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The family Amaryllidaceae, well known for their ornamental bulbous members and its chemically important and bioactive alkaloids, has attracted the interest of phytochemists. Among these alkaloids, galanthamine has been known as an anticholinesterase inhibitor and it is used in the treatment of mild to moderate Alzheimer's disease. Moreover, antitumor, anti-inflammatory and antiprotozoal alkaloids have been isolated from different Amaryllidaceae species (1). Among the Amaryllidaceae genera, *Galanthus* L. is represented in 14 taxa and one hybrid in Turkey (2). In this study, the aerial parts and bulbs of *Galanthus elwesii* Hook. was collected from Akseki, Antalya. The alkaloid profile of the samples was determined by gas chromatography-mass spectroscopy (GC-MS) using Thermo GC-Trace Ultra Ver: 2.0., Thermo MS DSQ II. A TR–5 MS (30 m x 0.25 mm x 0.25 μm) column was used and GC-MS system was operated in the electron impact mode (electron energy of 70 eV). Compound identification was performed with the help of the National Institute of Standards and Technology (NIST) standard library and, where available, by comparison with the retention times and mass fragmentation patterns of standard reference compounds. Totally, twenty-four different compounds were detected. Haemanthamine, galanthindole and 6-*O*-methylpretazettine have been found as major constituents, whereas galanthamine was detected in very small amounts in the tested samples.

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PM-234

Hexosomes based on cholesterol and phosphatidylcholine: a new drug delivery system for curcumin release.

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Hexosomes are lyotropic liquid crystalline systems with the ability to achieve a sustained release of the encapsulated drug, possess bio-adhesive properties, can solubilise both hydrophilic and lipophilic drugs. The LLC are complex systems with a thermodynamically stable morphology and with a wide range of sizes [1]; with the properties of both liquids (fluidity) and solids (organized crystalline structure and optical anisotropy) [2]. In the present study hexosomes were prepared and optimized using natural lipids, phosphatidylcholine and cholesterol. TEM analysis and Small angle X-ray diffraction profiles confirmed the presence of the hexagonal phase. Curcumin, the main biologically active component from the rhizome of Curcuma longa (Zingiberaceae), is a polyphenolic compound insoluble in water, having a low bioavailability [5]. 1, 5 and 10% w/w of curcumin was added to the hexosome formulation. TEM analysis showed that only 1% curcumin w/w was compatible with the hexagonal systems, while at higher concentrations a collapse of the system was evident. Addition of curcumin didn't change the size of the two populations (350±6.6nm and 100±4.5nm). Drug-entrapment efficiency was 51±0.12%. Release studies conducted at pH 1.2 demonstrated that the developed hexosomes are able to achieve a "sustained release" of curcumin. The developed new hexosomes suggest a potential application not only limited as drug carriers, but also for food, dietary supplements and cosmetic formulations.

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PM-235

The nutritional composition and anti-obesity effects of an herbal mixed extract containing *Allium fistulosum* and *Viola mandshurica* in high-fat-diet-induced obese mice

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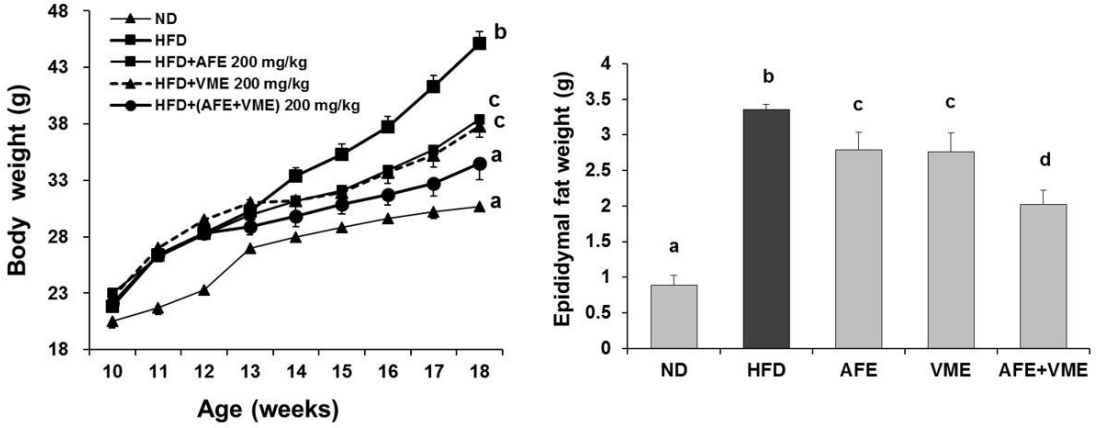
Obesity is a serious public health issue and plays a critical role in the pathogenesis of hypertension, dyslipidemia, and diabetes [1]. This study investigated the nutritional composition and anti-obesity effects of an herbal extract containing*Allium fistulosum* and *Viola mandshurica* (AFE+VME) in high-fat-diet (HFD)-induced obese mice. In traditional oriental medicine, *A. fistulosum* and *V. mandshurica* are considered to be effective in promoting blood circulation to remove blood stasis [2]. The nutritional analysis revealed that this mixed extract is high in carbohydrate (72.2 g/100 g) and protein (11.5 g/100 g); low in fat (1.7 g/100 g); rich in vitamins E (4.8 mg/100 g), B_1 (14.8 mg/100 g), B_2 (1.0 mg/100 g), niacin (7.9 mg/100 g), and folic acid (1.57 mg/100 g); and rich in minerals such as calcium (600 mg/100 g), iron (106.1 mg/100 g), and zinc (5.8 mg/100 g). The oral administration of AFE+VME in mice reduced body weight, tissue weight, adipocyte size, and lipid accumulation in the liver compared with HFD control mice. AFE+VME also decreased serum triglyceride, total cholesterol, and leptin concentrations. Furthermore, AFE+VME markedly increased the mRNA expression of peroxisome proliferator-activated receptor-γ (PPAR-γ), uncoupling protein-2 (UCP-2), and adiponectin and decreased leptin expression in the epididymal adipose tissue. Our results suggest that the extract containing *A. fistulosum* and *V. mandshurica* improved lipid metabolism via the up-regulation of PPAR-γ, UCP-2, and adiponectin expression and the down-regulation of leptin in HFD-induced obese mice. Therefore, the extract containing *A. fistulosum* and *V. mandshurica* may be a potentially effective therapy for obesity and its related metabolic disorders.



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PM-236

Population health risk due to the consumption of medicinal plant *Malva Sylvestris* collected in the contaminated sites by the toxic heavy metals, North Algeria

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In order to assess the possible health risks associated with consumption of medicinal plants collected from waste disposal sites (WDS), industrial site (IS) and waste site (WS), the state of metal traces in *Malva sylvestris* L., growing in contaminated sites have been the subject of study. Soil samples from different locations and different parts of the plant were collected and analyzed. The determination of trace levels was carried out in the different organs of the plant and crop soils. The samples were taken in winter, spring and summer. and analyzed for the evaluation of trace metals by atomic absorption spectrometer (AAS). From the leaves of plants, the copper concentration was found to be significantly higher than all other trace metals. The trend of trace metal accumulation of in the leaves was in order Cu, Zn, Pb, Cd and Cr. Consumption of the leaves of M. sylvestris in traditional medicine can be a health risk because the grades obtained are higher than those provided by WHO [1].

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PM-237

Comparison of the free radical-scavenging activity of Thai stingless bee propolis

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Propolis has been wildly applied as medicine and dietary supplement because of its broad biological activities including anti-inflammatory, immunomodulatory and antioxidant activity. Propolis from the stingless bees (Apoidae) such as*Lepidotrigona ventralis* Smith, *Lepidotrigona terminata* Smith, and *Tetragonula pagdeni* Schwarz, has been used as indigenous medicine and marketed in several preparations in Thailand. However, the biological activities of each type of propolis generally depend on the bee species. Thus, in this study, free radical scavenging activities of the propolis from these three stingless bee species in the same area of mangosteen garden were performed by FRAP, ABTS and DPPH assay. All extracts were observed as viscous crude and amber products. The propolis extracts from the stingless bee were able to exhibit free radical scavenging activity. The extract from *T. pagdeni*

illustrated significant highest activity with FRAP value 389.07±26.02 μmol Fe^{2+}/gram extract, and IC_{50} values were 59.52±10.76, and 122.71±11.76 μg/mL using ABTS and DPPH assay, respectively. In addition, the major compounds from *T. pagdeni* propolis extract was separated and identified as alpha mangostin and gamma mangostin that may be play an important role in the biological activity of the propolis. Therefore, *T. pagdeni* propolis was suggested as a suitable raw material for indigenous usage and further development as pharmaceutical products.

PM-238

Free radical scavenging activity of *Pluchea indica, Rhizophora mucronata* and *Rhizophora apiculata* edible leaf extracts

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Mangrove forest is valuable resource for food and herb. *Pluchea indica* Less, *Rhizophora mucronata* Lam. and *Rhizophora apiculata* Blume are wetland plants and have been used as vegetable, medicine and also applied as herbal tea for nutraceutical purpose. Thus, the study investigated the aqueous leaf extracts of these herbs on free radical scavenging activity. Total phenolic content, ferric reducing antioxidant power assay (FRAP), DPPH and ABTS scavenging assay were determined. All extracts exhibited free radical scavenging activity. *P. indica* extract demonstrated the strongest activity on every assay; 38.61 ± 0.62 g gallic acid equivalent/ 100 g extract for total phenolic content, 89.12±3.37 mmol $FeSO_4$equivalent/ 100 g extract for FRAP assay, IC_{50} 21.03±2.76 and 53.24±2.16 μg/ml, for DPPH and ABTS assay, respectively. From chemical fingerprint of *P. indica* aqueous extract, the present of abundant flavonoids and phenolics was possibly responsible for free radical scavenging activity.

PM-239

Anti-obesity effects of *Phyllostachys pubescens* and *Scutellaria baicalensis* complex

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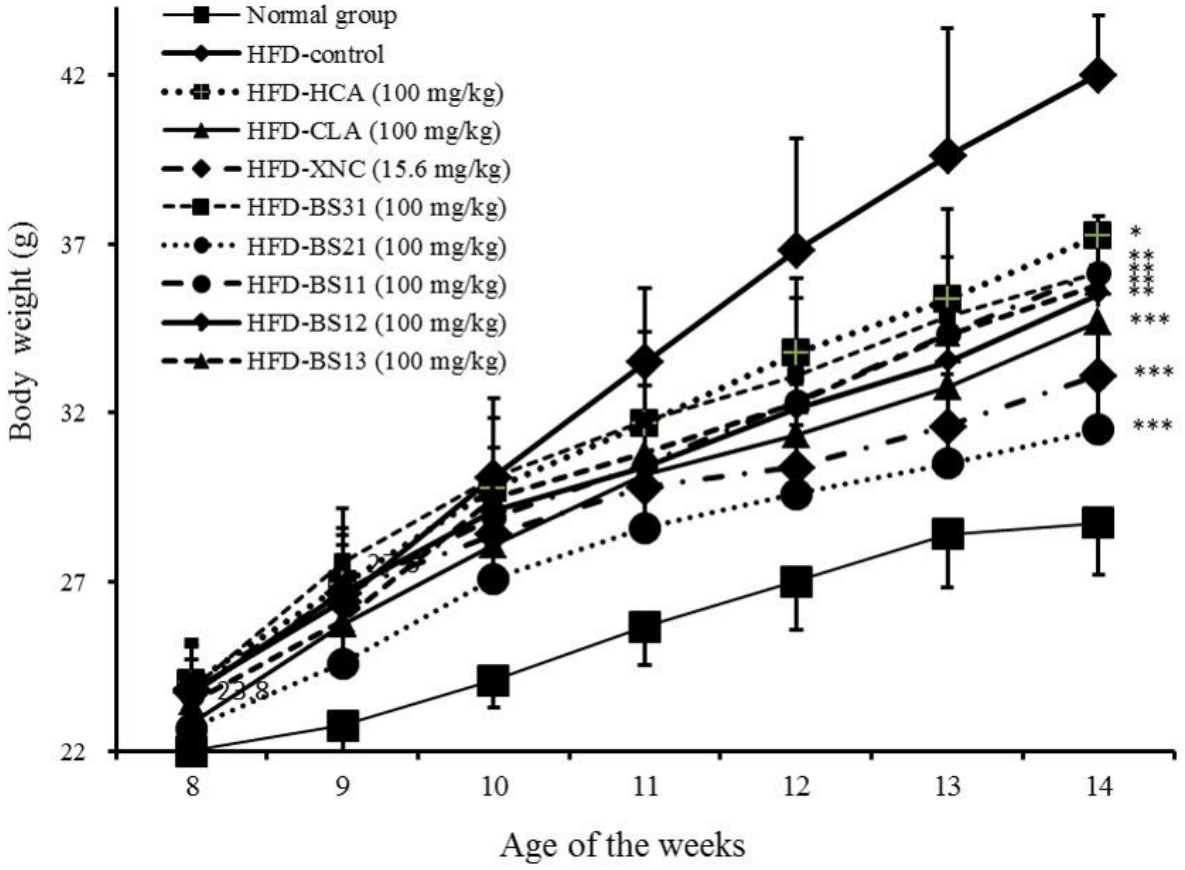
Anti-obesity drugs that have been developed so far have limited efficacies and considerable adverse effects affecting tolerability and safety. Therefore, most anti-obesity durgs have been withdrawn. We tried to develop an anti-obesity agent by combinations from herbs that are used in food ingredients as well as in traditional medicines. In our study, the ethanolic extracts from Bamboo (*Phyllostachys pubescens*) leaf (BL) and *Scutellaria baicalensis* (SB) and their 1:1 combination (BLSB) was evaluated on high fat diet induced obese mice. BLSB group showed significant reductions in body weight gain and fat weights of liver and epididymal adipose tissue compared to BL or SB alone as well as control. Total-cholesterol and LDL-cholesterol levels significantly decreased, and HDL-cholesterol level increased. In liver tissue, macrovesicular steaotisis was remarkably improved and its fat cell size was also significantly

decreased. We have further investigated the anti-obesity effects by various combinations of the BL and SB as follows: BS31, BS21, BS11, BS12, and BS13 were formulated by mixing B and S at a ratio of 3:1, 2:1, 1:1, 1:2, and 1:3. All of these combinations in obesity mice reduced body weight, lipid accumulation in liver and adipose tissue as well as adipocyte size, compared to the high fat diet control mice. Among these combinations, the 2:1 combination (BS21) showed a remarkably effect. These results suggest that the combination preparations of bamboo leaf and *S. baicalensis*, especially their 2:1 combination, have anti-obesity effects with synergistic effect compared to bamboo leaf or *S. baicalesis*.



PM-240

Curcumin complexed in a new PAMAM dendrimer to increase stability and solubility

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Dendrimers are one of the most promising innovative polymeric nanocarrier for different bioactives [1]. In continuing our studies on the development of innovative nanocarriers with curcumin, a very key natural product from turmeric with numerous biological activities but low bioavailability [2], we now report on the development and optimization of a new PAMAM

dendrimer. Dendrimers are core-shell nanostructures with precise architecture and low polydispersity, which are synthesized in a layer-by-layer fashion ("generation") around a core unit, resulting in high level of control over size, branching points and surface functionality. The new PAMAM dendrimer was synthesized through divergent method, using benzylamine and methylacrylate, followed by the synthesis of the amide with ethylendiamine and finally a Michael addition with methylacrylate [1]. Size was 176.7±7.3 nm, a Pd was 0.33±0.06. According to TEM, globular shape dendrimer aggregates of 96.66 nm are present. Curcumin complexation did not affect the structure of dendrimers. Encapsulation efficiency and loading capacity were very satisfactory, 88% and 21% respectively. Stability of curcumin complexed with the dendrimer was evaluated by HPLC and resulted satisfactory during three months analysis. The release studies of curcumin from dendrimer showed a slow release profile, which is essential for a sustained and prolonged activity. Dendrimer did not show cytotoxicity in MCF-7 cells while curcumin had an IC_{50} of $3x10^{-5}$ M. Studies with the complexed formulation are ongoing.

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PM-241

Polyphenol content and free radical scavenging activity of bee pollen collected in Castelo Branco, Portugal

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Bee pollen is a health food with nutritional and therapeutic properties.The aim of this work was to evaluate free radical scavenging activity (FRSA) in selected samples obtained from local beekeepers in Castelo Branco (Portugal). The identification of the floral origin was performed using acetolysis method.

Each sample of bee pollen (0.10 ± 0.01 g) was extracted with methanol, ethanol and water [1, 2] to evaluate which solution provided the best extract. All the experiments were analysed in quadruplicate. The total polyphenols content of bee pollen was analysed using

spectrophotometry at 725 nm using the Folin–Ciocalteu reagent with Ferulic acid as a standard. FRSA was evaluated according to the DPPH $^{\bullet}$ and ABTS $^{\bullet+}$ methods.

In relation to the content of total polyphenols, the FRSA values varied considerably. Different floral species present species-specific activity [1] but these are dependent on the analytical method and the extraction solvent. On average, the highest polyphenol content was observed in methanol bee pollen extracts with the exception of the mixture B and the *Echium* sp pollen (Table 1). For the total FRSA with DPPH and ABTS methods, ethanol pollen extracts show higher activity with the exception of *Trifolium* spp. where the aqueous extract gives the higher result (Table 1). Mixture B and C ethanolic extracts give the best FRSA values.

The ANOVA shows for the three methods that there are significant differences between solvent extracts and protocols, however the variation between the solvent extracts is similar in the different procedures.

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Table 1 - Polyphenol content and free radical scavenging activity of pollen extracts in different solvents

	Extract	*Echium* sp	*Trifolium* spp.	*Mixture A*	*Mixture B*	*Mixture C*
Polyphenol content (FAE/100 g pollen)	Water	51.38±0.21cB	29.85±0.17aA	86.75±0.19bE	72.90±0.18aD	62.50±0.12bC
	Ethanol	29.63±0.17aC	86.30±0.12bE	42.08±0.25aD	11.60±0.16aB	10.70±0.12aA
	Methanol	45.93±0.15bA	86.75±0.19bC	118.20±0.12cD	61.93±0.26bB	86.38±0.28cC
% DPPH Inhibition	Water	12.03±0.59aA	8.13±0.36aA	30.26±1.04aC	21.05±0.79cB	25.24±0.83bB
	Ethanol	10.78±0.45aC	41.62±1.11bE	28.03±2.23aD	4.02±0.30aA	2.53±0.12aA
	Methanol	11.72±0.24aA	61.91±1.51cC	64.30±2.84bC	15.68±0.98bA	45.09±1.74cB
% ABTS Inhibition	Water	17.10±0.67bA	20.38±0.72aB	37.37±0.67bD	33.89±1.63cC	32.82±0.76bC
	Ethanol	13.39±0.47aB	24.89±0.57bD	18.31±0.63aC	6.51±0.32aA	6.11±0.07aA
	Methanol	17.00±0.30bA	39.93±1.24cC	38.63±1.37bC	22.47±0.61bB	37.24±1.25cC

a, b, c mean values with different letters in a column for each method differ statistically ($p < 0.05$); A, B, C, D, E mean values with different letters in a line differ statistically ($p < 0.05$).
Mixture A: *Cistus* spp.; *Quercus* spp.; *Olea* spp; *Brassica* spp.; *Raphanus* spp.; ***Mixture B***: *Taraxacum* spp.; *Andryala* spp.; *Cistus* spp.; *Rhamnus* spp.; ***Mixture C***: *Cistus* spp. (majority); *Crepis* spp.; *Trifolium* spp. (minority).

PM-242

Hepatic protective and haematologic effects of Hepacare® in CCl_4 induced hepatic damaged rats

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Herbal formulations are plants parts used as raw materials for self-administered pharmaceutical remedies and many of them are being sold without any scientific validation on their potency and efficacy [1]. This study was aimed at evaluating the haematopoietic, biochemical and histological effects of Hepacare® against carbon tetrachloride (CCl_4)-mediated hepatic oxidative damage in rats. Male rats were randomly divided into five groups of eight animals per group. Group A was given only distilled water for 7 days. Group B was administered with vehicle on the first four days and with vehicle and CCl_4 on the fifth, sixth and seventh day. Groups C, D and E were respectively administered orally with 500, 1000 and 1500 mg/kg b.w. of the drug and distilled water for the first four days, and with distilled water, drug and CCl_4 on the last three days [3]. Haematological analysis showed significant reduction ($p<0.05$) in Hb, PCV, RBC and platelet counts in the CCl_4treated group when compared with the untreated control group. These parameters were however reversed across the groups treated with the drug. Levels of AST, ALT, ALP and total bilirubin were significantly ($p<0.05$) reduced after treatment of rats with the drug which were hitherto significantly ($p<0.05$) increased in the CCl_4 treated group when compared with the untreated group. CAT, SOD and GSH activities exhibited significantly different activities in the liver homogenates of CCl_4-administered rats. The level of MDA was lowered in the liver tissue samples of treated rats when compared with the CCl_4-exposed untreated rats. The groups treated with the drug showed signs of protection against this toxicant as evidenced by the absence of necrosis. Hepacare® showed reversal effects on the previously increased haematological parameters and damaged liver tissues with a potential to ameliorate oxidative stress in hepatic dysfunction.

PM-243

Growth stimulating effect of *Astragalus* extract HT042 in female Sprague-Dawley rats

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Astragalus extract HT042 is a mixture of *Astragalus membranaceus*, *Phlomis umbrosa* and *Eleutherococcus senticosus*which exhibits synergistic effects in promoting longitudinal bone growth rate. The growth stimulating effect of orally administered HT042 was determined with tetracycline as a marker of the longitudinal bone growth in adolescent female Sprague-Dawley rats. After intravital tetracycline labelling, longitudinal bone growth rate was determined by measuring the distance between the fluorescent band and epiphyseal end line of growth plate in the proximal tibia metaphysis. 5'-Bromo-2'-deoxy-uridine (BrdU) labelling was used for the quantitative analysis of chondrocyte proliferation. The width of the growth plate was also

determined. HT042 led to the increase of longitudinal bone growth rate up to 433.50±21.61 μm per day, compared with the control group, 410.03±17.40 μm. Width of the growth plate was also increased by 5.0% from control. A 1.5-fold increase of chondrocyte proliferation was measured by BrdU labelling in HT042 group compared to control. Consequently, HT042 increased longitudinal bone growth rate by stimulating chondrocyte proliferation in the growth plate. HT042 could be helpful for increasing height growth of children.

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PM-244

Applicability of plant extracts and essential oils as antifungal food additives

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The surface moulding of food products is a serious and challenging quality issue in the food industry. Fungal infection is not only an aesthetic problem, but also affects the food expiry date, food safety and consumer satisfaction as well.

The present study aimed at the analysis of plant extracts and essential oils with possible application as antifungal additives in the food industry. The primary aspect of the selection of the examined species was the legal and organoleptic compatibility with food products. Thirteen herbs and their extracts - prepared with food grade solvents - were screened for antifungal activity. As these additives are intended to be used on the surface of the products, a disc diffusion method was applied to imitate real application circumstances. To simulate various kinds of food milieus, and cover different food types, four modified microbiological substrates were used. For the trial the substrates were treated with the most abundant mischievous mould species (*Penicillium sp*. and *Eurotium sp*.) In addition eighteen volatile oils were tested on *Penicillium sp*. using modified substrates. To elaborate optimal taste and scent characteristics for food products and discover possible additive or synergistic effects of the compounds, dilution and mixing trials were also carried out.

Two out of the thirteen herb extracts and fifteen out of the eighteen volatile oils exhibited strong fungicidal properties. The most effective extracts derived from stinging nettle and hot pepper. Essential oils of cinnamon, caraway and oregano had the most significant fungicidal activity. Certain essential oils were active in combination and even after dilution. Moreover, a further six extracts, and two essential oils have shown moderate antifungal effect. These results confirmed that certain plant extracts and volatile oils may be applied as additives to prevent surface moulding due to their antifungal properties.

PM-245

The antimicrobial activity of mint flavour in candies, chocolates and food supplements

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The qualitative and quantitative composition of isolated mint flavour distillates in 45 commercially available mint flavoured candies, chocolates and food supplements were studied using GC-MS. The yields of flavour distillates varied from 11.2 to 847.2 mg/100 g. The most abundant flavour compounds were limonene, menthone, menthol, menthofuran, isomenthone, neomenthol, menthyl acetate and 1,8-cineol.

The antimicrobial activity of four candy flavour distillates were tested by agar dilution method. Bacterial strains chosen in the present study were those commonly used in microbial sensitivity tests. Gram-negative bacteria was represented by*Yersinia ruckeri*. Gram-positive bacteria were *Bacillus cereus, Bacillus subtilis, Bacillus pumilus* and *Micrococcus luteus*. The flavour distillates were tested at a concentration 10.0% (v/v) in n-hexane. Commercial peppemint (*Mentha* x *piperita*L.) oil served as the positive control (zones of inhibition 10, 18, 15, 15, 15 mm respectively). All the flavour distillates showed antimicrobial activity against the tested bacteria. The strongest bacteriostatic and bactericidal effect was observed on*B. cereus* and *B. subtilis* (zones of inhibition respectively 14-21 and 10-21 mm). All the tested distillates were bactericidal against *Y. ruckeri*.

The present study shows that commonly used mint flavour in candies, chocolates and food supplements not only possesses the food additive purpose. Instead it may also serve as a health promoting antimicrobial agent in the product. This is in agreement with our earlier study on peppermint teas and their effect on common respiratory tract pathogen *Chlamydia pneumoniae* [1].

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PM-246

Botanical health claims on foods: minimising the risk of misleading consumers during the evaluation period in the EU

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Health claims are an important tool when communicating health benefits of foods, particularly in specific food categories, such as food supplements. Currently the use of botanical health claims included in the EFSA's register (on hold) is tolerated, on the condition that they are used in line with the general requirements of EU regulation, not misleading for consumers,

and scientifically substantiated [1,2]. Considering the lack of pertinent controlled clinical trials on botanicals and the fact that traditional use has been in the past considered as insufficient evidence, meeting such criteria has presented a major challenge for both researchers and the food industry. However, recent successful use of data from EMA monographs for the substantiation of a health claim [3] has opened up a new chapter for botanical health claims. In accordance with this fact, the objective of our study was to compare the wordings of botanical health claims, for which the evaluation process was put on hold, with the evidence about their traditional use. Ten commonly used botanicals were included in our study, for which 64 applications for health claims have on hold status. We determined that evidence on traditional use exists for about 56% of claimed health-relationships. The presented concept can help food manufacturers avoid the risk of misleading consumers. The results will also contribute to the discussion in the scientific community and the authorities on the criteria for assessing botanical health claims.

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PM-247

Bioaccessibility and bioavailability *in vitro* of antioxidants from powdered green coffee beans

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Green coffee (*Coffea arabica*) beans (GCB) derived from Ethiopia, Kenya, Brazil and Colombia were studied. Phenolics profile and caffeine content were determined using UPLC-MS technique. Potentially bioaccessible and bioavailable phytochemicals were released during digestion in the simulated human gastrointestinal tract. Antiradical activity (AA), reducing power (RP), chelating ability (CHEL) and ability to prevent lipids against oxidation (LPO) were estimated.

The dominant compound identified in all analyzed samples was 5-caffeoylquinic acid. Significant amounts of other phenolic acids (3- caffeoylquinic acid; 4- caffeoylquinic acid; 3-feruloylquinic acid; 5- dicaffeoylquinic acids and 3,5- dicaffeoylquinic acid) were determined. Caffeine content averaged from 4.36 mg/ g dw to 4.99 mg/g dw. Digestion in vitro released chelating and reductive agents, free radical scavengers and lipid-preventers. The highest capacity for metal ions chelation was found for Brazilian GCB (EC_{50}=2.07 mg dw/mL), whereas the lowest for GCB from Ethiopia (EC_{50}=7.01 mg dw/mL). Importantly, the activity of extracts obtained after absorption in vitro was significantly higher than that determined for

samples obtained after simulated digestion. Particularly noteworthy is the fact that GCBs were an excellent source of potentially bioaccessible reductive compounds. The AA activity of samples obtained after digestion in vitro averaged about 4 mg dw/mL. The potential bioavailability of antiradical compounds differed significantly. The highest activity was found for Brazilian GCB whereas the lowest was found for GCB from Colombia. The LPO activity of bioaccessible in vitro phytochemicals was relatively low. Probably, this is because lipophilic compounds are less extractable in the gastrointestinal model system. Most importantly, the potential bioavailability of these phytochemicals was surprisingly high.

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PM-248

Robust method for the analysis of monacolin K in red yeast rice

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Red yeast rice (RYR) is produced by the fermentation of rice with *Monascus* species. During fermentation a variety of secondary metabolites are formed: pigments, citrinin, monacolin K and others. In Asia, the use of RYR in food and as a medicine exists for over of thousands years. More recently, RYR products are described in scientific reports mainly for the management of blood cholesterol. For companies providing food supplements with RYR, it is very important to control the quality of the RYR bulk product that is mostly produced in China. The extraction and analysis of monacolin K and lovastatin from RYR powder was already described in previous articles [1,2]. However, by analyzing RYR bulk products, we experienced problems related to the robustness of the methods. Causes we identified were incompleteness of extraction and pH dependent transitions. A robust analytical method using reversed phase high-performance liquid chromatography (RP-HPLC) with diode array detection (DAD) was developed and validated for the quantification of lovastatin and monacolin K in RYR bulk products. Tests on the composition of the extraction solvent to minimize transition, the time of extraction and the number of repetitions of extraction were evaluated. Monacolin K, lovastatin and minor monacolin peaks were separated on a C18 column (250 x 4.6 mm, 5 µm) using acetonitrile (ACN)/ 0.1% trifluoroacetic acid (TFA) as mobile phase. The standard lovastatin curve is linear over a concentration range of 6-119 µg/mL. The recovery of the method is 98.75%. The precision of the method with respect to time and concentration is acceptable, with relative standard deviation (RSD%) values of less than 5%.

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PM-249

Innovative formulations to enhance oral bioavailability of *Agnus castus* extract

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The main characteristic constituents of *Vitex agnus castus* are: diterpenes, iridoid glycosides, flavonoids, flavones, triglycerides. Therapeutic indications are premenstrual syndrome and menstrual cycle disorders [1]. *In vitro* and *in vivo*experiments have shown that Agnus castus extracts (ACEs) have dopaminergic, prolactin-inhibiting activity [2]. The project is aimed at developing new oral innovative formulations which can overcome the limited oral bioavailability of iridoid glycosides and flavonoids and thus increase the therapeutic efficacy. After optimization of a HPLC-DAD-QTOF analytical method for qualitative and quantitative characterization of consitutents of ACE, nanoemulsions were selected as lipid carriers. The preparations were performed by dissolving extract powder into the oil (triacetin)-surfactant (labrasol)-cosurfactant (Cremophor EL) mixture, adding water. The droplets in the microemulsion appeared dark, and the surroundings bright under Transmission Electron Microscopy with an average diameter of 11.15±0.07 nm. Polydispersity (PdI) was 0.07±0.01 measured by a Dynamic Light Scattering. The aqueous solubility of the extract was improved: the extract at the concentration of 40 mg/ml is completely soluble in the nanoemulsion, while the solubility of extract in water resulted less than 6 mg. Finally passive intestinal absorption was evaluated using a Parallel Artificial Membrane Permeation Assay. The test evidenced a good increase of total constituents of the ACE permeated from donor to acceptor compartement.

Acknowledgements: Bionorica Global Research Initiative has supported the study.

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PM-250

Interactions between ferulic and chlorogenic acids as a main factor determining antiradical activity of wholemeal wheat bread enriched with green coffee

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The hydroxyl radical (·OH) is primarily responsible for the cytotoxic effects of oxygen. Hydroxyl radicals can be produced from O2 under stress conditions and are involved in numerous disorders such as inflammations.Interactions between potentially mastication extractable (BE), potentially bioaccessible (DE) and potentially bioavailable (AE) compounds able to scavenge OH· radicals derived from green coffee and wholemeal wheat flour were studied. Green coffee (*Coffea arabica*) beans (GCB) and wheat bread flour (600 g), type 2000

(average 1,8 g/100g ash content, humidity14 g/100g) were used. Results were compared with those obtained for pure chlorogenic and ferulic acids (Sigma-Aldrich, Poland). For interactions determination the isobolographic analysis was used. BE, DE and AE phytochemicals were released during digestion in the simulated human gastrointestinal tract. For functional food preparation bread flour was replaced with GCB flour at 1 to 5% levels (GC1, GC2, GC3, GC4 and GC5, respectively).Taking into account the isobole shape it may be concluded that chlorogenic and ferulic acids acted synergistically. In the same way acted BE and AE compounds derived from tested raw materials. Only in the case of DE additive interactions were found. GCB addition significantly enriched wheat bread with antiradical compounds. The highest activity was found for extracts obtained after simulated digestion which indicates high potential bioaccessibility of active compounds. Most importantly, these phytochemicals were highly bioavailable in vitro. Due to fact that bread is one of the main products consumed in the cultural area of many countries and resignation from its consumption for many people is impossible, the proposed product is mainly targeted at this group of consumers – it is a compromise between "traditional" and pro-health food.

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New opportunities for biotechnology and cell biology

PM-251

Effects of N source concentration and ammonium-nitrate ratio on phenylethanoid glycosides of *Plantago lanceolata* tissue cultures: a metabolomics driven full-factorial study

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The metabolomic approach and data-mining gains increasing significance in phytochemical analysis.

The aim of the current work was to examine the effects of the N source concentration and ammonium-nitrate ratio on the pattern of phenylethanoid glycosides (PG) of the tissue cultures of *Plantago lanceolata* L. The experiment was carried out using a full factorial experimental design, the metabolome was investigated with LC-ESI-MS^3; Using modified Murashige Skoog media, 10, 20, 40, 60 mM total N source concentrations and 0, 0.11, 0.20, 0.33 ammonium-nitrate ratios were tested. Phenomena were sought using accepted data-visualization and data-mining methods. The effects of the treatment on the individual metabolites was also examined.

From the tissue culture, 16 natural products could be putatively identified as PGs. The concentration of these reacted very differently to the treatments, despite the compounds were of the same metabolite group. Plantamajoside and acteoside and were detected as major PGs.

Their maximal concentration was 3.54 ± 0.83% and 1.30 ± 0.40 %, respectively, on media 10(0.33) and 40(0.33).

Most of the examined 89 abundant natural products were affected by the composition of the media. The N source concentration and the ammonium-nitrate ratio significantly influenced the concentration of 42 and 10 compounds, respectively. For cumulation of many compounds, compositions, such as 10(0), 10(0.11) and 40(0.33) were found to be optimal, which significantly differ from the widely used Murashige Skoog medium – 60(0.33).

Computer simulation has shown, that using one-factor-at-a-time experimental design instead of full-factorial would have led to suboptimal yields with respect to many natural products. If the N source is optimized first, followed by the ammonium-nitrate ratio, this bias is significantly less during one-factor-at-a-time experimental design.

PM-252

Rational drug design tools for the discovery of novel microbial natural products with applications in cosmetics

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Cosmetics products can act based on their physicochemical properties (UV filters, colorants, etc.) or by binding to a specific receptor (tyrosinase, elastase etc). Since biology has augmented the number of known human skin's proteins, those proteins can be handled as drugable targets. Using common Rational Drug Design Tools such as Similarity Search, In Silico Screening and Prediction Models we can rationalize the bioprospection of natural sources producing appropriate metabolites for cosmetic use.

In the frame of the EU project "MICROSMETICS", we exploit the microbial global biodiversity to discover and develop novel cosmeceutical agents. Starting from the CosIng Repository, accurate functional prediction model was created for all known cosmetic functions. Next the homology models of specific cosmetic target receptors were constructed (tyrosinase, elastase, hyluronidase and collagenase) for which the appropriate in vitro tests have already been developed. About 40.000 known microbial metabolites were processed through a consensus scoring prediction protocol using: a)functional prediction model b)virtual screening procedure for the selected receptors c)similarity search based on all known molecules from literature that bind to the those receptors and d)toxicological profile filtering. From virtual screening, 93 metabolites ranked on the first 10% on all receptors, 125 on 3 receptors and 197 on 2 receptors. From similarity search 93 metabolites ranked on the top 10% for 3 targets (hyaluronidase lacks of known binders), 242 for 2 targets and 470 for 1 target. Combining those results, we selected 100 microorgnanisms that can produce those metabolites

or analogues. They are cultivated, extracted and tested in vitro in order to be prioritized for the isolation and identification of possible novel cosmeuceutical agents.

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PM-253

The occurrence of progesterone 5β-reductase is not limited to the Angiosperms, a functional enzyme was characterized from *Picea sitchensis*

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Early steps in cardenolide biosynthesis are catalyzed by 3β-hydroxysteroid dehydrogenase (3βHSD), Ketosteroidisomerase (KSI) and progesterone 5β-reductase (P5βR, [1]). Inspecting public data bases, a homologous cDNA sequence was detected with respect to Picea sitchensis. Following its transfer into E. coli, a recombinant enzyme was overexpressed owing the reduction of progesterone to 5β-pregnane-3, 20-dione in a stereo-specific manner [2]. The small substrate 2-cyclohexene-1-on was also found to be reduced. This enzymatic data provide first-time evidence concerning the occurrence of P5βR within the Coniferales.

It is presently unclear what a role the newly detected enzyme, belonging to the broadly documented SDR family [3], could play in planta since *P. sitchensis* does not produce cardenolides. However, P5βRs are also involved in the iridoid biosynthesis [4-5]. This hypothesis has to be proven for *P. sitchensis* in future.

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[3] Thorn A et al., 2008;

[4] Geu-Flores et al., 2014;

[5] - Munkert J et al. 2015;

PM-254

Influence of arsenic stress on growth, physiological activities and artemisinin production in two varieties of artemisia (*Artemisia annua*) differing in artemisinin yield

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Artemisinin, extracted from the leaves of *Artemisia annua* L., has been recognized as an effective and safe remedy against malaria. A pot experiment was conducted according to randomized block design using five replicates to screen the *A. annua*L. varieties, namely 'CIM-Arogya' and 'Jeevan Raksha' under arsenic stress. Four concentrations of arsenic [0 (control), 15, 30 and 45 mg kg^{-1} soil] were applied to the soil. Measurements for growth characteristics, physiological attributes, biochemical parameters, yield and quality attributes were carried out at pre-flowering [90 days after planting (DAP)] and flowering (120 DAP) stages. The variety 'Jeevan Raksha' was more adversely affected by arsenic stress than the 'CIM-Arogya', suggesting that the 'CIM-Arogya' was more arsenic tolerant than the 'Jeevan Raksha'. The highest arsenic concentration (45 mg kg^{-1} soil) proved most toxic dose as compared to other arsenic doses applied. It reduced the rate of photosynthesis and leaf-chlorophyll content significantly both at 90 and 120 DAP. The activities of CAT, POX and APX were also rapidly stimulated due to the highest concentration of arsenic at both the growth stages. Noticeably, all applied doses of arsenic significantly increased the production of artemisinin in the leaves. As compared to control, 'CIM-Arogya' contained the maximum artemisinin content under the highest arsenic stress, increasing the artemisinin content by 38.0 and 42.6% at 90 and 120 DAP, respectively; while, 'Jeevan Raksha' showed an increase in artemisinin content by 32.6 and 35.7% at the respective dates of sampling. At the highest arsenic stress, the yield of artemisinin was increased by 42.9 and 45.7% in 'CIM-Arogya' and by 37.5 and 40.5% in 'Jeevan Raksha'. Generation rate of H_2O_2 increased consistently when arsenic was applied irrespective of the varieties used, indicating the role of H_2O_2 in artemisinin biosynthesis in *A. annua*L.

PM-255

Effect of isoflavone compounds on the *in vitro* maturation of sheep oocytes

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Phytoestrogens are oestrogen-like but non-steroidal substances that are classified according to their chemical structure: flavones, flavanones, lignans, flavonols, chalcones, isoflavones. The serious problems caused by phytoestrogens in clovers on fertility in female sheep are well documented and led to the inclusion of 'duty of care' in the development of novel pasture species [1]. We therefore tested whether isoflavones (genistein, biochanin-A, formononetin) affected *in vitro*maturation or developmental competence of ovine oocytes, using a factorial design (3 isoflavones x 5 concentrations: 0, 2.5, 5, 10, 25 µg/ml) repeated 4 times. Cumulus-

oocyte-complexes from abattoir-sourced adult ovaries were randomly allocated to the treatments, then fertilized and cultured *in vitro* [2]. Cleavage and embryo development rates were recorded.

There was no significant effect of the lower concentrations (2.5-10 µg/ml) of any isoflavone on any measure of embryo development. However, the high concentration (25 µg/ml) caused significant effects for all 3 isoflavones: genistein decreased cleavage rate (92% vs 80%), blastocyst rate (62% vs 45%) and blastocyst efficiency (57% vs 36%); biochanin-A decreased cleavage rate (92% vs 57%) and blastocyst efficiency; (57% vs 32%) formononetin decreased blastocyst rate (62% vs 45%) and blastocyst efficiency (57% vs 42%). These outcomes *in vitro* suggest that isoflavones from fodder could cause reproductive failure *in vivo*. No effects were detected at low concentrations, but further investigation is needed with a focus on early morphogenesis and trophectoderm nuclei of the embryos.

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PM-256

Phytogenic substances modulate intestinal permeability - porcine intestinal epithelial cells as model system for gut health

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Antibiotic Growth Promoters (AGP) were banned in the EU in 2006. Since then, phytogenic feed additives are being studied with considerable focus on their potential application in livestock feeding. Phytogenics include a broad range of plant materials, able to improve gut health and performance in animals and are therefore conceived as an alternative to AGP.

The intestinal epithelial barrier serves as the first line of host defense against potentially harmful stressors taken up with feed from the environment. It is primarily formed by epithelial cells, connected by tight junctions (TJs). To test for intestinal integrity, an in vitro cell culture model, using an intestinal porcine epithelial cell line (IPEC-J2) has been established. Cells were differentiated for 8 days in Transwell® membrane inserts (1.12 cm^2), allowing the formation of TJs and the build-up of a representative intestinal layer which can be observed by an increased transepithelial electrical resistance (TEER). Phytogenic test substances were added and cells were incubated for 72 hours, while TEER was measured every 24 hours.

20 ethanolic plant extracts were tested in triplicate - three of them (grape seed, apple pulp, and *Echinacea* herb extract) were chosen, representative of positive, negative, and marginal effect on the TEER values, respectively. Grape seed extract increased TEER by ~ 26%. *Echinacea* extract decreased TEER by 6% at all tested time points and apple extract marginally affected

TEER causing a 7% increase. Cell viability was checked at the end of each experiment via the neutral red assay and always exceeded 90%.

Phytogenics include a broad range of plant materials, known to possess, amongst others, antimicrobial, antiviral, antifungal, and antioxidant properties. In this study, positive and negative modulation of gut health is shown by different phytogenic components. Further studies are needed to elucidate the underlying mode of action and the comprised active compounds.

PM-257

Clone selection of *in vitro* horseradish hairy root cultures

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Hungary is one of the largest horseradish (*Armoracia rusticana* Gaertn. Mey. et Scherb) harvesting countries. Its lacrimatory odor, taste and medicinal effects are due to isothiocyanates (ITC), which make up the essential oil of the horseradish (HREO). ITCs are hydrolytic products from glucosinolates. These compounds are intensively studied because of their strong anticarcinogen and antimicrobial effect [1].

Antifungal activity of HREO was previously analysed [2]. The two main compounds, allyl and phenylethyl ITC had the strongest antifungal effect. In our recent work we are studying these compounds produced by *in vitro* cultures.

Our aim was to set up *in vitro* genetically transformed hairy root cultures, and to select clones synthesizing antifungally active components in large amount, besides high biomass production.

Genetically different hairy root clones were created with *Agrobacterium rhizogenes* A4. Semiquantitative glucosinolate content was studied by LC-MS, while the ITC profile was analysed by GC-MS. The biomass growth was weighed.

From 35 hairy root clones 9 were selected. These possess the highest biomass production, considering the percentage scale results of the two main glucosinolates. Clone Ar9 contained the highest amount of sinigrin (20%), while DK34 had the highest content of gluconasturtiin (22%). Ar1 and ArL-101 had the antifungally best ratio of the two compounds. The antifungally active ITC content was supported by GC-MS analysis. Our further aim is to increase the ITC content in clones, through changing the cultivation conditions.

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PM-258

Exploitation of global microbial biodiversity for the discovery of novel comeceuticals using LC-HRMS based metabolomics

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MICROSMETICS, is an EU funded project aiming to discover and bring to development innovative anti-ageing cosmeceuticals, originating from microbial biodiversity, using emerging and state of the art technologies in the field of biotechnology, natural products chemistry and applied microbiology.

The proprietary microbial collection of Fundación MEDINA (over 116.000 strains) is being exploited by incorporating modern high throughput screening platforms (*in silico* & *in vitro*) for the rational and targeted selection of the most promising strains. Advanced analytical approaches and techniques are being applied for the efficient, accelerated and advantageous isolation and identification of natural constituents. A broad spectrum of bioassays and metabolomics approaches are being incorporated for the evaluation of anti-ageing, more specifically anti-oxidant, skin-protecting, and skin-whitening activity of all derived molecules.

In the frame of MICROSMETICS more than 110 potential candidate strains identified from a Rational Drug Design Tool (using a functional prediction model, virtual screening and similarity search) were selected to be studied. Among them 55 fungi and 55 actinomycetes were cultivated under "nutritional arrays". Approximately 1100 extracts have been generated and evaluated for their biological activity. Among them the top 100 were selected and a strategy combining UHPLC/Orbitrap-HRMS, in positive and negative modes, with multivariate statistical methods was applied. All derived chromatograms have been analyzed and a positive correlation between the profiles of the extracts with the aforementioned bioassays was observed. Thus, the 10 most promising extracts that represent the clusters generated in metabolomics have been forwarded for large-scale cultivation and bioassay guided isolation of novel molecules.

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PM-259

Sprouting and elicitation with natural elicitors as an effective tools for improving antioxidant activity of potentially bioaccessible wheat phytochemicals

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Antioxidant activity of potentially bioaccessible phytochemicals from 4-days-old sprouts of Polish winter wheat (*Triticum aestivum* ssp. *vulgare*) cultivars: Bogatka, Mulan and Muszelka were studied. Before germination seeds were treated with three kinds of natural elicitors: 0.1% (w/v) of *Saccacharomyces cerevisiae* (SC) extract, 0.1% (v/v) *Salix daphnoides* (SD) bark extract and their combinations (1:1, SCD) and distilled water (control) (6 h, 25°C) and were germinated at 20 °C in darkness. Potentially bioaccessible phytochemicals were released during digestion in the simulated human gastrointestinal tract. Antiradical activity (ABTS radical cation decolorization; AA), ferric reducing power (FRAP), Fe(II) ions chelating ability (CHEL) and ability to prevent linoleic acid against oxidation (LPO) were estimated. The analysis of variance (ANOVA) and Tukey's post-hoc test were used to compare groups (α=0.05). Sprouting significantly decreased AA activity of potentially bioaccessible phytochemicals from Mulan and Bogatka, whereas such impact on Muszelka sprouts was not observed. Elicitation with all elicitors significantly increased AA activity of Bogatka sprouts, whereas in the case of Muszelka and Mulan such impact was observed after treatment with SD and SCD. Sprouting significantly increased RP and CHEL in respect of wheat variety. Treatment not influenced on RP of Bogatka sprouts. Sprouting and treatments with all elicitors significantly increased LPO activity of potentially bioaccessible compounds from Bogatka and Muszelka sprouts. Elicitation significant increased LPO. Treatments with all elicitors increased CHEL activity of potentially bioaccessible compounds from Bogatka and Muszelka seeds, whereas in the case of Mulan SCD was not effective. Especially high effect of SD was obtained for antiradical activity, chelating power and ability to lipid prevention.

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PM-260

Biosynthesis of artemisinin in gamma-irradiated *Artemisia annua* plantlet proceeds via a mixed origin of isoprene units

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Artemisia annua is a traditional herb that used for antimalarial drug in China. The well-known antimalarial agent, artemisinin, is a sesquiterpene lactone bearing an endoperoxide and theoretically biosynthesized from the terpenoid pathway. The present study, the wild type *A. annua* shoots were irradiated with gamma ray 650 of cobalt-60, obtaining *A. annua* TBB strain

[1]. The *A. annua* TBB plantlets were then induced and propagated in Murashige and Skoog medium (MS) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar, under 16 h of photo-period (2000 lux). Plantlets containing high-yield artemisinin were selected from A. annua TBB and used as plant materials for isotopic glucose feeding experiment. Plantlet cultures of irradiated A. annua TBB (10-day old) from MS agar medium were transferred into 250-ml erlenmeyer flasks and grown for 30 days on shelf culture, in distilled water supplemented with 3%(w/v) glucose solution, a mixture of [1-13C]glucose (99% 13C enrichment) and unlabeled D-glucose (1:1 ratio). Artemisinin was isolated and their 13C-labeling patterns examined using quantitative NMR spectroscopy. Analysis of the patterns of 13C-enrichment revealed that all the isoprene units in the skeleton of artemisinin were supplied from both the DXP and the MVA pathways.

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PM-261

Screening of plant growth stimulating potential of several Iranian macroalgae by measurement of their auxin content

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Macroalgae are a very large and diverse group of eukaryotic organisms containing various primary and secondary metabolites of high biological activities such as polysaccharides, proteins, unsaturated fatty acids, polyphenols, minerals, pigments, phytohormones, etc. [1,2]. The positive growth effects of algal extracts for agricultural corps like wheat, maize and several medicinal plants are partly attributed to the presence of different phytohormones, mainly auxins [3]. Continuing our research work on the auxin contents of several Iranian microalgae [4,5], a screening study was conducted to identify the Iranian macroalgae with the highest contents of auxin which makes them potential plant biostimulants. For this purpose, concentrations of three major auxins, i.e. indole 3-acetic acid (IAA), indole 3- propionic acid (IPA) and indole 3- butyric acid (IBA) were measured in twenty red, brown and green macroalgae gathered from intertidal zones of southern coastal area of Iran. The ultrasonic extraction of active compounds was followed by HPLC analysis. The results showed the presence of auxins in 14 samples as follows: IAA in the range of 0.43-13.04 ng/g (dry weight, DW), IPA in the range 2-113 ng/g DW and IBA in the range of 1.99-7.97 ng/g DW which is in accordance with previously reported works[6]. The highest content of auxin was observed in the brown algae, *Nizamuddin zanardini*.

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PM-262

Monitoring of radiation risk by sensitive genes of *Tradescantia* species

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Some species of the genus *Tradescantia* are known to be sensitive to some environmental constraints. Exposure to ionizing radiation at doses of as low as 50 mGy can induce stamen hair color change to pink in the species, which might be a good biological indicator for monitoring radiation risk to humans. However, this perennial plant blooms within limited time periods, hence an alternative year-round monitoring system like genes sensitive to radiation leak from the related facility or instruments would be favourable. The objective of this study was to explore genes that respond to low radiation (~max 1000 mGy) and to validate the monitoring value of the genes. A total of 77,326 representative transcripts were obtained from the RNA-seq data from 50, 250, 500, and 1000 mGy irradiation, among which 32,000 genes were annotated to public genes reported in 39 plant species. Gene ontology analysis indicated that the molecular functions of the differentially up-regulated genes involved monooxygenase, transferase, carbon-oxygen lyase, and hem binding. Many of the heat shock proteins were up-regulated at 50 mGy, including chaperon protein, HSPs, and calnexin. Also, two transcription factors [GLOBOSA (GLO), DEFICIENS (DEF)] involved in flower development, including the stamen hair, were cloned with 567 and 678 bp lengths. These genes were functionally validated and evaluated for their potential as monitoring genes for *Tradescantia*, detecting radiation damage to humans and to ecosystems.

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PM-263

Hight throughput screening of microbial biodiversity for the discovery of novel cosmeuceutical agents

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MICROSMETICS, is an EU funded project aiming to discover and bring to development innovative products in the area of anti-ageing cosmeceuticals, originating from microbial biodiversity and using emerging and state of the art technologies in the field of biotechnology, natural products chemistry and applied microbiology.

In the frame of MICROSMETICS more than 110 potential candidate strains (fungi and actinomycetes) identified from a Rational Drug Design Tool (using a functional prediction model, virtual screening and similarity search) were selected to be studied. Approximately 1100 extracts have been generated and evaluated for their biological activity. A broad spectrum of bioassays and novel analytical approaches are being incorporated for the evaluation of anti-ageing, more specifically anti-oxidant, skin-protecting, and skin-whitening activity of all derived products. For the evaluation of antioxidant activity the DPPH and ABTS assays were used. Skin-protecting was evaluated by measuring spectrophotometrically the inhibitory properties of samples against enzymes which are related to the elasticity and moisture of the skin (elastase collagenase, and hyaluronidase). The skin whitening activities were determined by the tyrosinase assay, using L-DOPA as substrate. Finally for he safety of those extracts cytotoxicity was evaluated on A2058, CCD25sk, HepG2 cell lines by the MTT method. The results of the above bioassays showed that from the above extracts approximately 120 had significant activity in ABTS, 84 in DPPH, 65 in tyrosinase and 52 in the selected enzymes. All toxic extracts were eliminated and by the application of a consensus model, 100 extracts from the 1100 were selected as highly potent and have been submitted for LC-HRMS profiling, metabolomics analysis and dereplication.

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PM-264

LC and LC-MS studies on genetically transformed cultures of *Rubia tinctorum* cultivated in bioreactor

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Rubia tinctorum L. (european madder) is a perennial plant from the Rubiaceae family. It is a source of a natural dye; it produces a variety of anthraquinone pigments in its roots and rhizomes. The main components are di- and trihydroxy-anthraquinones, alizarin, and purpurin and their derivatives, ruberythric acid and pseudopurpurin. These substances show antimicrobial and spasmolytic activity and facilitate the loosening of kidney stones. Recent studies indicated that alizarin and purpurin have strong inhibitory effect on the genotoxicity of several carcinogens.

We have investigated the anthraquinone composition of the genetically modified hairy root cultures. Transformed root cultures of *R. tinctorum* were obtained by their inoculation with *Agrobacterium rhizogenes* (strain R-1601). After the elimination of bacteria, the hairy roots were cultured on liquid or solid Gamborg B5 and ½NMS media. In order to increase the biomass formation, we cultivated the hairy roots in a Braun Biostat S bioreactor (24 °C, pH 5.7).

For qualitative investigation of the anthraquinones produced by the hairy root cultures, various extracts were analyzed by LC-MS/MS (Agilent Triple Quadropole) method using electrospray ionisation in negative mode. A total of 11 anthraquinones were identified including ruberythric acid, lucidin-primeveroside and pseudopurpurin. For determination of anthraquinone aglycones, MeOH extracts were hydrolysed by refluxing with HCl. The hydrolysates were purified by SPE then investigated by HPLC method (Surveyor System) on Luna C-8 column [1], using a 45:55 (v/v) mixture of acetonitrile:20 mM ammonium formate (pH 3.00) as eluent. The cultures cultivated in bioreactor showed intensive biomass formation (fw: 175 g/3 L medium) and significant biosynthetic capacity (purpurin content: 5.76 mg g-1).

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PM-265

The *in vitro* propagation of *Helichrysum umbraculigerum*

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Stilbenoids are a specific class of phenolic compounds, some of which show medicinal potential. Novel stilbenoids have previously been isolated from the South African plant *Helichrysum umbraculigerum* [1]. These prenylated resveratrol analogues are well known for their applications, which have mainly been discovered in research conducted on *Cannabis sativa* [2]. The applications include possible anti-inflammatory, anti-microbial and anti-cancer effects. *H. umbraculigerum* is known to be used by South African traditional healers. Exploitation of medicinal properties will require large numbers of plants, but *H. umbraculigerum* has proved difficult to cultivate. The aim of this study was to optimise an *in vitro* propagation protocol for *H. umbraculigerum*. Leaf cuttings were sterilised using 70% ethanol followed by 2% sodium hypochlorite containing 1 drop of Tween-20®. They were then washed in distilled water and placed onto Murashige and Skoog (MS) media. Uncontaminated leaf cuttings were placed onto MS media supplemented with different combinations of plant growth regulators. Shoots were produced most efficiently on MS media containing a specific concentration of thidiazuron (TDZ). These shoots were subsequently moved into the light, after which they started producing roots. Plantlets were successfully acclimatised using Jiffy-7C® pellets. The *in vitro* propagation protocol developed will allow for the production of large amounts of plant material for future research conducted on *H. umbraculigerum.*

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PM-266

Comparing aryltetralin lignan accumulation patterns in four biotechnological systems of *Linum album*

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Linum album is a herbaceous plant used in the food and textile industries. It is also of medical interest due to its content of podophyllotoxin (PTOX), an aryltetralin lignan with anticancer activity.

Previous studies in our laboratory showed that cell suspension cultures of a *L. album* cell line produced more PTOX than methoxypodophyllotoxin (6-MPTOX), both lignans being formed

from the same precursor after divergence towards the end of the biosynthetic pathway. In contrast, hairy roots from this cell line produced more 6-MPTOX than PTOX.

Aiming to compare the lignan accumulation patterns amongst *L.album* plantlets obtained from seeds in *in vitro* flasks and different *in vitro L. album* systems of this cell line, we established four biotechnological platforms formed by wild type and transformed cell suspension cultures, adventitious roots isolated from *in vitro* plants and hairy roots, and determined the production of five aryltetralin lignans, PTOX, 6-MPTOX, deoxypodophyllotoxin (dPTOX), β-peltatin and yatein, using an HPLC-MS technique.

The results show that *in vitro* plantlets and wild type cells predominantly produced PTOX, production being 45-fold higher in the plantlets. Otherwise, the adventitious roots, hairy roots and transformed cells predominantly produced MPTOX, the hairy roots being the most productive, with MPTOX levels 17-fold and 400-fold higher than in adventitious roots and transformed cells, respectively. β-peltatin was found in all systems, while dPTOX and yatein were not detected at all.

Regarding the total lignans, the hairy roots were the most productive (675 μg/gDW), followed by plantlets (620 μg/gDW), adventitious roots (51 μg/gDW), wild type cells (21 μg/gDW) and transformed cells (3 μg/gDW).

We can infer from these results that in the studied cell line, both cell differentiation and transformation events promoted MPTOX over PTOX formation.

PM-267

New taxane biosynthetic gene encoding a β-phenylalanine-CoA ligase identified in jasmonate-elicited*Taxus* cells

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The data-generating power of "omics" technologies is increasingly being harnessed in studies of plant biology to shed light on aspects such as metabolite production patterns, gene sequences and protein expression.

Taxol and related taxanes, produced by several *Taxus* species, are high-value secondary metabolites widely used in cancer therapy due to their antineoplastic activity. To date, several biosynthetic steps of these compounds remain undefined.

After carrying out a genome-wide expression analysis of jasmonate-elicited *Taxus baccata* cell cultures using complementary DNA-amplified fragment length polymorphism (cDNA-AFLP), we found that the increased taxane production in the target cultures was correlated with an extensive elicitor-induced genetic reprogramming. Subsequent in silico analysis

allowed us to identify 15 genes with jasmonate-induced differential expression putatively involved in five unknown steps of taxane biosynthesis. Among them, the TB768 gene and its predicted 3D structure showed a strong homology with other genes previously reported to encode acyl-CoA ligases, suggesting it was involved in the formation of the taxol lateral chain. In vitro analysis confirmed that the TB768 gene encodes an acyl-CoA ligase, localized in the cytoplasm and able to convert b-phenylalanine into its CoA ester derivative. The latter is then attached to baccatin III in one of the last steps of the taxol biosynthetic pathway.

In determining that the TB768 gene encodes a cytosolic CoA ligase with an important role in the taxol biosynthetic pathway, we have corroborated that the combination of a cDNA-AFLP transcriptome profiling approach with bioinformatics analysis constitutes an efficient set of tools for the identificationof new genes involved in the metabolism of valuable bioactive compounds. The newly described gene will contribute to the establishment of sustainable taxol production systems through metabolic engineering or synthetic biology approaches.

PM-268

Influence of germination and elicitation on phenolic acids profile of wheat sprouts

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Seedlings of winter wheat cultivars were studied (*Triticum aestivum*, ssp. *vulgare*): Bogatka, Mulan and Muszelka. Before germination the seeds were placed in distilled water (control) and in distilled water containing 0.1% (w/v) of*Saccacharomyces cerevisiae* (SC) extract, 0.1% (v/v) *Salix daphnoides* (SD) bark extract and their combinations (1:1, SCD) for 6 h at 25 °C. Seeds were germinated for 4 days at 20 °C in darkness. Phenolic acids content were determined by reversed-phase high-performance liquid chromatography and electrospray ionization mass spectrometry (LC-ESI-MS/MS). Plant material was extracted using Dionex ASE 100 accelerated solvent extractor (methanol concentration 80%, temperature: 80˚C). Eleven phenolic acids (protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syryngic, *p*-coumaric, *cis*- and *t*-ferulic, salicylic, *t*- and *cis*-synapinic) were identified in the dormant seeds (control) and seedlings. Sprouting decreased protocatechuic and vanillic acids level. After sprouting significant increase (p=0.05) of *p*-hydroxybenzoic, syryngic and *p*-coumaric acids level was observed. In the case of Bogatka all elicitors increased protocatechuic, caffeic and *t*-ferulic acids content. The most effective elicitors were SD and SCD. In the case of Muszelka elicitation slightly, but significantly (p=0.05) decreased content of protocatechuic, *p*-hydroxybenzoic, vanillic and syryngic acids, whereas treatment with SD and SCD elevated caffeic, both ferulic, and *cis*-synapinic acids level. Treatment with SC increased caffeic and *t*-synapinic acids content. All elicitors increased caffeic, *cis*-ferulic and salicylic acids content in Mulan sprouts. Level of protocatechuic, *p*-hydroxybenzoic, vanillic and *t*-ferulic acids increased after SC and SCD treatment, whereas *p*-coumaric and both synapinic acids content increased after treatment with SD and SCD.

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PM-269

γ-terpinene synthase of *Thymus vulgaris*

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Thyme is a plant genus comprising over 300 species within the Lamiaceae family. For medicine, especially for the treatment of respiratory diseases, the essential oil is the most important ingredient. It shows antibacterial and antiviral effects. Essential oils are complex mixtures composed of active and inactive compounds [1-2]. In *Thymus sepyllum* (Ts) and six*Thymus* species the essential oils were isolated by hydrodestillation and analyzed by GC-MS. The γ-terpinene was identified as a characteristic monoterpene in all essential oils, albeit in varying concentrations. It is a naturally occurring monoterpene and a major component in most essential oils of citrus fruits and many aromatic plants. γ-terpinene is formed through cyclisation of geranyl diphosphate (GPP) by γ-terpinene synthase which belong to the monoterpene cyclase family [3]. Traces of several other monoterpenoids were formed in addition to γ-terpinene (product promiscuity). Using primers derived from *Origanum vulgare*, we here isolated and sequenced γ-terpinene synthase cDNAs (TPS) from *Thymus vulgaris*(Tv), *Thymus serpyllum* (Ts), *Thymus x citriodorus* (Txc) and *Thymus caespititius* (Tc). Sequence data were used to study the chemical-taxonomic relationship between the four species. The bastard plant Txc showed a close relationship to Tv, whereas Tc γ-terpinene synthase was closely related to that of *Origanum vulgare* [4]. The Tv γ-terpinene synthase gene 1 (TvTPS1), which encoded for a protein of 596 amino acids was expressed as a recombinant protein in *E. coli*. We succeeded in overproduction, purification and crystallization of the TPS from Thymus vulgaris for the first time.

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PM-270

Evaluation of genotoxicity of *Artemisia herba-alba* and *Jasmina montana* extracts against *Zucchini Yellow Mosaic Virus* in *Cucurbita pepo*

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Zucchini yellow mosaic virus (ZYMV) presents a critical problem in cucurbit production in Egypt and worldwide due to generation of aggressive viral strains. Therefore, novel approaches must be used for control of viral infections. In this study the effects of extracts from *Artemisia herba-alba* and *Jasmina montana* on ZYMV multiplication in *Cucurbita pepo* plants were evaluated. For this purpose, SDS-PAGE protein profiles and enzyme activities

were examined and induction of resistance by plant elicitors against ZYMV was studied. The results showed that application of the aqueous extracts of *A. herba-alba* or *J. montana* extracts prior to ZYMV inoculation gave 100% inhibition of virus infection. The C. pepo plants treated with mixture of A. herba-alba extract and virus inoculum were 100% resistant. Treatment of squash plants with extracts resulted in production of proteins and phenolic compounds and activated various defense-related enzymes in the squash plants. Genetic variability in DNA genomes of treated and untreated plants was compared. Random amplified polymorphic DNA (RAPD) and Inter-simple sequence repeat (ISSR) profiles for DNA samples differed among treatments and control plants with visible change as indicated by the appearance or disappearance of DNA amplicons. The changes in RAPD and ISSR profiles of representative squash plants can be exploited for an understanding of induced systemic resistance. These elicitors, when activated, induced resistance in squash plants. Consequently, elicitors should be considered in the breeding programs for protection against ZYMV.

PM-271

Production of *Tripterygium regelii* differentiated biomass as a sustainable source of celastrol

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Preparations of *Tripterygium* have been used in traditional Chinese medicine for years. Nowadays, close to 400 secondary metabolites have been reported from *Tripterygium* species, with 95% being terpenoids. One of the most active terpenoids isolated from *Tripterygium* is celastrol which has been used for the treatment of asthma, chronic inflammation, and autoimmune and neuro-degenerative diseases [1]. Celastrol has been shown to have very powerful anti-cancer activity in many cancer types, including leukemia, glioma, melanoma, prostate cancer, and breast cancer with various treatment mechanisms. Even with such a great potential, *Tripterygium* is not cultivated at large scale. Here we present several system of *in vitro* propagation for *Tripterygium regelii* to obtain a sustainable production of celastrol. We evaluate the importance of plant genotype and culture system and we identified the best organ (i.e. leaves or roots) to produce celastrol in vitro. Quantification and identification of celastrol was performed with HPLC-DAD and LC/MS along with standard compounds. After six weeks of culture in bioreactors, plant biomass was increased approximately nine times. Celastrol accumulation in leaves was four-fold increased in rooted plants in both genotypes. However, roots shown the highest amount of celastrol per weight of biomass, almost four times more than in leaves.

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PM-272

Hormonal effects of carbenicillin and cefotaxime on *Rhodiola rosea* callus culture

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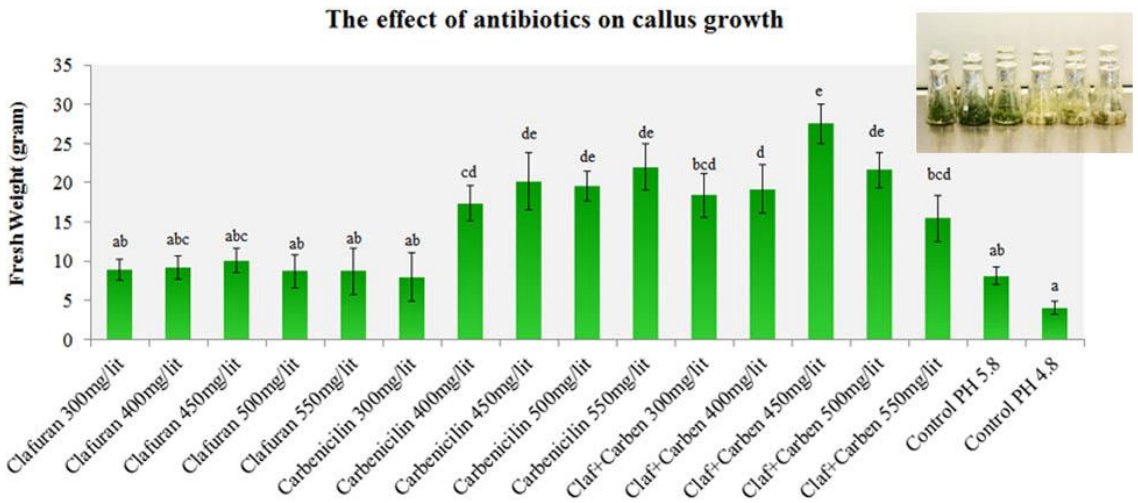
Rhodiola rosea L. is one of the well-studied medicinal plants for its valuable pharmaceutical significance. Roseroot is difficult to cultivate and develops very slowly in its natural environment, justifying development of new methods for production of its pharmaceuticals. An attempt was done to optimise the callus culture condition to obtain the better *in vitro*yield. Culture medium contained (Sucrose (30 g/L), MS (4.4 g/L), NAA (1 mg/L) and BAP (0.5 mg/L) [1]. Different concentration of Carbenicillin and Cefotaxime (claforan) (300, 400, 450, 500 and 550 mg/L) separately and in equal combination were applied for 1 mounth in 10 days intervals. No inhibitory effects were observed in all of the treatments compared to control. The highest fresh and dry weight was measured in the medium with 450 mg/l of both carbenicilline and claforan (Figure) with about 20 fold higher accretion rate comparing with control. 28 grams fresh callus from 1 gram of starting plant tissue was recorded in this treatment after 1 month. Whereas, when antibiotics were added individually, the highest callus yield was 10 grams for calaforan (450 mg/L) and 20 grams for carbenicillin (500 mg/l), respectively when compared with the control (8 grams). This remarkable increase in callus growth rate is very significant from an *in vitro*production stand point. This result if applied for engineered roseroot cell lines, would lead to a justifiable *in vitro*production in a large scale.



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PM-273

Comparison of different wound healing assays performed with lupeol-treated human keratinocytes

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Wound healing is a complex process comprising three different phases: inflammation, new tissue formation, and remodeling [1]. To avoid expensive animal models, where also ethical aspects have to be considered, *in vitro* assays with monocultures of human skin cells represent an alternative. There are several assays described in the literature focusing on the tissue formation phase where keratinocyte and fibroblast migration is essential [2]. In our studies, we tested five *in vitro*wound-healing assays and evaluated their advantages and limitations. We used human keratinocytes that were treated with HGF and the natural compound lupeol. HGF is known to promote the migration and lupeol is one of the main components of a triterpene enriched birch bark extract (TE1) that is known to improve wound healing [3,4]. We tested the "classical" scratch assay where a confluent cell monolayer is disrupted with a pipette tip and subsequently "wound" closure is observed over time. Additionally, the OrisTM-Cell Migration Assay and experiments with ibidi culture-inserts were carried out. For these assays keratinocytes were grown in a special chamber with a space holder that creates a defined cell-free gap in which the cells start to migrate after removal of the space holder. As a further assay the xCelligence method is described where cells have to migrate through a membrane in a modified Boyden chamber and the migration is measured by impedance. A more laborious approach was to track single cell movement using a fluorescence microscope. All these methods have their advantages but also their limitations; nevertheless, the simple "scratch assay" seems to be the one where the advantages predominate.

Acknowledgement: Financial support by Aif is gratefully acknowledged.

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PM-274

Polyketides from cultured lichen mycobionts of *Pseudopyrenula subnudata* and their biosynthetic origin

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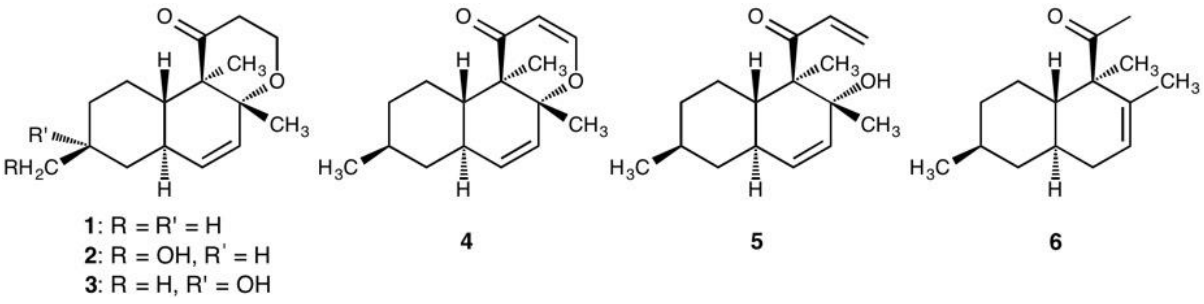
Lichens are symbiotic organisms, composed of a fungus (mycobiont) and one or more algae and/or cyanobacteria (phytobiont). Lichens are well known to produce a wide range of characteristic secondary metabolites, namely, lichen substances, some of which are potentially useful and biologically active compounds. The majority of lichen substances are secondary metabolites of the fungal component, in symbiosis or in the aposymbiotic state. Our previous studies indicated that aposymbiotically cultivated lichen mycobionts under axenic conditions produce novel substances that differ from the secondary metabolites of intact lichens, but are structurally similar to fungal metabolites [1,2]. Continuing our pursuit of novel bioactive metabolites from Vietnamese lichen-derived fungi [3], we cultivated spore-derived mycobionts of the crustose lichen *Pseudopyrenula subnudata* collected in Vietnam to isolate six new compounds from the cultures. The novel compounds **1**—**6** were polyketides closely related to versiol from the sponge-derived fungus, Aspergillus versicolor [4,5]. Their structures were elucidated by spectroscopic and chemical means. The assembly pattern of acetate units in their biosynthesis was also studied by administration of sodium [1-^{13}C]-acetate and sodium [1,2-$^{13}C_2$]-acetate to the culture.



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PM-275

Enhanced rosmarinic acid accumulation and rosmarinic acid synthase gene expression under drought stress in thyme (*Thymus vulgaris*)

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Rosmarinic acid (RA) is the ester of 3,4-dihydroxyphenyllactic acid and caffeic acid and widely occurs in the Lamiaceae. It has numerous biological effects such as antiviral, antibacterial, anti-inflammatory and antioxidant activity. In an earlier study RA accumulation correlated with the expression of rosmarinic acid synthase (RAS) gene in in vitro lemon balm (*Melissa officinalis* L.) cell cultures [1].

Our goal was to study the effect of irrigation and water deficiency in thyme (*Thymus vulgaris* L.) on RA accumulation and RAS gene expression.

The plants were grown in pots in 2014. The soil water capacity was 70% in the controls (irrigated) and it was 40% in the drought stress (water deficiency). Four genotypes were involved in the experiment; 'Varico' – thymol chemotype, TV17 candidate cultivar - thymol chemotype, TV115 - geraniol chemotype, TV143 - α-terpineol chemotype. The samples were collected 3 times at 3 weeks intervals. RA content was determined by HPLC. RNA was extracted with a CTAB-based method. RNA samples were DNase treated and cDNA was transcribed. Primers were designed based on the lemon balm RAS sequence and the amplified region was sequenced. The gene expression study was done by real-time PCR with actin and EF1 α reference genes.

According to the HPLC results the RA accumulation was 50-60% higher under drought stress. However each chemotypes reacted differently. The sequenced region of thyme showed 89% similarity to the corresponding region of the RAS sequence of lemon balm. The gene expression study showed twice as high relative RAS gene expression in the non-irrigated samples during the summer.

Based on these results RAS expression correlated with the RA accumulation in thyme plants. According to our results drought is favourable for higher RA content in thyme, while watering has an adverse effect on the RAS expression and hence on the RA accumulation.

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PM-276

RAPD marker linked *Potato Virus Y* resistant potato cultivars and treated with ribosome inactivating proteins (RIPs)

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Ribosome inactivating proteins (RIPs) isolated from *Phytolacca* sp. and *Mirabilis jalapa*, which inhibit the necrotic tuber necrosis strain of potato virus Y (PVYNTN) infection by catalytically removing a specific adenine residue from the 28S RNA. The primary objective of this study was the development of a simple and novel strategy for controlling PVYNTN by using of leaf extracts from *Phytolacca* sp., and *Mirabilis jalapa* plants. In addition, assessment of genetic variability among PVYNTN resistant, susceptible, and the healthy control potato plants was carried out by Random amplified polymorphic DNA (RAPD). Spraying extracts on five potato cultivars ('Selan', 'Spunta', 'Cara', 'Diamond' and 'Nicola') before virus inoculation resulted in 100% inhibition of PVYNTN infection; these results were confirmed by DAS-ELISA. Analysis of variance showed there were no significant differences in number, weight and volume of tubers between extract-treated potato plants and healthy control in all five potato cultivars. RAPD marker generated by five arbitrary decamers was used to determine DNA polymorphism among the PVYNTN resistant, infected and healthy control potato plants. A total of 65 reproducible fragments ranging from 100-1200 bp were scored using five primers. Thirty-three out of 65 (50.77%) bands were polymorphic, and the remaining 32 (49.23%) were monomorphic. The number of bands for each primer varied from 5 to 22 fragments. Among the 65 fragments, 18 (27.69%) turned out to be reproducible and regarded as reliable RAPD markers for further analyses. Extract treated potato cultivars and the PVYNTN resistance varied considerably using the five primers of RAPD-PCR, whereas the 'Selan' cultivar appeared to have the highest number of markers (seven), followed by 'Spunta' (six), while 'Cara' scored the lowest number from unique markers (one). This information should be taken into consideration in future breeding programs. The use of leaf extract sprays as those from *P. americana*, *P. acinosa* and *M. jalapa* on various crops are expected to prevent or control viral infection.

Poster session 2.

Ethnobotany and ethnopharmacology

PW-01

In vivo Toxicological Evaluation of *Syzygium malaccense* (L.) in Rats

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The study was aimed at evaluating sub-acute toxicity of the extract of *Syzygium malaccense* (L.) Merr. & Perry in albino rats. Five groups of eight rats per group were orally administered with graded 50, 100, 250 and 500 mgkg-1 body weight doses of the extract for 28 days [1]. Blood samples of the sacrificed rats were collected for biochemical and haematological studies while liver and kidney tissues were used for histopathological assessment. The results showed an LD50 of 1224.75 mg kg-1 body weight with no significant ($p>0.05$) changes in weight of organs tested. Biochemical parameters such as AST, ALP, protein and albumin levels in all the treated animals did not change significantly, however there was significant ($p<0.05$) change in the activity of ALT as well as some haematological parameters such as, RBC, WBC, HB, platelet counts, MCV and MCH when compared with the control group. The results from histopathology showed an inflammation of the liver cells at doses beyond 250 mg kg-1 body weight but there was no significant damage to the kidney tissue. It may be concluded that the extract of *S. malaccense* possesses the tendency of affecting the haematopoietic elements significantly. However, it may also alter the structural integrity of the liver tissue if ingested at higher doses.

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PW-02

In vitro studies on the anti-inflammatory potential of chamomile, myrrh and coffee charcoal – components of a traditional herbal medicinal product (Myrrhinil-Intest®)

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The herbal medicinal product Myrrhinil-Intest® consists of myrrh, chamomile flower dry extract and coffee charcoal. Clinical data prove the effectiveness of this herbal preparation for inflammatory intestinal disorders.

To further investigate the anti-inflammatory potential of the single components as part of a multi-target principle, an ethanolic (MY) and aqueous (MYA) myrrh extract, ethanolic chamomile flower extract (KA) and aqueous coffee charcoal extract (CC), were examined in an in vitro TNBS inflammation model using rat small intestinal preparations. The effect of the plant extracts on TNBS induced inflammatory damage was characterised based on TNFα-gene expression analysis, isometric contraction measurement and histological analysis. Furthermore, TNFα-release from LPS-stimulated THP-1 cells was determined. Budesonide was used as positive control. Additionally, microarray gene expression analysis was performed in LPS/IFNγ stimulated native human macrophages to determine potential underlying mechanisms.

The TNBS-induced overexpression of TNFα-mRNA was reduced after KA (0.1 mg/ml) and MYA (1 mg/ml) treatment down to 24% and 16% resp.; TNBS-induced loss of contractility and reduction of mucosal layer thickness was inhibited after KA (3 mg/ml) treatment by 26% and 25% resp.; after MYA (0.1-1mg/ml) treatment by 17% and 44% resp. LPS-induced TNFα release from THP-1 cells was inhibited concentration-dependently by MY (IC_{50}=60.65 µg/ml; 97% inhib.), KA (IC_{50}=439 µg/ml; 71% inhib.) and CC (IC_{50}=1886 µg/ml; 44% inhib.). Furthermore, KA (200 µg/ml) and CC (500 µg/ml) inhibited the LPS/IFNγ-induced expression of genes associated with chemokine signalling up to 100fold (for CXCL13). The presented study demonstrates further evidence for anti-inflammatory properties of the herbal components which contribute to the reported clinical effectiveness.

PW-03

Antioxidative effects of enzymatic hydrolysates of *Umbilicaria esculenta*

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Umbilicaria esculenta is a lichen of the genus *Umbilicaria* that grows on rocks. It can be found in East Asia including in China, Japan, and Korea. It is edible when properly prepared and has been used as a food source and medicine. The antioxidative effects of enzymatic hydrolysates from *Umbilicaria esculenta* (*U. esculenta*) was evaluated by measuring 1,1-diphenyl-2-picrylhydrazyl (DPPH) and alkyl radical scavenging activities using an electron spin resonance (ESR) spectrometer. In addition, protection effect on H_2O_2-induced DNA and cell damage. In our study, *U. esculenta* was enzymatically hydrolyzed by seven carbohydrases and eight proteinases. Flavourzyme hydrolysates from *U. esculenta*showed clearly superior alkyl radical scavenging activity and yield. In addition, flavourzyme hydrolysates from *U. esculenta*showed protective effect on DNA damage and cell death in PC-12 cells against H_2O_2-induced oxidative damage in a dose dependent manner. These results indicate that enzymatic hydrolysates of *U. esculenta* possess potential antioxidative activity.

PW-04

Ellagitannins, ellagic acid derivatives, stilbenes and flavonoids in antibacterial stem bark and leaf extracts of African medicinal plants *Combretum psidioides* and *Combretum fragrans*

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Combretum psidioides and *C. fragrans* are used as decoctions for diarrhea and wounds in African traditional medicine [1]. In Nigeria *C. fragrans* is used for the treatment of Blackleg disease in cattle among Fulani pastoralists. There exists no research on antibacterial compounds of these species but some phytochemical investigations have been performed on *C. psidioides*; fourteen phenanthrenes and stilbenes are known from the heart wood and pentacyclic triterpenoids have been characterized from the roots [2].

We have found that methanol and butanol extracts of the stem bark of *C. psidioides* gave good growth inhibitory effects against *Mycobacterium smegmatis*, *S. aureus* and *P. aeruginosa*. Our UHPLC-MS-QTOF results show that the stem bark of *C. psidioides* contains corilagin and punicalagin along with other ellagitannins as well as ellagic acid rhamnoside. These compounds have not previously been identified in *C. psidioides*. In addition, combretastatin B-2 along with its dihydrostilbene derivative were for the first time identified in the stem bark of C. psidioides.

A leaf extract of *C. fragrans* showed promising antibacterial effects, especially against *Micrococcus luteus* and *S. aureus*. Our preliminary HPLC-DAD results indicate that this extract contains luteolin and related flavonoids and three unknown ellagitannins. Mass spectrometric data is needed to confirm the identity of the compounds.

Acknowledgements: This study has been supported by Finnish Medical Association and Swedish Cultural Foundation in Finland. The first author is grateful for this support. This abstract is dedicated to the memory of Professor Raimo Hiltunen (1944-2014) who has greatly contributed to this work through his mentorship.

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PW-05

Relations between polyphenolics production and enzymatic antioxidant defense in *Pulsatilla montana* ssp.*balcana in vitro*

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Asian *Pulsatilla* species are utilized in traditional medicine as anti-inflammatory, spasmolytic, anti-enteritis and antitumor remedies. Little information is still available on the chemical composition and properties of the Balkan representatives of the genus.

Column and thin layer chromatographic separation of the methanolic extract of the aerials of Balkan endemic *P. montana* ssp. *balcana* led to isolation of miquelianin, caffeic and 3,5-dicaffeoylquinic acids as main components, which were determined by spectroscopic methods. In addition, tiliroside, hyperoside and isoquerctrin were confirmed by thin layer chromatography with authentic samples. Further on, shoot cultures of the plant were developed and treated with plant growth regulators in order to modify developmental patterns and study the enzymatic and non-enzymatic antioxidant defense of the plant *in vitro*. Indole-3-butyric acid stimulated the antioxidant enzymes phenylalanine ammonia-lyase, superoxide dismutase, catalase and glutathione peroxidase, but still increased oxidative stress (determined by the levels of hydrogen peroxide *in vitro*) and intensive callusogenesis were observed, worsening the quality of obtained explants. HPTLC screening showed reduced polyphenolics and DPPH scavenging capacity of these samples. On the contrary, 1-naphthaleneacetic acid led to inhibition of the activity of these enzymes, but stimulation of glutathione reductase and ascorbate peroxidase, as well as elevated non-enzymatic antioxidants as ascorbate and polyphenolics were observed, related to formation of normal rosette clumps and improved radical scavenging capacity of the plants. The results are indicative of the possible interrelations between enzymatic and non-enzymatic antioxidant defense in the plant which might be used as a tool for the optimization of polyphenolics production *in vitro*.

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PW-06

Phenylpropanoid metabolism and pharmacology of the blood lily, *Scadoxus puniceus*, a highly traded South African medicinal plant

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The current study utilised ultra-high performance liquid chromatography coupled with electrospray ionisation tandem mass spectrometry (UHPLC-MS/MS) to quantify the phenolic compounds in different organs of *Scadoxus puniceus*(Amaryllidaceae), an important South African medicinal plant. The antioxidant, antimicrobial and acetylcholinesterase (AChE) inhibitory activities of this species were also evaluated. UHPLC-MS/MS revealed a greater profusion of hydroxycinnamic acids (HCAs), accounting for 69.5% of the total phenolic acids as opposed to hydroxybenzoic acids (HBAs). Chlorogenic acid (CGA; 3-caffeoyl-D-quinic acid) was highly abundant (49.6% of HCAs) in the aerial organs suggesting a functional role of the compound against herbivory from the Amaryllis leaf borer which infested many of the stock plants. In addition to CGA, the current study is the first to report the presence of sinapic, gallic and m-hydroxybenzoic acids in the Amaryllidaceae. The accumulation of CGA in the leaves of S. puniceus substantiated the fact that leaf extracts exhibit significantly improved antioxidant activity (DPPH IC_{50}=0.07 mg/ml) as compared to the bulb. CGA, a known antifungal agent conferred potent antifungal activity (minimum inhibitory concentration; MIC<0.10 mg/ml) of leaf extracts against *Candida albicans*. Furthermore, ethanolic extracts of different organs of *S. puniceus* revealed AChE inhibitory activity in excess of 90% (IC_{50}=0.07-0.15 mg/ml), with no significant difference between bulb and leaf extracts.

PW-07

Antiviral phenolic compounds from the whole plants of *Zostera marina* against influenza A virus

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Zostera marina L. is a perennial seagrass in the family Zosteraceae that lives in the nearshores of East Asia, Europe, and North America [1,2]. *Z. marina* is known as a seaweed bed for fish and shellfish, and a traditional Chinese medicine [3,4]. Previous phytochemical studies on *Z. marina* have afforded several phenolic compounds and lipids [4]. In the course of a search for anti-influenza viral compound from natural products, we have found that EtOAc and BuOH fractions of the EtOH extract of *Z. marina* have antiviral activity against influenza A virus, (IC_{50}: 4.95 μg/mL, SI: 14.80; IC_{50}: 68.93 μg/mL, SI: 8.15, respectively) The EtOAc and BuOH fractions, which exhibited comparatively higher activity than the other fractions, were subjected to column chromatographic separation. Six phenolic compounds were isolated and their structures were identified as luteolin, apigenin 7-glucoside, luteolin 7-glucoside, p-sulfoxy cinnamic acid, luteolin 7-sulfate, luteolin 7,3'-disulfate by spectral analysis. Antiviral

activities for isolated compounds were evaluated using neutral red assay against influenza A/NWS/33(H1N1) virus and neuraminidase inhibition assay in influenza A/NWS/33 virus.

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PW-08

Ethnobotanical study on *Valeriana officinalis* in Bulgaria

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This study focuses on the Bulgarian folk botanical knowledge of *Valeriana officinalis* L. and its aim is to present data about traditional herbal remedies and to give an impression about their contemporary use in relation to the state of the modern herbal market. The study gathered data from more than 35 ethnobotanical and ethnographical sources which provide information for the end of 19th to the middle of the 20th century, in addition to field data collected through semi-structured interviews (2012-2014). Two models of use of valerian were outlined: "ritual-leaves (dry, fresh)-external application" and "medical-roots (dry, fresh)-herbal teas". A total of 28 folk remedies were found which cover a wide range of symptoms ranging from antiseptic, spasmolytic and sedative to cures for edema, cramps and faintings. The main components are medicinal plants (more than 25) from Lamiaceae, Leguminosae and Compositae. The significant participation of species such as *Urtica dioica*, *Ocimum basilicum*, *Malva sylvestris*,*Gentiana cruciata*, *Ruta graveolens*, *Inula helenium*, *Tussilago farfara* and *Juglans regia* (green husks) sheds new light on the list of species that are traditionally used in valerian-based remedies [1]. Valerian roots are ingredients of several panacea-remedies, including quinine, "locmaruhu" and honey.

The research found significant loss of traditional knowledge for valerian (less than 10% is preserved today) and indicates the need to discover some of the folk remedies as traditional herbal medicinal products and as a source of new compounds for drugs.

Acknowledgement: The financial support of the National Science Fund of Bulgaria (project DFNI T02/23) is greatly acknowledged by the authors.

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PW-09

Plants used during pregnancy, chirdbirth and postpartum healthcare in Eastern Azerbaijan, Iran

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In many Southeast Asian cultures the activities and diet during the postpartum period are culturally dictated and a period of confinement is observed. Plants play an important role in recovery during the postpartum period in diet, traditional medicine, steam bath and mother roasting (where mother and child placed on a bed above a brazier with charcoal embers on which aromatic plants are laid).

This research focuses on the use of plants during pregnancy, parturition, postpartum recovery and infant healthcare in Eastern Azerbaijan, Iran. It aims to identify culturally important traditions that may facilitate implementation of culturally appropriate healthcare. Data were collected in 5 different regions in Eastern Azerbaijan province, through group and individual interviews with women by female interviewers.

A total of 90 different plant species are used in women's healthcare, of which belong to 43 genera and 85 species. Most of the plants belong to the Asteraceae, Apiaceae or Rosaceae family. Medicinal plant use is common among villagers of eastern Azerbaijan province and widely prescribed by herbal supplements stores to facilitate childbirth, alleviate menstruation problems, assist recovery after miscarriage, mitigate postpartum hemorrhage, aid postpartum recovery, and for use in infant care.

The wealth of novel insights into plant use and preparation will help to understand culturally important practices such as confinement, dietary restrictions, mother roasting and herbal steam baths and their incorporation into modern healthcare.

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PW-10

Quantitative ethno-botanical analysis and conservation issues of medicinal flora of Northern areas of Pakistan: present and future prospects

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This is a first comprehensive study of Northern areas of Pakistan concerning ethnobotany and conservation status of important medicinal plants. Locals residing in mountainous areas belonging to various ethnic groups are traditionally utilizing plants over many generations.The data was analyzed and compared by quantitative ethnobotanical indices such as Jaccard index (JI), Informant Consensus Factor (ICF), use value (UV) and Relative frequency of citation (RFC). In the present study a total of 325 belonging to 123 families were recorded.11 species of to Gymnosperm,17 species of Pteredophytes and 297 plant species of Angiosperm. The used value showed that *Aconitum violaceum* (UV = 0.68), *Achillea millefolium* (UV = 0.67) and *Paeonia emodi* (UV=0.63). IC value showed that digestive system and respiratory system ailments were ranked highest (FIC = 0.75). According to DMR output, *Juglans regia* ranked first due to multipurpose uses among all species and was found most threatened with higher market value. In comparison, highest similarity index is recorded in these studies with JI 17.72 followed by 16.41. 59 threatened species in northern areas, of which 24 are endangered, 19 vulnerable and 16 are rare.These medicinal plants are used in local community. *Caltha alba*, *Colchicum luteum*, *Paeonia emodi*, *Podophylum emodi* are some of the most threatened species and need special attention. Unwise use of plant resources combined with improper harvesting and post harvesting techniques have intensified pressure on plants of area. The major factors contributing towards plant diversity loss found were poverty, grazing of pasture, forest encroachment, hunting, lopping of trees for fodder, medicinal plant collection, fuel collection, forest fire, invasive species intensify the environment. For sustainable use, *in situ* and *ex situ* conservation, controlled harvesting, and aforestation may be the solution .Further extensive field conservation research is needed.

PW-11

Effects of medicinal plants of the genus *Leonurus* and seventeen of their isolated constituents on the activity of PPARα, β/δ, and γ in an *in vitro* luciferase reporter gene assay

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Previously, we reported the effects of *Leonurus japonicus* Houtt. – used in TCM against the metabolic syndrome – and its N-containing constituents leonurine and stachydrine on the

PPAR system [1]. However, no active constituents could be identified. Here we describe the isolation of 17 further dominant constituents of *L. japonicus* and the related European herb*Leonurus cardiaca* L. – namely 7*R*-chloro-6-desoxy-harpagide [2], ajugol, campneoside II, chicoric acid, ferulic acid, harpagide, isoacteoside, isocampneoside II, isoleosibirin, lavandulifolioside, leonoside A, rutin, and verbascoside, as well as four new phenylethanoids described here for the first time with the preliminary names LC139C, LC138C, LC141B, and LC140A1 – and their screening for activity on the metabolic syndrome related targets PPARα, β/δ, and γ in the above mentioned luciferase reporter gene assay [3]. All 17 isolated constituents (at 6.25, 25, 50, and 100 µg/ml each), and GW0742 (positive control, 0.1 nM) were dissolved in DMSO and added to the medium of the COS-1 cells, transfected as described at [1,3]. For PPARα and γ, the positive controls WY14643 (50 µM) and troglitazone (10 µM), respectively, were used. In this assay, only 7*R*-chloro-6-desoxy-harpagide, which was recently isolated by our group for the first time [2], displayed significant activity in the PPARβ/δ assay at 50 µg/ml while the result for 100 µg/ml was even higher than for the GW0742 positive control. Furthermore, rutin at 100 µg/ml showed weak PPARα agonistic activity. For PPARγ no significant effects were observed. This activity of extracts of medicinal plants of the genus *Leonurus* and especially of their active constituent 7*R*-chloro-6-desoxy-harpagide on the PPARβ/δ subtype of the PPAR system strongly indicates their potential for anti-obesity therapy.

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PW-12

Cytotoxic activity of the Native Austratlian plant *Acacia ligulata*

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This study forms part of an ongoing investigation into the phytochemistry and pharmacological activities of the native Australian plant *Acacia ligulata*. Leaves and bark of the plant has been used as a traditional medicine for cough, colds and general illness whereas the seeds have been eaten and described to "make your hair fall off". Only a few bioactivity studies have included *A. ligulata*, however none have investigated the plant for its cytotoxic activities.

Crude extracts of leaves, bark and different developmental stages of seedpods and seeds were prepared using 80% ethanol. The extracts were tested against a panel of cancer cell lines

including melanoma, breast and leukaemia. A tetrazolium-based (MTS) assay was used to screen for active extracts and calculate IC_{50} values and active extracts were further validated using the Sulforhodamine B cell viability assay.

Bark and seedpod extracts showed cell toxicity effects against a melanoma cell line with IC_{50} values from 37.9-131.8 μg/mL for the MTS assay with similar results obtained from the Sulforhodamine B assay with IC50 values of 38.4-134.7 μg/mL. Chromatographic profiles of the crude extracts were obtained using Liquid Chromatography/Mass Spectrometry (LC/MS), which indicated a similar chemical profile of the different stages of seedpod growth. Further activity-guided fractionation was performed with the mature seedpod extract that gave the best IC_{50} value in the screening.

The crude extract has further been fractionated by solid phase extraction (SPE) and centrifugal radial thin-layer chromatography to identify active fractions. Additional fractionation by HPLC will be performed in order to purify active compounds, which will be analysed by structural elucidation techniques. Through this study is has been shown that crude extracts of bark and seedpods of *A. ligulata* have cytotoxic activity against melanoma cells and may contain novel cytotoxic compounds.

PW-13

Development of anti-ageing natural products based on biodiversity of the Greek flora by employing environmentally friendly technologies and anti-ageing biological research

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Ageing is a complex process driven by diverse molecular pathways and biochemical events. It has been defined as the time-dependent decline of functional capacity and stress resistance and affects most of tissues and organs of the body. The aim of this study is the development of anti-ageing natural products by employing state-of-the art environmentally friendly technologies and anti-ageing biological research. Specifically, a high number of plants (600) from Greek flora was selected and extracted by using "green technologies" (SFE, ASE and MWE). The extracts were investigated for their chemical profile and for their *in vitro* antioxidant activity (DPPH• and ABTS assays). Subsequently, the most promising extracts were applied to human diploid skin fibroblast cells and their antioxidant capacity was recorded by using the DCFH-DA assay. Based on these results 25 plant extracts were selected for further investigation. Assays were mainly based on normal human cells and refer to targets known to

contribute cell protection from age-related damage. Specifically, they were tested for their efficacy against UV protection, as well as for their ability to modulate the proteasome and/or the autophagy-lysosome pathways functionality. "Multi-functional" extracts that apart from exerting antioxidant activity, are also activating main cellular pathways were identified. Plants affording the most bioactive extracts were cultivated, in order to protect the Greek biodiversity, while their chemical profile and biological activity was afresh confirmed. Among others, *Rosa damascena* R. and *Sideritis scardica* L. preparations showed the ability to reduce the ROS levels (to 24% and 51% compare to the control, respectively), while at a non-toxic concentration induced the activation of the proteasome LLVY/β5 peptidase activity. Finally, the results of the aforementioned biological research revealed these extracts as promising anti-ageing agents for potential usage as cosmeceuticals.

PW-14

Medicinal plants in Benin: An analysis of traditionally used Beninese plants indexed in the Prota Database

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There are approximately 7000 useful plants that could play a vital role in food security, health care and income improvement for the 1.2 billion people living in sub-Saharan Africa. PROTA (Plant Resources of Tropical Africa) is a unique project under which the available information on these useful African plants is collected, validated and made available in books and an interactive database. A systematic database search was performed in the Prota database to analyze the indigenous knowledge about herbs in Benin. The Boolean operators were utilized to link the search-terms and the entry field 'countries' was used to limit the search geographically. The results where then evaluated referring to species and families. 724 plant species were listed for medicinal usage in Benin. The dominant plant families were: Fabacaceae (138), Euphorbiaceae (114), Rubiaceae (66), Combretaceae (70) and Asteraceae (70). Species found most often were: *Acacia* (15), *Ficus* (14),*Euphorbia* (9) and *Crotalaria* (9). An analysis of diseases mainly treated with the plants indexed in the database was difficult and did not lead to clear results because many plants are used for various indications and in some cases the indication is unclear. Especially in Benin there was developed list of medicinal plants for the treatment of malaria including instruction for application. An herbal pharmacopeia providing an official list of medicinal plants for other indications is still lacking. Only few Beninese plants applied as herbs have been studied in in-vitro experiments or in clinical trials. The use is based on traditional experiences. Further research referring to safety and efficacy as well as the identification of the active components within the herbs is necessary.

PW-15

Laticifers in the Leaves and Stems of *Gomphocarpus physocarpus*: Distribution, Structure and Chemical Composition

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Gomphocarpus physocarpus (E.Mey.), commonly known as balloon milkweed and *umbababa*, is a short shrub of the Apocynaceae family native to southern Africa. In South Africa, all parts of the plant are used in traditional medicine to produce ointments for the treatment of warts, and the seeds are used in indigenous rituals. The leaves and stems produce a milky latex that is toxic, yet it has never been described in detail, particularly with regards to the anatomy of lactiferous cells. The present study was undertaken to examine the distribution and structure of the laticifers and the chemical composition of the secretions in the leaves and stems of *G. physocarpus*. Histochemical methods and light microscopy were used. The laticifers in the leaves were associated with the vascular system of the midrib and the larger lateral veins. Stem longitudinal and cross sections stained with safranin and fast green revealed the presence of laticifers in the pith, cortex and external to the phloem. Laticifers in the stems were branched, non-articulated and parallel to the longitudinal axis. The main compounds accumulated in the laticifers were phenols and alkaloids. Phytochemical screening of leaf and stem crude ethanolic extracts displayed positive reactions for the presence of carbohydrates, cardiac glycosides, steroids/terpenoids, alkaloids and phenols. The compound groups identified could potentially be contributing to the toxic nature of this plant. The biological and medicinal applications of *G. physocarpus* are yet to be assessed; therefore this study provides a foundation for future work. In addition, the knowledge of laticifer systems of *G. physocarpus* could be of taxonomic importance, considering that this study is the first on laticifers in this genus.

PW-16

An ethnobotanical study on wild medicinal plants sold in the local markets at both sides of the Bulgarian - Turkish border

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Local markets in urban areas nowadays are refuges for traditional knowledge (TK) and places for disseminating of new knowledge and practices about medicinal plants. [1,2]. This study aims to investigate the diversity of wild medicinal plants sold in local markets at both sides of the Bulgarian-Turkish border and to understand their role for the local people in prevention, treatment and healing. Data was collected through 2011-2014 from local markets by free-listed observations and semi-structured interviews. A total of 39 wild medicinal plants belonging to 18 families were documented (Bg: 28, Tr: 26), which were sold mostly dry as mono-component herbal teas. The plants were used mostly as antiseptic, to treat skin diseases, for stomach problems, cough, cold, asthma and against diabetes. Among the 17 species that overlapped in the study area, the most common species were *Cotinus coggygria*, *Rosa canina*, *Urtica dioica*, *Hypericum perforatum*, *Sideritis scardica* (endemic) and *Tilia* spp. The specific use of plants for border sub-regions can be outlined as follows: as ready prepared herbal

mixtures (Bg), as sale of more fresh herbs (Tr), *Urtica urens* (Tr) as a cultural distinctive plant, wild fruits for healthy and ritual food (hosaf) (Tr) and some rare herbal substances (*Fragaria* sp., calyx) (Tr). *Prunus spinosa* was marked as the diabetes perspective plant. It was found in the study that there was confusion and dual use of herbal substance (dried leaves) of *Thalictrum aquilegifolium*, and registered herbal product, caused by insufficient or lost TK and gaps in the current processes of sustainable use of medicinal plants and management of traditional herbal medicinal products.

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PW-17

Toxicity of *Acacia nilotica* (Garad) to Nubian goats

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Acacia nilotica Delile (Leguminosae) is a spiny tree growing in western and central Africa and Arabia. Its flat brown pods are widely used in some African countries and the Middle East for the treatment of many conditions including diarrhea, cough, fever, common cold and influenza [1]. It is taken either orally as an aqueous extract, applied topically as a powder or inhaled as fumes evolving from heated pods. Chemical analysis of the pods revealed the presence of tannins and some galloyalated flavans. No known data about its toxicological effects.A recent study reported analgesic, antipyretic and anti-inflammatory activity of an aqueous extract of *A. nilotica* administered orally to rats [2].

The clinical, patological ,haematological and biological changes in Nubian goats given daily oral doses of 1 or 5 g/kg body weight of Acacia nilotica .Other than the dose co-related mortality rates, the clinical signs were observed to be salivation ,staggered gait, intermittent loss of voice and low appetite. On histopathological testing ,the main lesions were hepatic centrolobular necrosis and fatty changes associated with the significant changes in GGT and ALP are indicating hepatic dysfunction.Renal malfunction is indicated by haemorrhages in addition to the change in the urea concentration.The congested , haemorrhagic, emphysematous, edematous and cyanotic lungs may contribute to the development of dyspnoea.*Acacia nilotic*a poisoning may lead to an immunosupression pointed out by the lymphocyte infiltrarion.

On evaluation of the above results *Acacia nilotica* was considered toxic to Nubian goats at the above mentioned doses.

[1] Al-Gazali et al., 1987

[2] Dafallah and Al-Mustafa, 1996

PW-18

The establishment of a natural products database from the Brazilian Biodiversity

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The diversity of natural products provides unique chemical scaffolds that have been used as templates for medicinal chemistry and drug discovery. Nevertheless, the lack of organized data is still one of the drawbacks for the new advances on natural products and medicinal chemistry. Recently we reported the organization of the first database containing botanical, chemical, and biological information of the secondary metabolites isolated from species of Cerrado and Atlantic Forest, by NuBBE during 15 years of research ($NuBBE_{DB}$, http://nubbe.iq.unesp.br/nubbeDB.html) [1]. The compilation of secondary metabolites data is of great value, especially concerning several molecular parameters, useful for natural products drug discovery. This database has recently established a link with the Royal Society of Chemistry, and thus all information available in $NuBBE_{DB}$ can additionally be accessed in ChemSpider database. Considering the impact of the $NuBBE_{DB}$ [2], our recent efforts concern the inclusion of the secondary metabolites of the Brazilian biodiversity species. The new platform has an additional functionality that will provide simulated spectroscopic data (^{1}H and ^{13}C NMR) for all the reported compounds, which is developed using the highly accurate algorithms of the ACD/NMR Predictors together with an integration algorithm in Matlab. $NuBBE_{DB}$ is an original initiative aimed at cataloguing natural products of the Brazilian biodiversity, providing all this knowledge in a standardized and useful manner for studies on dereplication, metabolomics, computational screening and design of novel bioactive compounds.

Acknowledgement: Cnpq, Fapesp and Capes.

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PW-19

New insights into aromatic medicinal plant use by Australian Aboriginal People

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Medicinal plants used by Australian Aboriginal people include many essential oil yielding species. In many cases desired therapeutic effects may be directly mediated by volatiles. In addition, volatile components may act indirectly as carriers of fixed components or even as aromatic markers guiding harvest selection by the designated healer or shaman in species with

extensive widespread chemovariation. Here we review our recent research concerning the chemistry and bioactivity of volatile and fixed components of native Australian plants selected on an ethnopharmacological basis, particularly concerning members of the genus *Eremophila* (Scrophulariaceae). Therapeutic usage modalities often involved ritualistic smoking ceremonies (smudging), or alternatively, topical treatments using lipophilic volatile and fixed components extracted into animal fats. We have developed several techniques for laboratory 'smudging' simulation producing greatly enhanced activity in smoke condensates by comparison with volatiles produced by hydrodistillation alone. Other medicinal plants including *Pittosporum* spp. (Pittosporaceae), *Callitris* spp. (Cupressaceae) or *Geijera* spp. (Rutaceae), were similarly employed and are further explored herein. Comprehensive investigation into the pharmacology, chemistry and clinical value of endemic medicinal Australian plants has hitherto involved only a small number of species of known commercial value including *Eucalyptus* and *Melaleuca* species. Informed by an ethnopharmacological approach, our research extends the range of species with potential therapeutic and commercial value and casts light on the importance of traditional custom usage modalities, providing the basis for longer term government and private investment in partnership with Aboriginal communities in an emerging health care industry addressing the imperatives of a globalised market while providing employment opportunities for Aboriginal people in marginalised regional communities.

PW-20

Targeted isolation of prenylated isoflavonoids from *Erythrina excelsa* using LC-MS and HSCCC techniques

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Erythrina excelsa Baker of Leguminosae family is geographically confined in sub-Saharan Africa, where is widely used to alleviate menopausal symptoms. Despite its large utilization, scientific reports of its phytochemistry and pharmacology are very scarce. Therefore, the present study was aimed firstly at investigating the estrogenic properties of ethanol extract of the stem bark of *E. excelsa in vitro* using the yeast estrogen screen-YES, and *in vivo* using a 3-day uterotrophic assay. Secondly, a phytochemical investigation of this extract was performed using both LC-MS and HSCCC techniques in order to identify the potential active components.

As results, the ethanol extract of *E. excelsa* showed a dose-dependent estrogenic activity in YES. Likewise, in rats, this extract increased significantly the uterine wet weight and uterine epithelial height at the dose of 50 mg/kg/day. The tentative identification of *E. excelsa* active secondary metabolites was performed on a UHPLC-LTQ-ESI-Orbitrap system. Chromatographic and spectrometric features such as retention time (Rt), UV-Vis spectra, suggested elemental composition (EC), Ring Double Bond equivalent (RDBeq) values as well as the HRMS/MS spectra were incorporated for the identification procedure. The extract

revealed a rich content in prenylated derivatives of genistein. Subsequent targeted fractionation with step-gradient HSCCC led to the isolation of 6 prenylated isoflavonoids namely erythrinin B, erythrinin C, laburnetin, alpinum isoflavone, warangalone, erysenegalensein M, and 2 pterocarpans, coumestrol and demethyl medicarpin.

In conclusion, the results of this study provided scientific evidence of the estrogenic potential of *E. excelsa* both *in vitro* and*in vivo* verifying the ethnopharmacological use. Moreover, LC-MS and HSCCC techniques were successfully applied for the rapid identification and targeted isolation of active isoflavonoid constituents.

PW-21

In vitro antileishmanial activity of Brazilian plant species

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Leishmaniasis is an infectious disease still widely spread in Brazil. It is a public health problem for the country due to its magnitude and geographic expansion leading to a complex, expensive and laborious control system [1,2]. There is an urgent need to search for new medicines to treat leishmaniasis. Natural products can provide unlimited opportunities for the discovery of new lead compounds targeting this neglected disease. In this work, we investigated the antileishmanial activity of 22 plant extracts, from seven different species belonging to Apocynaceae, Asteraceae, Ebenaceae, Primulaceae and Rutaceae families. Samples were evaluated against promastigotes from *L. infantum* (strain BH46). The antileishmanial activity was measured 24 h after exposition to the sample using the resazurin-based viability assay [3]. The toxicity of test samples is currently being investigated using dye exclusion method for cell viability using non-infected macrophages. According to preliminary results, four extracts from *Aspidosperma* spp. showed antileishmanial activity. The dichloromethane (DCM) extract from the stem showed an IC_{50} value of 89.5 ± 4.9 µg/mL. The IC_{50} of the remaining active extracts is currently being determined. The antileishmanial activity of this species will be further investigated, using intra- and extracellular parasite forms from other *Leishmania* species. The metabolic profiling and targeted isolation of bioactive constituents are in progress aiming for the identification of new lead compounds with antileishmanial activity.

Acknowledgement: To CNPq for the financial support to Dr. VL Almeida (ref. 249299/2013-5).

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PW-22

Exploration of apoptotic effect in cancer cells treated with stingless bee *Trigona incisa* propolis native to East Kalimantan, Indonesia

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The challenge in the fight against cancer is the non-specific nature of current treatments. The search for specific drugs that are non-cytotoxic to normal cells and can effectively target cancer cells has led some researchers to explore the potential anti-cancer activity of natural compounds. Propolis from honey bee is one of alternative resources that have been traditionally used in indigenous people. However, wild propolis from stingless bee species is not commonly known. This study provides a detailed look at the effect of major compound isolated from *Trigona incisa*, native stingless bees from Indonesia on cancerous cells. Extraction and partition was by n-hexane, ethyl acetate and methanol, respectively. Then, the cytotoxic activity was checked in lung cancer (Chago), breast cancer (BT-474), liver cancer (Hep-G2), colon cancer (SW620) and gastric (KATO-III) cancer cell lines to lead an active fraction. Then, it was purified by chromatography and analyzed by NMR to identify the compound. We found that the active compound was alkylresorcinol ($C_{21}H_{36}O_2$) or cardol as a major compound from this stingless bee propolis. Our results indicated that cardol induced apoptosis in cancer cells with IC_{50} of 0.81±0.18 μg/mL (Chago), 4.28±0.14 μg/mL (BT-474), 0.71±0.22 μg/mL (Hep-G2), 4.51±0.76 μg/mL (SW620) and 6.06±0.39 μg/mL (KATO-III). One of the mechanisms by which chemotherapeutics destroy cancer cells is by inducing apoptosis. Here, cardol showed program cell death at early apoptosis (2 h, 4 h and 6 h of incubation) on SW620. Cell arrest in G0/G1 sub phase was observed. This compound specifically in cancer cells may lead to the development of new and more effective cancer fighting agents.

PW-23

Antimicrobial activity of the extracts from some *Phyllanthus* species cultivated in vitro

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In subtropical regions the extracts from *Phyllanthus* species are used in the treatment of bacterial, fungal and parasitic diseases (e.g. *P. niruri*, *P. amarus*). Literature data showed that antimicrobial activity of *Phyllanthus* species results from synergistic effects of occurring secondary metabolites.

The MIC and MBC values were determined to show antibacterial activity of extracts from shoot cultures of *P. glaucus*, *P. multiflorus* and *P. juglandifolius* and shoot culture and non-transformed root culture of *P. amarus*. The dry extracts dissolved in either DMSO or methanol were examined against strains of Gram (+) bacteria – *Streptococcus G*, *S. aureus*, *S. epidermidis*, *B. subtilis*, *C. sporogenes*, *E. hirae*, strains of Gram (-) bacteria – *M. catarrhalis*, *K. pneumoniae*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, *H. pylori* as well as a yeast *C. albicans*. As controls, the alkaloid securinine, present as a dominant compound of the alkaloid fraction in the shoot culture of *P. glaucus*, and flavan-3-ol – catechin present in all tested species, were used.

Using DMSO to dissolve dry extracts higher MIC values were observed than when dissolved in methanol. The obtained results showed the differences in bacterial susceptibility depending on the *Phyllanthus* species. For the methanol extracts antimicrobial activity expressed as MIC was in a range from 0.01 mg/ml to 5.0 mg/ml, while the MBC value was determined from 0.01 mg/ml to 10.0 mg/ml. Securinine and catechin were active in a range from 0.03 to 0.25 mg/ml showing the highest activity against *Streptococcus G* and *P. aeruginosa* (securinine) and *H. pylori* (catechin).

All extracts and standards were inactive against *E. hirae* while the most susceptible strain was *M. catarrhalis* (MBC 0.01 mg/l).

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PW-24

DNA authentication of tulsi (*Ocimum tenuiflorum*) using the nuclear ribosomal internal transcribed spacer (ITS) and the chloroplast intergenic spacer *trnH-psbA*

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DNA barcoding, a technique used to identify species based on short regions of DNA [1], can discriminate between different species, and identify contaminants and adulterants. *Ocimum tenuiflorum* L., commonly known as holy basil or tulsi, is native to Asia. Three types of tulsi are recognised in the Hindu culture (figure 1); Raam and Shyam (Krishna) are varieties of *O. tenuiflorum*, whilst Vana tulsi is *O. gratissimum* L. [2]. Following migration, tulsi plants are widely grown in South Asian (SA) households across the UK. The aim of this research is to use DNA barcoding techniques to identify tulsi plants collected from SA families, using ITS and *trnH-psbA* regions.

A variety of tulsi samples were collected for authentication: community samples from SA families in the UK, commercial samples, and vouchered specimens. DNA analyses discovered that samples described as Shyam tulsi collected from communities in the UK were *O. tenuiflorum*, but Raam tulsi samples were *O. gratissimum*. Commercial samples proved to be recalcitrant to DNA extraction; this could be because the DNA had been degraded during manufacturing processes. Vouchered specimen obtained were used to create reference DNA barcodes which were not available in the current DNA databases.

Both ITS and *trnH-psbA* regions were successfully used to distinguish between *O. tenuiflorum* and *O. gratissimum*samples. The plastid *trnH-psbA* primers were more efficient for amplification and sequence analysis than the ITS region. The results exemplify how with the transmission of traditions from one country to another, confusion of species is occurring. Both the ITS and *trnH-psbA* regions had their strengths and limitations which reinforces the importance of using multiple primers for species authentication.

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Figure 1: Tulsi plants in India

PW-25

Antimicrobial and Toxicological Studies with Extracts of *Ricinodendron heudelotti* (Euphrobiaceae)

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The use of plants as medicines is as old as the ancient man. The isolation of quinine from the Cinchona bark by the French scientists led to the beginning of the study of active principles from plants [1]. This research work was designed to assess the phytochemical, biochemical and antimicrobial activity of leaf extract of *Ricinodendron heudelotti*. The antimicrobial activity of the extract was investigated against 10 different species; *Klebisella* sp, *Pseudonomas* sp, *Shigella* sp, *E.coli*, *Staphylococcus* sp, *Bacillus* sp, *Micococcus* sp, *Streptococcus* sp, *Salmonella* sp and *Candida* sp. by agar well diffusion method and minimum inhibitory concentrations were determined. The effects of oral administration of the extract on biochemical parameters were investigated in albino rats for 28 days. Five groups of seven male rats per group were used for the study. Animals in group A were administered with normal saline while rats in groups B, C, D and E were treated with 250, 500, 750 and 1000mg/kg b.w.of the extract. Animals were anaesthetized, blood samples were collected for biochemical assays; organs were isolated and weighed, while the liver was processed for histopathological studies. The result showed that the extract of *R. heudelotti* contains tannins, polyphenols, terpenoids, glycosides and alkaloids and was active against 7 of the 10 microorganisms tested

with zone of inhibition ranging from 14–36mm and minimum inhibitory concentrations of 31.25 and 62.5mg. ALT, AST, cholesterol, HDL cholesterol and total bilirubin were significantly ($p< 0.05$) increased in all treated groups. ALP, urea and creatinine were significantly ($p<0.05$) reduced when compared to the control. Histopathological studies indicated extensive areas of hepatocytes containing micro-globules of fat deposit across the treated groups. The results suggest that constituents of the plant might be used as antibiotics but the prolonged use of extracts may induce liver injury.

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PW-26

Antimicrobial and antioxidant activity of the crude leaf extracts of *Pycnostachys urticifolia* Hook (Lamiaceae)

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Pycnostachys urticifolia is an ethnomedicinal plant indigenous to South Africa. Its crude leaf extracts possess phenolics, alkaloids, flavonoids, terpenoids and saponins amongst other bioactive compounds. This study compared the antimicrobial and antioxidant activities of young and mature leaf extracts obtained by soxhlation and cold sonication. Evaluation of the antimicrobial activity of hexane, chloroform and methanol extracts were performed against Gram-positive and Gram-negative bacteria and fungi by the microdilution method [1]. The antioxidant activity of 50% methanol extracts was assessed using the ferric-reducing antioxidant power (FRAP), DPPH radical scavenging and β-carotene-linoleic acid model assays [2]. Good antimicrobial activity against all bacteria and fungi was exhibited by the soxhlated chloroform extracts of young and mature leaves (between 0.156 and 0.625 mg/ml). The FRAP activity of soxhlated extracts was greater than cold sonicated extracts in a dose-dependent manner (between 0.078 and 1.25 mg/ml). Mature leaves showed greater FRAP activity than young leaves irrespective of the extraction method (<0.3125 mg/ml). A greater DPPH radical scavenging activity was observed in mature leaf extracts compared to that of the young. All extracts presented noteworthy antioxidant activity compared to butylated hydroxytoluene based on the rate of β-carotene bleaching (73.1-95.9%). *Pycnostachys urticifolia* possesses considerable antimicrobial and antioxidant activity that validates the traditional use of this species.

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PW-27

Antiviral Phlorotannin from *Eisenia bicyclis* against Human Papillomavirus *in vitro*

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The edible brown alga *Eisenia bicyclis* belongs to the family Lessoniaceae and is distributed along the coastal area of Korea and Japan [1,2]. It has been used as an industrial source of alginic acid, and an edible alga [2]. Previous studies on *E. bicyclis* have afforded several phlorotannins, and have investigated various biological activities. Genital human papillomavirus (HPV) infection is the most common sexually transmitted infection, and virtually most of cervical cancers are attributable to HPVs infection. In the course of a search for anti-HPV compound from natural products, we have found that the EtOH extract of *E. bicyclis* has anti-viral activity against HPV16PVs and HPV18PVs. The EtOH extract was consecutively partitioned with CH_2Cl_2, EtOAc and *n*-BuOH, and the EtOAc fraction, which showed comparatively higher anti-viral activity than the other fractions, was subjected to column chromatographic separation. Five phlorotannins were isolated from the EtOAc fraction, and their structures were identified as eckol, dieckol, 8,8'-bieckol, 6,6'-bieckol, and phlorofucofuroeckol-A. Anti-viral activity for isolated compounds was evaluated using bio-luminescence (SEAP) assay on HPV16PVs and HPV18PVs infected 293TT cells. All compounds exhibited reduction of HPV16PVs and HPV18PVs at concentration 50 μg/ml.

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PW-28

The Sapotaceae as a source of antitubercular metabolites and isolation of antimycobacterial pentacyclic triterpenes from *Sideroxylon inerme*

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Tuberculosis (TB), a chronic infectious disease caused by *Mycobacterium tuberculosi*s, infects approximately one-third of the world's population. Other *Mycobacterium* species including the zoonotic *M. bovis* and nontuberculous mycobacteria such as *M. kansasii* and *M. fortuitum* also infect humans, especially those with compromised immunity. Preliminary investigations showing good *in vitro* antimycobacterial activity in plants of the Sapotaceae family encouraged further study for novel antitubercular chemicals. Ten Sapotaceae species were tested for antimycobacterial efficacy against a panel of non-pathogenic mycobacteria as well as *M. bovis* and *M. tuberculosis* strains. The extracts were screened for cytotoxicity against Vero kidney and C3A human liver cells. From a bulk extraction of *Sideroxylon inerme* leaves, active

compounds were isolated using bioassay-guided fractionation. The extract was tested for mutagenicity in the Ames and comet assays.

All Sapotaceae extracts had some antimycobacterial activity, with highest activity against *M. smegmatis*. *M. fortuitum* was relatively resistant while *M. aurum* and *M. bovis* BCG were moderately susceptible. Activity against the infectious *M. bovis*and *M. tuberculosis* aligned best with results against *M. bovis* BCG, supporting the use of this species as a non-pathogenic, fast-growing model organism for investigating antitubercular activity of plant extracts. Two pentacyclic triterpenes, alpha-amyrin and 3-beta-hydroxyolean-12-en-27-oic acid, isolated from *Sideroxylon inerme* had good activity against *M. smegmatis* and low cytotoxicity. The extract of this species showed no mutagenicity in the Ames test and relatively little genotoxicity in the comet assay. This appears to be the first time that these compounds have been reported from *S. inerme*.

PW-29

A comparative study on the protective effect on human fibroblast cell line NHDFof *Olea europaea* and*Ligustrum vulgare* leaves extracts

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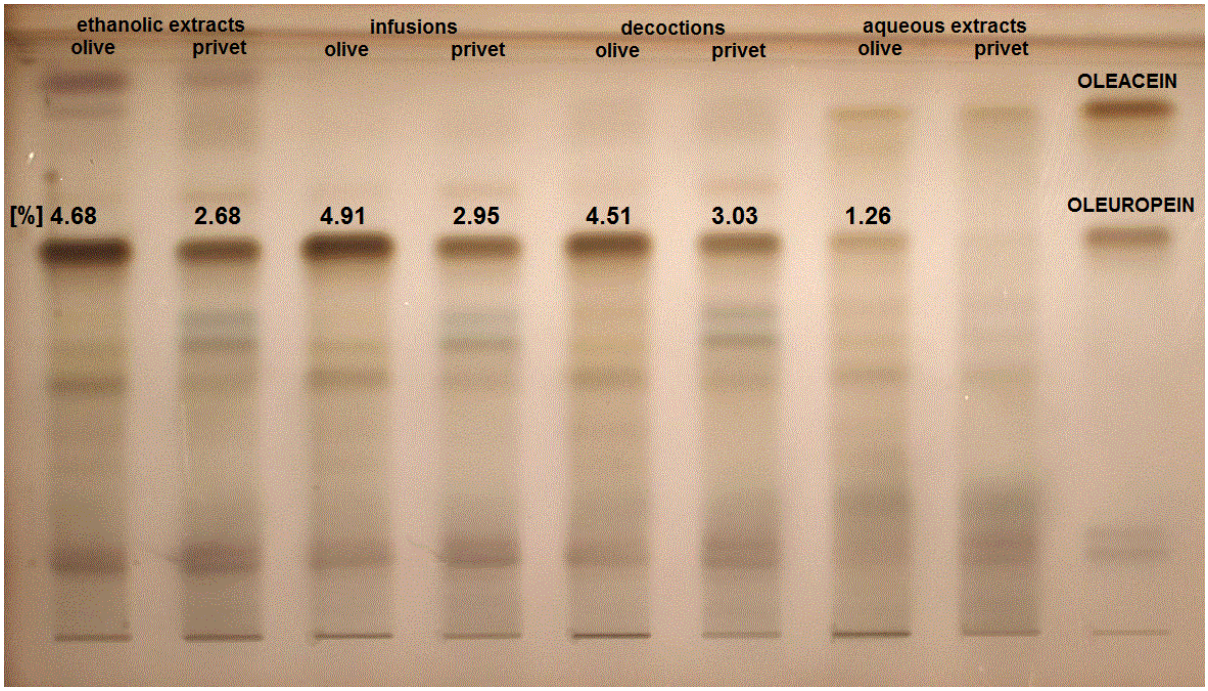
Considering the traditional use of olive (*Olea europaea* L., Oleaceae) leaves on cuts, wounds and burns, we have studied the protective effect of extracts from olive leaves in fibroblast line (NHDF) in vitro. Furthermore, due to the limited knowledge on wound healing activity of common privet (*Ligustrum vulgare* L., Oleaceae) leaves, it has been justified to compare the composition and activity of these two species. Firstly, we determined the content of the main secoiridoid glucoside oleuropein in different extracts (aqueous, ethanolic, infusion, decoction) using HPTLC-photodensitometry method (Fig. 1). Secondly, we aimed to study the protective effect of extracts on human fibroblast cell (NHDF) viability (MTT assay), apoptosis rate (Annexin V/iodide propodium staining) and ROS production after UVA-irritation and H2O2-induced (2.5 mM) oxidative stress. It was established that none of extracts at the concentrations of 5, 25 and 50 μg/ml affected cells viability. The UVA-irritation triggered the reduction of cell viability by 51.4±4.9%. Aqueous extracts, infusions and decoctions of both plants have protective effect on NHDF cell against UVA. Olive leaves extracts inhibited ROS production more significantly than privet leaves extracts after UVA-irritation. The percentage of ROS generation by cells treated with ethanolic and aqueous extracts, infusion or decoction of olive leaves (50 μg/ml) was 104.0±0.4%, 72.5±16.7%, 67.6±18.0%, 75.9±3.7% vs. (+)UVA control 100.0±20.6%, respectively. In conclusion, O. europaea leaves extracts characterised by higher content of oleuropein showed more significant inhibition of ROS production and protection against UVA-irritation than L. vulgare extracts.

Fig. 1. Stationary phase - Silica Gel F254; Eluent - dichloromethane:methanol:formic acid:water (80:25:4:1.5, v/v/v/v); Visualisation - anisaldehyde (0.5%, methanolic) and sulphuric acid (5%, methanolic).



PW-30

New phenolics from *Eriosema laurentii*

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The African plant *Eriosema laurentii* De Wild. is widely used in Cameroon for the treatment of infertility and gynaecological and menopausal complaints. Pharmacological studies have proven the potential of an extract from this plant for the treatment of menopausal complaints and identified isoflavones as active compounds [1,2]. In continuation of the phytochemical investigation, two new natural phenolics with a hitherto unknown substitution pattern, namely 3,4',6,8-tetrahydroxyflavon-7-C-glucoside (I) and 3,4',6,8-tetrahydroxy-flavanon-7-C-glucoside (II), were isolated from the methanolic extract and structurally elucidated.

The isolation was performed by liquid-liquid partition of the extract, column chromatography on silica gel and Sephadex LH-20 and by high performance counter current chromatography of the fractions. For dereplication thin layer chromatography, high performance liquid chromatography and liquid chromatography-mass spectrometry were applied. Mass spectrometry and one- and two-dimensional nuclear magnetic resonance techniques were used for the structure elucidation of the new compounds.

Besides the two new substances by these methods several known phenolic constituents were identified as 2''-*O*-a-rhamnosyl-6-C-fucosyl-3´-methoxy-luteolin, 2'-hydroxygenistein-7-*O*-glucosid, genistein-8-C-glucosid, syringaresinol, 2,6-dihydroxybenzoic acid and 3,4-dihydroxybenzoic acid, all for the first time in E. laurentii.

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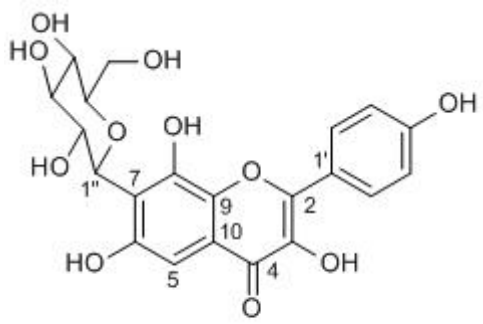

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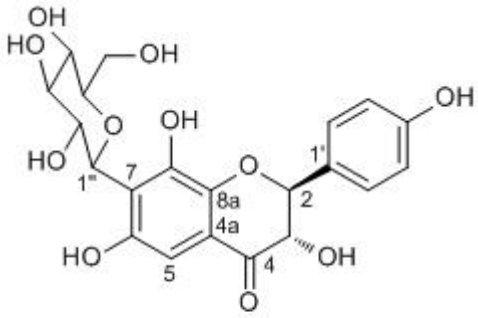

II

PW-31

Cytotoxicity of two medicinal plants commonly used in the management of diabetes in Eastern Cape South Africa using Chang liver cell lines

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Albuca bracteata and *Albuca setosa* are two of the commonly used medicinal plants by traditional healers in Eastern Cape Province of South Africa. These plants have been reportedly used for the management of diabetes, ulcer, wounds and inflammation. However, there are few reports on the toxicity of these plants.This study therefore, investigates the cytotoxicity of the two plants.

The aqueous, methanolic and acetonic extractions were evaluated in Chang liver cells for cytotoxicity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and crystal violet assays. Different concentrations of the extracts were added into 24 h cultured cells and incubated for 72 h at 37 °C and 5% CO_2.These were compared with nordihydroguaiaretic acid (NDGA) and 2,4-dinitrophenol (DNP) as standards.The cell proliferation was then accessed in MRHF and INS-1 cells incubated for 72 h at 37 °C and 5%

CO_2 by comparing the extract treated cells with the untreated cells while the percentage cell viability was compared to the controls.

The aqueous extracts of *Albuca setosa* and *Albuca bracteata* enhanced cell proliferation and were considered relatively not cytotoxic in both MTT and crystal violet when compared with NDGA and DNP. *Albuca bracteata* acetonic extract was the most toxic while *Albuca bracteata* methanolic extract, *Albuca setosa* acetonic and methanolic extracts all showed weak toxicity compared with the controls in the MTT and crystal violet assays.

These findings showed that the aqueous extracts of these plants are not cytotoxic supporting their folkloric usage. However, the cell proliferation property of these plants raises serious concern suggesting that they be used with caution.

PW-32

Estrogenic and anti-estrogenic properties of tropical African plants traditionally used in folk medicine

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An amazing diversity of traditionally used plants is found in the tropical areas of Africa and many of these plants have not or only insufficiently been studied. The purpose of this study was to evaluate eight African tropical plants, belonging to miscellaneous plant families, for estrogenic and anti-estrogenic properties and to isolate and characterize single compounds obtained from these herbal extracts.

Plant materials of *Brillantaisia owariensis* (BO), *Palisota schweinfurthii* (PS), *Ricinodendron heudelotii ssp. africanum* (RH), *Thonningia sanguinea* (TS), *Hymenocardia ulmoides* (HS), *Garcinia mannii* (GM), *Alchornae cordifolia* (AC), and*Xylopium aethiopicum* (XA) were extracted by methanol after defatting with petroleum ether. Experimentally, all extracts were tested using estrogen receptor subtype specific transactivation assays in human bone-derived U2OS cells. Cytotoxicity was measured by using MTT assay in U2OS cells. Overall, we identified two of the herbal extracts (RH, TS) with high and one (AC) with moderate estrogenic activities, three (BO, PS, HS) with moderate anti-estrogenic properties. One extract (GM) showed preferential estrogen receptor subtype specific anti-estrogenic properties. A strong cytotoxicity was detected for one extract (XA).

With this study on the estrogenic properties of plants of tropical origin, we provide evidence for agonistic activity of three plant extracts and antagonistic properties in four cases which support the traditional use for gynecological or reproductive purposes of some investigated plants. In order to prevent intoxications of the local population the observed cytotoxic extract has to be investigated in detail.

Acknowledgements: We thank Dr. Thea Lautenschläger and Prof. Christoph Neinhuis in collaboration with their partners in Angola for collecting and providing the plant material.

PW-33

Ethnobotanical evaluation of oregano (*Origanum vulgare*) in Latvia

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The research of Latvian folk songs showed that oregano (*Origanum vulgare* L.) has been utilized in folk medicine for thousands of years [1]. About 40 names of this plant are mentioned in written sources [2]. Oregano can be defined as a priority species of local medicinal plants. The wide use of oregano is the main reason why the local populations are severely depleted [3]. The aim of this research was to evaluate the ethnobotany of oregano in Latvia.

It was found that infusions of leaves traditionally are used for improvement of digestion, for reducing headaches and coughs, to promote menstruations and as a remedy for toothache. The infusion of flowers helps to calm nerves, to prevent seasickness and to remedy hangovers (one of the name of oregano is "labdusa" or "good health"). It is believed that oregano aroma dispels worry and concern, joys mind and increases vitality. Oregano is used in sauna whisks, for bathing. In some Latvian districts oregano is named "peppermint" due to its intensive aroma.

As culinary herb, oregano historically is called "sausage grass" ("desu zale"). It is used as an ingredient in production of sausages, for aromatisation of beverages and cucumbers marinating. It fits well together with parsley, thyme, caraway and garlic. Oregano is well-known as honey plant and as natural insecticide. It is mentioned that oregano can be used for colouring wool.

It is necessary to explore the biochemical content of oregano accessions from different Latvian places to confirm the traditional use of this plant in local environment.

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PW-34

Antibacterial activity of *Matricaria chamomilla* flower and leaf extracts

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Matricaria chamomilla L., also known as chamomile, is used as an anti-inflammatory, emmenagogue, analgesic, sedative, and antiulcer, among others. In Mexico flowers and leaves

are used to treat different ailments. Therefore, the aim of this work was to evaluate the *in vitro* antibacterial activity of flower and leaf extracts from *M. chamomilla*.

Leaves and flowers were macerated and centrifuged (3000 rpm/20 min/4 °C), the supernatant was called aqueous extract (AE). Flower (F) and Leaf (L) were extracted with acetone and ethanol. The solvent extracts were evaporated to dryness using rotary flash evaporator. Dry residues were dissolved in acetone (AE) or ethanol (EE) (1:10 w/v); extracts without pigments were macerated and centrifuged (EWP). The antimicrobial activities of extracts obtained were tested by Kirby-Bauer disc diffusion method and the time-killing curve method [1] against six bacterial pathogens. The AE presented antibacterial activity against *Escherichia coli* ATCC 25922, FAE MIC of 0.110 mg/ml and LAE MIC of 0.169 mg/ml. FAE was bactericidal against *Staphylococcus aureus* ATCC 27543, with *Bacillus cereus* (MIC 2 mg/ml), and against*Shigella sonnei* (MIC 0.5 mg/ml). LAE had an effect on *S. aureus* ATCC 27543 (MIC 2 mg/ml), *B. cereus* ATCC 14579 (MIC 4 mg/ml) and *S. sonnei* (MIC 0.5 mg/ml). The FEE was active against *S. aureus* ATCC 27543 and ATCC 25923 (MIC 2 mg/ml), *B. cereus* ATCC 14579, *S. sonnei* and *P. aeruginosa* ATCC 27853 (MIC 4 mg/ml), *Listeria monocytogenes* ATCC 7644 (MIC 0.5 mg/ml). The FEE is bactericidal against *P. aeruginosa* 27853. The EWP did not exhibit antibacterial activity. This is the first report were the flowers and leaf extracts antibacterial activity are analyzed simultaneously. *M. chamomilla* flowers and leaves extracts exhibit antibacterial properties; the active compounds are found in the acetonic and alcoholic extracts.

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PW-35

Effects of heat and mechanical processing on release of ibotenic acid from *Amanita muscaria* in traditional preparations used for catching flies in Slovenia

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We investigated the traditional use of *Amanita muscaria* in Slovenia and analyzed the effects of heat processing (HP) and mechanical processing (MP) on the release of ibotenic acid (IBO) from *A. muscaria* in different preparations for catching flies. From October 2013 to September 2014 we interviewed 59 people and 16 respondents described the preparations used for catching flies, which they remembered from their parents or neighbors. However, there were no reports about the present use. The methods for preparing the cap of *A. muscaria* included soaking in milk or water and combinations of HP or MP with soaking. Three caps of *A. muscaria* were harvested in October 2014 in Črni Vrh, Slovenia, and sliced into 9 equal pieces, 2 were used for the analysis of IBO content and 7 were prepared following the traditional methods in 50 ml of milk or water. Preparations were: soaking in milk or water, cooking in milk or water and toasting on a hot plate, tapping or slicing before soaking in milk. Preparations were sampled at 5 time points (0.5, 1, 2, 3, 24 h) and the concentration of IBO was analyzed with HPLC. Our results suggest that the release of IBO from the fungal material did not depend on the solvent used in the experiment, but was time dependent with the proportion of released IBO gradually increasing over time. HP and MP led to faster extraction of IBO into milk or water. In the first 30 min the soaked fungal material released up to 10% of IBO content, the mechanically processed from 10 to 25% and the heat processed at least 60%.

HP also weakly influenced the yield of extraction. After 24 h the heat processed fungal material released at least 85% of IBO content, but when prepared with other methods the proportion approached but not exceeded 80%. In conclusion, soaking the fungal material in milk or water resulted in the lowest proportion of the released IBO; on the other hand, HP and MP of fungal material led to faster release of IBO into milk or water.

PW-36

Cytotoxicity of medicinal plants used in cancer prevention in Thailand

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Currently, it is almost impossible to treat advanced cancer patients. Preventive approaches which attempt to control carcinogenesis have been adopted based on the idea that fixing small impairments is easier than larger ones [1]. It is important to prevent cancer development by modulating different targets and maintaining cell homeostasis. Thai traditional medicine (TTM), a holistic medicine which focuses on maintaining the balance of the four elements of the body provides a good framework for strategies for cancer prevention. The aim of this study was to evaluate cytotoxicity of medicinal plants used in cancer prevention by local healers/ traditional medical practitioners as a first step of a systematic study of these plants.

An ethnopharmacological approach was used to obtain information for selecting potential species. Each plant was extracted with water or 70% ethanol. Alamar blue assay was used to evaluate cytotoxicity of the potential plant extracts (6.25 – 200 μg/ml) in HepG2 cells. Based on a field survey, 18 plant extracts were selected. After 48 hours, ethanol extracts of *Aucklandia lappa* DC. (Compositae) (AUE), *Ligusticum striatum* DC. (Apiaceae) (LSE), and *Derris scandens* (Roxb.) Benth. (Leguminosae) (DSE) inhibited the cell proliferation with IC50 of 17.28, 91.13, and 145.70 μg/ml, respectively. For AUE, its phytochemical were reported to induce apoptosis and Nrf2 [2]. Maximum non-toxic concentration of AUE, LSE, and DSE are 7.58, 36.02, and 65.84 μg/ml, respectively.

In our model AUE exhibited potent cytotoxicity. It should be further evaluated for other cancer chemopreventive properties in order to discover a novel and practical cancer preventive extracts.

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PW-37

Screening of 19 traditional Mexican medicinal plants for effects on the metabolic syndrome related targets PPARα, β/δ, and γ in an in vitro luciferase reporter gene assay

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In the present study, the effect of traditionally prepared infusions of 19 traditional Mexican medicinal plants on the metabolic syndrome related peroxisome proliferator-activated receptors (PPAR) α, β/δ, and γ was investigated using a novel luciferase reporter gene assay [1]. COS-1 cells were co-transfected with the luciferase reporter plasmid p17m2G, containing a GAL4 upstream activating sequence in the promoter region, an expression vector for the human PPARβ/δ ligand-binding domain fused to the GAL4 DNA-binding domain pPPARβ/δ-GAL4, and the secreted alkaline phosphatase control vector pSEAP. The results are relative to the luciferase expression levels, which were normalized using secreted alkaline phosphatase (SEAP) activity. All extracts (all at 6.25, 25, and 100 µg/ml), and GW0742 (positive control, 0.1 nM) were dissolved in DMSO and added to the medium of the transfected cells. The same approach was used for PPARα and PPARγ in which case the COS-1 cells were transfected with pPPARα or γ-GAL4 and p17m2G, respectively (positive controls 50 µM WY14643 / 10 µM troglitazone). In the above described assay, significant effects could be detected for both *Aloe vera*(L.) Burm.f. (commercial whole leaf preparation) and *Larrea tridentata* (DC.) Coville while no activity above DMSO vehicle negative control levels could be detected for any of the other samples. In the case of *A. vera* leaf juice, significant effects as compared to the negative controls were observed at all three PPAR targets hinting to its high potential for metabolic syndrome therapy. For the dried leaves of *L. tridentata*, significant activity was only observed for the PPARα target, which is mainly associated with a potential for the treatment of anti-hyperlipidaemia.

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PW-38

Pharmacological and phytochemical evaluation of crude seed extracts of *Protorhus longifolia*

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Protorhus longifolia (Anacardiaceae) was selected for the present study as it is endemic to South Africa and is well known for the medicinal properties of its bark, stem, roots and leaves. However, there is a paucity of information on the potential medicinal value of the seeds. Various seed extracts (methanol, ethanol, ethyl acetate, hexane, chloroform and distilled water) of *P. longifolia* were evaluated for phytochemical composition and biological activity. Phytochemical analyses of the extracts via GC-MS revealed the presence of phenols, flavonoids, saponins and alkaloids. *In vitro* antiproliferative activity against a breast adenocarcinoma cell line (MCF-7) and cytotoxicity (Vero cells) was evaluated using the MTS assay. A noteworthy growth inhibitory effect was observed for the methanol, ethanol and hexane extract (IC_{50} values of 110, 183, 124 µg/ml respectively). The agar diffusion method [1] and determination of MIC using the broth micro-dilution assay [2] was used to determine *in vitro* antimicrobial activity using three antibiotic susceptible Candida species, three Gram-positive and two Gram-negative bacteria. Antibacterial activity was noted for all extracts. Furthermore, the *in vitro* antioxidant activity was investigated using the DPPH radical scavenging method [2]. A pronounced radical scavenging effect was observed at low concentrations of the methanol extract with an IC_{50} value<6.25 µg/ml. These results scientifically validate the use of *P. longifolia* seeds as a good candidate for pharmaceutical plant-based products.

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PW-39

Anti-inflammatory activity of the aerial parts of *Platycodon grandiflorum* and its constituents in LPS-stimulated RAW264.7 macrophages

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Platycodon grandiflorum A. DC. (Campanulaceae) grows as a perennial herb, and is widely distributed or cultivated in Korea, Japan and China. Platycodi Radix, the dried root of *P. grandiflorum* has been used as an antitussive, expectorant and drainage medicine in the traditional herbal medicine [1]. Lipopolysaccharides (LPSs) induce the production of inflammatory mediators such as iNOS, COX-2, TNF-α, IL-1, and IL-6 in macrophages. Therefore, reducing the expression levels of LPS-inducible inflammatory mediators is considered as an effective way to evaluate anti-inflammatory activity [2]. In order to compare the anti-inflammatory activity of solvent fractions from the aerial parts of *P. grandiflorum* on LPS-stimulated macrophages, the EtOH extract was consecutively partitioned with CH_2Cl_2,

EtOAc and n-BuOH, and the levels of inflammatory mediators such as NO, TNF-α and IL-6 were evaluated. The anti-inflammatory activity potential of the solvent fractions was in the order of *n*-BuOH>EtOAc>CH_2Cl_2>H_2O. The BuOH fraction strongly regulated NO, TNF-α and IL-6, and especially showed potent inhibition of the production of NO and IL-6 in LPS – stimulated RAW264.7 cells (IC_{50}: 6.7 and 8.1 μg/ml, respectively). Seven known phenolic compounds were isolated from the BuOH and EtOAc fractions, which showed comparatively higher activity than the other fractions. The known compounds were identified as luteolin 7-glucoside, 3'-rhamnoside, apigenin 7-glucoside, 4'-rhamnoside, luteolin 7-(6''-*O*-acetyl)-glucoside, luteolin 7-glucoside, apigenin 7-glucoside, chlorogenic acid, and luteolin. The isolated compounds revealed inhibitory effects on NO, TNF-α and IL-6 production at concentrations of IC_{50} below 25, 60 and 38 μg/ml, respectively.

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PW-40

Phytochemical constituents and antioxidant activities of the whole leaf extract of *Aloe ferox* Mill.

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Aloe ferox Mill. is traditionally used for the treatment of constipation in South Africa. There is no information on the antioxidant properties of the plant. Therefore this study focused on its phytochemical and antioxidant activities. Butylated hydroxytoluene (BHT) and rutin were used as positive controls while methanol and water without plant samples were used as negative controls. Phytochemicals were determined by spectrophotometry [1]. The composition (mg/g of plant material) of phenols (70.33), flavonols (35.2), proanthocyanidin (171.06) and alkaloids (60.9) were significantly higher ($p=0.02$) in the acetone extract. Both flavonoid and saponin contents were higher in both the methanol and ethanol extracts, while tannin levels were, however, not significantly different ($p=0.10$). At 0.5 mg/ml, the ABTS scavenging activity of the acetone extract ($IC_{50}=0.05$) was higher than those of BHT ($IC_{50}=0.10$) and rutin ($IC_{50}=0.07$) while the DPPH scavenging activity of the aqueous extract ($IC_{50}=0.25$) was higher than those of the other extracts and was not significantly different ($p=0.10$) from that of rutin ($IC_{50}=0.25$). At all the tested concentrations, nitric oxide scavenging activity was highest in the methanol extract ($IC_{50}=0.14$) but lower than those of BHT and rutin while lipid peroxidation was higher in the ethanol ($IC_{50}=0.22$) and acetone ($IC_{50}=0.18$) extracts. Hydrogen peroxide scavenging activity of the aqueous extract ($IC_{50}=0.24$) was higher than that of BHT ($IC_{50}=0.14$) at concentrations greater than 0.05. The study validates the ethnotherapeutic claim of the plant.

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PW-41

Microscopic characterization of medicinal plants commonly used in Ethiopia

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In Ethiopia the traditional use of medicine plants has a long history. Numerous ethnopharmaceutical publications document widely used plants in this country. These reports were developed by questioning of community members, healers, traders and collectors. Based on these publications we focus on commonly used medical plants. In pharmacognosy, the exact taxonomic classification should be combined with a microscopic monograph for an unambiguous identification. Here we present the microscopic characterization of five plants collected in different regions of Ethiopia.

Dodonaea viscosa ssp. angustifolia (leaf and fruit, Sapindaceae): The leaves have a bifacial structure. Glandular trichomes are visible on both epidermis sides. The fruits show an epidermis like exocarp with glandular trichomes, a netlike sclerified mesocarp and a sclereid layer as an endocarp. *Syzygium guineense* (bark, Myrtaceae): The bark shows stone cells and fiber bundles. Young twigs are characterized by oil cavities typical for a Myrtaceae. *Ruta chalepensis* (leaf, Rutaceae): The leaves are bifacial with a bilayer palisade parenchyma. Anomocytic countersunken stomata are countable in the lower epidermis only. Rutaceae-typical oil cavities are located in the mesophyll. *Solanum incanum* (root and fruit, Solanaceae): The roots contain the Solanaceae-typical idioblasts with calcium oxalate sand. *Embelia schimperi* (fruit, Primulaceae): The pericarp shows an epidermis like exocarp. The mesocarp contains sclereids and oil cavities. The endocarp is a bilayer of highly packed stone cells. In the case of Embelia schimperi the microscopic features of fresh plant material were compared to dried material from a traditional marketplace.

Acknowledgement: This presentation on microscopic characterisation of Ethiopian medicinal used plants is part of the project “Welcome to Africa” which is supported by the DAAD.

PW-42

In vitro antioxidant and anti-inflammatory activity, and determination of chemical composition of aqueous and ethanolic extracts of *Stellaria media* herb.

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Stellaria media L. (Vill.) (Caryophylaceae) is common weed used as food and medicinal plant. It is used to treat various diseases such as inflammations of the digestive, renal and respiratory tracts. Moreover extracts are applied topically to treat dermatological diseases [1,2].

The aim of our study was determination of antioxidant and anti-inflammatory activity, and chemical composition of aqueous and ethanolic extracts of *Stellaria media* herb. Scavenging of DPPH radicals, hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{-\bullet}$) in xanthine-xanthine oxidase system as well as nitrogen oxide ($NO^{\bullet}$) and peroxynitrite ($ONOO^-$) were evaluated. To examine the anti-inflammatory activity of the studied extracts, inhibition of hyaluronidase,

lipoxidase and collagenase activity by the extracts was tested. The range of the extracts concentrations was: 50-500, 10-200, 5-50, 100-500 μg/mL for DPPH, H_2O_2 and $O_2^{-\bullet}$, $NO^{\bullet}$ and $ONOO^-$, and enzymes, respectively. All mentioned assays were made in in vitro cell-free systems. Studies of composition of the extracts were performed using high-performance thin layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) coupled with Diode Array Detector (DAD) and Ion Trap Mass Detector. Main components of both extracts were apigenin glycosides.

Stronger scavenging activity against tested radicals showed ethanolic extract (SC50: 132.8±3.9, 16.5±0.4, 11.9±1.1 μg/mL for H2O2, $NO^{\bullet}$ and $ONOO^-$, respectively), only against superoxide anion activity of aqueous extract was more potent (SC_{50}= 62.7±8.1 μg/mL). Enzymes inhibition activity of tested extracts was rather weak, but in those cases also ethanolic extract showed stronger activity.

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PW-43

The antimicrobial and antioxidant potential of *Lithops lesliei*, a South African medicinal plant

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The Mesembryanthemaceae account for approximately 63% of the southern African succulent flora [1]. Lithops, known as "living stones" represents one of the most popular and widespread genera of the family. *Lithops lesliei* (N.E. Br) has gained prominence for its use as an ornamental and in traditional medicine [1]. This study investigated the antimicrobial and antioxidant properties of whole plant crude extracts. The antimicrobial activity of plant extracts on a range of Gram-positive and Gram-negative bacterial and fungal species was determined using the broth microdilution assay[2]. Antioxidant activity was determined by DPPH radical scavenging activity, β-carotene-linoleate bleaching assay and the ferric reducing antioxidant power (FRAP) assay using extracts obtained via soxhlation and sonication [2]. Antimicrobial activity was obtained in chloroform and methanolic extracts, particularly against *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus pyogenes* (MIC values of 0.625 mg/ml). All extracts exhibited poor antifungal activity against *Candida albicans* and *Cryptococcus neoformans*. The antioxidant activity of soxlation-derived, methanolic plant extracts was appreciable when compared to BHT (83.5 % and 71 % respectively in the β-carotene linoleic acid assay). Dose dependent FRAP activity was obtained for both sonicated and soxhlation-derived methanolic extracts at comparable levels to BHT and ascorbic acid. This study supports the use of *L. lesliei* as an antibacterial and antioxidant agent in several applications and strengthens the use of this succulent as a source of novel, biologically active phytocompounds.

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PW-44

Antimicrobial and antiproliferative activities of some North African desert plants

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The landscape of southern Tunisia areas is dominated by shrubs and woody plant species. Their morphological and physiological features being suitable for arid and Saharan bioclimate. It can be estimated that their phytochemical characteristics and metabolic activities are in accordance with the environment, and the plants have special spectrum of secondary metabolites, and offer a great medical and pharmaceutical potential.

The present study aims the investigation of the biological activities on some plant species from the Tunisian region of Sahara. The studied species were: *Anthyllis henoniana* (Coss.), *Centropodia forskalii* (Vahl.), *Cornulaca monacantha*(Delile), *Ephedra alata* var. *alenda* (Stapf.) Trabut, *Euphorbia guyoniana* (Boiss.&Reut.), *Helianthemum confertum*(Dunal), *Henophyton deserti* (Coss.&Durieu), *Moltkiopsis ciliata* (Forssk.) and *Spartidium saharae* (Coss.&Durieu).

The antibacterial activity was evaluated on 19 strains of microbes by disc-difussion method with determination of the values of inhibition zones (7-14,5 mm) and minimum inhibitory concentrations (MIC=0,1-5 mg/ml). Amoxicilin+clavulanic acid and vancomycin were applied as positive controls in the experiments. In vitro antiproliferative effect of the extracts was evaluated against 4 human adherent cell lines (HeLa, A431, A2780 and MCF7) using the MTT assay and cisplatin as reference compound. The aqueous-ethanolic (1:1) extracts of six desert plants showed antimicrobial activity against *Bacillus subtilis*, *Moraxella catarrhalis*, *Staphylococcus aureus* or methicillin-resistant *Staphylococcus aureus*. Extracts of *E. guyoniana* and *H. confertum* showed antiproliferative activity on breast adenocarcinoma (MCF7), and ovarian carcinoma (A2780) cell lines.

Our screening study proved that Saharan plant species are promising sources of potential antibacterial and antitumor agents. Our findings serve as starting points for selection of plant species for further investigation.

PW-45

Analysis of volatile constituents of *Sideritis raeseri*

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The genus *Sideritis* L. was known in Ancient Greece already, mentioned by Theophrastus and Dioscurides. Popularly known as Ironwort, it is a traditional beverage in Balkan countries, one of the most used species is *Sideritis raeseri* Boiss. & Heldr., Lamiaceae (Sideritis raeseri herba). The EMA-HMPC is currently working on a Community Herbal Monograph. The aim of this study was to analyse the volatile compounds in Sideritis raeseri herba by SPME GC-MS. The plant material originated from Greece (Othrys Mountains). The harvests were carried out in June of 2011, 2012, and 2013, respectively, i.e. during the optimal phenophase of blooming. We identified 10 components in Sideritis raeseri herba (2011), that is 88.2 % of the volatile components. The components identified in highest percentage were: γ-elemene (26.5%), β-caryophyllene (15.8%), and spathulenol (10.8%). Nine components were identified in the 2012 herb, that is 94.2% of the volatile components. These volatile were components identified in highest percentage: γ-elemene (32.3%), β-caryophyllene (14.9%) a spathulenol (9.7%). The analysis of the 2013 herb showed the presence of 10 components, and these represent 91,4% of the volatile components, and the volatile compounds present in highest percentage were: γ-elemene (29.5%), β-caryophyllene (15.4%) a spathulenol (10.1%). Further materials analysed included the Aetheroleum (2013), obtained by hydrodistillation, that showed the presence of 7 components that make up to 98.5% of volatiles. The main volatile compounds differed significantly in comparison to the herbal samples, the highest percentage showed: carvacrol (77.1%), nuciferol (8.2%), and α-bisabolol (6.6%). The aqua aromatica (2013), a side product of hydrodistillation, showed the presence of 4 components, i.e. 62.8% volatiles were identified. Essential oil compounds identified in highest percentage include: nuciferol (45.2%), spathulenol (11.6%) a caryophyllene oxide (5.6%).

Medicinal plants and natural products in animal healthcare and veterinary medicine

PW-46

In vitro antimycobacterial activity and cytotoxic activity of the acetone extract, fractions and compounds from *Oxyanthus speciosus* (Rubiaceae)

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The acetone extract, fractions and two compounds isolated from *Oxyanthus speciosus* DC. were screened for their antimycobacterial activity against three non-pathogenic mycobacteria namely: *Mycobacterium aurum*, *M. fortuitum* and *M. smegmatis* and a pathogenic strain, *M. tuberculosis* (8104) using a microdilution assay. Bioautography was used to determine the presence of antimycobacterial compounds of the plant extracts and fractions. Cytotoxicity was determined using the tetrazolium-based colorimetric cellular assay (MTT) against C3A human liver cells and Vero kidney cells. The selectivity index (SI) values of the extracts were calculated. The extract had significant activity against the 4 tested organisms with minimum inhibitory concentration (MIC) values ranging from 60 - 170 µg/ml. Two fractions out of 11 had significant activity against *M. smegmatis* with MIC values of 39 µg/ml. Compound 1 had moderate activity against all the tested mycobacterial strains with MIC values ranging from 12.5 - 50 µg/ml while compound 2 had weak activity (MIC value of 100 µg/ml). The acetone leaf extracts of *O. speciosus* and 4 fractions had relatively low cytotoxicity with LC_{50}values ranging from 0.160 - 0.383 mg/ml against C3A human liver cells. Compound 1 showed no cytotoxicity against Vero kidney cells even at the highest concentration (200 µg/ml) while compound 2 had an LC_{50} value of 33.77 µg/ml. The crude extract and all the fractions had selectivity index (SI) values (LC_{50}/MIC) ranging from 0.03-3.27. Compound 1 had the best SI value ranging from 4-16. The promising activity of compound 1 in vitro suggests its potential as an anti-TB drug candidate. Further work is continuing on structure elucidation and more intensive activity studies. The next phase of this work is to test the activity of promising extracts against *M. bovis* an important zoonotic pathogen causing large problems in wildlife and bovine management in Africa.

PW-47

The value of *in vitro* cytotoxicity testing in identifying suitable plant-based preparations for use in animal healthcare

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Searching for bioactive chemicals in plant extracts for development into animal health products is a fruitful area of research, reflected by increasing global interest. As with human medications, investigation of potential toxic effects at an early stage is required to exclude less promising candidates. The mechanism of action of most toxic chemicals is associated with basic biochemical processes common to all cells, and correlations exist between toxic concentrations determined *in vitro* to those noted *in vivo*.

Many parameters need to be taken into account when assessing toxicity of plant extracts and compounds. Limitations of cytotoxicity tests include difficulties in extrapolating results to *in vivo* situations where pharmacokinetic aspects, particularly metabolism, play a role. For mixtures like plant extracts it is challenging to predict interactions between different components as well as varying degrees of bioavailability.

Biological efficacy is generally not due to cytotoxicity when the selectivity index (SI, ratio of cytotoxicity to biological activity) is greater than or equal to 10. In antimicrobial and anthelmintic investigations of plant extracts in our research group, SI values are used to highlight active extracts with low toxicity, and also those selectively active against certain test organisms. This provides good leads for continuing research on promising extracts that may be sources of interesting biologically active and therapeutically useful preparations with excellent activity and low toxicity. For example, research in our group has led to identification of plant extracts and compounds that inhibit the hatching of eggs and development of larvae of *Haemonchus strongyloides*, one of the most important nematodes affecting sheep production in the world. In another example to be discussed, a crude leaf extract of *Loxostylus alata* had the same antifungal efficacy as amphotericin B in lungs of chicks infected with *Aspergillus fumigatus*.

PW-48

An overview on a variety of medicinal plants with respect to their potency against pests of disease vectors

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Within a multidisciplinary research program, the work was directed to investigate eco-friendly pesticides based on local botanical resources against some pests of disease vectors. The targeted pests included mosquitoes, house flies and the intermediate host snail (*Biomphalaria alexandrina*) of Schistosomiasis disease "Bilharzias". The protocol of assessment was carried out according to a "Bioassy-Guided Fractionation Scheme". Of special concern to the mosquitocidal activity of Thyme plant (*Thymus capitatus*), the volatile oil and certain compounds isolated from the unsaponifiable fraction (e.g., thymol, α-amyrin, carvacrol+ β-caryophyllene) showed high larvicidal and adulticidal potency. Sterols, hydrocarbons and β-amyrin isolated from Nigella sativa seeds induced potentiation with the insecticide Malathion against Cx. pipiens larvae. The potency of the herb, *Conyza aegyptiaca*-essential oil against *M. domestica* and *An. pharoensis* was mainly referred to the presence of limonene. Successive TLC fractionation of the chloroformic extract of the plant chicory (Cichorium intybus) was resulted in isolation and identification of two biologically active compounds: Lactucopicrin-15-oxalate and Chicoralexin; both showed high toxicity towards mosquitoes and house flies. Interestingly, essential volatile oils extracted from different plants synergized the potency of the bacterial endotoxins of *B. thuringiensis* (Bti) and *B. sphaericus* (Bs) against *Cx. pipiens* and *M. domestica*. Some plant extracts including *T. capitatus*, *N. sativa*, *Syzigium aromaticum* and*Ammi majus* showed moderate therapeutic effects against Schistosoma mansoni infection in mice, compared to Praziquantel as a specific antischistosomal drug. The results of these studies revealed the broad-spectrum toxic properties of the used botanical extracts against the tested insects and thus could be exploited for integrated pest management (IPM) programs, especially to combat pests of medical importance to human health.

PW-49

Effects of low inclusion levels of dried drumstick tree *Moringa oleifera* leaves into the diets of rainbow trout, *Oncorhynchus mykiss*, on growth, feed conversion and some blood parameters

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The drumstick tree, *Moringa oleifera* (MO), is a multi-purpose plant containing high amounts of plant secondary compounds like total phenolics, tannins, saponins and phytic acid.

Moringa leaves have been tested for various applications so far. They contain high amounts of protein (around 25% CP of untreated, whole, dried leaves in the dry matter), carotenoids, ascorbic acid and minerals and have been tested as fish meal replacement for *Nile tilapia* in several studies. Defatted MO seed meal has also been used as feed additive for sheep with indications of improved rumen fermentation. However, up to now, MO leaves have not been tested for their effects in carnivorous fish. Therefore an experiment was conducted in which rainbow trout, *Oncorhynchus mykiss*, were fed 0 (control, C), 0.625 (M 0.625), 1.25 (M 1.25), 2.5 (M 2.5) and 5% (M 5) of dried MO leaves in a fish meal based diet for a total duration of eight weeks. Each diet was fed to six replicate 55 l aquaria containing 25 fish each (mean initial body mass = 3.92 ± 0.08 g) in a flow-through system with approximately a 10-fold water exchange rate per day. Growth, feed conversion, fin index, blood glucose and hematocrit were evaluated for the five groups.

No differences were observed in any of the evaluated parameters. The fish grew between 428% (M 5) and 447% (M 0.625), the feed conversion ratios were between 0.95 (M 0.625) and 0.97 (M 5), the blood glucose levels were between 4.23 (M 1.25) and 4.42 (M 0.625), the hematocrit values were between 45.1 (M 2.5) and 50.7 (M 1.25) and the fin indices of dorsal and caudal fins were around 1 (Table 1). In conclusion it can be said that even comparatively high levels of 5% untreated MO dried leaves in the diet had no negative impact on fish performance and can thus be utilized for aquaculture as potential protein source.

Table 1: Growth performance, feed conversion, blood glucose, hematocrit and fin index of the rainbow trout.

	C	M 0.625	M 1.25	M 2.5	M 5
Initial body mass (g)	3.92 ± 0.07	3.92 ± 0.09	3.89 ± 0.08	3.91 ± 0.08	3.98 ± 0.09
Final body mass (g)	17.0 ± 0.63	17.5 ± 0.99	17.0 ± 1.21	17.1 ± 0.87	17.0 ± 0.50
Growth (%)	435 ± 19.1	447 ± 30.2	437 ± 27.9	437 ± 16.9	428 ± 12.6
SGR (% day^{-1})	2.33 ± 0.07	2.37 ± 0.11	2.34 ± 0.10	2.34 ± 0.06	2.31 ± 0.05
Feed conversion ratio	0.96 ± 0.03	0.95 ± 0.05	0.96 ± 0.05	0.96 ± 0.03	0.97 ± 0.03
Blood glucose (mmol/l)	4.36 ± 0.18	4.42 ± 0.22	4.23 ± 0.34	4.39 ± 0.48	4.37 ± 0.31
Hematocrit	46.2 ± 2.57	48.2 ± 8.13	50.7 ± 5.61	45.1 ± 3.14	47.0 ± 0.62
Dorsal fin index	0.96 ± 0.10	0.81 ± 0.30	1.13 ± 0.21	0.92 ± 0.36	1.10 ± 0.23
Caudal fin index	0.88 ± 0.21	0.79 ± 0.16	0.88 ± 0.19	0.67 ± 0.20	0.79 ± 0.13

Values are expressed as mean ± SD, n = 6, SGR = specific growth rate

PW-50

Medicinal plants and animal health care; experiences from India

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India has ancient traditions of animal care using plants. From Ayurveda to folk medicine, plants have been used in treating animals. Recently there has been a shift towards the use of chemicals in treating animals with varied effects on the health and welfare of animals and humans consuming these animals. This paper captures experiences using plant based medicine by a study which ran for several years.

The research study began in 1995 with an annotated bibliography. Subsequently, an action research project was initiated in 6 districts of India to document, validate and disseminate knowledge related to animal health care for different livestock rearing communities. Over 1000 treatments using over 500 plants for about 114 conditions affecting livestock were initially collected using participatory methods. These treatments were screened carefully and a few were selected based on specific criteria including empirical observation , safety, availability and ease of collection & preparation. These were again categorized into three categories based on secondary literature support. Finally about 165 treatments used for about 15 conditions which occurred frequently were shortlisted for conducting trials for validation. Over a 1000 ruminant cases and 4000 heads of poultry have been successfully treated using these plant remedies. The selected preparations have since then been handed back to the community for further use and continued validation.

The project proved that there were several practices and therapies using plants which can very easily solve a number of common health problems of livestock. The challenge is to find ways to sustain and promote these practices in equitable and environmentally sustainable ways. Today, the relevance of plant based medicine in India has increased with challenges posed by multi drug resistance accelerated by the misuse of chemotherapy. There is a need to find ways to reintroduce these plant based therapies in livestock rearing.

PW-51

Plant species reported from Swiss farmers to treat bovine respiratory diseases

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Bovine respiratory disease (BRD) causes high morbidity in cattle, and extensive antibiotic treatment is leading to increasing resistance of BRD pathogens. Medicinal plants (MP) used traditionally by Swiss farmers for BRD might be a potential future therapeutic option. Since 2011 ethnoveterinary surveys have been conducted in Switzerland, with some 200 interviews leading to more than 1'500 use reports (UR). From this dataset all URs referring to BRD were

extracted and analyzed with respect to plant parts used, preparation of remedies, administration, and oral daily dose (ODD). A total of 54 URs were documented (Table 1). The most common of the 15 reported MPs were *Thymus vulgaris* L. (TA), *Picea abies* L. (PA) and *Ilex aquifolium* L. (IA) representing some 70% of all URs. Common ways of administration of MPs were by direct feeding of the herb, as herbal teas, by inhalation, and as a liniment. For 25 of the 32 oral applications an ODD could be determined. ODDs reported for TA varied widely, but were comparable (median 0.19 g/kg^{0,75}) to commonly used human ODDs of 0.13-0.26 g/kg^{0,75}. The use of this plant was reasonable considering known antimicrobial, anti-inflammatory and spasmolytic properties. PA was administered by feeding of fresh twigs, but no detailed information on dosage could be obtained. However, antibacterial properties of spruce resin has been reported in literature. IA is commonly considered as toxic, but five UR corresponded to an average ODD of 0.41 g/kg^{0,75}. Whether this ethnoveterinary use can be rationalized by the content in triterpene saponins is not clear at the moment. More research is needed, and the use of IA cannot be recommended at present. The 54 URs for BRD represent less than 5% of total URs documented so far from Swiss farms. In contrast to dermatological and gastrointestinal diseases the treatment of BRD with MPs seems less common. Nevertheless, several of the 15 documented MPs are interesting starting points for further investigations.

Tab. 1: Plant species used by Swiss farmers to treat respiratory diseases in cattle

No of UR	Plant species	Used plant part	Route of administration	ODD (g/kg^{0,75}) (min/median/ max)
23	*Thymus vulgaris L.*	herb (20), leaves (2), essential oil (1)	o (15), t (2), i (4), et (2)	0.002/0.19/0.78
9	*Picea abies* L.	branches (6), resin (3)	o (6), t (3)	-/-/-
6	*Ilex aquifolium* L.	branches (3), leaves (3)	o (5), et (1)	0.01/0.41/1.04
2	*Origanum vulgare L.*	herb (2)	o (2)	0.16/-/0.39
2	*Allium cepa* L.	bulb (2)	o (1), i (1)	1.65/-/-
2	*Abies alba* MILL.	branches (2)	o (1), et (1)	-/-/-
2	*Juniperus communis* L.	branches (2)	o (2)	0.61/-/1.55
1	*Armoracia rusticana* B.GAERTN., *Avena sativa* L., *Camellia sinensis* KUNTZE., *Eucalyptus* ssp., *Matricaria chamomilla* L., *Pelargonium sidoides* DC., *Plantago lanceolata* L., *Salvia officinalis* L.			

UR = use reports; o = oral; t = topical; i = inhalative; et = environmental treatment
ODD = g dried plant equivalent per kg metabolic body) weight (g/kg^{0,75})

PW-52

An *in vitro* cell culture model using IPEC-J2 to assess the anti-inflammatory and anti-oxidative effects of phytogenic feed additives

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Disproportionate inflammatory reactions in the intestine of livestock are a threat in livestock production, as they contribute to gut disorders and animal health impairment. Driven by the resulting reduction of animal growth performance and consequently economic losses, the investigation of inflammation restricting feed additives is of particular importance. The pro-inflammatory transcription factor NFkB is regarded to be a key factor in the development of intestinal inflammation, whereas the anti-oxidative transcription factor Nrf2 features cytoprotective activity. Thus, the study evaluated the anti-inflammatory (NFkB down-regulation) and anti-oxidative (Nrf2 activation) potential of three phytogenic (plant based) feed additives (PFAs): PFA1 (including thymol and cinnamaldehyde as major active substances), PFA2 (including menthol and eugenol as major active substances) and PFA3 (including trans-anethole and carvone as major active substances), on intestinal porcine epithelial cells (IPEC-J2), a non-tumorigenic and non-transformed cell line from the jejunum of a neonatal, unsuckled piglet.

IPEC-J2 were differentiated in 100 mm dishes for eight days and then stimulated by TNF-α [10 ng/ml] to activate pro-inflammatory NFkB. Ethanolic extracts of the test products were added to see putative anti-inflammatory effects (TransAM NFkB p50 ELISA).

Non-cytotoxic test concentrations (Neutral red assay) were tested and PFA2 and PFA3 significantly decreased NFkB transactivation. None of the PFAs showed NFkB transactivation when applied without stimulation by TNF-α. Up-regulation of anti-oxidative Nrf2 was observed for PFA1.

All three PFAs exhibited protective properties on IPEC-J2. Further experiments to elucidate the component(s) most relevant for the observed activity of the PFAs are in progress and confirmation of the effects in vivo is planned.

PW-53

In vivo antiviral activitiy of *Sanguisorba officinalis* roots against viral hemorrhagic septicemia virus in olive flounder *Paralichthys olivaceus*

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Viral hemorrhagic septicemia (VHS) causes significant economic losses for the flounder aquaculture in Korea and its causative agent is VHSV, a negative sense ssRNA virus in the family Rhabdoviridae [1]. Although several kinds of experimental vaccines for VHS have been developed, none of them are commercialized. In our on-going research to discover natural products to be used as antiviral agents for VHS, we previously found that an 80% methanolic extract of *Sanguisorba officinalis* L. (Rosaceae) roots and its methylene chloride fraction (SOMC) showed significant antiviral activity against VHSV in FHM cells. Here, we evaluated in vivo antiviral efficacies of SOMC in olive flounder and its effect on immune gene

expression. Olive flounders orally pre-administered with SOMC at doses of 3, 10 and 30 mg/kg/day for 2 weeks were challenged by VHSV ($10^{6.8}$ $TCID_{50}$/fish) and then observed for 29 days post challenge. The SOMC groups showed cumulative mortalities of 63, 63 and 40.5% ($P< 0.01$) at doses of 3, 10 and 30 mg/kg/day, respectively, while the virus (VC)- and naïve control (NC) groups showed cumulative mortalities of 85.5 and 0%, respectively. To investigate the mechanisms of anti-viral effects of SOMC in olive flounders, immune gene expression analyses using real-time qPCR were carried out for kidney samples from fishes of the dose of 30 mg/kg/day collected on each day (1, 2, 4, 7, 10 and 14 day since oral administration was started). SOMC significantly up-regulated transcriptions of IL-1β, IL-6, IL-8, TNFα, IFNγ, ISG15, Mx than naive control showing that immune-enhancing roles of SOMC could exert as antiviral effects in VHSV-infected olive flounders. These results suggest that Sanguisorba officinalis roots and SOMC can be proposed as antiviral materials for VHS in olive flounders.

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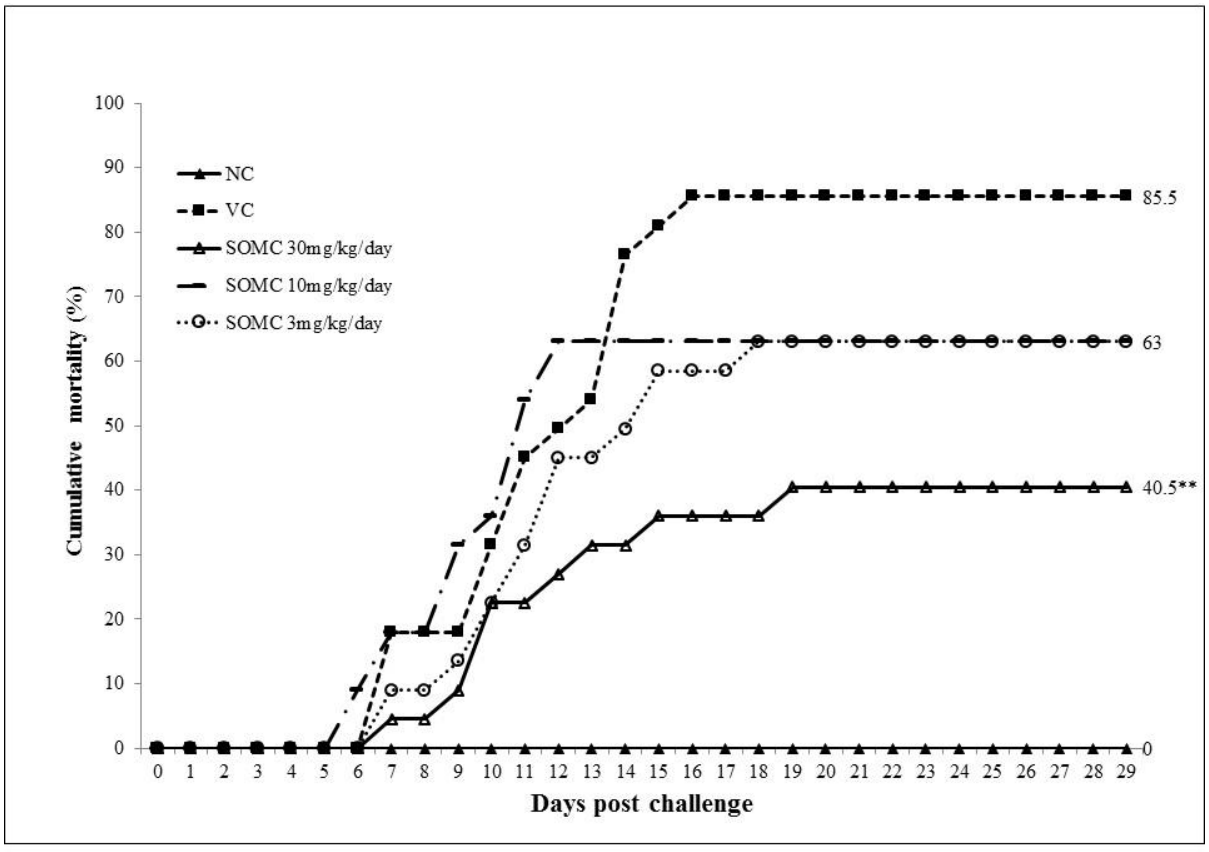

Fig. 1 Anti-VHSV activity in olive flounder *Paralichthys olivaceus* fed a diet supplemented with SOMC for 2 weeks.
** $p < 0.01$

PW-54

Cytotoxic effect of *Croton sphaerogynus* extracts against *Rhipicephalus microplus* cell line

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Croton (Euphorbiaceae) comprises approximately 1,300 species in tropical regions. In almost all Brazilian ecosystems*Croton* species occur, and some are used in folk medicine for various purposes [1]. The diversity of medicinal uses of*Croton* species is related to a wide diversity of secondary metabolites. The cattle tick *Rhipicephalus microplus* is one of the economically most damaging livestock ectoparasites, and its widespread resistance to acaricides is a considerable challenge to its control. The aim of this study was to evaluate the cytotoxic effect of hexanic fraction of *C. sphaerogynus* (HEF) against the embryonic cells of *Rhipicephalus microplus* (strain BME26). Leaf material was collected in Itanhaém, São Paulo, dried, powdered and 1 kg was subjected to maceration in ethanol for seven days. Crude extract was fractionated using methanol and hexane, in order to obtain a fraction rich in diterpenos (HEF). Tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was used to evaluate the cytotoxicity of different HEF concentrations. Percentage of viable cells treated with HEF was normalized to the untreated cells, which were considered to be 100% viable. At a concentration of 500 µg/mL HEF cell viability was 12.16%. At the lowest concentration tested (0.975 µg/mL) 60.05% of viable cell was observed. Morphological features of the treated cells suggested apoptosis as the mode of action. These data demonstrated that HEF can lead to cell death following a polynomial dose-response curve (IC_{50} 103.7 and 0.63 µg/mL, respectively). Croton species are potential sources for the discovery of natural products with acaricidal activity.

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PW-55

A herbal feed additive reduces the urea content in milk of dairy cows

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Digestion of fibers and transformation of plant protein into microbial protein is the main nutritive source for dairy cows. The aim of this study was to determine if a herbal feed additive (HFA) containing mainly *Urtica dioica* L. (herba), *Silybum marianum* (L.) Gaert. (fructus),

Artemisia absinthium L. (herba) and *Achillea millefolium* L. (herba) can improve the digestive functions of the foregut of dairy cows.

A stratified (farm, age, calving date) randomized placebo (PL) controlled field study was conducted including 106 cows from eleven German and Swiss farms (6-12 per farm). From 14 days before calculated calving date until sampling day (mean 206 (+/-40) day of lactation) 100g HFA or 100g green meal as control (PL) were fed daily to each cow.

Within each farm, faeces samples were collected on the same date and under same feeding condition, connected to a milk recording day. To evaluate different particle contents of the total faeces dry matter (SF: smaller particle fractions: 0.315mm - 2mm; LF: larger particle fractions: > 2mm) a wet fractionation was conducted. Milk recording data include the contents of fat (%), protein (%), urea (mg/dl), and quantity of milk (kg). A mixed model (ANOVA) was applied whereby the feeding group (HFA and PL) was defined as a fixed factor, the farm as random effect, and the individually differing concentrate levels as a covariate (level of significance $p<0.05$; trend $p<0.15$).

In the mixed model, cows receiving HFA showed significantly lower milk urea content, and a trend towards a lower LF than cows receiving PL (Tab.1). Milk urea represents the liver detoxified rumen ammonium, based on microbial degraded feed protein which is not metabolized to microbial protein due to a lack of microbial available energy. An improved rumination (leading to lower LF, connected to a better microbial energy supply) could be possibly due to bitter substances of *Artemisia absinthium* L. and *Achillea millefolium* L., but needs further confirmation..

Table 1 Influence of a Herbal Feed Additive (HFA) on faeces (faecal particle fractions (g/100gr faeces dry matter): SF - smaller particle fractions: 0.315mm - 2mm; LF - larger particle fractions: > 2mm) and milk (quantity (kg) and contents of fat (%), protein (%) and urea (mg/dl)) parameters compared to a Placebo (PL); Significance (p-value) and standard error (S.E.)

Faeces and milk parameter	Other factors	Feed additive		p-value
		HFA (SE)	PL (SE)	
Faeces dry matter	farm			0.02
	concentrates			0.38
		13.1 (0.2)	13.0 (0.2)	0.76
SF	farm			0.014
	concentrates			0.44
		29.0 (0.7)	28.5 (0.6)	0.47
LF	farm			< 0.001
	concentrates			0.21
		7.8 (0.6)	8.8 (0.6)	0.13
Milk kg	farm			< 0.001
	concentrates			< 0.001
		23.4 (1.0)	23.7 (1.1)	0.96
Fat	farm			0.006
	concentrates			0.83
		4.4 (0.1)	4.3 (0.1)	0.71
Protein	farm			< 0.001
	concentrates			0.16
		3.5 (0.1)	3.5 (0.1)	0.86
urea	farm			< 0.001
	concentrates			0.95
		19.0 (1.0)	21.0 (1.0)	0.03

PW-56

The association between antifungal activity and antiparasitic activity of plant extracts against helminths

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Haemonchus contortus is globally the most important helminth that causes tremendous losses in small animal production. Treatment cost with anthelmintics to manage infestations was more than $175 million in South Africa, Kenya and India in 2004 [1]. Many poor farmers cannot afford the cost of the anthelmintics. The situation is aggravated by the strong development of resistance by the parasite against currently used anthelmintics. Plants have been used to treat helminth infections by rural farmers with some positive results. The isolation of anthelmintic compounds is challenging due to the demanding assays required for bioassay-guided fractionation. A class of the important anthelmintics the benzimidazoles have good antifungal activity [2]. We separated plant extracts into different fractions based on polarity and determined the activities. When the ethylacetate fraction was omitted there was an excellent correlation between egg hatch assay and minimum inhibitory concentration (MIC) (R^2=0.9) and a good correlation between larval development and MIC, (R^2=0.6). We determined the anthelmintic activity of 13 tree leaf extracts with known antifungal activity. In many cases there appeared to be a good association between egg hatch assay and the larval development assay of *H. contortus*. When we analysed the data there was some correlation between the MIC of *Cryptococcus neoformans* and the egg hatch assay (R^2=0.15) and also between *Aspergillus fumigatus* and the egg hatch assay (R^2=0.15). The correlations between the MICs and the larval development was much lower (R^2=0.01). By deleting outliers the correlation was improved. We conclude that if the mechanism of antifungal activity is related to the anthelmintic activity it may be worthwhile to isolate antifungal compounds and then determine their anthelmintic activities.

[1] Peter J. Waller and Chandrawathani P Tropical Biomedicine 22(2): 131–137 (2005)

[2] Adamu et al. 2012, 12:213 doi:10.1186/1472-6882-12-213

PW-57

Plants from Brazilian savannah possessing activity against bacteria causing dermatophilosis in animals

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Dermatophilosis, also known as streptothrichosis or "lumpy wool disease" in sheep is a skin disease caused by the Gram-positive bacterium *Dermatophilus congolensis*. This disease affects many species of animals, such as cattle, sheep, goats, horses, pigs, dogs, domestic cats, and occasionally humans. Dermatophilosis is seen in animals at all ages but is most prevalent in the young, in animals chronically exposed to moisture, and in immunosuppressed hosts. The objective of this study was to test ethanolic extracts from leaves of three plant species of the Brazilian savannah (cerrado), *Anacardium humile*, *Annona crassiflora*, *Byrsonima crassifolia* and *Eugenia dysenterica* for in vitro antimicrobial activity against *Dermatophilus*

*congolensis*strains. Plant material was harvested in UPIS, Lagoa Bonita Farm, Planaltina - DF, Brazil. This leaves were dried at 37 °C, transformed into powder by a grinder and macerated with ethanol for five days with stirring, at room temperature. Then the extracts were filtered and concentrated using an evaporator at 50 °C. The microbiological tests were performed according to the Bauer – Kirby method, with modifications. After 24h of incubation zones of inhibitions were seen with extracts of *A. humile* (29 mm), *A. crassiflora* (24 mm), *B. crassifolia* (19 mm) and *E. dysenterica* (16 mm) at a test concentration of 0,5mg/ml. These preliminary results suggest that the extracts may be of interest for further investigations.

PW-58

In vitro effect of seven seaweed extracts on gastro-intestinal nematodes

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The exploitation of seaweed for animal feed (i.e. protein) and health promoting purposes is becoming more and more important – especially in countries where large amounts of biomass can be produced (e.g. northern countries such as Norway). Many seaweed species contain plant secondary compounds (PSMs) with broad bioactivity and potential animal health and welfare impacts. We report preliminary data on the effect of 7 seaweed extracts on the gastro-intestinal nematode species *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* which are responsible for considerable morbidity in sheep. For each seaweed species, 50 g of dried and grinded (1 mm) seaweed was extracted with 500 ml of EtOH/H_20 (50:50 v/v) for 2h. After elimination of all particulate matter, EtOH was evaporated and the EtOH free extract was mixed with 50g Amberlite XAD-2 in order to absorb PSMs. After that, the extract was washed again to eliminate NaCl and iodine (present because of marine origin) which both interfere with larval stages (L3) used for in vitro tests. After eluting with 100% EtOH, water was added and the EtOH/H_2O extract was subjected to evaporation and lyophilisation. A larval exsheathment test as described by Brunet et. al. (2007) [1] was used for in vitro evaluation of the respective extract efficacies. The test dose (table 1) was calculated considering both the respective extract yield and an estimate of extract concentration available within the rumen of sheep having consumed 25% of dry seaweed within a daily ration. None of the extracts reduced the exsheathment of either *T. colubriformis* or *T. circumcincta* (table 1) significantly.

[1] S. Brunet, J. Aufrere, F. El Babili, I. Fouraste and H. Hoste. The kinetics of exsheathment of infective nematode larvae is disturbed in the presence of a tannin-rich plant extract (sainfoin) both in vitro and in vivo. Parasitol. (2007), 134, 1253-1262

Table 1. Seaweed species and extract concentrations testet for larval exsheathment of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta*. Larval exsheathment results are given as percent reduction compared to the respective controls (controls not shown). Six replicates were run per tested seaweed and parasite species as well as for respective controls.

Seaweed species	Extract (mg/ml)	GIN species	larval exsheathment reduction (%)
Acrosiphonia sp.	0.282	*T. circumcincta*	0,0
Acrosiphonia sp.	0.282	*T. colubriformis*	5,5
Alaria esculenta	0.293	*T. circumcincta*	0,0
Alaria esculenta	0.293	*T. colubriformis*	0,6
Laminaria sp.	0.564	*T. circumcincta*	0,5
Laminaria sp.	0.564	*T. colubriformis*	0,0
Mastrocarpus stellatus	0.487	*T. circumcincta*	8,2
Mastrocarpus stellatus	0.487	*T. colubriformis*	0,0
Palmaria palmata	0.762	*T. circumcincta*	0,0
Palmaria palmata	0.762	*T. colubriformis*	0,0
Pelvetia canaliculata	0.339	*T. circumcincta*	1,4
Pelvetia canaliculata	0.339	*T. colubriformis*	1,6
Porphyra sp.	0.285	*T. circumcincta*	3,3
Porphyra sp.	0.285	*T. colubriformis*	0,0

PW-59

Antioxidative properties of pure compounds and phytogenic feed additives in a chemical (ORAC) and a cell-based assay (yeast)

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Antioxidants in animal feed prevent oxidation of essential components like vitamins or fatty acids, confer benefits to animal health by scavenging excess free radicals, and improve storage stability of animal products. Phytogenic feed additives (PFAs) are derived from plant material and may contain considerable amounts of antioxidants. Chemical assays to assess antioxidative properties usually involve reduction of oxidants or radical scavenging. Innate antioxidative defence mechanisms are observed in living cells and yeast serves as a model organism for testing antioxidants in a cell-based assay comprising a wide range of potential modes of action.

In this study, the oxygen radical absorbance capacity assay (ORAC) and an assay based on growth arrest of *Saccharomyces cerevisiae* exposed to oxidative stress caused by hydrogen peroxide were applied. Both assays were employed to test natural and synthetic antioxidants and PFAs.

Antioxidative effects of gallic acid, quercetin and butylhydroxytoluene determined by the ORAC assay corresponded well to expected values, while ascorbic acid surprisingly did not show activity up to 25 µM. Three PFAs (see Table 1), which are complex mixtures of herbs, spices, extracts and essential oils, displayed values of 226, 320 and 603 µmol trolox equivalents/g. In the yeast assay, 8 mM ascorbic acid successfully countered growth arrest induced by 4 mM hydrogen peroxide, as expected. Other commonly used antioxidants like butylhydroxyanisole and ethoxyquin however were unable to reverse growth arrest, even at

high concentrations. The same phytogenic feed additives which showed clear antioxidative activity in the ORAC assay were unable to prevent growth arrest in the yeast assay.

Well-established antioxidants and PFAs yielded diverging results in a chemical and a cell-based assay for antioxidant activity. Depending on the chemical environment, the same substance may act as pro- or antioxidant, which might explain the observed results.

Table 1. Composition of tested phytogenic feed additives (PFA)

PFA	Major ingredients
1	Preparation from *Thymus vulgaris, Cinnamomum verum,* and *Allium sativum*
2	Preparation from *Carum carvi, Mentha* × *piperita* and *Citrus sinensis*
3	Preparation from *Mentha x piperita, Syzygium aromaticum* and *Pimpinella anisum*

PW-60

Virus inhibitory activity of methanol extracts of *Halimodendron halodendron* Voss.

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This paper explores the effect of methanol extract of Halimodendron halodendron Voss. leaf against bird flu virus (strain A/FPV/Rostock/34 (H7N1)). Plant material (dry leaves of H. halodendron) was extracted with methyl alcohol after chloroform treatment. The methanol extract contains 8-10% of chlorophyll, 10-11% of flavonol glycosides with the rutin structure as it was shown by High-Performance Liquid Chromatography and triterpenoid glycosides as the main part of the extract. When studying the virus inhibiting properties of H. halodendron extract it is established that in doses from 0,5 to 4 mg on 1 kg of weight of a chicken embryo the preparation didn't possess ability to suppress a flu virus reproduction. The increase in a dose of a preparation to 5 mg/kg led to suppression of a reproduction of a virus for 20%. The further increase in a dose of a preparation to 50 mg/kg of weight of an animal leads to full suppression of a reproduction of a virus. The current findings have clearly demonstrated that H. halodendron leaf extract has anti-H7N1 avian influenza properties [1].

[1] Gubareva LV, Molecular mechanisms of influenza virus resistance to neuraminidase inhibitors. Virus Research 2004; 103: 199–203

PW-61

In vitro antimicrobial activity of *Annona muricata* against *Staphylococcus intermedius*

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Staphylococcus intermedius is part of the normal skin and oral flora of dogs. This bacterium is the predominant coagulase-positive *Staphylococcus* isolated from canine skin and mucous membranes. Case reports of human infections are rare, but the true incidence is unknown because the pathogen is frequently misidentified as *S. aureus*. Here, we tested the in vitro activity, of an ethanolic extract of "araticum" *Annona muricata* L. leaves against *S. intermedius* strains. Plant material was harvested in UPIS, Lagoa Bonita Farm, Planaltina - DF, Brasil. The leaves were macerated with ethanol for five days and the extracts were concentrated using an evaporator at 50 °C. MICs were determined in 96-well microplates using a tetrazolium salts as an indicator of bacterial growth and corresponded to 5 mg/ml. In addition, antioxidant activity was determined with the ABTS method and polyphenol content was measured with the Folin-Ciocalteau method. The total polyphenols content in *A. muricata* extract was 192 mg/100 g while the antioxidant activity corresponding to 190 mM/g. These findings suggest an antimicrobial potential for *A. muricata* leaves. Further research needs to be carried out to identify the active molecules and evaluate the in vivo antibacterial activity as well as sub-acute or chronic toxicities.

PW-62

Susceptibility of animal pathogenic bacteria to an ethanolic extract from jambolan (*Syzygium cumini*) leaves

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Pyoderma is a prevalent disease in dogs and its treatment usually involves antimicrobial therapy. Due to the development of resistant bacterial strains, there is an increased interest to study alternative antibacterial compounds. *S. cumini* L. Skeels is popularly used to treat infectious diseases. This work was aimed at testing the antimicrobial properties of an ethanolic extract from jambolan leaves against *Staphylococcus intermedius* and *Pseudomonas* spp. isolated from canine skin infections showing antibiotic resistance. Leaves of *S. cumini* were collected in Lagoa Bonita Farm, Federal District, Brazil. Air-dried leaves were powdered and exhaustively extracted with ethanol at room temperature for 5 days. Extracts were filtered and the solvent removed by evaporating at 50ºC. The antimicrobial activity the extract was evaluated in 96-well micro dilution plates, and MICs of 0,3 µg/µL and 0,08 µg/µL were found for *Pseudomonas* spp. and *S. intermedius*, respectively. Hence, *S. cumini* should be further investigated with respect to its potential against important veterinary bacterial strains.

PW-63

Antimicrobial and antioxidant activities of an ethanol extract from *Genipa americana*

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Genipa americana is widely distributed throughout the tropical, parts of the subtropical areas of Latin America and the savannah transition zone. This plant is a natural source of iron, riboflavin, and anti-bacterial substances. We studied the presence of polyphenols and tested the antimicrobial and antioxidant activities of ethanol extract of *G. americana*. Leaves were harvested in UPIS, Lagoa Bonita Farm, Planaltina-DF, Brazil. This material was macerated with ethanol for five days. The ethanolic extract was tested against clinical isolates of *Staphylococcus intermedius* and *Pseudomonas aeruginosa* which were obtained from dogs presenting dermatitis. MICs were determined in a 96-well assay using a tetrazolium salts as readout. The ABTS method was used for determination of antioxidant activity and the polyphenol content was determined with Folin-Ciocalteau. The total polyphenol content in *G. americana* extract was 111,73 mg/100g while the antioxidant activity correspondied to 89 mM/g . MIC were to 1,25 mg/ml for both bacteria strains. *G. americana* may have some potential as an adjunctive topical treatment against dermatitis. However, are needed to substantiate these findings.

PW-64

Antimicrobial activity of *Eugenia dysenterica* against *Staphylococcus intermedius*

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In veterinary dermatology, *S. intermedius* is one of the principal agents of canine bacterial skin infections, such as otitis, abscesses and pyodermas. However, this organism is increasingly reported to be resistant to many antibiotics, and failures in treatment are a cause of problems in small animal veterinary practices. *S. intermedius* can be transferred from dogs to humans, leading to a risk of human infections with the resistant bacteria. In the aim to test plant extracts against this important zoonotic bacteria, *Eugenia dysenterica* growing in the Brazilian Cerrado was selected because of multiple popular uses and some published scientific data. Leaves of *E. dysenterica* were collected in Lagoa Bonita Farm, Federal District. Air-dried leaves were powdered and exhaustively extracted with ethanol at room temperature for 5 days. Extracts were filtered and the solvent removed by evaporating at 50 º C. The in vitro antimicrobial activity of the extract was determined in 96-well plates, and the bacterial growth was measured in an MTT assay, with MIC 0,009 µg/µL. The *S. intermedius*bacterial strain used was an isolate from canine skin infection. The potent antimicrobial effect of this plant against these zoonotic important bacteria increased the interest to investigate the content of the extract. The phenolic content of the plant extract was determined by Folin-Ciocalteau method, showing 201 mg/100g. The antioxidant activity of this plant, also determined by Folin-Ciocalteau, corresponding to 729 mM/g. Further studies are needed to identify the constituents responsible for the antimicrobial activity.

PW-65

Potent anthelmintic activity of galloylated proanthocyanidins from shea meal (*Vitellaria paradoxa*)

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Proanthocyanindins (PAs) from shea meal (SM), a by-product obtained after lipid extraction of the nuts, contained B-type linkages, had a high ratio of prodelphinidins (73%) and were galloylated (42%). The average polymer size was 8 flavan-3-ol subunits (≈2384 Daltons) and epigallocatechin gallate was the major subunit.

Purified PA fractions from SM were tested in vitro for anthelmintic properties against gastrointestinal nematodes from ruminants (*H. contortus and T. colubriformis*) [1] by the larval exsheathment inhibition assay and from pigs (*A. suum*) by the larval migration inhibition assay.

Results showed that PAs from SM have a potent anthelmintic activity against those parasites similar to white clover (*Trifolium repens*) flowers (WCF) [1,2] (EC_{50} µg/ml; SM: 55.1, 16.5, 75.9; WCF: 37.4, 14.5, 110.1 for *A. suum, H. contortus and T. colubriformis* respectively).

WCF PAs are constituted almost exclusively of prodelphinidin (PD) compared to SM (98% vs. 73%) but do not contained galloylated PAs. Studies [1,2] have shown that anthelmintic activity of PAs was mainly associated with their PD ratio but our current results suggest that galloylation can be a major factor to anthelmintic activity and SM as a potential nutraceutical anthelmintic feed for controlling parasitic nematodes.

[1] Quijada J, Frygana C, Ropiak H, Ramsay A, Mueller-Harvey I, Hoste H. Contrasted structural factors of tannin-rich fractions explained the anthelmintic activity against *H. contortus or T. colubriformis*. JAFC. in press.

[2] Williams A R, Fryganas C, Ramsay A, Mueller-Harvey I, Thamsborg S M. Direct anthelmintic effects of condensed tannins from diverse plant sources against *A. suum*. PLoS ONE 2014; 9: 1-16.

PW-66

Mangifera indica actives key the metabolic master switch enzymes SIRT-1 and AMPK

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Emerging research suggests that physical activity and calorie restriction prevent age-associated metabolic disturbances by maintaining the function of mitochondria, the power plant of cells, and by activating the evolutionary conserved metabolic sensors sirtuin-1 (Sirt-1) and AMP-activated protein kinase (AMPK) [1-3].

Mangifera indica L. fruit preparation, used in this study, is a 100% pure mango fruit powder obtained from fruits harvested in India at a special degree of ripeness. The fruits are characterized by a higher level of secondary plant ingredients Mangiferin and a lower level of sugar compared to fully ripe fruits.

Activation of human recombinant SIRT1 isoenzyme was investigated using both, a fluorescence and a luminescence assay. Activation of AMPK was investigated in HepaRG™ cells. AMPK activation was analyzed by detection and quantification of AMPK protein phosphorylated at threonine residue 172.

At a concentration of 500 µg/ml an SIRT1 activation of 30% was detected. The positive control resveratrol showed activation of 50%. Further, at a concentration of 300 µg/ml the *Mangifera* fruit extract increased AMPK activity by 50 % compared to the positive control resveratrol and metformin, which increased AMPK activity up to 100%.

In conclusion, our in vitro results demonstrate that a fruit preparation of *Mangifera indica* could be useful for the treatment of metabolic disturbances.

[1] Zhang BB, Zhou G. AMPK: an emerging drug target for diabetes and the metabolic syndrome. Li C. Cell Metab 2009; 9(5):407-16

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PW-67

Epigallocatechin-3-gallate alters the metabolic phenotype of human Sertoli cells but protects from oxidative damage: a possible role for male fertility?

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Epigallocatechin-3-gallate (EGCG) is the most abundant catechin in tea (*Camellia sinensis* (L.) Kuntze, Theaceae). Most of the medicinal properties attributed to tea are suggested to be due to EGCG. Herein, we evaluated the effect of EGCG (5 and 50 μM) on human Sertoli cells (hSCs) metabolism, mitochondrial functionality and oxidative profile.

Exposure to 5 μM EGCG did not cause major alterations on hSCs, but 50 μM of EGCG induced a higher consumption of glucose and pyruvate to sustain the same production of lactate. In fact, hSCs exposed to 50 μM of EGCG presented a decrease in LDH and MCT4 levels, as well as LDH activity, suggesting that the efficiency of lactate production (essential for germ cell development) could be compromised. These metabolic alterations promoted by 50 μM EGCG on hSCs were accompanied by an increased lactate/alanine ratio, which is linked with the NAD^+/NADH ratio. This alteration on cellular redox status might be associated with mitochondrial dysfunction observed in hSCs of this group. Interestingly, we verified that oxidative damage to proteins and lipids were decreased in hSCs exposed to 50 μM EGCG.

This study suggests that EGCG is a metabolic modulator of hSCs but it appears to be safe to men in reproductive age. Interestingly, our study also illustrates that despite EGCG (50 μM) induces mitochondrial dysfunction in SCs, the oxidative damage is lower than is control cells. Since SCs are responsible for the nutritional support of spermatogenesis and present a Warburg-like metabolism, these findings suggest that EGCG may protect SCs from oxidative damage which may be relevant to male reproductive health.

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PW-68

The *Echinacea purpurea* extract Echinaforce® is effective as a preventive in a model of bacterial superinfection

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Outbreaks of influenza infections are associated with an increased risk of bacterial co-infections, turning normally mild infections to fatal conditions. Respiratory viral infections may facilitate secondary bacterial infections which increase host immunopathology through the overproduction of inflammatory cytokines. Here we tested in a new in vitro model with respiratory virus and subsequent bacterial co-infection the effectiveness of an echinacea extract as preventive of pathological effects of secondary bacterial infections.

Different cell lines (monocytic leukemia THP-1 cells, BEAS 2B bronchial epithelial cells, and A549 lung epithelial cells) were infected with H3N2 influenza virus. After 24h, infected cells were stimulated with LPS (10 µg/ml). Incubations were performed with our without an ethanol extract (65% V/V) from fresh Echinacea purpurea roots (5%) and herba (95%) (Echinaforce®). Supernatants were collected from each sample after 48 hours and IL6, IL8 and RANTES were measured.

In all the cell lines, the echinacea extract reduced the inflammatory parameters after bacterial co-stimulation significantly by around 70% when a concentration of 1/100 was used. Lower concentrations still showed significant reduction of these parameters.

These results show that the echinacea extract is able not only to reduce inflammatory responses after viral infections as shown previously [1]. This is the first time exhibited that echinacea can reduce bacterially induced subsequent inflammations effectively and may be a prevention of bacterial superstimulation.

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PW-69

Phenolic metabolites from *Acacia albida* leaves and evaluation of antihyperglycaemic effect of its extract

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Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. The present study deals with the isolation and identification of the phenolic constituents from *Acacia albida* leaves and evaluation of antihyperglycaemic effect of aqueous methanol extract. The aqueous alcoholic extract (MeOH-H_2O, 7:3) of *A. albida* leaves was subjected to extensive repeated column chromatography on polyamide, cellulose and Sephadex LH-20 resulted in

quercetin-3-***O***-β- glucopyranoside, kaempferol 3-***O***- β-glucopyranoside, quercetin-3-***O***-α-L-rhamnopyranoside, kaempferol 3-***O***- α-L-rhamnopyranoside, quercetin-3-***O***--α-L - arabinoside, quercetin and kaempferol. The structures of the isolated compounds were elucidated on the basis of spectral analysis. The extract was tested in two dose levels 100 and 200 mg/kg for 4 weeks. Fasting blood glucose levels of streptozotocin-diabetic rats elevated to be 378.16±25.27 comparing with control value. Treatment with the plant extracts (100 and 200 mg/kg) significant reduced blood glucose in a dose dependent manner in streptozotocin-diabetic rats. Also, *A. albida* significantly decreased total cholesterol (120.50±3.26; 102.33±1.56 mg/dl), triglyceride (144.66±2.74; 113.33±3.48 mg/dl) and increased HDL (52.50±1.66; 55.16±2.00 mg/dl) in lower and higher dose compared with streptozotocin-treatment. The extract failed to produce hyperglycemic activity in normal treated rats. The chemical constituents of the plant especially phenolics and other compounds present in the plant may be involved in the observed hypoglycemic effect of the plant extract [1]. The results show that the oral administration of *A. albida l*eaf extract on the diabetic state reducing hyperglycemia.

[1] Resurreccion-Mago M, Villase I, Harada N, Monde K. Antihyperglycaemic flavonoids from Syzygium samarangense (Blume) Merr. and Perry. Phytother Res 2005, 19; 246-251

PW-70

Isolation of iridoids and flavones from the anti-inflammatory, antioxidative and antimicrobial extract of*Melampyrum barbatum*

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The genus *Melampyrum*, belonging to the Scrophulariaceae family, represents about 25 species of herbaceous annual plants found in the Northern hemisphere. In traditional medicine, aerial parts of *Melampyrum* species have been used to alleviate rheumatic complaints, symptoms of gout and to treat different skin infections in decoction form [1-2].

The aim of the present work was the detailed phytochemical and pharmacological analysis of aboveground parts of*Melampyrum barbatum* Waldst. & Kit. ex Willd. Previously no data on the chemical constituents of this species have been reported; only antioxidant and free radical scavenging activities of red and yellow forms were investigated [3].

The plant material, collected from wild stock in Hungary, was extracted with methanol-water 7:3, and then liquid–liquid partition was performed with chloroform, ethyl acetate and *n*-butanol. These fractions were examined for antioxidant activity using DPPH and FRAP methods, and for antimicrobial effect against 6 Gram+ and 4 Gram- bacterial strains by a disc-diffusion method. The *in vivo* anti-inflammatory activity was studied in rats using the carrageenan-induced paw oedema test after intraperitoneal administration. It was found that the MeOH extract possesses a remarkable antioxidative property, and the EtOAc fraction

showed an antibacterial effect against *Staphylococcus epidermidis* and *Moraxella catarrhalis*. In the antiphlogistic test the *n*-butanolic fraction displayed a significant effect (27.3±2.03% inhibition).

Isolation was carried out by a combination of different chromatographic methods, including CC, VLC, preparative TLC and HPLC. The structures of the compounds were determined by analysis of their spectroscopic data (^{1}H- and ^{13}C-NMR,^{1}H,^{1}H-COSY, NOESY, HSQC and HMBC). As a result, apigenin, luteolin, and the iridoid glycosides, mussenoside, 8-epi-loganin, aucubin and loganic acid were identified, besides benzoic acid and galactitol. All compounds were isolated for the first time from this species.

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[2] Mogosan C, Munteanu MF. A comparative study on antiinflammatory effect of the tinctures from *Melampyrum bihariense* Kern and Melampyrum cristatum .(Scrophulariaceae). Farmacia, 2008; 56: 389–392

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PW-71

Phytophenolics composition, hypolipidemic, hypoglycemic and antioxidative effects of the leaves of*Fortunella japonica*

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The majority of research programs are directed towards the investigation of *Fortunella japonica* (Thunb.) Swingle fruits and peels volatiles constitutive due to their acetylcholinesterase inhibitor, anti-inflammatory, and antiproliferative effect. There have been little detailed phytophenolics composition reports on this genus [2] and the antidiabetic effect of plant was not evaluated.

Structures of compounds were elucidated by UV, NMR and MS spectra. Preliminary in vitro evaluation of antidiabetic activity (α-amylase, α-glucosidase, and β-galactosidase) of crude extract and the major compounds (**3**-**6**) were carried out. Blood glucose level, activities of liver enzymes, total protein content, serum lipid profiles, antioxidant parameters and some glucolytic and gluconeogenic enzymes in streptozotocin (STZ)-induced diabetic rats were determined. The evaluation also carried out through determination of liver disorder biomarkers and histopathological examination of liver, kidney and pancreas.

β-sitosterol (**1**), xanthotoxin (**2**), isopimpinellin (**3**), umbelliferone (**4**), apigenin-7-glucopyranoside (**5**), apigenin-7-rhamnoglucoside (**6**) and cirsimaritin (**7**). These compounds were isolated for the first time from the genus *Fortunella*except compound (**1**). Compounds **3**-**6** exhibited weak to moderate carbohydrate metabolizing enzymes inhibitory effect. Treatment with leaves extract effectively ameliorated antioxidant markers, glucolytic, glycolytic

enzymes. The histopathological analyses also confirmed the experimental findings. These results suggesting that this plant extract supplementation can be useful in preventing diabetic complications associated with hyperlipidemia and oxidative stress.

[1] Ogawa K, Kawasaki A, Omura M, Yoshida T, Ikoma Y, et al. Phytochemistry 2001; 57: 737-42.

[2] Barreca D, Bellocco E, Caristi C, Leuzzi U, Gattuso G. Food Res Int 2011; 44: 2190-7.

PW-72

In vitro antioxidant activity of *Retama monosperma*

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The relationship between the antioxidant activity and the phenolic contents (total polyphenol, flavonoid and condensed tannin) of *Retama monosperma* (L.) Boiss. (Fabaceae)**,** used commonly in the traditional medicine of Mediterranean regions [1,2], was investigated. The antioxidant activities of the various fractions (toluene, chloroform, ethyl acetate and butanol) of the hydromethanolic extract of the seeds, stems and flowers have been evaluated using in vitro 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging activities and phosphomolybdic acid assays and were compared to ascorbic acid. A significant high Pearson's correlations between flavonoid content and antioxidant activities (r=0.91) with phosphomolybdic acid assays and (r= -0.79) with IC_{50} DPPH radical scavenging activities. However, there was no correlation between condensed tannin and antioxidant activities. The results obtained in the present study indicate that the ethyl acetate fraction of seeds is a potential source of natural antioxidant for *R. monosperma*.

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PW-73

Crataegus ssp. promotes late-stage cardiac differentiation and regeneration

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The minimal and clearly insufficient ability of the adult heart to regenerate after ischemic injury is a great appeal for identifying biological mechanisms, substances and factors that improve this process [1]. Two main sources for cardiomyocyte renewal and regeneration have emerged in the field: a) adult multipotent progenitor cells and b) pre-existing cardiomyocytes [2].

Based on the many positive effects on the myocardium after infarction and the overall cardiovascular protective activity of*Crataegus ssp*. (extract WS®1442) [3], we aimed at studying whether also mechanisms of cardiac differentiation and regeneration could possibly play a role.

Here, we show that WS®1442 efficiently stimulated cardiomyocyte differentiation from murine and human ESCs in a dose-dependent manner after mesoderm was formed. This activity was thoroughly validated in a mESC-based (CGR8-*Myh6*-GFP) spontaneous differentiation assay. First bioassay-guided fractionations of the extract suggested that this activity is reserved for specific compound classes.

According to the observed activity profile, we hypothesize that the identified active fractions in WS®1442 could possibly target multipotent progenitors, stimulate their differentiation towards the cardiac lineage but also expand their pool. Further elucidation of the underlying cellular and molecular mechanisms might lead to novel targets that can be exploited for *ex vivo*expansion of cardiac progenitor cells. Eventually, it will be interesting to see whether (and how) our *in vitro* findings translate to *in vivo* regeneration.

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PW-74

Separation of neutral and acidic triterpenes from Mastic gum using Centrifugal Partition Chromatography (CPC) and Supercritical Fluid Chromatography (SFC-CO_2)

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Pistacia lentiscus L var chia is a native shrub in southern Chios (Greece) and is cultivated for the production of Mastic gum, a natural resin obtained after "hurting" the trunk and branches.This resin is collected for more than 2,500 years and is used in traditional medicine for various gastrointestinal disorders such as gastralgia, dyspepsia, and peptic ulcer [1]. Moreover this natural resin is used in perfumery, dentistry and the production of chewing gum, as well as a spice and flavoring in Mediterranean cuisine. The therapeutic properties of mastic gum are due to their bioactive triterpenes that constitute about 75% of the resin [2]. The present work aims to develop a rapid and effective process for the fractionation of mastic gum leading to the isolation of the main triterpenes (neutral and acidic) in pure form. The first step of the procedure was the separation of triterpenic fraction from the polymeric part by liquid-liquid extraction method. The obtained triterpenic fraction was then elaborated by centrifugal partition chromatography (CPC). Initially the CPC technique was used in the pH-zone refining displacement mode. 7 g of the initial crude extract without polymer were successfully separated into fractions selectively enriched inionizable triterpenes isomers (masticadienolic acid and isomasticadienolic acid, masticadienonic acid and isomasticadienonic acid, oleanonic acid and moronic acid). Neutral triterpenes were, subsequently fractionated by step-gradient elution CPC method resulting to a good separation of the major constituents (oleanolic aldehyde, tirucallol, dammaradienone). In order to isolate pure isomers in sufficient quantities from the enriched acidic triterpenes CPC fractions, an original preparative technique, Supercritical fluid Chromatography SFC-CO_2, was used. The method was successfully developed to separate the major acidic triterpenes including the production of pure isomers using a chiral column. Identification and structure elucidation of the isolated compounds was achieved by LC-HRMS, GC-MS and NMR analysis. Since that mastic gum was recently added to the European Union's products with a Protected Designation of Origin (PDO) all these isolated compounds could be used for the chemical profiling and quality control of this row material and their products.

Acknowledgement: This work was supported by a Marie Curie Industry-Academia Partnerships and Pathways (IAPP) Fellowship within the 7th European Community Framework (286287).

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PW-75

Influence of urolithins on inflammatory response of RAW 264.7 murine macrophages.

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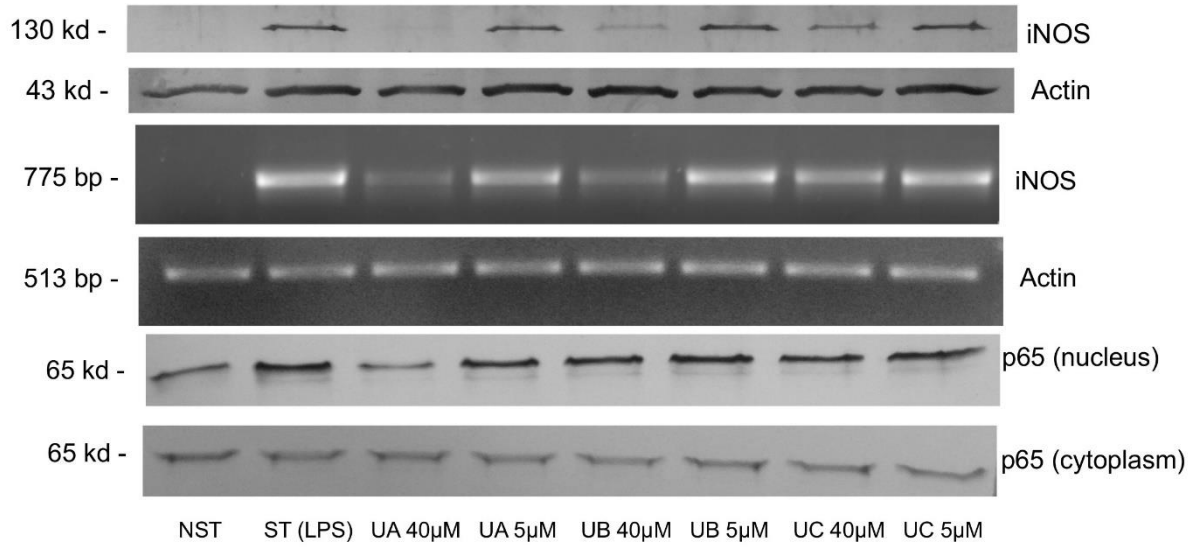
Ellagitannin-rich medicinal plants and food products are known to express beneficial effects towards chronic intestinal inflammation. Due to not fully established bioavailability of ellagitannins currently their gut microbiota metabolites-urolithins are indicated as the potential factors being responsible for observed *in vivo* activities.

The aim of the study was to determine the influence of the three most abundant bioavailable gut microbiota metabolites-urolithins A, B and C on the LPS-induced inflammatory response of RAW 264.7 murine macrophages taking part in pathogenesis of the intestine inflammation.

Urolithins A, B and C decreased LPS-induced NO production. The strongest impact was observed in the case of urolithin A, which dose dependently inhibited NO production at the concentration range 2.5-40.0 μM with IC_{50}=9.8±2.1 μM. The determined effects were shown to depend on the inhibition of iNOS protein and mRNA expression. Moreover all tested urolithins at the concentration of 40 μM inhibited IL-1β, TNF-α and IL-6 mRNA expression in LPS challenged RAW 264.7 macrophages. Inhibition of NF-κB p65 nuclear translocation contributed to the observed anti-inflammatory activity of urolithins.

The anti-inflammatory effects demonstrated for urolithins A, B and C at the concentration range (≥40 μM) potentially reachable in the gut tissues, support observed in *in vivo* studies beneficial effects of ellagitannin-rich products in intestine inflammation.



Acknowledgments: The author was financially supported by the National Science Centre Center scholarship ETIUDA decision number DEC-2013/08/T/ NZ7/00011.

PW-76

The effects of *Cassia tora* against the photo-oxidation of A2E laden retinal pigment epithelium

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Age-related Macular Degeneration (AMD) is the leading cause of blindness among the elderly. The death of retinal pigmented epithelium (RPE) cells caused by excessive A2E accumulation is crucial determinants of AMD according to recently research papers. A2E, a pyridinium bis-retinoid derived from all-trans-retinal and ethanolamine, can intercede light mediated damage to RPE cells by generation of singlet oxygen and self-oxidation at carbon-carbon double bonds. *Cassia tora* L. (Leguminosae) is traditional medicinal plant in Asia countries which is well known to have bioactivities such as hypotensive, anti-oxidation, anti-inflammatory, anti-microbial and anti-mutagenic activities. The *C. tora* 70% EtOH extract and its fractions by liquid–liquid partition using hexane, ethyl acetate, n-butanol and water were evaluated for the blocking on RPE cell damage from A2E photo-oxidation. The hexane fraction exhibited remarkable anti-photo-oxidant efficacy. These results demonstrate that *C. tora* may have protective effect on ARPE-19 cell death caused by blue light induced A2E photo-oxidation.

PW-77

Anti-inflammatory and antioxidant properties of methanol extracts of *Hilleria latifolia* and *Laportea ovalifolia*

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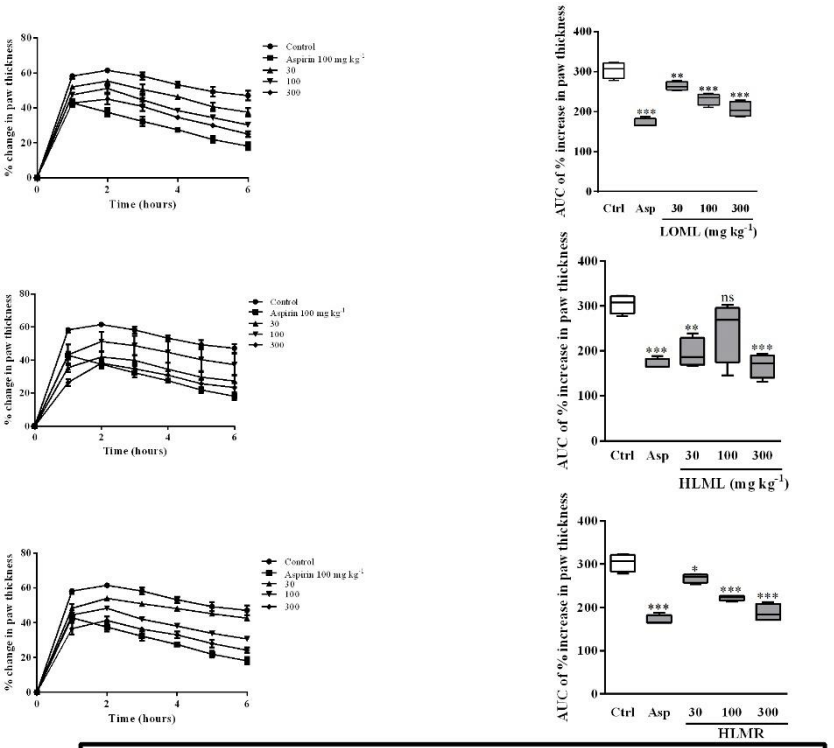
Hilleria latifolia (Lam.) H. Walt. and *Laportea ovalifolia* (Schumach.) Chew are herbs used for treatment of rheumatism, boils, skin diseases and as haemostatic on wounds [1,2].

To investigate the antioxidant and anti-inflammatory activities of methanol leaf and root extracts of *H. latifolia* (HLML and HLMR respectively) and methanol leaf extract of *L. ovalifolia* (LOML).

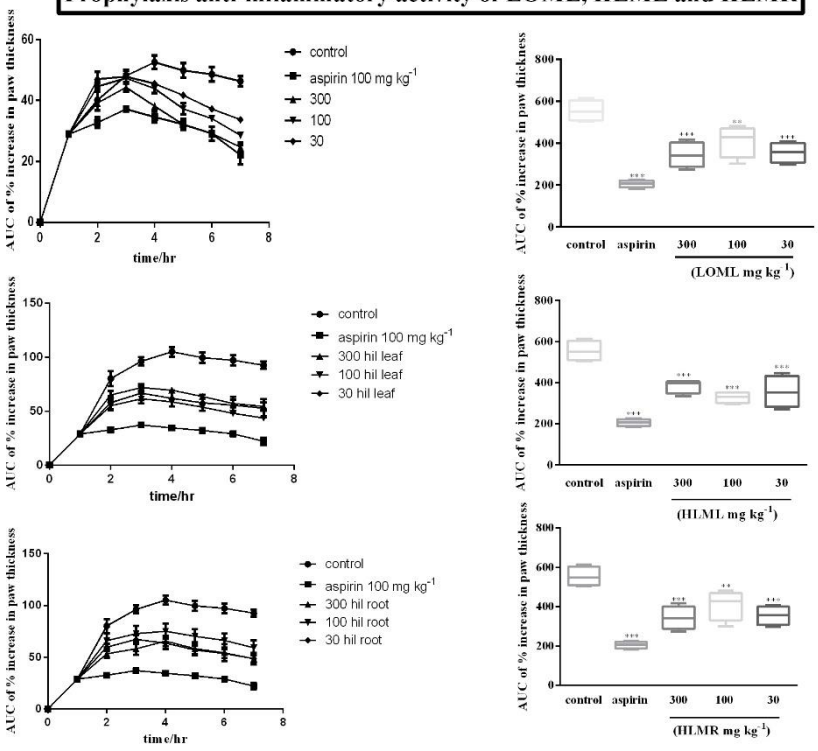
DPPH free radical scavenging activity, total phenol and antioxidant content assays were employed to determine antioxidant activity of extracts. Acute anti-inflammatory activity of extracts (30, 100, 300 mg/kg) was assessed using the carragenan induced foot oedema in rats [3].

HLML, LOML, HLMR and α-tocopherol had IC_{50} values of 0.299, 0.178, 0.306 and 0.01886 mg/mL, respectively. The total phenolic and antioxidant content of the extracts were concentration dependent. Total phenol content of HLML, LOML and HLMR were 103.0±1.335, 56.75±0.3220 and 91.32±4.258 mg tannic acid equivalent per gram of extracts respectively. Total antioxidant capacity of HLML, LOML and HLMR were 410.4±4.732, 337.6±6.961 and 408.0±18.70 mg α-tocopherol equivalent per gram of extracts respectively. All extracts significantly reduced paw oedema in prophylaxis and therapeutic models. LOML 300 and 100 mg/kg ($p<0.0001$), 30 mg/kg ($p<0.001$); HLML 300 and 30 mg/kg ($p<0.0005$) and ($p<0.005$) respectively; HLMR 300 and 100 mg/kg ($p<0.0001$), 30 mg/kg ($p<0.01$) (Figure 1).

HLML, LOML and HLMR exhibited antioxidant and anti-inflammatory activities.



Prophylaxis anti-inflammatory activity of LOML, HLML and HLMR



Therapeutic anti-inflammatoryactivity of LOML, HLML and HLMR

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PW-78

Evaluation of *in vitro* anti-inflammatory effects of the remaining water subextract of *Cistus laurifolius*leaves

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Cistus laurifolius L. (Cistaceae) is known as 'laden' in Anatolia and the leaves of the plant are used against rheumatismal diseases traditionally [1]. The ethanol extract of the leaves were submitted to solvent-solvent extractions and *n*-hexane, chloroform, ethyl acetate, *n*-butanol, remaining water subextracts were obtained. The extract and subextracts were investigated for their inhibitory effects on Nuclear Factor kappa B (NF-κB) transcrption factor on lipopolysaccharide induced Raw 264.7 macrophages. Only remaining water subextract exerted 20% inhibition. The subextract was fractionated by chromatographic methods but the fractions were found to be not effective. Thus, remaining water subextract was directed for further *in vitro* anti-inflammatory investigations. The effects on nitric oxide (NO), prostaglandine E_2 (PGE_2), tumor necrosis factor (TNFα) and interleukins (IL-1α, IL-1β, IL-2, IL-6) were studied by Griess and ELISA. The effects on iNOS and COX-2 protein levels and the phosphorylation levels of mitogen activated protein kinases (ERK1/2, JNK, p38) and I kappa B alpha (IκBα) were examined by Western Blot method. The subextract was applied to Raw 264.7 macrophage cells at 25, 50 and 100 μg/ml concentrations and it provided up to 57% decrease of NO production and up to 55% decrease of PGE_2 production of cells. The iNOS and COX-2 protein levels were decreased accordingly. The subextract exerted inhibitory effect on TNFα (37%). This subextract inhibited the phosphorylation of IκBα at 50 and 100 μg/ml concentrations while JNK phosphorylation was concentration-dependently inhibited.

Acknowledgements: This study is supported by TUBITAK-SBAG (Project no: 110S197) research grant

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PW-79

The ulcer protective effect of *Gleditsia triacanthos* methanolic fruit extract and its saponin-containing fraction

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Genus *Gleditsia* (family Fabaceae) comprises fourteen species that have been widely used in folk medicine. *Gleditsia triacanthos* L. was reported to be anodyne, mydriatic and experimentally oxytocic. Moreover, the pods have been used as a folk remedy for dyspepsia and measles among the Cherokee tribe. Saponins proved to be the main constituents of *G. triacanthos* fruit extract. In this study, we describe the ulcer protective activity of the metahnolic fruit extract of *G. triacanthos* (MEGT) and the saponin fraction derived from this extract (SFGT). Acute toxicity test was carried out for both MEGT and SFGT and the results showed no mortality in test animals up to the dose level 2000 mg/kg thus, indicating that these substances were non toxic. Forty eight rats were divided into eight groups (I-VIII) each comprising six animals. Group I was orally administered saline and served as negative control. Ulcers were induced by oral administration of 80% ethyl alcohol into groups (II-VIII). Group II was administered saline and served as positive control. Groups III-V orally received MEGT at three dose levels 100, 200 and 400 mg/kg while groups VI-VIII were given SFGT at the same previous dose levels. The number of ulcers was counted by morphological examination using a magnifying lens. Arbitrary scoring system was used to grade the incidence and severity of lesions. The results showed that MEGT at its highest dose level and SFGT at the three dose levels displayed significant decrease in ulcer count compared to the control group in a dose dependant manner. In conclusion, saponin content may be responsible for the ulcer protective activity.

PW-80

Curcumin induces apoptosis in hepatic stellate cells via inhibition of the MyD88 pathway

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Turmeric (*Curcuma longa* L.) and its constituent curcumin exert positive effects on hepatic fibrosis in rat-models, inducing apoptosis and inhibiting proliferation of hepatic stellate cells (HSCs) [1]. Although some studies implicated a role for MyD88 in the development of fibrosis [2], the interaction of curcumin with this pathway is still unknown. HSCs were divided into four groups, namely control group (A), MyD88 siRNA group (B), curcumin group (C), and curcumin + MyD88 siRNA group (D). Groups B and D were subjected to transient transfection with siRNA for 48h. Groups C and D were incubated with curcumin (25 μmol/L) for 24h.

MyD88 protein expression was observed by Western Blot, apoptosis was detected by flow cytometry, mRNA expression was detected by RT-PCR. Treatment with curcumin or MyD88 siRNA significantly reduced the expression of MyD88 in HSCs (both $P<0.05$ vs control), even more so if cells were treated with both agents simultaneously ($P<0.01$ vs control). Both curcumin and MyD88 siRNA inhibited the expression the cytokines TLR2, TLR4, NF-κB, TNF-1α, and IL-1B on the mRNA level. For curcumin these effects were significant for all five (all $P<0.05$ vs control). For MyD88 siRNA only the effects on NF-κB, TNF-1α, and IL-1B were significant (all $P<0.05$ vs control). For cells receiving both treatments, a significant inhibition of the expression of all five cytokines was observed (all $P<0.01$ vs control). Correspondingly, a significant induction of apoptosis was observed for both agents with apoptosis rate of 20% for the controls, 40% ($P<0.05$ vs. control) for cell incubated with curcumin, 41% ($P<0.05$ vs. control) for cells treated with MyD88 siRNA, and 47% ($P<0.01$ vs. control) for cells receiving both treatments. This study shows that curcumin promotes apoptosis of HSCs by inhibiting the expression of Myd88 pathway related cytokines on the mRNA level.

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PW-81

In vitro and *in vivo* anti-inflammatory effects of the chloroform fraction from *Trapa japonica* pericarp

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In this study, we found that the chloroform fraction (CF) from Trapa japonica pericarp (TJP) ethanolic extract inhibited lipopolysaccharide (LPS)-induced production of nitric oxide (NO) and intracellular ROS in RAW264.7 cells. In addition, expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) genes were reduced, as evidenced by Western blots. Our results indicate that CF exerts anti-inflammatory effects by down-regulating expression of iNOS and COX-2 genes through inhibition of MAPK (ERK, JNK and p38) and NF-κB signaling. Similarly, we also evaluated the effects of CF on LPS-induced acute lung injury. Male Balb/c mice were pretreated with dexamethasone or CF 1 hr before intranasal instillation of LPS. Eight hours after LPS administration, the inflammatory cells in the bronchoalveolar lavage fluid (BALF) were determined. The results indicated that CF inhibited LPS-induced TNF-α and IL-6 production in a dose dependent manner. It was also observed that CF attenuated LPS-induced lung histopathologic changes. In conclusion, these data demonstrate that the protective effect of CF on LPS-induced acute lung injury (ALI) in mice might relate to the suppression of excessive inflammatory responses in lung tissue. Thus, it can be suggested that CF might be a potential therapeutic agent for ALI.

PW-82

Isolation of anti-inflammatory compounds from *Sambucus ebulus* leaves through *in vitro* activity-guided fractionation

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The *in vitro* anti-inflammatory effects of subextracts (*n*-hexane, chloroform, ethyl acetate, *n*-butanol, remaining water) of the methanol extract of the leaves of *Sambucus ebulus* L. (Adoxaceae) were investigated for their inhibitory activities on the activation of Nuclear factor kappa B (NF-κB) on lipopolysaccharide induced Raw 264.7 cells. The *n*-hexane, chloroform and ethyl acetate subextracts inhibited NF-κB activation at 50, 100 and 100 μg/ml concentrations respectively. Two flavonoid mixtures [quercetin-3-*O*-β-D-glucopyranoside, quercetin-3-*O*-β-D-galactopyranoside], two flavonoids [isorhamnetin-3-*O*-β-D-glucopyranoside (**1**), isorhamnetin-3-*O*-rutinoside (**2**)] were isolated from ethyl acetate subextract. 10-*O*-acetylpatrinoside (**3**) and a new iridoid [Sambulin B (**4**)] was obtained from chloroform and n-hexane subextracts respectively. Structures were elucidated by NMR and MS. The compounds exerted inhibitions between 30-80% on NF-κB. Flavonoids were applied to cells at 25, 50, 75 and 100 μg/ml concentrations. Sambulin B was applied at 6,25, 12,5, 25 and Sambulin A applied at 12,5, 25 and 50 μg/ml concentrations. The effects on nitric oxide (NO), prostaglandine E_2 (PGE_2), tumor necrosis factor (TNFα) and interleukins (IL-1α, IL-1β, IL-2, IL-6) were investigated by Griess and ELISA. The effects on iNOS, COX-2 protein levels and phosphorylation levels of mitogen activated protein kinases and I kappa B alpha (IκBα) were examined by Western Blotting. **1**, **2**, **3** and 4 inhibited NO productions (between 59-84%), iNOS levels were decreased. 2, 3 and 4 exerted inhibitions on PGE_2 between 39-84%, COX-2 protein levels were decreased. **1** and **2** prevented p38/IκBα phosphorylations while 3 inhibited JNK/p38. Compound 4 inhibited JNK phosphorylation. All compounds (except mixtures) inhibited TNFα more than 29% and only 4 inhibited IL-6.

Acknowledgements: This study is supported by TUBITAK-SBAG (Project no: 110S197) research grant.

PW-83

Chemical profile and cytotoxic activity in B16 melanoma and C6 glioma cell lines of the extracts of old man's beard obtained by different methods

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In the current study, a comparative screening of chemical profile and cytotoxic activity of the extract obtained by novel supercritical CO_2 extraction, SCE (1) compared to the conventional extracts: Soxhlet extract with ether (2) and ethanol (3) and maceration with ethanol (4) of old man's beard has been performed. High performance liquid chromatography allowed identification and quantification of the major chemical constituent - usnic acid in the investigated extracts, while their cytotoxic activity was assessed by cell viability measurements using acid-phosphatase method in tumor (B16 melanoma and C6 glioma) and normal cells (HaCaT human keratinocytes). As seen in Table 1, content of usnic acid was the lowest in the extracts prepared with ethanol (4 and 3), followed by its noticeable increase with non-polar ether employment (2), while the highest usnic acid content was detected in the sample 1, obtained by SCE. With the exception of sample 3, cytotoxic activity assessment indicated B16 to be more susceptible cell line compared to C6, and also, based on significantly less toxic effect to HaCaT cells, suggested certain selectivity of the extracts towards cancer cells (Table 1). Additionally, IC_{50} values of the tested extracts were in good correlation with their usnic acid content i.e. extraction method used (Table 1), extricating sample 1 as the most active in both tested cancer cell lines. Our results imply SCE of old man's beard to be superior technique compared to the conventional solvent extractions in terms of the higher yield of usnic acid, which is further connected to the increased cytotoxic activity against B16 melanoma and C6 glioma cancer cells.

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Table 1. Results of the chemical analysis (usnic acid content (expressed as %(w/w)) and IC_{50} values (expressed as µg/mL) in tested cancer (B16 melanoma and C6 glioma) and non-tumor (HaCaT normal human keratinocytes) cell lines of the investigated old man's beard extracts (1-supercritical CO_2 extract; 2- Soxhlet extract prepared with ether; 3- Soxhlet extract prepared with ethanol; 4- macerate prepared with ethanol).

Sample	Chemical analysis	IC_{50} values for tested cell lines (µg/mL)		
	Usnic acid content (%(w/w))	B16	C6	HaCaT
1	81.41	31.21	43.40	278.75
2	67.09	58.20	69.10	380.31
3	2.43	466.10	395.60	543.44
4	1.39	391.76	457.24	637.10

PW-84

Antidiabetic activity of *Eremophila maculata* leaves methanol extract and its major secondary metabolites

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Eremophila maculata Muell, Spotted Emu Bush or Native Fuchsia, is a well-known member of family Scrophulariaceae. It was popular among the Australians as an effective cure of cold sores; however, nowadays it is cultivated as an ornamental plant worldwide [1]. The biological assessment of*E. maculata* has not been fully explored. Thus, the foregoing study aimed to evaluate the antidiabetic activity of *E. maculata* leaves methanol extract (EMM) *in vivo* according to Pari et al. 2000 [2]. Herein, diabetes was induced in the rats by a single intraperitoneal injection of streptozotocin (STZ) in saline (60 mg/kg b.w.).

The administration of glibenclamide or E MM to STZ-diabetic rats elicited significant declines in fasting blood glucose level (FBG) by 28.57 and 65.60%, respectively associated with a marked increase in ser um insulin level by 22.22 and 88.89%, respectively. A detailed phytochemical investigation of EMM resulted in the isolation and identification of six lignans. Among them a rare naturally-occurring lignan glycoside namely pinoresinol 4-*O*-[6"-*O*-(*E*)-feruloyl]-β-D-glucopyranoside was isolated from the nature for the second time in addition to pinoresinol 4-*O*-β-D-glucopyranoside. The antidiabetic activity was explained byvirtual docking of the major compounds to the main sites on both human α-glucosidase & aldose reductase. Pinoresinol 4-*O*-β-D-glucopyranoside showed a relevant binding mode in the aldose reductase active sites with a binding energy equals -57.93 Kcal/mol. It was concluded that

EMM exhibited a marked *in vivo* antidiabetic activity that could be attributed to its polyphenolics and lignans.

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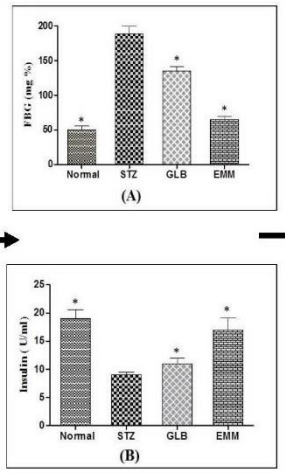
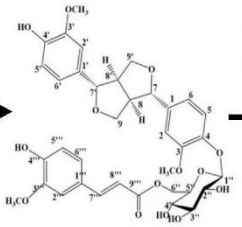
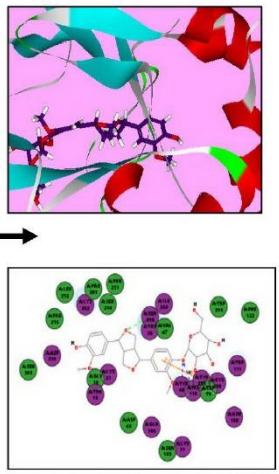
A photograph of *Eremophila maculata* (Scrophulariaceae).



Influence of oral intake of EMM (20 mg/kg) or GLB (600 µg/kg) to STZ-diabetic rats on FBG (A) and serum insulin (B).



Pinoresinol-4-*O*-[6''-*O*-(*E*)-feruloyl]-*β*-D-glucopyranoside



Binding of pinoresinol 4-*O*-*β*-D-glucopyranoside in the active site of human aldose reductase (A) and its 2D diagram binding mode (B).

PW-85

Olive, boldo, roselle, and rosemary support diet

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Olive, boldo, roselle, and rosemary are known plants in folk medicine [1-3]. Several phenolic compounds are responsible for their anti-inflammatory, anticancer, antimicrobial, antiviral, hypolipidemic or hypoglycemic activities [4].

Methanolic extracts of olive leaves (*Olea europaea* L.), boldo leaves (*Peumus boldus* Molina), rosemary leaves (*Rosemarinus officinales* L.), and roselle blossoms (*Hibiscus sabdariffa* L.) showed inhibitory effects on pancreatic lipase and *α*-amylase activity. For the determination of lipase inhibitory activity, an enzymatic *in vitro* assay based on the hydrolysis of an oleate ester of 4-methylumbelliferone was used (IC_{50}: 36-217 µg/mL). The EnzChek® Ultra Amylase Assay Kit (Molecular Probes™) was used to determine *α*-amylase activity. IC_{50} values ranged from 29 µg/mL to 1.53 mg/mL. The activity assays used are fluorescence-based. Flavonoids, anthocyanins, catechins, and other polyphenols don`t allow photometric measurements because of high self-absorptions. High Performance Liquid Chromatography analysis of the

four extracts show which compounds are probably responsible for inhibition of the both enzymes α-amylase and pancreatic lipase.

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PW-86

Cholesterol lowering effect in the gall bladder of dogs by *Herniaria hirsuta*

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Infusions of *Herniaria hirsuta* L., *H. glabra* L. and *H. fontanesii* J.Gay (Caryophyllaceae) are well known in Moroccon folk medicine for the treatment of biliary dyskinesia, (uro)lithiasis or as a diuretic, and in Europe as an urological drug [1,2]. An *in vivo* experiment to evaluate the cholesterol lowering effect of a decoction of *H. hirsuta* in the gall bladder of dogs was carried out. Three groups of dogs i.e. control dogs (CG), dogs treated with ursodeoxycholic acid (UDCA) (2x7.35 mg/kg body weight/day) and dogs treated with the standardized decoction (HG) (2x48.5 mg/kg body weight/day) were fed a fatty diet during 120 days after which a diet without additional fat was introduced till day 180 [3]. Treatment started 30 days after the start of the fatty diet and lasted until the end of the experiment. A bile and blood sample of each dog was collected every 30 days, after which the cholesterol level was determined. The *in vivo* experiments already showed a minor difference for bile cholesterol between CG and HG after 30 days of treatment with the decoction, and a more pronounced difference after 90 days of treatment. Even 30 days after discontinuation of the cholesterol-rich diet a significant difference remained between CG and HG. There was no significant difference in blood cholesterol between the groups. Prolonged use of this standardized *H. hirsuta* extract resulted in a cholesterol-lowering effect in the bile of dogs. Since this pharmacological effect prevents the formation of gallstones and can contribute to solving existing gallstones, a standardized decoction of *H. hirsuta* may have a positive effect in the treatment of gallstones in human patients.

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PW-87

Influences of STW 5, a multi-component herbal preparation, on motility and inflammation challenges in gut and enteric nervous system (ENS)

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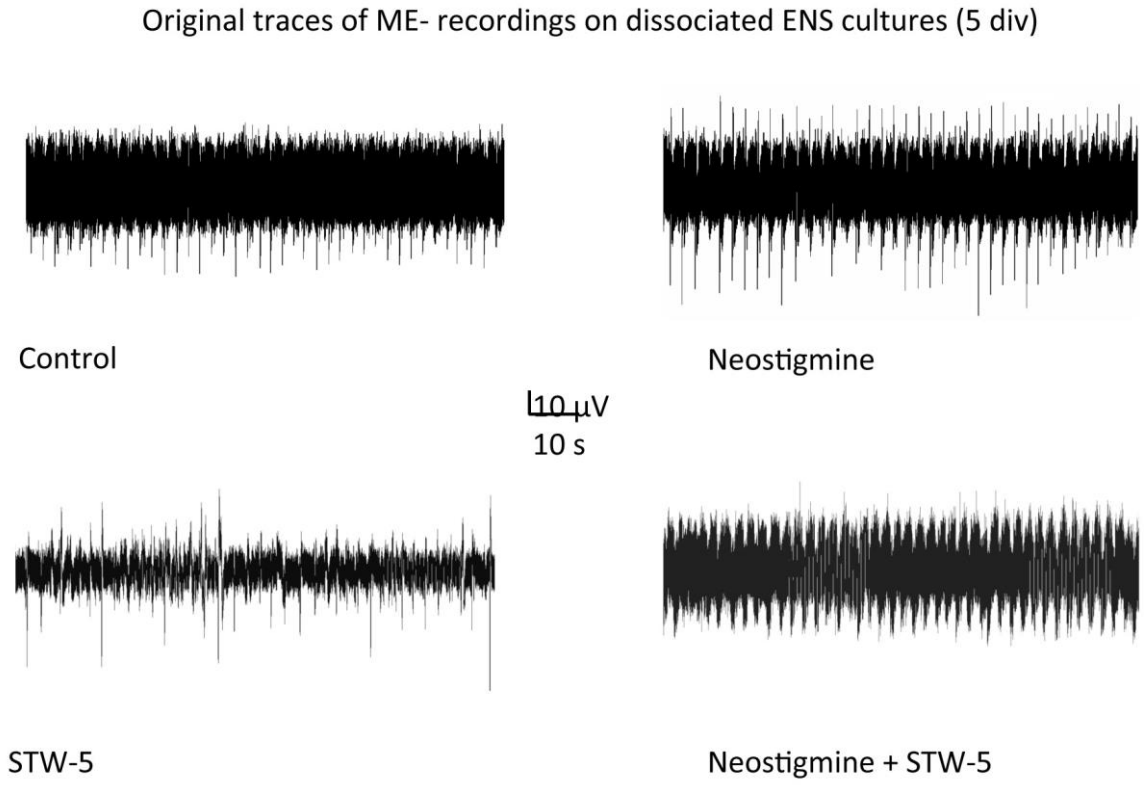
The enteric nervous system is responsible for the undisturbed regulation of gut motiliy, secretion or resorption. Whenever the gastrointestinal tract is affect by diseases, the ENS is also part of the problem. Especially during inflammation, the ENS can be stimulated and challenged by inflammatorysignaling molecules such as cytokines or hormones.

We investigated the impact of STW 5 on induced motility and inflammation in the gut, respectively the enteric nervous system. Gut segments were kept in an organ bath under perfusion conditions and motility increased by neostigmin application. STW 5 was added after the stimulation. To investigate whether the ENS was the target for the compound, we also performed electrophysiological measurements of enteric neuronal networks on microelectrode arrays.

Inflammation was simulated in isolated ENS tissue. Myenteric plexus from adult mice from both jejunum and colon was isolated and either stimulated with a cytokine cocktail of Interferon-gamma, IL-1ß and TNF-alpha alone, or in combination with the multi-component herbal preparation STW 5.

The plexus tissues were kept for 24 hrs in tissue culture medium and supernatant was collected the following day. Cytokine liberation was measured using Multiplex-ELISA. Application of STW 5 reduced significantly the neostigmin-induced motility in a dose dependent manner. The electrical activity of the neuronal networks could also be increased by neostigmine and was reduced to basal activity by addition of STW 5. In the inflammation approach, the supernatants from colonic and jejunal myenteric plexus showed different responses. While many cytokines were released after cytokine stimulation in both colonic and jejunal preparations, STW 5 led to a complete downregulation of the release only in the colonic myenteric plexus.

STW 5 does regulate both motility and inflammation via a direct interaction with the enteric nervous system.



PW-88

Hepatic protective effect of *Trapa japonica* pericarp *in vitro* and *in vivo*

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In this study, the hepatic protective effect of the *Trapa japonica* pericarp ethanolic extract were evaluated. The ethyl acetate fraction (EF) showed protective effects against *tert*-butylhydroperoxide (t-BHP)-induced oxidative damage *in vitro* and *in vivo*. *In vitro* experimental results showed that the EF suppressed t-BHP-induced damage in Chang cells by inhibiting reactive oxygen species generation and regulating the mitochondrial membrane potential. Furthermore, western blot analysis showed that the EF effectively inhibited *t*-BHP-induced apoptosis by suppressing caspase-3, caspase-7, caspase-8, and caspase-9. *In vivo* study, the EF significantly prevented serum increases in glutamate oxaloacetate transaminase and glutamate pyruvate transaminase and hepatic malondialdehyde levels caused by *t*-BHP. Furthermore, the EF markedly increase hepatic superoxide dismutase, catalase, and glutathione levels. Histopathological examinations further confirmed that the EF could protect the liver from *t*-BHP-induced oxidative injury. These findings indicate that the EF could be developed as a medicinal plant for the therapy and prevention of hepatic injury.

PW-89

Endopleura uchi: a Medicinal Plant with Antidiabetic Potential

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Endopleura uchi is an Amazonian tree and its bark is used as tea against arthritis, cholesterol, diarrhea and cancer. In spite of its large use, the antidiabetic, anti-cholinesterase, antioxidant and cytotoxic against Caco-2 of this species have not been assessed before and its chemical composition is less studied. In this work two different extracts (infusion and hydroethanolic) were studied concerning phenolic composition and biological potential. Five compounds were determined by HPLC-DAD, being bergenin the major one. In general way, infusion presents a greater richness in these metabolites. The antioxidant, acetylcholinesterase, butyrylcholinesterase, α-glucosidase and antibacterial activities were checked by *in vitro*assays. A dose-dependent response was noticed against DPPH·, superoxide and nitric oxide radicals, acetylcholinesterase, butyrylcholinesterase and in the α-glucosidase inhibitory assay. In the latter case, hydroethanolic extract (IC_{50}=2.2 μg/mL) and infusion (IC_{50}=2.4 μg/mL) showed remarkable activity as compared to the control acarbose (IC_{50}=284 μg/mL). Antibacterial capacity of both extracts was investigated against Gram-positive and Gram-negative bacteria, being more effective against the first one. The concentrations of extracts tested here showed no toxicity on intestinal (Caco-2) cells. These results suggest that the extracts of *E. uchi* may be interesting for incorporating in pharmaceutical preparations, since it may suppress hyperglycaemia and inhibit cholinesterases, or can be utilized as food additive due to its antioxidant and antibacterial activities.

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PW-90

Phytochemical investigation and cytotoxic characterization of bioactive constituents from *Conyza dioscoridis*

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A follow-up chemical investigation to methanol extract of *Conyza dioscoridis* (Family Asteraceae) led to the isolation of seven known compounds (**1**-**7**). The isolated compounds identified as 2-(3,4-dihydroxyphenyl)ethyl-2-*O*-[6-deoxy-α-L-mannopyranosyl-4-(3,4-dihydroxyphen-yl)-2-propenoate]-β-D-glucopyranoside (**1**), rutin (**2**), isoquercitin (**3**), E-caffeic acid (**4**), gallic acid (**5**), quercitin (**6**), β-sitosterol-β-D-glucoside (**7**). The methanol and chloroform extracts of the plant showed cytotoxic activity against brine shrimp in a

preliminary assay and inhibitory activity against colon carcinoma cells (HCT-116) with LC_{50} values; 20, 32 µg/ml and IC_{50} values; 25, 35.3 µg/ml respectively. Brine shrimp lethality test was conducted on the seven isolated compounds at six different concentrations 400, 200, 100, 20, 10 and 5 µg/ml. Compounds **1**, **2**, **3**, **5**, **6** and **7** showed significant cytotoxicity with LC_{50} values; 9, 10, 11, 22, 8 and 19 µg/ml respectively; while compounds **1**, **2**, **3** and **6** exhibited inhibitory activity against colon carcinoma cells with IC_{50} values; 25, 30, 34.2 and 10 µg/ml respectively. These results indicate that; *Conyza dioscoridis* has biochemical activity as potential pharmaceuticals. The chemical structures of active constituents were unambiguously determined by analysis of ^{1}H NMR,^{13}CNMR, ESI-MS, as well as by comparison with literature data and physical methods.

PW-91

Metabolite profile of *Salix reticulata* methanolic extract and its antiproliferative effect on immortalized human keratinocytes

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Traditionally, *Salix* species (Salicaceae) have been used for their anti-inflammatory, analgesic and antipyretic properties. The pro-apoptotic effect of *Salix* extracts on cancer cells has been reported [1]. In the search for herbal remedies for skin diseases with cellular hyperproliferation, like psoriasis, such anti-inflammatory and pro-apoptotic effects would be highly desirable.

Salix reticulata L. is a mountain dwarf shrub. To our knowledge, no previous phytochemical investigation has been reported on this plant. In this study, the metabolite profile of the methanolic extract was characterized and its effect on immortalized human non-tumorigenic keratinocytes (HaCaT) was investigated.

The leaves were extracted successively by dichloromethane and methanol. Fractionation of the methanolic extract by a combination of column chromatography on Sephadex LH-20, and preparative and semipreparative HPLC afforded several flavonoids including luteolin and apigenin glycosides, and phenolic glucosides typical of *Salix* species such as triandrin and salicortin. Cell viability was determined using fluorescein diacetate-propidium iodide staining. Quantification of viable (green fluorescence) and inviable (red fluorescence) cells was performed using ImageJ software. Cell proliferation was assessed on HaCaT cells by a BrdU incorporation ELISA assay. Proliferation of HaCaT cells decreased with increasing concentrations of the extract. At a concentration of 200 µg/mL, BrdU incorporation was inhibited by 50%. Investigation of the possible molecular pathways leading to the observed anti-proliferative effect is ongoing.

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PW-92

Cytotoxic activity of water extract fractions of *Hypericum androsaemum* L. on colorectal cancer cells

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Hypericum androsaemum L. (HA) grows wild in shadowy sites, namely in northern region of Portugal, where it is widely used as a medicinal herb. According to some authors, this species is used in popular medicinal preparations as a cholagogue, hepatoprotector, and diuretic and in kidney failure [1]. In this work, the composition of water soluble and volatile components of HA infusions was characterized and the total extract (HA-T) fractioned in order to identify the active compound(s) responsible for their cytotoxic effects. HA-T and each of its seven fractions (HA-A to HA-G) were tested against CO115 cells, at 40 µg/ml. Effects on cell viability/proliferation and apoptosis were assessed by MTT and nuclear condensation assays, respectively. The expression of intermediates of the MAPK/ERK and PI3K/AKT signalling pathways and the expression of apoptotic markers were evaluated by western blot. HA-E and F showed decreases in cell viability in relation to control. Of these fractions, only HA-F showed to slightly increase apoptosis. The effects of individual fractions did not explain the 40% apoptosis reached in response to the total and the reconstituted extract, suggesting that the synergistic effects of the extract's constituents are needed for the HA cytotoxic properties against the CO115 cells. In agreement with this, only HA-T showed significant increases in the apoptotic markers cleaved caspase-9 and cleaved PARP-1. Relative to the total extract (HA-T), only slight decreases in BRaf (HA-E), pERK (HA-E) and pAKT (HA-E and HA-F) were observed. Only HA-T reduced significantly the expression of BRaf, pErk and pAKT. These results support the hypothesis that there is more than one compound in the HA-T responsible for the HA toxicity on the colorectal cancer cells.

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PW-93

Anti-inflammatory and antioxidant activity of white lupin (*Lupinus albus)* aerial parts

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Lupinus albus L. (white lupin), is a member of the family Fabaceae, known for high contents of secondary metabolites with potential biological activities. In this paper, antioxidant and anti-

inflammatory potential of aerial parts were investigated, since little is known about its aforementioned properties.

80% MeOH herb extract was prepared by maceration. Anti-inflammatory potential was examined in human platelets, using method based on determination of inhibition ability of icosanoid production [1]. For examination of antioxidative capacity, spectrophotometric methods were used. DPPH˙ neutralization and NO˙ scavenging ability was determinated, and reduction potential was examined using the FRAP assay.

Anti-inflammatory activity of is expressed in IC_{50} values of icosanoid production inhibition: 12-HETE (12-hydroxyeicosatetraenoic acid) 7.13 mg/mL, 12-HHT (12(S)-hydroxyheptadeca-5Z,8E,10E-trienoic acid) 5.66 mg/mL and TXB_2 (thromboxane B_2) 4.94 mg/mL, while for PGE_2 IC_{50} value was not reached. IC_{50} value for DPPH˙ neutralization is 100.3 µg/mL, and for NO˙ scavenging 1.95 mg/mL. Reduction potential is 15.0 mg vitamin C equivalents per g of dry weight. High reduction potential and free radical scavenging ability makes the white lupin herb extract a good source of antioxidant agents, while it has shown only moderate anti-inflammatory activity.

Acknowledgement: This work was supported by a research grant from the Ministry of Education and Science, Republic of Serbia (Grant No. 172058).

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PW-94

High-resolution assays combined with HPLC-HRMS-SPE-NMR for identification of antidiabetic constituents in Vietnamese plants

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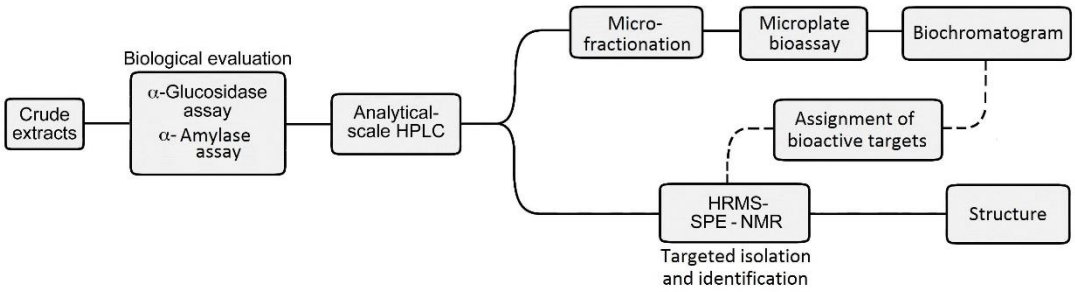
Type-2 diabetes is affecting 246 million. Patients with type-2 diabetes suffer from complications as high blood pressure, blindness, kidney failure, lower limb amputation, heart disease and stroke.

20 medicinal plants traditionally used in Vietnam for the management of diabetes were collected and investigated for inhibition of carbohydrate-hydrolysing enzymes.

Chloroform, ethanol and water extracts of 20 plants were evaluated for α-glucosidase and α-amylase inhibitory activity. Analytical-scale HPLC was then used to investigate the most active extracts, where samples without tannins were identified and fractionated into 96-well microplates, followed by α-glucosidase [1] and α-amylase [2] inhibition assays.



Ethanol and water extracts of *Phyllanthus amarus, Phyllanthus urinaria, Lagerstroemia speciosa, Nepenthes mirabilis, Syzygium cumini, Rhizophora mucronata* and *Kandelia candel* had IC_{50} below 40 μg/mL in the α-glucosidase assay. Ethanol extracts of *Kandelia candel* and *Ficus racemosa* inhibited α-amylase (IC_{50} 7.66 and 46.70 μg/mL, respectively).

Having no tannin constituents, *P. amarus*, *P. urinaria*, *L. speciosa* water extracts and *F. racemosa* ethanol extract were chosen for fractionation followed by α-glucosidase and α-amylase inhibition assays. Biochromatograms of *P. amarus* and*P. urinaria* water extracts showed several active compounds against α-glucosidase.

Biochromtogram of *P. amarus* and *P. urinaria* water extracts have many promising peaks with more than 90% inhibitory activity. The biochromatograms constructed from these assays allowed fast identification of active compounds responsible for antidiabetic activity. Subsequent HPLC-HRMS-SPE-NMR experiments will allow the isolation and structural elucidation.

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PW-95

Effect of plant extracts on rat basophilic leukemia (RBL-2H3) cells sensitized with IgE

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In this study, the inhibitory activities of one hundred plant extracts were analyzed against the IgE-induced allergy reaction in the rat basophilic leukemia (RBL-2H3) cells. Release of IL-4 and beta-hexosaminidase from the IgE-sensitized RBL-2H3 cells treated with the plant extracts were measured. Additionally, the effects of the plant extracts on the cell viability were tested. From the analysis, 17 extracts including *Rhamnus davurica* Pall. (branch) significantly inhibited beta-hexosaminidase release at 20 μg/mL. And 41 plants such as *Artemisia absinthium* (leaf and stem) showed high suppressive activities on IL-4 release. All of the

extracts proliferated the sensitized cells above 80% compared with control, which was not treated with plant extract samples but treated only with the buffer used for sample preparation.

In conclusion, the result suggests that the plants with the inhibitory activities on allergy reaction at cell level are candidates for developing useful anti-allergy materials and need to be study more.

PW-96

Palm-oil derived vitamin E as prophylaxis against osteoporosis in chronic glucocorticoid excess: An *in vivo* study.

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Palm oil from the fruit of the palm tree, *Elais guineensis* is rich in vitamin E, a strong antioxidant. Palm vitamin E (PVE) is composed of 80% tocotrienols (γ, α, δ-isomers), and 20% α-tocopherol. Our earlier studies showed that oxidative stress induced by ferric ions in a male rat model resulted in reduced bone density. This was prevented by supplemention with oral PVE extract. Osteoporosis is a known side effect of longterm treatment with systemic glucocorticoids. In this study we determined the effects of PVE on cellular bone histomorphometry and biomechanical strength in a rat model of glucocorticoid-induced bone loss. 3 month old male Sprague-Dawley rats were divided into groups of 10: (i) Adrenalectomised (Adrx) + 120 μg/kg/day intramuscular (IM) dexamethasone (Dexa) + oral PVE 60 mg/kg/day. (ii) Adrx + Dexa + vehicle oral palm olein 0.1 ml/kg/day. (iii) Sham operated + vehicle palm olein 0.05 ml/kg/day IM + 0.1 ml/kg/day orally. The PVE was a gift from Sime Darby Ltd. and its composition was similar to Gold Tri-E™. The treatments were given for two months. The left femora were analyzed for cellular bone histomorphometry and the right femora were tested for biomechanical strength (3-point bending test). The results showed that longterm glucocorticoid treatment significantly decreased Osteoblast Surface (Ob.S) [Sham 39.71+1.88, Adrx+Dexa 15.33+2.77, p=0.011], while Osteoid Surface /Bone Surface (OS/BS) significantly increased compared to Sham [Sham 3.64+0.55, Adrx+Dexa 10.54+ 1.52, p=0.01]. Supplementation with PVE maintained the Ob.S (Adrx+Dexa+PVE 40.31+7.6, p=0.907) and the OS/BS (Adrx+Dexa+VE 6.9+0.72, p=0.121) comparable to Sham. Biomechanical strength (Load to fracture) were reduced in the glucocorticoid group compared to Sham [Sham 133.13+5.94, Adrx+Dexa 108.18+4.15, p=0.016]. Supplementation with PVE maintained bone strength comparable to Sham [118.45+0.071' p=0.718]. Thus PVE may be developed as a prophylactic anti-osteoporotic agent.

PW-97

Antihyperglycemic effect of anthocyanin fraction of *Berberis integerrima* on STZ induced diabetic rats

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Berberis integerrima, a species of Berberidaceae, found in most regions of Iran. Different compound and metabolites in this plant make it as a candidate for medicinal applications [1]. Fruits of *Berberis* are one of the richest sources of anthocyanins [2].Recently results of several studies indicated various biological activities of anthocyanins, such as antioxidant, anti-inflammatory anti-mutagenic and anticancer activities [3]. The aim of present study was evaluation of antidiabetic potential of anthocyanin-rich fractions obtained from Berberis integerrima fruits on STZ-induced diabetic rats. Antioxidant activity of the anthocyanin fraction was determined through DPPH assay. Anthocyanin fraction (200,400,1000 mg/kg), glibenclamid+ anthocyanin fraction(3 mg/kg + 1000 mg/kg) and metformin+anthocyanin fraction(15 mg/kg + 1000 mg/kg) were fed intra-esophageally to STZ-induced diabetic rats for 21 days and blood glucose, liver glycogen and body weight were measured parameters. Antioxidant assay showed there is no significant difference between the IC_{50} of anthocyanin fraction and quercetin. Treatment of diabetic rats with anthocyanin fraction (200,400,1000 mg/kg) significantly decreased blood glucose as compared with control moreover anthocyanin fraction (400,1000 mg/kg) significantly increased liver glycogen in compared to control. Anthocyanin fraction (400,1000 mg/kg) significantly increased body weight. The results of this study indicate that anthocyanin fraction of Berberis integerrima fruits possesses antioxidant and hypoglycemic effects in STZ- induced diabetic rats but there were no synergistic effects between anthocyanin fraction and metformin or glibenclamid on blood glucose, liver glycogen and body weight.

PW-98

White tea *(Camellia sinensis)* consumption improves heart and brain glycolytic and oxidative profiles in prediabetic rats

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Diabetes mellitus (DM) is a major public health problem and its incidence is dramatically rising. Heart and brain are particularly susceptible to glucose fluctuations and hyperglycaemia-induced oxidative stress. Tea is widely consumed and studied; however, the antidiabetic properties of white tea (WT) remain largely unexplored. In this work, we hypothesized that

the regular consumption of WT by prediabetic rats improved cardiac and cerebral cortex glycolytic and oxidative profiles.

WT composition, and rats' heart and brain cortex glycolytic and oxidative profiles were determined.

WT phytochemical profile was composed by glucose, sucrose, (-)-epicatechin, (-)-epigallocatechin, (-)-epigallocatechin-3-gallate, caffeine, L-theanine, alanine, and lactate, being (-)-epigallocatechin-3-gallate the major one.

Prediabetic rats drinking water developed mild glycaemia, glucose intolerance and insulin resistance. In addition, prediabetes decreased lactate and acetate contents and lactate dehydrogenase (LDH) activity in cardiac tissue; while in brain cortex decreased lactate content and increased LDH activity. Prediabetes also decreased heart and brain cortex antioxidant capacities, increasing lipid peroxidation and protein oxidation.

Consumption of WT improved glucose tolerance and insulin sensitivity in prediabetic rats. In the heart, WT increased alanine content and antioxidant capacity, and normalized LDH activity and lipid peroxidation. In the cerebral cortex, WT decreased lactate and alanine contents and normalized the antioxidant capacity, lipid peroxidation and protein oxidation.

In conclusion, the regular consumption of WT improved the heart and brain metabolic and oxidative profiles in prediabetic rats, suggesting it as a good, safe and inexpensive strategy to prevent DM-related effects.

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PW-99

Heme oxygenase-1-mediated anti-inflammatory effect of tussilagonone in RAW 264.7 macrophages

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The dried flower buds of *Tussilago farfara* have been used in traditional medicine, mainly as antitussive in treatment of coughs and other respiratory problems. In the present study, we investigated the anti-inflammatory signaling pathway by up-regulation of heme oxygenase-1 (HO-1) expression in response to tussilagonone (TGN), a sesquiterpene compound isolated from Tussilago farfara. Treatment of RAW 264.7 cells with TGN induced HO-1 protein and mRNA expression. TGN increased protein but not mRNA level of nuclear factor-E2-related factor 2 (Nrf2), suggesting that TGN regulated Nrf2 at the translational level. Nuclear translocation of Nrf2 by TGN is also increased in a time- and dose-dependent manner indicating that TGN induced HO-1 via Nrf2 pathway. Consistent with the notion that HO-1 has anti-inflammatory properties, TGN suppressed inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) and also reduced the mRNA expression of pro-inflammatory cytokines, including iNOS, COX-2, TNF-α and IL-6, as well as nitric oxide (NO) and

prostaglandin E2 (PGE2) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. Furthermore, the LPS-induced SEAP expression in NF-κB-SEAP-NPT construct-transfected RAW 264.7 macrophages was inhibited by TGN. Moreover, TGN inhibited the phosphorylation and degradation of inhibitory κB (IκB)-α and the nuclear translocation of NF-κB. However, a specific inhibitor of HO-1, SnPP, reversed TGN-mediated suppression of NO production and knockdown of HO-1 by small interfering RNA abrogated inhibitory effects of TGN on iNOS and COX-2 protein expression and NF- κB nuclear translocation in LPS-stimulated RAW 264.7 cells. Taken together, these findings suggest an important role of TGN-induced HO-1 activation in regulating inflammatory responses and TGN is a potent therapeutic candidate targeting the crosstalk between Nrf2/HO-1 and NF-κB signaling pathway for preventing or treating inflammation-associated diseases.

PW-100

Phytochemical, free radical scavenging and hepatoprotective activity of *Acacia nilotica* leaves

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The current study was designed to evaluate the phytochemical, free radical scavenging and hepatoprotective effects of*Acacia nilotica* leaf extracts against deltamethrin (Delta) induced liver damage in male mice. The chemical constituents of*Acacia nilotica* leaves were studied. Total phenolics and flavonoids were determined. The results revealed that *Acacia nilotic*a leaves extract exhibited antioxidant capacity manifested by inhibitory effects on DPPH, ABTS, hydroxyl radical and reducing power *in vitro* in a concentration dependent manner. Male mice were divided into six groups (6 mice each): control group (I); extract groups (II&III) received an extract at doses of 150 & 300 mg/kg body weight; Delta group (IV) received Delta (3.40 mg/kg b.wt, 1/10 LD_{50}) in corn oil; groups (V&VI) simultaneously received Delta along with the two doses of extracts via oral route for 28 consecutive days. Delta caused a significant increase of liver biomarker serum enzymes e.g., alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) and decreases in superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities and increase in the level of lipid peroxidation (LPO) in liver of male mice accompanied by histopathological alterations. Co-administration of *Acacia nilotica* extract ameliorated the above-mentioned parameters. The ultimate effect was achieved by the highest dose of the extract. It could be concluded that co-administration of *Acacia nilotica* leaves extract attenuated the harmful effects of Delta, which may be attributed to its antioxidant potential. Results indicated that *Acacia nilotica* leaves could be used for therapeutic option against hepatic injuries resulting from pesticide intoxication.

PW-101

Effects of *Zilla spinosa* on liver fibrosis induced by carbon tetrachloride in rats

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Hepatic stellate cells (HSCs) are key mediators of fibrogenesis, and the regulation of their activation is now viewed as an attractive target in the treatment of liver fibrosis. The aim of

this work was designed to evaluate the ability of aqueous methanol extract of *Zilla spinosa* Prantl. (Brassicaceae) to regulate the activation of HSCs, to prevent liver fibrosis, and to inhibit the oxidative stress. The two doses (100 or 200 mg/kg) of *Z. spinosa* significantly improved liver function (ALT and AST) and increased the activities of SOD (19.40±0.98; 24.10±1.25 U/mg protein), CAT (3.24±0.43; 3.85±0.06 U/min), GSH (412±7.2; 543±5.41 mol/mg tissue), while MDA decreased significantly (71.20±1.35; 77.50±1.72 nmol/mg protein) in lower and higher dose compared with CCl_4 treatment. A comparative histopathological study of liver of rat treated with *Z. spinosa* exhibited almost normal architecture, and reduced the liver damage including steatosis and fibrosis in a dose dependent manner, as compared to CCl_4 treated group. Image analysis of liver revealed a marked reduction damage area and quantity of collagen distribution after treatment with *Z. spinosa* (100 or 200 mg/kg) compared with CCl_4 treated group. In addition, the *Z. spinosa* extract significantly decreased pro-fibrotic markers of hydroxyproline and hyaluronic acid in liver content. Furthermore, the immunohistochemical study showed that the extract markedly reduced the numbers of α-smooth muscle actin positive cells and transforming growth factor-β1 (TGF-β). These results suggested that the extract significantly inhibited the progression of hepatic fibrosis induced by CCl_4, and the inhibitory effect on hepatic fibrosis might be associated with its ability to scavenge free radicals, decrease of TGF-β, inhibit collagen synthesis and proliferation in HSCs. Our results indicate that treatment with *Z. spinosa* after the establishment of CCl_4 induced hepatic fibrosis significantly reduces the fibrosis in rats.

PW-102

Osteoprotective effect of extract from *Panax ginseng* in ovariectomized rats

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This study was carried out to investigate the effects of the *Panax ginseng* extract on serum level of hormones from osteoporosis induced ovariectomized rats. Two month-old rats were ovariectomized (OVX), remained untreated for 8 weeks, and were subsequently administered *P. ginseng* (200 mg/kg) every day for 8 weeks. We examined the effects of treated *P. ginseng* every 10 days on ovariectomy-related changes in Insulin-like Growth Factors (IGF), Insulin-like Growth Factor binding protein-3 (IGBF-3), Estrogen, Calcium, and Phosporus After 8 weeks serum levels of IGF-I, -II, and IGFBP-3 were heigher after *P. ginseng* extract treatment on OVX rats as compared to the other two groups ($p<0.05$). Bone alkaline phosphatase levels were increased through *P. ginseng* extract treatment in OVX rats compared to the other two groups. There were no differences between OVX and *P. ginseng* extract treated OVX rats in serum levels of estrogen, but estrogen levels for the sham group were higher than for the other two groups. *P. ginseng* extract is increased to serum levels of IGFs and IGFBP-3 of osteoporosis induced by ovariectomized rats. Thus, the results reveal that the *P. ginseng*extract is a possible role for improvement of osteoporosis induced-ovariectomized rats and has a great potential as an alternative tool for the treatment of osteoporosis.

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PW-103

In vitro and *in vivo* evaluation fo antioxidant activity of *Annona muricata* stem bark extracts in *Rattus Novergicus*

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The objective of this study is to evaluate the *in vivo* antioxidant potential of ethanol extract of *Annona muricata* against CCl_4 induced toxicity in rats as well as its *in vitro* antioxidant effect and lipid peroxidation. The extract was prepared by cold maceration using absolute ethanol. The invitro antioxidant properties of the extract was determined using DPPH (2,2-diphenyl-1-picrylhdrazyl) radical and invivo antioxidant enzymes were assayed to evaluate the biological activities of the extract. The polyphenol content are alkaloids, tannin, flavonoids and phenol . In the invivo studies, the animals were grouped into three groups of 15 rats each. Group 1 served as control and received 1ml/kg b.w of olive oil orally for 28 days. Group 2 rats were orally administered 1ml/kg CCl_4 mixed with olive oil (1:10) daily for 28 days while group 3 rats were administered 1ml/kg CCl_4 and 200 mg/kg b.w of *Annona muricata* stem extract. Three of the rats from each group were sacrificed on days 1, 8, 15, 22 and 28. The plant extract showed remarkable hepatoprotective and antioxidant activity against carbon tetrachloride (CCl_4) induced oxidative stress as revealed from serum enzyme markers. CCl_4 induced a significant rise ($p<0.001$) in aspartate amino transferase , alanine amino transferase , alkaline phosphatase and malondialdehyde level in the serum with a reduction in catalase activity. Treatment of rats with the plant extract (200 mg/kg b.w) significantly altered both serum enzymes activities and oxidant levels to near normal against CCl_4-treated rats. The *in vivo* and *in vitro* rapid radical scavenging studies were positive for the stem bark extract. This study suggests that the possible mechanism of the exhibited biological activities of the extract may be due to free radical scavenging owing to the presence of polyphenols in the extract. The plant extract possesses, antioxidant, anti-lipid peroxidation effect and it is hepatoprotective.

PW-104

Effects of STW 5, STW 5-II and STW 6 on rat ileal and colonic preparations: Region specific effects

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STW 5 (Iberogast®) is a fixed combination of nine plant extracts with *Iberis amara* (STW 6) as one of its components. It is successfully used for treatment of functional dyspepsia and irritable bowel syndrome (IBS). Moreover, STW 5-II is a preparation without *Angelica archangelica, Silybum marianum* and *Chelidonium majus*. In this study we compared the effects of STW 5, STW 5-II and STW 6 on tone and acetylcholine (ACh)-induced contractions

in intact and inflamed intestinal preparations. We used 1-1.5 cm long ileum and colon preparations of male Wistar rats to analyze region specific differences. The inflammation was induced by intraluminal instillation of 2,4,6-trinitrobenzene sulfonic acid (TNBS, 10 mM, 30 min). Incubation with STW 5 (512 µg/ml) reduced the tone and decreased ACh-induced contractions of untreated ileal and colonic preparations concentration dependently (64-512 µg/ml). The effects of STW 5-II in a concentration of 533.2 µg/ml were comparable to those of STW 5. STW 6 in equivalent concentrations (3-24.1 µg/ml) neither affected the tone nor the contractility. TNBS-induced inflammation was accompanied by a significant reduction of ACh-induced contractions. Co-incubation of TNBS with STW 5 (512µg/ml), STW 5-II (533.3µg/ml) or STW 6 (24.1µg/ml) partially normalized the TNBS-induced attenuation of tone as well as of ACh-induced contractions in ileum preparations. In inflamed colon segments the co-incubation of TNBS with STW 6 in a high concentration (24.1 µg/ml) revealed protective effects whereas STW 5 as well as STW 5-II had no effects.
In conclusion, STW 5 and STW 5-II influenced ACh-induced contractions and tone in untreated ileal and colonic preparations, whereas STW 6 did not contribute to these effects. In TNBS-inflamed ileum preparations STW 5, STW 5-II as well as STW 6 normalized contractile disturbances, while in colon preparations only STW 6 was effective. Our study confirms region specific effects of these plant extracts.

PW-105

In vitro anti-inflammatory and free radical scavenging activities of crude saponins extracted from *Albuca bracteata* bulb

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Albuca bracteata Jacq. is a medicinal plant traditionally used in the management of diabetes mellitus in the Eastern Cape of South Africa. The purpose of this study was to evaluate the antioxidant and anti-inflammatory activities of saponins extracted from the bulb of *Albuca bracteata* and compared with the crude methanolic extract. The methanolic extract of *Albuca bracteata* was fractionalized to obtain the crude saponins. The *in vitro* antioxidant activity of the isolated saponins and the crude methanolic extract were detected using free radical scavenging assays such as DPPH, ABTS and NO_2 while the anti-inflammatory potential was tested using inhibition of protein denaturation of egg albumin as a model of anti-inflammatory capacity.

Both the crude methanolic extract and saponins showed inhibition of DPPH, ABTS and NO_2 scavenging activity, the free radical scavenging activity of isolated saponins compared favourably with rutin and BHT, however, the crude methanolic extract showed higher inhibition percentage of protein denaturation compared with the saponins at the concentration investigated. This study indicates that saponins from *Albuca bracteata* bulb possesses potent anti-inflammatory activity and is also a good source of natural antioxidant.

PW-106

Effects of root bark extracts from *Dictamnus dasycarpus* on ICAM-1 expression and immune cell infiltration in human keratinocytes

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The root bark of *Dictamnus dasycarpus* Turcz. (Rutaceae) is a well known anti-inflammatory agent for skin diseases such as eczema, pruritus and urticaria in Eastern countries.

We investigated the effects of methanolic extract of *D. dasycarpus* root bark (MEDD) on Intercellular Adhesion Molecule-1 (ICAM-1) expression, epidermal hyperplasia and immune cell infiltration in 1-fluoro-2,4-dinitrofluorobenzene (DNFB)-induced contact dermatitis (CD) mice. We also investigated its effects on the expression of ICAM-1, binding capacity to THP-1 cells, and phosphorylation of NF-κB in human keratinocytes (HaCaT cells).

Topical application of MEDD effectively inhibited ICAM-1 expression and epidermal hyperplasia in inflamed tissues. MEDD treatment also inhibited immune cell infiltration induced by DNFB. In addition, treatment with MEDD reduced the total amount of ICAM-1 in HaCaT cells and effectively lowered the capacity to bind to THP-1 cells. Finally, MEDD treatment prevented activation of the NF-κB pathway induced by TNF-α in HaCaT cells.

These data indicate that root bark of *D. dasycarpus* has the potential for treatment of inflammatory skin diseases as a complementary or alternative medicine to corticosteroids. In addition, they suggest that the anti-inflammatory effects of *D. dasycarpus* on CD are involved in the regulation of ICAM-1 expression through down-regulation of the NF-κB signaling pathway in keratinocytes.

PW-107

The anti-inflammatory action of the herbal preparation STW5-II in normal human colonic mucosal cells

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Inflammatory bowel diseases (IBD) are chronic relapsing intestinal disorders characterized by an up regulation of pro-inflammatory cytokines followed by an invasion of immune cells. Standard therapies consist of anti-inflammatory or immunosuppressive drugs. Since clinical efficiency of the commonly used drugs is not satisfactory and most of them cause massive side effects, the search continues for new treatment options.

We investigated the protective effect of the fixed combination herbal preparation STW5-II and the contribution of its single components in an in-vitro model of colonic inflammation. The normal human colonic epithelial cell line NCM460 was treated with STW5-II or its single components for 4h prior to induction of inflammation. A pro-inflammatory cocktail consisting of TNF-α, IL-β and IFNγ was used to simulate inflammatory conditions normally caused by immune cells. The effect on NCM460 cells was investigated by enzyme linked immunoassay (ELISA) and Proteome Profiler®. Levels of IP-10, MCP-1, I-Tac, Gro-α and IL-8 were

increased in inflammatory state and significantly reduced by STW5-II. The effect of its individual constituents was much less pronounced. Further we investigated the effect of STW5-II on pro-inflammatory transcription factor nuclear factor-kappaB (Nf-κB) by analyzing nuclear extracts of treated NCM460 cells using Western blot and DNA binding ELISA. In Addition HEK-Blue-Null-1 cells stably expressing HEK-Blue-Null1 vector and secreted alkaline phosphatase (SEAP) on a NF-κB promoter were treated with various concentrations of STW5-II for 4h before induction of SEAP with TNF-α for another 24h. Effects on Nf-κB activity were inhibited only at high concentrations of STW5-II in both cell lines, hence the effect of STW 5-II on cytokine release was not mediated through NF- κB in the current experimental setting.

The herbal preparation was superior to single extracts highlighting the use of the combination. These results support a possible usefulness of STW5-II in treating IBD.

PW-108

Preservation of gastric functional activity in a novel stress model of functional dyspepsia by the herbal preparation, STW5.

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We report novel findings regarding modulation of gastric emptying and function by the herbal preparation STW5 (Iberogast®) in a recently established model of functional dyspepsia (FD) [1] simulating the clinical situation where FD is attributed to emotional stress in early life [2], followed by later stress [3]. STW5 consists of standardized extracts of *Iberis amara* (Brassicaceae), *Melissa officinalis* (Lamiaceae), *Matricaria chamomilla* (Compositae), *Carum carvi* (Apiaceae),*Mentha piperita* (Lamiacea), *Angelica archangelica* (Apiaceae), *Silybum marianum* (Compositae), *Chelidonium majus*(Papaveraceae), and *Glycyrrhiza glabra* (Leguminosae). Rats were subjected to neonatal maternal separation at intervals during the first 3 weeks of birth followed by restraint stress in adulthood. Restraining sessions were carried out 90 min/day for 1 week during which they were given STW5 in daily doses of 2 and 5 ml/kg p.o. One day after the last session, gastric emptying time was determined using phenol red [4]. Stressed rats showed marked delay in gastric emptying, an effect which was counteracted dose dependently by STW5. We tried to correlate changes in gastric emptying with changes in stress hormone levels and gastric reactivity towards smooth muscle stimulants and relaxants. We had shown earlier that giving STW5 ahead of stress prevented changes in fundus strip sensitivity to carbachol, serotonin, adrenaline and KCl. Here we extend our findings by giving STW5 alongside with stress. STW5 prevented the rise in corticosterone and changes in fundus sensitivity to these agents induced by the model. The preservation of gastric functional activity by STW5 in the face of stress contributes to our understanding of its beneficial use in FD [5].

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PW-109

Anti-inflammatory activity of a new triterpenoidal saponin from *Crotalaria madurensis*

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The genus *Crotalaria* belonging to the family leguminnosae. Several *Crotalaria* species have already been chemically studied and found to contain mainly flavonoids, chalcones and triterpenes. A new triterpene saponin, namely sophradiol 3-*O*-α-L-rhamnopyranosyl-(1'''→4")-*O*-β-D-galactopyranosyl- (1"→6')-*O*- β-D-glucopyranoside (**1**) together with ten known constituents, have been isolated from the leaves of *Crotalaria madurensis* L. The structure of a new compound was established by chemical and physochemical analysis (UV, ESI-MS, ^{1}H-, ^{13}C-NMR, ^{1}H-^{1}H COSY, HSQC and HMBC). The known compounds were identified as quercetin (**2**), isoquercetin (**3**), quercitrin (**4**), 8-hydroxy quercetin 3-*O*-β-D-arabinopyranoside (**5**), quercetin 3-*O*-neohespridoside (**6**), myo-inostol (**7**), sophradiol 3-O-β-D-glucuronopyranoside (**8**), 23-hydroxy-3α-[(*O*-α-L-rhamnopyranosyl-(1"→4')-*O*-α-L-arabinopyranosyl)-oxy]olean12-en-28-oic acid 3-*O*-α-L-rhamnopyranosyl-(1''''→4'''')-*O*-β-D-glucopyranosyl-(1''''→6''')-*O*-β-D-glucopyranosyl ester (**9**), sophradiol 3-*O*-β-D-glucopyranosyl-(1"→4')-*O*-β-D-glucopyranoside (**10**), 23-hydroxy olean 12-en-28-oic acid 3-*O*-α-L-rhamnopyranosyl-(1"→6')-glucopyranoside (**11**). Moreover, as a conclusion for the biological study on male Swiss Albino mice (18-20 g), it was found that the new saponin (**1**) is non-toxic (LD_{50} 1000 mg/kg b. wt.) and has a significant anti-inflammatory activity in comparison to indomethacine. A significant anti-inflammatory effect was concluded from the reduction recorded in granuloma diameter on treatment with (**1**) (18.2% & 19.9%) in comparison to indomethacine (32.1 & 35.5%) at 32 and 48 days after ova injection, respectively relative to control group. PGE_2 level in granuloma was also reduced after treatment with (**1**) (36.3 & 21.9%) with respect to that of indomethacine (44.6 & 32.6%) at 32 and 48 days after ova injection, respectively.

PW-110

The estimation of antioxidant potential of oil extracts of wild apple fruit

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Wild apple fruit (*Malus sylvestris* (L.) Mill., Rosaceae) contains a large number of biologically active antioxidant substances (including phenolic compounds). Their extracts can be potentially used in dermopharmaceutical products for prevention and/or treatment of many skin's diseases caused by oxidative stress (contact dermatitis, phototoxity, photoaging, cancer). Therefore, the aim of our study was to estimate the antioxidant activity of oil extracts of wild apple fruit originated from Serbia, obtained by two extraction methods using olive and sunflower oil. Method 1 comprised the maceration of the wild apple fruit in olive or sunflower oil on a water bath for 4 hours (Samples S1 and S2). Method 2 comprised the maceration dry wild apple fruit in 96% ethanol followed by maceration with the olive or sunflower oil extraction, with removing ethanol throughly at same time (S3 and S4). The ratio of drug:extract was 1:5. Total phenolic content (TPC) was determined by Folin-Ciocalteu test and expressed as gallic acid equivalents (GAE). Antioxidant activity was determined by DPPH test and test with linoleic acid and expressed as %RSC (Radical Scavening Capacity) and %AOA (AntiOxidant Activity), respectively. Type of used solvent didn't have significant influence on antioxidant activity of oil extracts, but the extraction method influenced TPC content, that was 400.47 and 172.91 mgGAE/100g dry weight in S1 and S2, and 729.90 and 996.49 mgGAE/100g dry weight in S3 and S4, respectively. %RSC ranged from 14.94 - 35.20% and %AOA from 61.21 - 66.48%. The use of 96% ethanol additionally increased antioxidant capacity (S3 and S4), compared to the extraction with oil solvents only (especially noticed by DPPH test). All extracts showed better ability to prevent lipid peroxidation and low to neutralize free radicals. Oil extracts of wild apple fruit demonstrated a satisfying antioxidant activity and phenolic compounds might be responsible for their activity.

PW-111

In vitro screening of Traditional Chinese Medical (TCM) plants for pancreatic lipase and α-amylase inhibitory activities

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Inhibition of digestive enzymes is one of the most widely studied mechanisms for the treatment of obesity and its associated diseases as diabetes mellitus, coronary heart diseases or sleep-breathing disorders [1,2]. The use of plant based resources as a potential platform for discovery and development of new drugs has become a lucrative research field in this context.

For finding new compounds with pancraetic lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) and α-amylase (1,4-alpha-D-glucan glucanohydrolase, EC 3.2.1.1) inhibitory activities several TCM plants have been screened. Methanolic and water extracts of the plants were evaluated. To determine the pancreatic lipase activity, an enzymatic *in vitro* assay based on the hydrolysis kinetic of 4-methylumbelliferyl oleate (4-MUO) was used. For the determination of α-amylase activity the fluorescence-based EnzChek® Ultra Amylase Assay Kit (Molecular Probes™) was used. This *in vitro* enzyme assay is based on the hydrolytic cleavage of a modified starch derivative. Methanolic extracts from *Crataegus pinnatifida* Bunge Var. Major N.E.Br. (Rosaceae), *Rehmannia glutinosa* Libosch (Plantaginaceae), *Cornus officinalis* Siebold &

Zucc. (Cornaceae) and *Dioscorea opposita* Thunb. (Dioscoreaceae) showed the best inhibitory effect to both enzymes (IC_{50}<2.0 mg/mL). Further identification, isolation and characterization of active compounds responsible for enzyme inhibitory effects is required to evaluate the therapeutic potential of these plants.

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PW-112

Spirogyra neglecta, freshwater green alga, modulates the early stages of 1,2-dimethylhydrazine-induced colon carcinogenesis in rats

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Spirogyra neglecta (Hassall) Kützing is an edible freshwater green macroalga found in northern Thailand. Our studies found that the hot water extract of *S. neglecta* showed antimutagenic effects against several environmental mutagens in *Salmonella*mutation assay and anticarcinogenicity in diethylnitrosamine-induced hepatocarcinogenesis in rats. This study focused on the chemopreventive effects of the hot water extract of *S. neglecta* (SNE) and dried *S. neglecta* mix diet (SND) on 1,2-dimethylhydrazine (DMH)-induced preneoplastic lesion of colorectal carcinogenesis. Male Wistar rats were divided into 8 groups. Groups 1-5 were subcutaneously injected by 40 mg/kg bw of DMH, while Groups 6-8 were injected by 0.9% NaCl instead. Groups 1 and 6 were a positive control and a negative control, respectively. Groups 2 and 4 were fed with low dose of SNE and SND, respectively. Groups 3 and 7 were given by high dose of SNE, while Groups 4 and 8 were fed with high dose of SND. To study their effects on the initiation stage, SNE and SND were fed a week before DMH injection. The 5 week treatment of SNE significantly decreased number of aberrant crypt foci (ACF) in the colon of DMH treated rats. The extract also modulated the activities of some hepatic detoxifying and antioxidant enzymes including UDP-glucuronyl transferase, glutathione-*S*-transferase and glutathione peroxidase when compared to a DMH alone group. To study the effect on the post-initiation stage, SNE and SND were administered for 10 weeks after DMH injection. The SNE significantly declined number of colonic ACF in DMH-treated rats. It also significantly reduced number of proliferating cell nuclear antigen (PCNA) positive cells and increased number of apoptotic cells in colonic crypts of DMH-treated rats. In conclusion,*S. neglecta* modulated the early stages of DMH-induced colon carcinogenesis in rats via modulation of xenobiotic metabolizing enzymes and inhibition of cell proliferation as well as induction of apoptosis.

PW-113

Cytotoxic activity of some Libyan medicinal plants against human breast cell lines MCF-7 and colon cell lines HCT-116 *in vitro*.

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Breast cell carcinoma is a series public health in Libya with increasing incidence and mortality rates. The present study aimed to investigate the cytotoxic activities of crude ethanol extracts of a total of 4 plants used in Libyan folklore medicine against breast and colon carcinoma. Cytotoxic activities of the plant extracts against the cancerous cell lines compared with normal cell line were assessed using Sulfo-rhodamine-B assay method. The extracts from four plant species *(Capparis spinosa, Juniperus phoenicea, Ruta graveolens and Artemisia herba alba*) which are growing in Al-jabal Al-akhdar {The Green Mountain} in Libya exhibited promising activity against breast cell lines MCF-7 and colon cell lines HCT-116 *in vitro*. Measurement of the potential cytotoxic activity was concluded according to the calculated IC50 values [µg/ml]. The total alcohol extract obtained from*Juniperus phoenicea* showed the most potent effect against both breast cell lines IC_{50}=6.98 µg/ml. and colon cell lines IC_{50}=9.38 µg/ml. followed by that of *Artemisia herba-alba* IC_{50}=10.1 and 15.2 µg/ml., then that of *Ruta graveolens* IC_{50}=15.2 and 16.3 µg/ml. and finally the alcohol extract of*Capparis spinosea* IC_{50}=17.0 and 18.8 µg/ml. respectively.

PW-114

Potential role of acid sensing ion channels and 5-hydroxytryptamine receptors in a rat model of gastro-esophageal reflux disease

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An ongoing week acidic reflux is well established for patients of gastro-esophageal reflux disease (GERD) with persistent symptoms in spite of a treatment with proton-pump inhibitors (PPIs). The mechanisms of acid induced activation of esophageal afferent nerves are not well understood. We investigated in a rat model of GERD described earlier [1] the expression of acid sensing ion channels (ASIC) and of 5-hydroxytryptamin receptor (HTR) subtypes in esophageal tissue homogenates by whole genome microarray analysis. Tissues of animals suffering from GERD showed a small, but significant increase in the expression of the ASIC-subtype 4 (3.8f, $p<0.001$) and of HTR2A (3fold), HTR2B (6.6f), and HTR7 (9.3f) (<0.001) compared to "normal" tissue. Thus, both – ASICs and distinct 5-HTR subtypes – were up-regulated during the inflammatory process in our rat model. The higher expression of ASIC4 was not found in tissues of animals treated with either STW5 (2 ml/kg) or with the PPI omeprazole (O) (30 mg/kg). Minor differences, especially in the magnitude of down regulation, were seen for the 5-HTR subtypes after treatment with STW5 and O. Interestingly serotonin is not only the ligand for 5-HTR subtypes, but also an inflammatory mediator which

can activate ASICs in peripheral nerve tissue to activate a central pain response [2]. Thus results could explain pain sensations without ongoing acidic reflux via serotonin. We hypothesize that a down regulation of ASIC4 and 5-HTR-subtypes by the treatments plays a role for the fast pain relief in responders to PPIs and to STW5. Both receptor types form a communication network involved in pain signaling and are candidate targets likely to be important for a succesful pain treatment in GERD.

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PW-115

Anti-inflammatory and antioxidative constituents from *Trapa natants*

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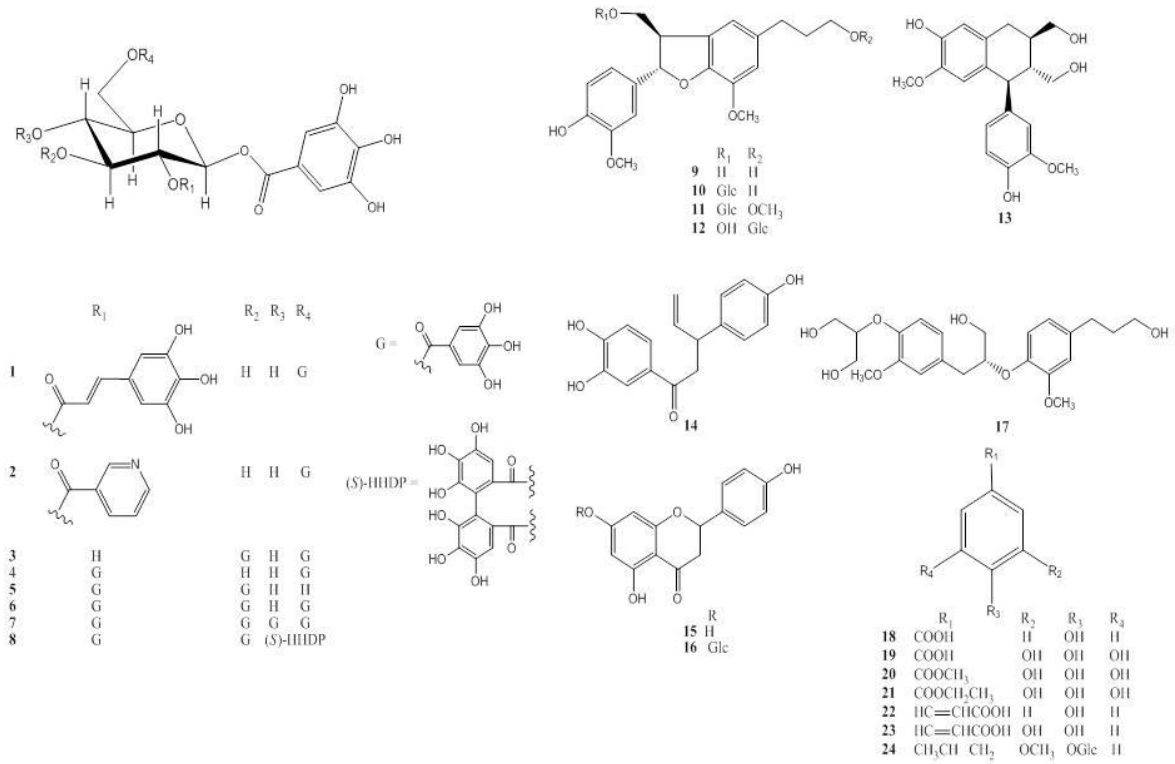
Trapa natans L., commonly known as water chestnut or water caltrop, is an aquatic floating plant native to Eurasia, Africa and Asia, grown as a food or medicinal plant in Taiwan, China and parts of South East Asia. There are advantages for utilizing the waste pericarps chestnut as sources of valuable products and decreasing the negative impact of these wastes. In the continuing phytochemical investigation on *T. natans* L., we further identified two new tannins (**1** and **2**), one new lignan (**14**), and one new phenolic compounds (**17**), together with six tannins (**3**-**8**), six lignans (**9**-**14**), two flavonoids (**15**-**16**), and seven phenolic compounds (**18**-**24**) from the EtOH extract of the fruit pericarps and the structural determination of these substances using extensive spectroscopic methods. Compounds **3**-**8** were screened for anti-inflammatory activity by their inhibition of LPS-induced nitric oxide production in RAW364.7 marcophages and further tested for their antioxidant scavenging effects on DPPH. Compounds **3** and **6** significantly decreased NO production in a dose-dependent manner and exhibited potent inhibition activities with IC_{50} of 11.91±0.19 and 17.10±0.94 ug/mL, respectively. In addition, **3**, **6**, and **8**exhibited significant antioxidant effects with ED_{50} values of 6.68±0.08, 4.97±0.24, and 4.42±0.06 ug/mL, respectively.



PW-116

Effects of Samul-tang on hepatic lipid accumulation and hyperlipidemia in a postmenopausal rat model

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Samul-tang has been used as a traditional Chinese remedy for the treatment of various diseases. In this study, we examined the phytoestrogenic effects of Samul-tang on breast carcinoma, lipid accumulation in methyl-β-cyclodextrin (MβCD)-induced HepG2 cells, and lipid-related disease in a rat model of postmenopausal hyperlipidemia. An E-screen assay was used on MCF-7 cells, to identify phytoestrogens, especially those with anti-estrogenic activity. Oil Red O staining and intracellular cholesterol analyses were used to quantify cellular cholesterol levels. A 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGCR) assay was used to measure enzyme activity. The levels of phosphorylated AMP-activated protein kinases (AMPK) and products of genes involved in cholesterol synthesis were measured via Western blotting. Thirty rats were either ovariectomized (OVX) or sham-operated (Sham) and then randomly assigned to four groups (n=5): Sham, OVX, or OVX with simvastatin, or OVX with Samul-tang (50, 150, 450 mg/kg) for 8 weeks. A number of targets associated with lipid-related diseases were examined to confirm the estrogenic effects of Samul-tang. Samul-tang showed anti-estrogenic activity in MCF-7 cells. Samul-tang decreased lipid accumulation, total cholesterol, and low-density/very-low-density lipoprotein levels in HepG2 cells. Moreover,

Samul-tang reversed the effects of MβCD on cholesterol synthesis regulators, and inhibited the activity of HMGCR. Phosphorylation of AMPK was stimulated by Samul-tang. In ovariectomized rats, Samul-tang reduced retroperitoneal and peri-renal fat accumulation, serum lipids, atherogenic index, cardiac risk factors, intima-media thickness, and nonalcoholic steatohepatitis scores. These results indicated that Samul-tang inhibits lipid accumulation without estrogenic activity in the breast. Therefore, Samul-tang may have potential as a therapeutic agent for the treatment of hyperlipidemia in postmenopausal females.

PW-117

Greek flora as a source for the detection of natural compounds potentially effective in preventing post-menopausal osteoporosis

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Osteoporosis is the most common metabolic bone disorder and remains an increasingly significant problem affecting post-menopausal women. It results from an imbalance between the processes of bone formation and resorption leading to decreased bone mass and increased risk of fracture. Treatments against osteoporosis include hormone replacement therapy (HRT) with estrogens, that unfortunately is combined to an increased hormone-dependant cancer risk, and selective estrogen receptor modulators (SERMs). Nowadays, numerous women use plant derived supplements to prevent postmenopausal diseases including osteoporosis. Despite Greece constituting one of the richest floral diversity regions, there are scarcely any reports connecting Greek flora herbs with prevention of osteoporosis. Therefore, the aim of this work is to identify and characterize plant extracts deriving from Greek flora, and isolated compounds that display significant SERM activity. Considering our research experience on natural products with estrogenic activities and traditional medicine sources, 44 plant species were selected and 91 extracts were prepared using conventional (maceration) and modern-green techniques (ASE, MAE and SFE). Preliminary phytochemical and biological screening revealed *Rhamnus species, Lupinus album, Psoralea bituminosa, Ceratonia siliqua* and *Cytisus villosus* between the most capable extracts of inducing MC3T3-E1 differentiation to osteoblasts. Consequently, we proceeded to the isolation of the major compounds, which were structurally elucidated using NMR spectroscopy, and in vitro evaluated using a cell–based screening, concerning the differentiation of MC3T3-E1 cells to osteoblasts and their estrogenic-antiestrogenic properties. The obtained results, revealed derivatives of genistein, kaempferol and its glycosides, as the most promising agents for the potential prevention and treating of post-menopausal osteoporosis.

PW-118

Area Under Curve (AUC) as a validating index for the hypoglyceamic effects of *Sida acuta* (Fam. Malvaceae) ethanolic leaf extract in experimental diabetes

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The heterogeneity of diabetes has placed a demand on the search for newer hypoglyceamics from medicinal plants and reports [1] on the anti-hyperglycemic effects of *Sida acuta* are suggestive. This work aims to evaluate the hypoglyceamic effect of *Sida acuta* ethanolic leaf extract in alloxan-induced diabetes and establish the predictability of AUC as a monitoring and validating index for hypoglyceamia. Experimental diabetes was induced in wistar rats of either sex with alloxan (150 mg/kg, i.p), screened 72 hours post-induction and treatments with extract and metformin for 21days were administered as follows: Group I (negative control); Group II (diabetic); Group III (diabetic + metformin 2.57 mg/kg, p.o); Group IV (diabetic + *S. acuta* 200 mg/kg, p.o); Group V (diabetic + *S. acuta* 400 mg/kg, p.o); Group VI (diabetic + metformin + *S. acuta* 200 mg/kg, p.o) and Group VII (diabetic + metformin + *S. acuta* 400 mg/kg, p.o). Blood glucose levels (BGLs) were measured at 0hr, 1hr, 3hr, 6hr, 24hr, 48hr, 72hr, 7days, 14 days and 21 days and the AUC estimated using the trapezium method [2]. Statistical analyses of ANOVA and student's paired t-test ($p<0.01$) were done using GraphPad Prism 5.0.

Statistical significant reduction in BGLs was seen in all treatment groups with *Sida acuta* showing a better hypoglyceamic activity at lower dose [3] than metformin and the drug-herb combinations. The percentage reduction in AUC trended similarly as the percentage reduction in BGLs, suggesting a perfect correlation. Thus, AUC can be used to monitor BGLs and validate the anti-diabetic potentials of *Sida acuta*.

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PW-119

In vitro antifungal activity and GC/MS profiling of ten selected Sudanese essential oils against *Madurella mycetomatis*, the causative agent of black-grain mycetoma

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Madurella mycetomatis is one of the most prevalent causative agents of black-grain eumycetoma. Infection caused by this fungus is notoriously difficult to treat due to absence of adequate therapy and frequently leads to surgical excision of the infected limb. Prolonged follow-up after surgery with currently available antifungals, however, might improve the clinical outcome [1]. Nevertheless, the increasing recurrence and resistance of these pathogenic organisms to existing antifungals warrant the development a reproducible in vitro assay for discovery of novel bioactive agents [2].

The new 96-well microplate's assay based on resazurin dye, which was developed by our group to assess the antifungal activity against *Madurella mycetomatis* [3] is presently applied to 10 essential oils. GC/MS analysis was carried out with an HP 5890 Series II gas chromatograph (FID) and the identification of the compounds was based on comparisons with published MS data and a computer library search.

In vitro, M. mycetomatis appears to be highly susceptible to essential oils, hence the MICs of the essential oils ranged between 0.625-2.5 mg/mL, while the standard antifungal, itraconazole exhibited MIC of 0.354 µM. More than 300 mono- and sesquiterpenes were identified and a correlation between the bioactivity and the identified compound has been hypothesised.

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PW-120

Retrospective treatment outcome analysis on the use of medicinal plants to alleviate diarrhoea in the Thaba Nchu area of the Free State Province, South Africa

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The aim of this study was to identify at least one medicinal plant commonly used by participants in the Thaba Nchu area, South Africa, to reduce/alleviate diarrhoea. Diarrhoea continues to be one of the leading causes of mortality and morbidity in developing countries, including South Africa, especially in children under 5 years [1]. It is estimated that 88% of diarrhoeal-related deaths are caused by inadequate sanitation and poor hygiene [2]. The major cause of intestinal infection is due to foodborne infections caused by Salmonella and *Campylobacter jejuini*, and waterborne infections due to contamination of the water supply with the cysts of *Giardia lamblia*, *Cryptosporidium parvum* and *Escherichia coli* [3]. The main cause of death is dehydration [4].

This investigation is a qualitative RTO based study with one-on-one interviews using a questionnaire for data collection. Each participant received an information leaflet and informed consent form in the language of choice (English, Afrikaans, Sesotho). Participants were recruited from the Thaba Nchu area in the Free State province, South Africa, and were 18 years and older, able and willing to provide written informed consent, a user of traditional medicine and had diarrhoea in the last three months.

Results showed that the traditional remedies are effective for treating diarrhoea and, since no adverse effects were reported, the remedies have low toxicity. *Xysmalobium undulatum* is indicated most frequently to treat diarrhoea in the Thaba 'Nchu area and thus merits further phytochemical and phytopharmacological investigation.

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PW-121

Inhibitory potential of 40 medicinal plant extracts from Madagascar against enzymes linked to type 2 diabetes

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Worldwide, type 2 diabetes affects 246 million people. Management of blood glucose by inhibition of carbohydrate-hydrolyzing enzymes is important to avoid diabetic complications. The aim of this study was to assess whether inhibition of α-glucosidase [1] and α-amylase [2] by ethanol extracts of 40 Madagascarian plants is the scientific rationale behind their traditional anti-diabetic use.

The ethanolic extracts were generally inactive against pancreatic α-amylase, and only bark extracts of *Psidium guajava* and*Vangueria madagascariensis* showed good activity with IC_{50} of 10.6 and 11.6 μg/mL respectively. Several extracts showed strong inhibition of yeast α-glucosidase. *Psidium guajava* bark and leaf extracts showed IC_{50} of 0.5 and 1.0 μg/mL, respectively. Bark extract of *Antidesma madagascariensis* showed IC_{50} of 1.7 μg/mL, whereas bark extract of *Vangueria madagascariensis* and leaf extract of *Rhizophora sp* showed an IC50 of 1.8 μg/mL.

All extracts with IC_{50} below 12.6 μg/mL were investigated for their content of tannins, known to give false-positive results in enzyme-based in vitro assays due to their non-specific enzyme binding. All active extracts had large amounts of tannins in the HPLC chromatogram, except aerial parts of *Euphorbia hirta* and leaves of *Artocarpus heterophylla*, *Ravenala madagascariensis*, and *Zanthoxylum tsihanimposa*, which contained only low levels of tannins. These four species were subjected to high-resolution α-glucosidase bioactivity profiling [2], in order to determine whether they contained specific enzyme inhibitors. It was, however, found that the α-glucosidase inhibitory profile correlated fully with the elution profile of the tannins.

In conclusion, the 40 plant species traditionally used on Madagascar to treat diabetes do not hold promise as specific inhibitors of the carbohydrate-hydrolyzing enzymes α-amylase and α-glucosidase.

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PW-122

Enhancement of glucose uptake by steroidal alkaloids from *Veratrum nigrum*

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Chemical investigation of the roots and rhizomes of *Veratrum nigrum* led to the isolation of five new steroidal alkaloids jervine-3-yl formate (**1**), veramarine-3-yl formate (**2**), jerv-5,11-diene-3β,13β-diol (**3**), (1β,3β,5β)-1,3-dihydroxyjervanin-12(13)-en-11-one (**4**), and veratramine-3-yl acetate (**5**). Compounds **1** and **5** exhibited potent inhibitory activity against protein tyrosine phosphatase 1B (PTP1B). Based on their PTP1B inhibitory activity, the compounds were evaluated for their potential to enhance glucose uptake in C2C12 skeletal muscle cells. The insulin-stimulated glucose uptake was enhanced upon treatment with compounds **1** and **5** (10 μM) by 49.9±6.5% and 56.0±9.7%, respectively. Our results suggest that steroidal alkaloids serve as practical anti-diabetes mellitus leads capable of enhancing glucose uptake.

PW-123

Investigation of α-amylase inhibitory activities of herbal extracts with a HPLC-based assay

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Inhibition of α-amylase, an enzyme that plays a key role in digestion of starch, is considered as a strategy in the treatment of type-II diabetes. Several herbal extracts and natural products with remarkable amylase-inhibiting activity have been identified so far. Although in the human medicine α-glucosidase inhibitors have greater importance, inhibition of pancreatic amylase is a potential target for antidiabetic drugs. Moreover, the widely applied α-glucosidase inhibitor acarbose exerts inhibitory activity on both enzymes, therefore the identification of α-amylase inhibitors may contribute the development of acarbose-type drugs as well.

Screening of plant extracts for α-amylase inhibitory activity is usually based on the application of spectrophotometric methods. The aim of our work was the development of a HPLC-based method which provides quick, specific and reliable determination of enzyme activity. For this purpose, a 2-chloro-4-nitrophenyl β-glycoside was synthesized and introduced as an a-amylase substrate [1].After incubating with the enzyme, the reaction mixture was analyzed by HPLC. Assessment of enzyme inhibitory activity was carried out based on the kinetic of formation of the major chromophor containing degradation product. HPLC analysis was done on a C18 column, using MeCN-H_2O as eluent and UV detection at 300 nm with a running time of 20 min.

This method was tested by the analysis of 10 plant extracts, some of which have reported to haveα-amylase inhibitory activities. Water extracts of 5 plants (bay leaves, cranberry, cinnamon, cloves, green tea) exhibited remarkable activities, with IC_{50} values in the range of 0.92-35.17 μg/ml. Results reassured that the our HPLC-based method is an appropriate tool for the quick assessment of specific α-amylase inhibitory activity of herbal extracts.

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PW-124

Phytochemical screening, total phenolic content and free radical scavenging activity of *Bruguiera sexangula* and *Connarus semidecandrus* extracts in Kung Krabaen Bay.

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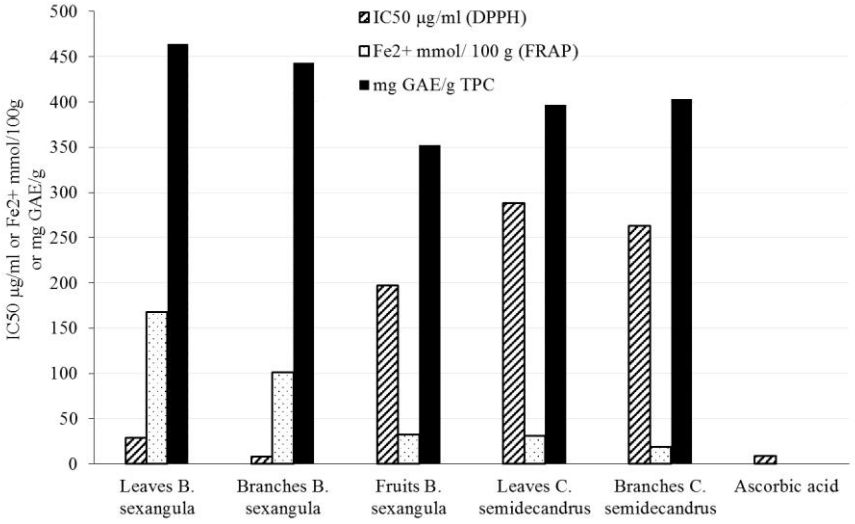
Phytochemical investigation of *Bruguiera sexangula* (Lour.) Poir and *Connarus semidecandrus* Jack, medicinal plants found in mangrove forest of Thailand, revealed the presence of tannin, terpenoids, saponins, flavonoids, alkaloids, and anthraquinones in various plant parts. The total phenolic content was determined by Folin-Ciocalteu assay, expressed in gallic acid equivalent (mg of GAE/g of extract). The free radical scavenging activity was estimated as IC_{50} values using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods. The result showed high phenolic content, varying from 350 to 465 mg GAE/g extract. The highest level of total phenolic content was found in the leaves (464.7±0.4 mg GAE/ g) followed by the branches (443.4±0.4 mg GAE/g) of *B. sexangula* extracts. The strongest free radical scavenging activity was exerted by extracts of branches of *B. sexangula* with IC_{50} of 8.62±0.37 µg/ml (DPPH) and 167.90±18.13 mmol Fe^{2+} equivalent/gram extract of leave using FRAP method. This study confirmed that medicinal plants with high phenolic content could possess strong free radical scavenging property thus indicated that the methanolic extracts of *B. sexangula* and *C. semidecandrus* could be significant sources of natural antioxidant.



- Figure 1. Free radical scavenging activity (▨ DPPH and ▩ FRAP assay) and total phenolic content (■) of the methanolic extract from different part of *B. sexangula* and *C. semidecandrus*.

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[2] Lima AA, Parial R, Das R and Das AK. Phytochemical and pharmacological studies of ethanolic extract from the leaf of mangrove plant *Phoenix paludosa* Roxb. Malay J Pharm Sci 2010; 8: 59–69.

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PW-125

Two new flavone glycosides from the aerial parts of *Platycodon grandiflorum* and their cytotoxic activity against human tumor cell lines

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Platycodon gradiflorum A. DC., which belongs to the family Campanulaceae, is a perennial herb and distributed in Korea, China and Japan [1]. The root of *P. grandiflorum*, a well-known traditional Chinese medicine, has been used for treatment of cough, bronchitis, sore throat and purulent disease [2]. It is widely cultivated in Korea, and has been used more often for food than for medicinal purposes since old times [2]. In the course of a search for bioactive compounds from natural products, we have found that the EtOH extract of *P. grandiflorum* has cytotoxic activity against human tumor cell lines (MCF-7, SK-OV-3 and Ishikawa) with IC_{50} below 73 μg/ml. Two new flavone glycosides (**1**, **2**) and six known flavonoids (**3**-**8**) were isolated from the EtOAc and *n*-BuOH fractions of the EtOH extract. The structures of two new compounds, named dorajiside A and B, were determined as luteolin 7-*O*-(6''-*O*-acetyl-ß-D-glycopyranoside), 3'-*O*-α-L-rhamnopyranoside (**1**) and apigenin 7-*O*-(6''-*O*-acetyl-ß-D-glucopyranoside), 4'-*O*-α-L-rhamnopyranoside (**2**) from spectral data and chemical evidence. The known compounds, luteolin 7-*O*-ß-D-glucopyranoside, 3'-*O*-α-L-rhamnopyranoside (**3**), apigenin 7-*O*-ß-D-glycopyranoside, 4'-*O*-α-L-rhamnopyranoside (**4**), luteolin 7-*O*-(6''-O-acetyl)-ß-D-glucopyranoside (**5**), luteolin 7-*O*-ß-D-glucopyranoside (**6**), apigenin 7-*O*-ß-D-glucopyranoside (**7**), and luteolin (**8**) were identified by comparing their spectral data with literature values. Cytotoxicity of isolated compounds were evaluated by using MTT assay against human tumor cell lines (MCF-7, SK-OV-3 and Ishikawa), and most of compounds revealed moderate activity at concentrations below 50 μg/ml.

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PW-126

Anti-allergic and anti-inflammatory potential of *Typhonium blumei* and *Typhonium roxburghii*: inhibition of degranulation via calcium influx modulation

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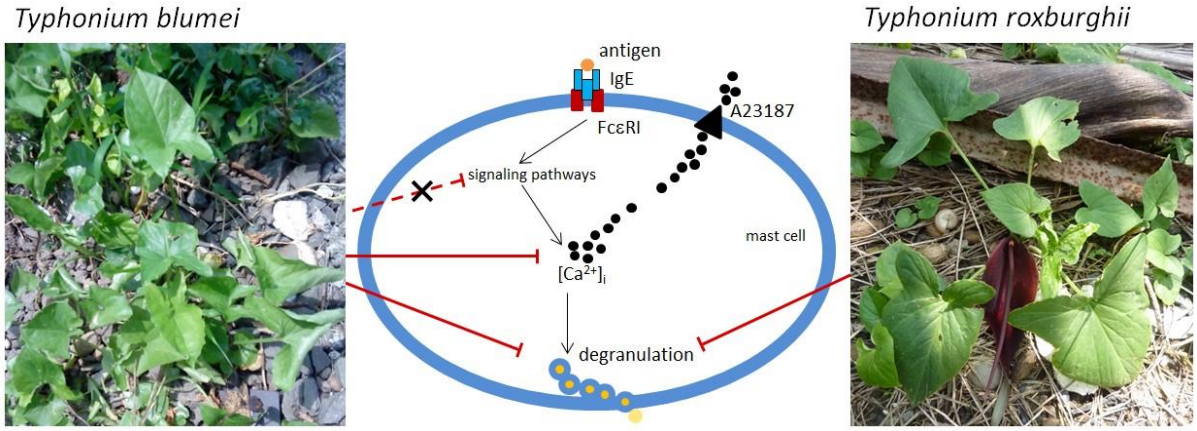
Typhonium blumei Nicolson & Sivadasan (Araceae), a traditional Chinese medicinal herb, is used in Taiwan as a folk medicine to treat cancer and inflammatory diseases. *Typhonium blumei* is usually not distinguished from *Typhonium roxburghii* Schott and they are commonly used interchangeably.

We evaluated and compared the anti-allergic and anti-inflammatory properties of *T. blumei* and *T. roxburghii* in relation to their composition profiles.

The methanolic extracts of leaves, rhizomes or whole plants were partitioned with different solvents to obtain the nonpolar fractions. The anti-allergic activity of the nonpolar fractions was assessed by A23187 and antigen-induced degranulation assays using RBL-2H3 mast cells. The effect of these fractions on the anti-allergic molecular targets including FcεRI receptor expression, calcium influx, cytokines mRNA expression and protein expression was investigated. The anti-inflammatory activity was evaluated using superoxide anion and elastase release assays in human neutrophils. The results revealed that both species possess potent anti-allergic and anti-inflammatory activities. The inhibition of degranulation in mast cells was attributed to calcium influx modulation. The active fractions were rich in fatty acids, as revealed by NMR analysis. Palmitic, linoleic and α-linolenic acids were identified as the major fatty acids in both plants by GC-MS.

The obtained results support the traditional use of *T. blumei* in the treatment of inflammatory diseases as well as its substitution with *T. roxburghii.*



PW-127

Chemopreventive potential of the pentane-based fraction of wild carrot oil against chemically-induced squamous cell carcinoma

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Daucus carota L. ssp. *carota* (wild carrot) was recently shown to exhibit *in vitro* and *in vivo* anticancer activities [1,2]. DCOE was chromatographed to yield F1 (pentane; 100%), F2 (pentane-diethyl ether; 50:50), F3 (diethyl ether; 100%) and F4 (chloroform-methanol; 93:7) fractions. The fractions were tested in vitro against several cancer cell lines and F1 was found to possess the highest activity.2 Therefore, the present study aimed to evaluate its activity using the DMBA/TPA skin carcinogenesis model in mice. Skin papilloma were initiated by DMBA and promoted by TPA. F2 was administered to three experimental groups (10 mg/kg, 50 mg/kg, 200 mg/kg) via intraperitoneal injections thirty min prior to TPA promotion for a period of 21 weeks. Papilloma incidence, yield, and volume were compared with those of a non-treated control group. Treatment with F2 caused an inhibition in papilloma incidence, being highest (50%) at a dose of 200 mg/kg at the end of the experiment (week 21). Also, there was a dose-dependent decrease in papilloma yield during the study period. Additionally, F2 treatment with the three doses significantly decreased the papilloma volume at weeks 15, 18 and 21 ($p<0.05$, $p<0.01$ and $p<0.01$, respectively). In conclusion, these findings clearly demonstrate that F2 has a remarkable antitumor activity against DMBA-TPA induced skin cancer and suggest the need for further studies to isolate the active chemotherapeutic agent.

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PW-128

Mucilage of *Cordia dichotoma* seeds pulp: Isolation, purification and a new hypolipidemic agent in normal and hyperlipidemic rats

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The *Cordia dichotoma* mucilage (21 g/kg) was of brown-yellowish color, soluble in water and forms viscous solution. Various parameters such as determination for carbohydrates, protein, monosugars, alkaloids, phenolic compounds amino acid and other properties, swelling index and viscosity were evaluated for characterizing the mucilage. It has a good swelling index of 76.35%. The mucilage was examined for purity by performing different phytochemical tests and showed that carbohydrates, alkaloids and amino acids were found to be present. Mucilage of *C. dichotoma* seeds pulp (CDM) was studied as a hypolipidemic agent on blood lipid status and oxidant stress in liver. Male albino Wistar rats were fed standard diet and others fed on high-fat diet for 10 weeks. Feeding on high-fat diet caused significant increase in serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) as well as significant decrease on high density lipoprotein cholesterol (HDL-C). Treatment with CDM by two doses; 0.50 and 1.00 g/kg normalized the lipid profile. Liver and kidney function were improved in normal and hyperlipidemic rats by CDM. Antioxidants enzymes, glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) were decreased significantly by feeding on high-fat diet. CDM with two doses increased those significantly. Pathological study showed that feeding on high-fat diet caused fatty changes on liver and kidney of hyperlipidemic rats. These fatty changes were improved significantly by CDM. Administration CDM for normal rats caused improvements in all obvious parameters.

PW-129

Inhibitory effect of ginsenoside on UVB-induced melanocyte proliferation

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UVB-exposed keratinocytes secrete various paracrine factors which are known to play an important role in differentiation and proliferation of melanocytes. Among these factors, granulocyte-macrophage colony-stimulating factor (GM-CSF) and basic fibroblast growth factor (bFGF) are known to stimulate the proliferation of melanocytes. In this study, we are trying to find the inhibitory mechanism of saponin from Korean red ginseng (SKRG) on UVB-induced GM-CSF expression in keratinocytes and bFGF-induced proliferation of melanocytes. It was reported that expression of GM-CSF is regulated by epidermal growth factor receptor (EGFR) and protein kinase C (PKC) pathway. We found that treatment of SKRG decreased the phosphorylation of EGFR and ERK in UVB-irradiated keratinocytes and both ginsenoside Rc and Rh3, components of SKRG, decreased the phosphorylation of PKC. Previous studies verified that bFGF binds to the FGFR, and this leads to activation of the MAPK pathway. We identified bFGF increased melanocytes proliferation through ERK phosphorylation.

Moreover, We observed that co-treatment of ginsenoside Rh3 and F1 strongly inhibited bFGF-induced ERK phosphorylation in melanocytes. Taken together, we found that inhibition of GM-CSF expression was derived from decreased phosphorylation of EGFR and PKC by SKRG and ginsenoside Rc and Rh3 could be major compounds in this inhibitory mechanism. We also found that ginsenoside Rh3 and F1 could inhibit synergistically the bFGF-induced melanocytes proliferation by downregulating EKR phosphorylation.

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PW-130

Phytochemical and biological activity studies on *Enterolobium contortisiliquum* pods

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Enterolobium contortisiliquum (Vell.) Morong is a tree belonging to family Leguminosae. Nevertheless, it is widely spread in Egypt; the chemical composition was not yet investigated. GC-MS analysis of unsaponifiable matter of *E. contortisiliquum* revealed that α- and β-amyrin and 4-methyl 2,6-di-tert-butylphenol to be its main components, while palmitic and 9,12-octadecadienoic acids were the major fatty acids. Nine sugar components were identified in hydrolysate of mucilage with glucose (34.89%), xylose(6.78%) and rhamnose (5.98%) being the predominant sugars by GLC. Fourteen amino acids have been identified in protein fraction. The phenolic fraction was chromatographed over polyamide to yield gallic acid, protocatechuic acid, quercetin-7-rutinoside, catechin, isovitexin and quercetin, which were characterized by the comparison of their physical and spectral data with those in the literature. Further, HPLC analysis of the phenolic fraction revealed the presence of pyrogallol, syringic and p-coumaric acids.

The crude extract (70% alcohol) and the saponin fraction exhibited potent cytotoxic activity on HepG2 (IC_{50} 14 and 29 μg/ml) and MCF7 (IC_{50} 16 & 31 μg/ml) cell lines. The mucilage and petroleum ether fractions showed cytotoxicity activity on HepG2 with (IC_{50} 19 & 61 μg/ml), while phenolic fraction showed cytotoxicity towards MCF7 cells with IC_{50} value of 79 μg/ml.

The antibacterial activity of different fractions were evaluated against seven Gram-positive and six Gram-negative microorganisms using agar well diffusion assay method. Maximum inhibition was observed with compounds at 1 mg/ml; catechin and protocatechuic acid against

Pseudomonas aeruginosa (-ve) (14.5 and 17 mm, respectively) while, the crude and petroleum ether extracts showed antimicrobial activity against *Micrococcus luteus* (+ve) (inhibition zone 12 and 10 mm, respectively). Whereas, polysaccharide and protein exhibited antimicrobial activity against *Klebsiella pneumonia* (-ve) (16 and 13 mm, respectively).

PW-131

Antibacterial and anti-inflammatory activities of poly-herbal mouthwash formulated using *Breynia nivosa, Moringa oleifera, Azadirachta indica*, and *Psidum guajava* stems.

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Breynia nivosa, *Moringa oleifera*, *Azadirachta indica*, and *Psidum guajava* stems are used as chewing stick in different part of Nigeria and have significant protection against tooth diseases. Methanol extract of each plant was evaluated for phytochemical content. Methanol extracts of the plants were used in combination to prepare three batches of mouthwash formulation using x2, x4 and x8 MIC of their crude extract. The antibacterial potential of the herbal mouthwash formulation and the crude extracts of the plants in oral hygiene was assessed *in vitro* using the standard method for oral antiseptic. A standard mouthwash (Minty Brett) served as control. The mouthwash formulation was screened for antibacterial activity against culture of *Streptococcus mutans* obtained from extracted decayed tooth and extinction time of each concentration was determined. Antiinflammatory activity of the three formulations was evaluated using xylem induced ear inflammation in albino rat, and standard topical anti-inflammatory preparation, diclofenac cream (Voltaren) and standard mouthwash (Minty Brett) as control. The stability of three formulations was monitored over six months. Tannins, saponins, alkaloids and glycosides were present in all the plants. The three batches of the mouthwash showed a significant activity against *S. mutans* as compared to the standard marketed mouthwash. The MIC (*Breynia nivosa* 8.75 mg/ml, *Moringa oleifera* 17.5 mg/ml, *Azadirachta indica*50 mg/ml, and *Psidum guajava* 12.5 mg/ml, mouthwash formulation 11.3 mg/ml). The extinction time of the three batches obtained were as follows 25, 18 and 15 min respectively, for batch 1, 2 and 3. The anti-inflammatory activity was significantly higher than the standard diclofenac cream (voltarene cream) and mouthwash (Minty Brett). The three formulations were stable over six months. The formulated mouthwash is good as it meets the minimum requirements and could be scaled up for commercial mouthwash.

PW-132

Curcumin sensitizes human U-87 glioblastoma and MCF-7 breast cancer cells to the endocannabinoid reuptake inhibitor OMDM-2

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Δ9-Tetrahydrocannabinol (THC), active constituent of *Cannabis sativa* L., possesses potential antitumor activity. Modulating endogenous levels of cannabinoids is a new strategy to avoid clinical and ethical considerations which limit the use of direct agonists. As dietary interventions may improve the effectiveness of cancer chemotherapy, we investigated the combinatory effect of the endocannabinoid reuptake inhibitor, OMDM-2, and the natural product curcumin, derived from*Curcuma longa* (L.), on cytotoxicity.

The *in vitro* antiproliferative activities of OMDM-2 alone or in combination with curcumin were evaluated against both, breast cancer (MCF-7) and glioma (U-87) cells, using resazurin assay. The effect of curcumin on OMDM-2 chemosensitivity was determined by comparing IC50-values of OMDM-2 in absence and presence of curcumin. The additive, synergistic or antagonistic activity of the combination was evaluated by combination index (CI) and isobologram analyses.

OMDM-2 by itself showed antiproliferative effects against both MCF-7 and glioma with IC_{50} of 4.9 and 2.7 μM, respectively. Co-exposure to curcumin increased the sensitivity of both cell lines to OMDM-2 in a dose dependent manner. The exposure to the ratio 1:8 of OMDM-2/curcumin decreased the IC_{50} of curcumin to 1.8 in both cell lines. Isobole and CI analyses at different IC levels revealed that drug interaction was predominantly synergistic against MCF-7 cells. While in case of glioma cells, this combination could be synergistic or antagonistic depending on the ratio and concentration of drugs.

These findings provide experimental support for the use of curcumin as a modulator of tumor cell chemosensitivity in cannabinoid based therapies.

PW-133

Protective effects of *Punica granatum* peels on cisplatin-induced oxidative stress and nephrotoxicity in rats

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Cisplatin (cis-dichlorodiammineplatinum (II)) is a synthetic anticancer drug extensively used clinically for the treatment several human malignancies. Cisplatin-induced nephrotoxicity results in the depletion of renal antioxidant defense system. In this study, we investigated the protective effect of *Punica granatum* L. (Punicaceae) peels extract against cisplatin-induced renal injury in rats.

Twenty four rats were divided into four different groups. Group 1 was used as control; groups 3 and 4 were orally treated with the extract (200 and 400 mg/kg bw, respectively) for 15 consecutive days. Groups 2, 3 and 4 received a single intraperitoneal dose of cisplatin (7.5

mg/kg bw), on the 10th day of the experiment. Cisplatin caused marked renal damage, characterized by a significant ($P<0.05$) increase in serum creatinine (2.51±6.34), blood urea nitrogen (78.12±11.66) and MDA (95.55±3.61), comparing with the control values. Treatment with the plant extract (200 and 400 mg/kg) significantly decrease serum creatinine, blood urea nitrogen and MDA, in dose-dependent manner in cisplatin-nephrotoxicity rats. Also,*P. granatum* extract significantly increased the activities of SOD (19.42±3.56), U/mg protein), GSH (14.38±0.69 mol./mg tissue) and CAT (22.00±7.61 mol./mg tissue) compared with a cisplatin-treatment. Cisplatin treatment caused prominent morphological alterations, including renal tubules with protein casts, swelling, vacuolization and proximal tubule necrosis. The treatment with *P. granatum* reduced the lesion induced by cisplatin. The expression of TNF-α, COX-2, iNOS and caspase-3 was markedly suppressed by *P. granatum* indicating the inhibition of inflammatory response. The present study demonstrates that *P. granatum* has a protective effect on cisplatin induced experimental nephrotoxicity and is attributed to its potent antioxidant. The findings in the present study suggest that *P. granatum* can be used as a combinatorial regimen in cancer chemotherapy.

PW-134

Multi-step evaluation of data on the multi-target effect of STW 5

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A multitude of concomitant causes and likewise also targets for therapeutic interventions have been identified in functional gastrointestinal diseases (FGDs) [1]. Therefore, a multi-target approach is a promising therapeutic strategy, as is exemplified by the herbal combination medicinal product STW 5, which has been proven to be effective in a large number of clinical studies [2]

All data from about 100 studies including STW 5 alone, or STW 5 and its components, were retrieved and studies, study methods and numeric results transferred into a data base. In a second step, study results were sorted according to types of study models and respective etiologic mechanisms related to FGDs and visualized by means of 2D histograms.

The evaluation of the data shows that STW 5 is active in response to multiple etiologic factors involved in functional dyspepsia and irritable bowel syndrome, like hyper- and hypomotility, acidity, inflammation and hypersensitivity, but also in inflammatory gastrointestinal diseases. The clustering by multiple steps allows the conclusion that all components are, with a respective specific profile of activities, involved in these actions, with the 2D histograms allowing an overview of the highly complex body of data within one figure.

Multi step clustering allows the transformation of complex data sets necessary to show the multi-target action of medicines, especially those with a complex composition, like STW 5, so also giving support to its clinical use in patients with different symptoms.

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[2] Ottillinger et al. 2013, WMW 163:65;

PW-135

The *in vivo* and *in vitro* diabetic wound healing effects of two wild plants growing in Kazakhstan and its mechanisms of action

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Diabetic complications, such as foot ulcer is a global health problem. Therefore, much attention has been paid to find biological active substances from plants that help to solve this problem. In our previous studies were shown antimicrobial and antioxidant properties of two plants growing in Kazakhstan (*Vexibia alopecuroides* (L.) Jakovl. and *Salvia deserta*Shang). In this study they were examined for the ulcer healing effect in vivo, and its potential mechanisms of action in vitro.

A streptozotocin induced diabetic foot ulcer rat model was used for studying the wound healing effect. The proliferative effects of crude extracts on MDCK cells were evaluated by the MTT assay. The migration of MDCK cells was examined using the scratch wound healing assay.

Our *in vivo* results demonstrated a significant reduction of wound area at day 9 in both treated groups as compared to control group ($p<0.05$). But only methylene chloride extract obtained from *Vexibia alopecuroides* could significantly stimulate proliferation in a dose dependent manner ($p<0.05$). Besides, this extract could significantly increase the cell migration ($p<0.01$). However, due to the complexity of a wound healing process *in vivo*, the methylene chloride extract obtained from *Salvia deserta* may still influence the healing through other pathways.

Our study presents for the first time scientific evidence towards the efficacy of the two plants growing in Kazakhstan in enhancing diabetic wound healing.

PW-136

Anti-diabetic activity of phlorotannin from *Eisenia bicyclis* in Zebrafish, a model of type 1 and 2 diabetes

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Eisenia bicyclis (Kjellman) Setchell is an edible perennial brown alga in the family Lessoniaceae and is widely distributed in the coastal area of Ulleung and Jeju islands in Korea, and in Japan [1,2]. Previous studies on *E. bicyclis*, have investigated several biological

activities including anti-inflammation, antioxidative, and neuroprotective effects. In continuation of our search for anti-diabetic compound from natural products, we have found that the EtOH extract of *E. bicyclis* has antihyperglycemic activity in the zebrafish model for type 1 and 2 diabetes. Type 1 diabetes zebrafish model was induced by alloxan, which cause pancreatic β-cell necrosis [3]. In addition, type 2 diabetes zebrafish model was induced by insulin. Exposure to excess insulin can induce insulin resistance typical of type 2 diabetes [4]. Following alloxan or insulin treatment, pancreatic islet size and fluorescence intensity were measured. The EtOH extract was consecutively partitioned with CH_2Cl_2, EtOAc and *n*-BuOH to give four fractions. Among these fractions, the EtOAc fraction which showed antihyperglycemic activity was subjected to activity-guided fractionation and isolation. Three phlorotannins, eckol, dieckol and phlorofucofuroeckol-A, were isolated from the EtOAc fraction. The isolated compounds revealed anti-diabetic activity for type1 and 2 in the zebrafish model.

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[2] Kwon T-H et al. Antioxidant activity of various solvent fractions from Edible brown alga, Eisenia bicyclis and Its Active compounds. J. Food Sci. 2013; 78: C679-684

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[4] Yang X et al. Exposure to excess insulin (glargine) induces type 2 diabetes mellitus in mice fed on a chow diet. Journal of Endocrinology, 2014, 221(3), 469-480.

PW-137

Total phenolics, antioxidant, antidiabetic, anti-gout and anti-cholinesterase effect of ethyl acetate extracts from bitter gourd (*Momordica charantia)*

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In the present study, antioxidant potential, α-amylase, α-glucosidase, cholinesterase, xanthine oxidase, and tyrosinase inhibitory activity of peel, pulp and seed extracts of bitter gourd (*Momordica charantia* L.) were investigated. The contents of total phenolics and total flavonoids ranged between 502.2 and 768.4 mg gallic acid equivalent (GAE)/L and 158.8 and 221.2 mg rutin equivalent/L, respectively. The antioxidant activity of each extracts was evaluated by employing different *in vitro* assays such as reducing power assay, DPPH•, ABTS•+ and •OH radical scavenging capacities, peroxidation inhibition activity, and antihemolytic assay. In addition, the α-amylase inhibition was found 90.7% (peel), 78.2% (pulp), and 82.5% (seed); whereas α-glucosidase inhibition was 85.5% (peel), 77.6% (pulp), and 78.2% (seed) under *in vitro* starch digestion bioassay. Also, all extracts (peel, pulp and seed) exhibited significant xanthine oxidase (68.6-82.1%), tyrosinase (72.5%–80.9%), acetylcholinesterase (68.1%–75.4 %) and β-glucuronidase inhibitory activity (58.1%–65.3%). These results indicated that peel; pulp and seed extracts of bitter gourd (*Momordica charantia* L.) could be used as functional food and nutraceuticals as good source of antioxidant, antigout, and antidibetic agents.

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PW-138

An investigation into consumed South African plant species as potential hERG channel blockers

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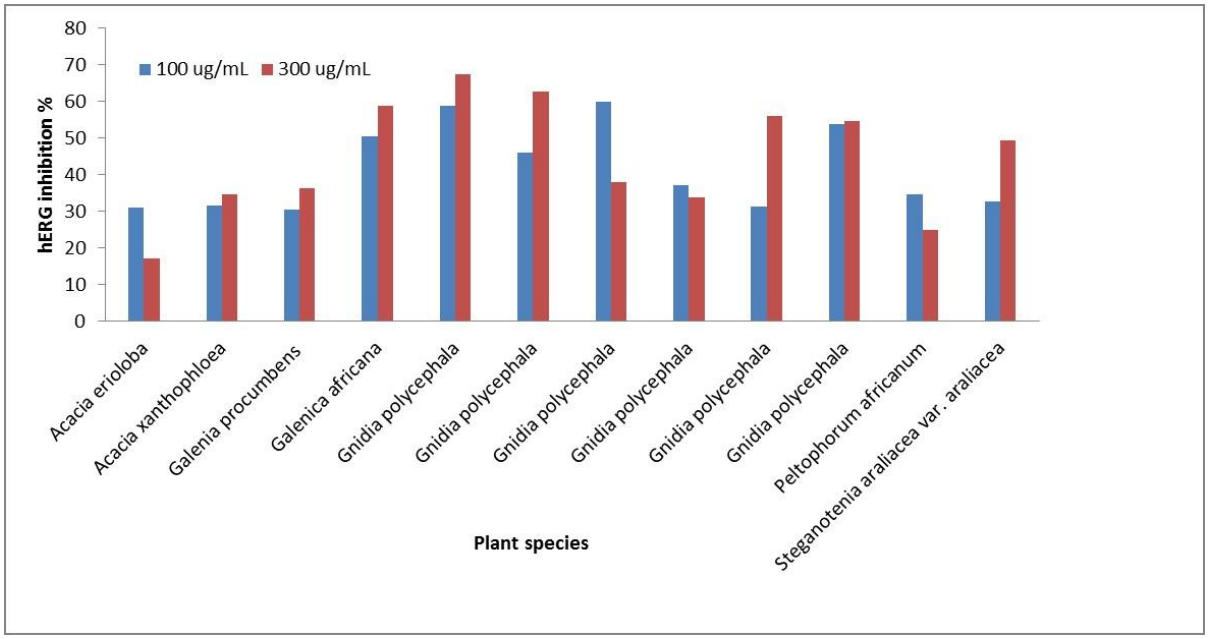
hERG (human Ether-à-go-go Related Gene) is a gene that encodes the pore-forming α-subunit of a voltage-gated potassium K^+ channel expressed in the heart and in nervous tissue [1]. It is responsible for channels mediating the 'rapid' delayed rectifier K^+ current (IKI) which plays a critical role in ventricular repolarisation phase of the cardiac action potential [2].

Mutations in hERG can lead to loss of function, prolong the ventricular action potential and may cause an inherited cardiac arrhythmia, long QT syndrome (LQTS) which is associated with torsade de pointes (TdP), a ventricular arrhythmias that can degenerate into ventricular fibrillation, leading to sudden death [3].

129 South African botanicals, distributed in 48 families were investigated for their potential to block hERG K^+ channels which afforded 350 plant extracts of both DCM and MeOH. Plant extracts which reduced the peak tail current hERG by ≥30% were considered as positive hERG channel blockers. Ten extracts showed inhibitions between 30-60%. The highest inhibition percentage was shown by *Gnidia polycephala* species.



We have demonstrated that a virtual screening approach (two-microelectrode voltage clamp) was successful in identifying novel hERG blockers. This experimentally validated model represents a valuable predictive tool in the assessment of potentially cardiotoxic consumed natural botanicals.

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PW-139

Treatment with the EtOH extract of B.H. stimulated glucose transporter and inhibited Protein-tyrosine phosphatase 1B (PTP1B) expression in muscle of streptozotocin-diabetic mice

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Diabetes mellitus is one of most common metabolic disorders in the world [1] and is characterized by hyperglycaemia resulting from defects on insulin action, secretion or both [2]. Genus *Bauhinia* (B.H. is a special extract to be patented of a *Bauhinia* species) is used in folk medicine to treat many diseases like inflammation, infections and diabetes [3]. Previous studies from our laboratory showed that the treatment with the EtOH extract of B.H. for 14 days at a dose of 400 mg/kg was able to decrease fasting glycaemia, modulating processes as gluconeogenesis, glycogenolisis and glycogenesis. The aim of this study was to verify others mechanism of action for the observed hypoglycaemic effect of the extract of B.H. Male Swiss mice (90 days old, 40 g) were divided in 4 groups: CTLSAL (normal mice treated with saline), CTLEXT (normal mice treated with the extract of B. H, 14 days, 400 mg/kg.day), STZSAL (diabetic mice treated with saline) and STZEXT (diabetic mice treated with the extract of B.H, 14 days, 400 mg/kg.day). The chemical screening showed glycosides of flavonols, as quercitrin and guajavarin in addition to other flavonoids, such as myricitrin. Our results showed that the treatment with the EtOH extract of B.H. increased the AKT, PI3K and GLUT4 expression in muscle leading to an increase in glucose transport and decreased PTP1B expression, an important down regulator of insulin receptor. Furthermore, the treatment also increased the AMPK expression, which probably contributes for stimulation of lipids oxidation and glucose intake.

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PW-140

Asiaticoside reduces TNF-α-induced increased soluble platelet endothelial cell adhesion molecule -1 (sPECAM-1) levels in human aortic endothelial cells

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In early atherogenesis, increased expression of surface cellular adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and platelet endothelial cell adhesion molecule-

1 (PECAM-1) facilitates adhesion of monocytes to the endothelium and subsequent monocyte infiltration into the intima. Therefore, the soluble form of cellular adhesion molecules in plasma serves as important biomarker to predict cardiovascular risk factors. Asiaticoside is an active compound derived from *Centella asiatica* (L.) and has been reported to possess anti-inflammatory and anti-pyretic effects [1]. The mechanism underlying anti-atherogenic effect of asiaticoside remains unknown. This *in vitro* study aimed to evaluate the effect of asiaticoside on TNF-α-induced increased soluble VCAM-1 (sVCAM-1) and soluble (sPECAM-1) levels as well as increased monocyte adhesion and migration in human aortic endothelial cells (HAECs). HAECs were pretreated with asiaticoside (6.25-50μM) for 30 min followed by TNF-α stimulation (10 ng/mL) for 6 h. The levels of sVCAM-1 and sPECAM-1 were measured by using FlowCytomix multiplex kit purchased from eBioscience. Monocyte adhesion and migration assays were assessed in static condition by co-incubating HAECs with fluorescently labeled monocytes. It was shown that asiaticoside at the doses of 6.25, 12.5 and 25μM significantly reduced TNF-α-induced increased sPECAM-1 level by 62.3%, 92.3% and 61.3%, respectively (P<0.05). However, all tested doses of asiaticoside failed to suppress the increased sVCAM-1 level, increased monocyte adhesion and increased monocyte migration augmented by TNF-α. Simvasatin (2 μM) was used as a positive control. Simvastatin inhibited the sPECAM-1 level by 91.4% and the sVCAM-1 level by 60.2% (P<0.05) but it failed to inhibit monocyte adhesion and migration elicited by TNF-α.

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PW-141

Effect of chronic intake of *Salvia libanotica* infusion on glycemia and lipemia in rats fed a high fat diet.

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Salvia libanotica is a commonly used herb in folk medicine in Lebanon and the Middle East. The present study aimed to assess the scientific basis of the use of *S. libanotica* aqueous extract in glycemia and lipemia by adopting a protocol simulating its human consumption. Animals were fed a high-fat diet and allocated into a control and three experimental groups receiving 3 doses of the plant extract 50, 150 and 450 mg/kg body weight respectively for six weeks. Results showed that the intake of *S. libanotica* extract was associated with a significant decrease in normal fasting serum glucose (GII and GIII), an increase in fasting serum insulin (GIII) and liver glycogen content (GII and GIII). Additionally, the plant extract intake produced a significant improvement in serum HDL and HDL/LDL cholesterol ratio. The freeze dried aqueous extract was partitioned between methanol soluble and methanol insoluble fractions almost equally by mass. Surprisingly, none of the obtained fractions produced a significant effect on either lipemia or glycemia. In conclusion, the current study is the first to demonstrate the benefits of the chronic intake of *S. libanotica* infusion on lipemia and glycemia and supports its traditional use as a remedy for the prevention of type 2 diabetes. The results also suggest that the efficacy is confined to the whole extract and not fractions.

PW-142

Adenosine receptors in inflammation on rat colon preparations

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The purine nucleoside adenosine, which is involved in several physiological functions, regulates a variety of immune and inflammatory responses and acts as modulator of gut functions. Although it is present at low concentrations in the extracellular space, stressful conditions, such as inflammation can markedly increase its extracellular level up to micromolar range. Recent evidence suggests a prominent role of A2A receptors (A2AR) in the anti-inflammatory effect of herbal preparations as e.g. in STW 5 (Iberogast®). In the current study we investigated the role of A2AR and A2BR to regulate contractility in untreated and inflamed rat colon preparations using specific receptor agonists and antagonists. Inflammation was induced by intraluminal instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS, 0.01/0.1 M, 30 min). mRNA-expression was determined using RT-PCR. Contractions were measured isometrically in an organ bath setup. All four adenosine receptor subtypes were expressed in colon preparations. Activation of A1, A2B, and A3 receptor with specific agonists reduced the acetylcholine (ACh, 10 µM) contractions, while activation of A2BR enhanced it. After incubation with TNBS morphological damages in colonic mucosa and muscle walls were detectable followed by reduced ACh-contractions. The TNBS-mediated decrease of ACh-contractions as well as the morphological damages were partially normalized by co-incubation of TNBS with the A2AR agonist 2-p-[carboxyethyl]phenethylamino-5'-*N*-ethylcarboxamido-adenosine (CGS 21680, 10µM) or the A2BR antagonist 4-(2,3,6,7-tetrahydro-2,6-dioxo-1-propyl-1H-purin-8-yl-benzenesulfonic acid (PSB 1115, 100 µM). Using an *in vitro* inflammatory model, we demonstrate that the A2AR agonist CGS 21680 or the A2BR antagonist PSB 1115 effectively conteracted the development of TNBS-induced disturbances in colon preparations. This study therefore opens new options for uncovering anti-inflammatory mechanisms of action of herbal medicinal products.

Natural products in CNS-related diseases

PW-143

Herbal remedies for neuropathic pain in Traditional Persian Medicine

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Nerve-originated Pain was a known concept in traditional Persian medicine (TPM) [1]. Different therapeutic strategies were used for management of nerve-originated pain in TPM.

The goal of this study was to review the herbal remedies used for nerve originated pain in TPM as potential options for current researchs on new drugs for neuropathic pain.

In this study, we reviewed TPM literature including Avicenna's *Canon of Medicine* regarding this topic, to find herbs suggested for theraputic use in nerve originated pain.

Topical oil-based ointments' of herbs with hot temperaments such as pepper, chamomile, lavender, thyme, bitter apple and flax were used for nerve-originated pain. Among these herbs *Capsicum annuum* and *Citrullus colocynthis* have been investigated in clinical studies with promising results [2-3]. Others are only investigated in pre-clinical studies. Herbal remedies used in the treatment of nerve-originated pain and potential mechanisms for their effects in-light-of current medical literature are summarized in Table 1.

These herbs can be more investigated as potential agents in the field of drug discovery in neuropathic pain.

Table 1. Medicinal plants mentioned in the traditional Persian medical books for managing the nerve originated pain.

COMMON NAME	TRADITIONAL NAME	SCIENTIFIC NAME	ROUTE OF ADMINISTRATION	PHARMACOLOGIC EFFECT
Pepper	*Felfel*	*Capsicum annuum* L.	Oral, Topical (Oil based formulation)	Antinociceptive, Antiinflammatory, Antioxidant, Neuroprotective
Colocynth	*Hanzal*	*Citrullus colocynthis* (L.) Schrad.	Topical (Oil based formulation)	Analgesic, Antiinflammatory, Antioxidant
Olive	*Zeyton*	*Olea europaea* L.	Topical (Olive oil)	Anti-inflammatory, Antioxidant, Neuroprotective
Castor	*Karchak*	*Ricinus communis* L.	Topical (Castor oil)	Anti-inflammatory, Antioxidant
Flax	*Katan*	*Linum usitatissimum* L.	Topical (Flax oil)	Analgesic, Antioxidant, Neuroprotective
lavender	*Iranian Ostokhudus*	*Nepeta menthoides* Boiss. & Buhse	Oral (Syrup)	Neuroprotective
Shirazi Thyme	*Sa'atar*	*Zataria multiflora* Boiss.	Oral (decoction)	Analgesic, Antiinflammatory
Garlic	*Sir*	*Allium sativum* L.	Oral, Topical (Oil based formulation)	Analgesic, Antioxidant
Citron	*Balang*	*Citrus medica* L.	Topical (Oil based formulation)	Analgesic, Antiinflammatory
Saffron	*Zaferan*	*Crocus sativus* L.	Topical (Oil based formulation)	Antinociceptive, Antiinflamatory

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PW-144

Evaluation of the antioxidant and anticholinesterase activity of extracts from *Aspidosperma* spp.

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Alzheimer's disease (AD) is a progressive neurodegenerative disease associated with a deficiency in cholinergic transmission, affecting the CNS [1]. Many natural products have been shown to inhibit acetylcholinesterase (AChE) as well as exhibiting antioxidant, anti-amyloid and anti-inflammatory activities, indicating a promising source of new alternatives for the treatment of AD [1]. In this work, we evaluated the antioxidant and anticholinesterase activity of the extracts of leaves and stems of *Aspidosperma* spp. (Apocynaceae) using the system of co-oxidation of β-carotene/linoleic acid [2], TLC [3] and microplate assays [4], respectively. The extracts showed antioxidant activity with IC_{50} values of 39.02 μg/mL for the EtOH extract of stems, and 61.73 μg/mL for the EtOH extract of leaves. Quercetin and rutin were used as positive standards (IC_{50} of 0.44 μg/mL and 21.56 μg/mL, respectively). In the AChE inhibition assay using TLC most of the extracts showed inhibition halos of different intensities. In the microplate assay, the DCM extract of stems and leaves and the EtOH extract of stems showed AChE inhibition greater than 50% (59%, 67% and 54% respectively). Physostigmine was used as a positive control (90 % inhibition). The antioxidant activity of the stem extract can be correlated with the phenolic content while the presence of alkaloids and triterpenes may contribute to the anticholinesterase activity observed in the extracts of *Aspidosperma* spp. Further studies will be developed aiming for the isolation of bioactive substances, which can be used for the development of novel therapeutic strategies for the treatment of neurodegenerative diseases.

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PW-145

Kopsanone and *N*(4)-oxide-kopsanone: two β-carbolinic indole alkaloids with monoamine oxidase A inhibitory activity

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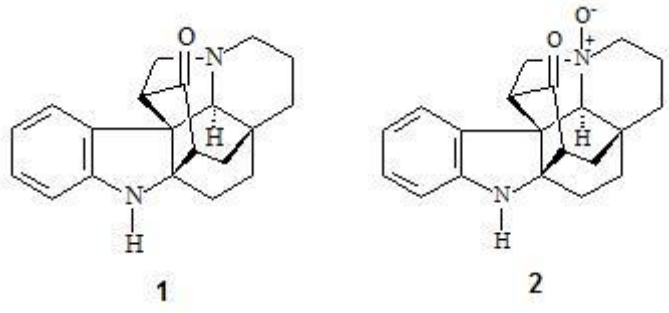
Indole alkaloids (IAs) are known for their pharmacological activities, including for neurodegenerative diseases (NDs) treatment. In fact, IAs have been calling the attention due to their multifunctionality, interacting with different targets mainly related to NDs. In this study, two β-carbolinic IA (βCIA), namely kopsanone (**1**) and *N*(4)-oxide-kopsanone (**2**), were evaluated for their monoamine oxidase (MAO) inhibitory potential. Both βCIAs were obtained from *Aspidosperma macrocarpon*. For isolation, the leaves' methanol extract was submitted to acid-base extraction. The alkaloid fraction (0.47 g) was chromatographed in silica open column using mixtures of CHCl3:MeOH, affording 1 (85 mg) and 2 (14.7 mg). The in vitro assays were carried out using kynuramine as non-selective substrate of human MAO-A and MAO-B supersomes (BD Gentest). Compound **1** was identified based on data comparison to previous studies. Compound **2** analysis, afforded a mass spectrum with a characteristic $[M-16]^+$ fragment and downfield shift in some signals in NMR analysis, indicating a*N*-oxide derivative of **1**. It was observed that **2** impaired MAO-A activity with an IC_{50} value of 4.8 μM, while **1** exhibited ten times higher potency. Molecular docking (GOLD, version 5.2, CCDC) was performed aiming at understanding the binding of **1** in MAO-A. Compound **1** was able to bind the MAO-A active site in the same pocket occupied by harmine. Its indole moiety faces to the FAD co-factor, making π-π stacking interactions with Y407. Van der Waals contacts are observed between **1** and Y69, Q215, I335, L337, and F352, contributing to stabilize the protein-ligand complex. The GoldScore retrieved for the docking of **1** in MAO-A was 51.6 while the value obtained for the re-docking of harmine (IC_{50} = 0.002 μM) was 79.1, suggesting that the *in silico* scores are in agreement with the experimental data. These results corroborate to the fact that IAs can be used as scaffolds aiming the inhibition of ND related enzymes.



1 2

PW-146

Virtual screening for new lead compounds for Alzheimer's disease with dual mode of action

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Alzheimer's disease (AD) is the most common form of dementia which affects people over the age of 65. The exact mechanism of the disease is still unknown what makes development of new drug leads more complicated. First treatment against AD introduced to general use were inhibitors of acetylcholinesterase (AChE). The drugs help patients in daily life but are not able to reverse the progression of the disease and some of them have been withdrawn because of their serious side effects. New and safer drugs with multi-target activity are needed. Galanthamine is a plant alkaloid isolated from Snowdrop (*Galanthus sp.*) and approved as a drug for the treatment of Alzheimer's disease. It has a dual mode of action - it is an inhibitor of AChE as well as an "allosterically potentiating ligand" at nicotinic acetylcholine receptors (nAChRs). This activity is of great interest in the search for new lead compounds for Alzheimer's and other neurodegenerative diseases. In the search for dual-action enhancers of ACh-mediated neurotransmission natural products including alkaloids isolated from Icelandic club mosses (Lycopodiaceae) were screened using high throughput virtual screening (HTVS) in an X-ray structure of the AChE and in a homology model of the *human* α7-nAChR. Results of the homology model optimization along with results from the virtual screening will be presented.

PW-147

Protective effect of *Gastrodia elata blume* extracts on cerebral injury induced by middle cerebral artery occlusion in rats

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Stroke is one of leading cause of death and long-lasting disability. Ischemia-reperfusion (IR) is very initial events of ischemic stroke and leads to neuronal damage. *Gastrodia elata blume* (GEB) is an herb traditionally used for the treatment of cerebrovascular related diseases in

Korea. In this study, we investigated the effects of aqueous extracts of GEB and 4-hydroxylbenzyl alcohol (HBA), an active ingredient of GEB, on focal cerebral IR injury induced by middle cerebral artery occlusion (MCAO) for 2h followed by reperfusion for 24h or 72h. Male SD rats were randomly divided into 4 groups: Sham group; IR group; GEB-treated group (24 mg/kg); and HBA-treated group. Animals were administered with distilled water (Sham and IR group), GEB or HBA for 28 days. One day after last treatment, all animals were underwent MCAO and sacrificed at 24 or 72 hr after reperfusion. The recoveries of neurological function were estimated by behavioral tests, neurological defect scoring and 2,3,5-triphenyltetrazolium chloride staining. Serum malondialdehyde (MDA) and superoxide dismutase (SOD) were assayed by colorimetry. Histological structures were observed by hematoxylin and eosin staining. Immunohistochemistry was performed to detect the TNF-α, IL-6 and apoptosis. Behavior tests showed that GEB or HBA-treated groups got better scores than IR group. GEB or HBA administration significantly reduced the neurological defect scores and lessened the cerebral infarction volume. The treatment of GEB or HBA lowered MDA content and up-regulated SOD levels. Histological examination indicated that dense neuropil and more surviving neurons were seen in GEB- or HBA-treated rats. GEB or HBA administration decreased TNF-α, IL-6 and TUNEL positive cells significantly. The results suggest that GEB or HBA demonstrates a strong and ameliorative effect on cerebral IR damage and its mechanisms are associated with its properties of anti-apoptosis and anti-oxidation.

PW-148

Withanolides and alkaloids from *Withania somnifera* roots with binding affinity to opioid, cannabinoids and GABAergic receptors

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Withania somnifera Dunal (Solanaceae) is a plant commonly used in Ayurvedic medicine to treat several diseases [1]. Interestingly, Kulkarni and Ninan [2] demonstrated that, in mice chronically treated with morphine, a methanol extract of *W. somnifera* roots prevented the development of tolerance to the antinociceptive effect of morphine. These studies suggested a possible anti-nociceptive effect of *W. somnifera*. Using behavioral approaches we demonstrated for the first time the ability of a *W. somnifera* methanol extract (WSE) pre-treatment to prolong analgesia and to prevent the development of rebound hyperalgesia in mice treated with morphine [3]. Consequently, we examined the affinity of WSE and a soluble ethyl acetate fraction obtained from WSE towards opioid, cannabinoid, GABAergic and glutamatergic receptors using radioligand receptor binding assays. WSE exhibited the highest affinity for the $GABA_A$ receptors (K_i = 13 μg/ml) while the ethyl acetate fraction showed good binding affinity for the opioid and cannabinoids receptors (K_i = 12-44 μg/ml). From the active extracts four withanolides (withanolide A, withanone, 12-deoxywithastramonolide and withaferin A), four alkaloids (anaferine, (+)-sedridine, tropine, choline) along ferulic acid

methyl ester have been isolated. Some compounds exhibited moderate binding affinity (K_i = 15-40 μM) for opioid and cannabinoids receptors.

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PW-149

Ameliorating effect of swertisin in MK-801-induced prepulse inhibition deficits and cognitive impairment

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Schizophrenia is a chronic neuropsychiatric disorder characterized by positive symptoms, negative symptoms and cognitive disturbance. Because of the narrow therapeutic effects and other adverse effects in schizophrenia patients, developing novel candidates for treating schizophrenia is required. *Swertia japonica* is a herbaceous plant within the family Gentianaceae. Extracts of *S. japonica* inhibited the dopamine D2 receptor signaling on gastrointestinal tracts, but its antipsychotic effects have not been studied until yet. In a pilot study, we found that ethanol extract of *S. japonica* ameliorated MK-801-induced sensorimotor gating impairments. Further studies were conducted for which ingredients would have the similar antipsychotic effect of *S. japonica* and isolated swertisin. We investigated the effects of swertisin on schizophrenia-like behaviors induced by MK-801 in mice. In the acoustic startle response test, the MK-801-induced prepulse inhibition deficit was significantly attenuated by the administration of swertisin (30 mg/kg, p.o.). Swertisin itself did not have any effects on the acoustic startle response or prepulse inhibition (PPI) level in normal naïve mice. In the novel objects recognition test, recognition memory impairments induced by MK-801 were reversed by the administration of swertisin (30 mg/kg, p.o). These results indicate that swertisin may be useful for the treatment of several symptoms of schizophrenia, including sensorimotor gating disruption, and cognitive impairment.

PW-150

In vivo eurobiological Activity of Inhaled Juniper essential oil

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Although *Juniperus communis* L. is known as a traditional remedy, common juniper is used especially as food spice for food & beverage industry. In aromatherapy, juniper essential oil is recommended for anxiety, nervous tension, mental exhaustion and stress-related problems. In our study, we assessed the biological properties of inhaled *Juniperi aetheroleum*(200 µl, either 1% or 3%, 60 minutes/day for 21 continuous days) on oxidative stress parameters in the hippocampus of amyloid beta (1-42) (400 pmol/rat) – induced rat model of Alzheimer's disease using superoxide dismutase, glutathione peroxidase, catalase and acetylcholinesterase specific activities, the total content of the reduced glutathione, protein carbonyl and malondialdehyde levels. Additionally, the animals were behaviorally tested for assessment of memory, anxiety and depression responses. The results suggest positive effects on spatial memory formation. Moreover, the inhalation of juniper oil significantly increased antioxidant enzymes specific activity, while reducing glutathione peroxidase and acetylcholinesterase potential in a dose-dependent manner. The co-existence of anxiolytic, antidepressant-like effects plus antioxidant properties suggest that juniper oil is a potential candidate for the development of therapeutic agents to manage oxidative stress associated with Alzheimer's disease.

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PW-151

In vivo analgesic activity and phytoconstituents of *Scrophularia kotscyhana*

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Scrophularia L. genus (Scrophulariaceae) is represented by 60 species (23 endemic) in Turkey [1]. Some *Scrophularia*species are used in different folk medicines for the treatment of many skin diseases, constipation, neuritis and bacterial-viral infections [2-3]. In the present study we aimed to evaluate the analgesic activities of aerial parts and roots of *Scrophularia kotscyhana*

Bentham separately. The plant materials were first extracted with MeOH. The MeOH extracts were dispersed in H_2O and then subjected to partition with *n*-hexane, dichloromethane, ethyl acetate and n-butanol consecutively to obtain subextracts. Analgesic activities of all extracts at the doses of 5, 10, and 30 mg/kg (i.p.) were examined using hot plate test in mice. Diclofenac (15 mg/kg; i.p.) was used as reference analgesic agent. Among the tested extracts, MeOH extract prepared from the aerial parts and in the group of the subextracts prepared from the active crude MeOH extract, the n-butanol subextract showed analgesic activity both at the doses of 5, 10, and 30 mg/kg while n-hexane and dichloromethane subextracts displayed activity at 30 mg/kg. Phytochemical studies on n-butanol subextract led to the isolation of two new iridoid glycosides as an inseparable mixture along with two known compounds, β-Sitosterol 3-*O*-β-glucopyranoside and apigenin 7-*O*-β-D-glucopyranoside. The structures were identified on the basis of 1D- and 2D-NMR experiments as well as MS. The structures of the new compounds were elucidated as 8-*O*-acetyl-4'-*O*-(*E*)-*p*-coumaroylharpagide and 8-*O*-acetyl-4'-*O*-(*Z*)-*p*-coumaroylharpagide.

Acknowledgements: This research was supported by TUBITAK (SBAG-113S252).

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PW-152

Alkaloids from *Berberis vulgaris* and their biological activity connected to Alzheimer's disease

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Alzheimer's disease (AD) is neurodegenerative disease with specific neuropathological changes, which are observed at molecular level. Nevertheless, ethiopathogenesis is still not clear and therapy is only symptomatic.

Berberine, an isoquinoline alkaloid from *Berberis* sp. is known agent, which interferes with many processes present within the course of the disease. The aim of this phytochemical study was to isolate tertiary alkaloids with potential neuroprotective activity, which could have less cytotoxic effect and better bioavailability.

The primary extract was acquired from dried barberry root bark by extraction with ethanol and then was subjected to liquid/liquid extraction with different pH and treated by standard chromatographic methods. Alkaloid structures were determined by spectroscopic methods (MS, NMR). Isolated alkaloids were subsequently tested *in vitro* for their inhibition activity in term of human acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and prolyl oligopeptidase (POP); IC_{50} values were determined. The most active alkaloids were tested for type of cholinesterase inhibition and ability to cross blood brain barrier (BBB).

Some of the alkaloids were weak AChE inhibitors: the berlambine, bersavine, obamegine and berbostrejdine (IC_{50} ranged from 55.3 to 97.4 μM). The last one inhibited also BuChE (IC_{50} 6.9±1,0 μM). The most potent inhibitor of BuChE was aromoline with IC_{50} value of 0.82±0.1 μM. The type of inhibition of aromoline was determined at horse plasma BuChE model, it acted via a mixed mechanism in a dose-dependent manner. Based on parallel artificial permeation assay it is not able to cross BBB by passive permeation.

The promising inhibition activity against POP was shown by bersavine (with IC_{50} of 67.3 ± 6.2 μM), inhibition potency of aromoline and berlambine was comparable to the standard berberine (IC_{50}=142.3±21.1 μM).

PW-153

Sideritis scardica extracts inhibit the aggregation of α-synuclein and β-amyloid peptides in *Caenorhabditis elegans* used as a model for neurodegenerative diseases

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Sideritis scardica Griseb. is one of over 150 species of the genus Sideritis L. (Lamiaceae) and endemic to the Balkan peninsula, where it is traditionally used as tea. Recent studies show an activity of *S. scardica* extracts in the CNS *in vitro*and *in vivo*. An influence on the reuptake of monoamine neurotransmitters [1], a stimulating effect on EEG patterns in rats [2] and a cognition enhancing activity in mice [3] have been reported. To further investigate CNS effects of the plant we used the nematode *Caenorhabditis elegans*, a well investigated model organism, to explore the influence of four *S. scardica*extracts of different polarity (H_2O; 20, 50, 70% EtOH) on characteristics of neurodegenerative diseases like Alzheimer's and Parkinson's *in vivo*. Deposits of human β-amyloid formed by the transgenic *C. elegans* strain CL2006 were stained with thioflavin S and counted. All groups treated with *S. scardica* showed a significant lower number of plaques (max. 21% reduction vs. control). Furthermore, the extracts were examined concerning an alleviation of Aβ-oligomer-induced neurotoxicity by observing paralysis progression of strain Cl4176. Treated worms paralyzed significantly later with a delay of the PT_{50} value of max. 9% vs. control. Additionally, we included human α-synuclein, a histopathological hallmark of dementia, which is expressed by *C. elegans* strain NL5901. It is fused to the yellow fluorescent protein enabling to observe and quantify its aggregation. Here the treated groups showed significant lower fluorescence intensity (max. 37% reduction vs. control). In all assays *S. scardica* showed a dose-dependent effect

influenced by the extraction medium (most potent 50% EtOH). The results confirm the potential of *Sideritis scardica* preparations for the treatment of neurodegenerative diseases.

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PW-154

Inhibitory potential of *Rosa canina* and *Rosa sempervirens* fruit extracts towards acetylcholinesterase

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Acetylcholinesterase (AChE) is a key enzyme catalysing the hydrolysis of acetylcholine in the nervous system. The inhibitors of AChE are used in treatment of Alzheimer's disease. Due to numerous side effects of synthetic AChE inhibitors, there is a great need for new inhibitors, preferably from natural sources. Thus, the aim of this study was to examine AChE inhibitory potential of water and methanol fruit extracts of *Rosa canina* L., traditionally used in folk medicine, and poorly investigated *Rosa sempervirens* L. The inhibitory effect of the extracts was evaluated using a modified in vitro spectrophotometric Ellman´s method [1]. During the method optimization, it was tested how buffers (Tris-HCl and phosphate buffer), frequently used in the assay, and solvents used for plant extract dilution (DMSO, methanol, ethanol, water) affect the enzyme activity. The results suggest that the use of 20 mM Tris-HCl pH 8 as a buffer and water as a solvent had the lowest influence on AChE activity, so they were chosen as optimal. All investigated extracts of *R. sempervirens* expressed greater activity than those of *R. canina*. Methanol dry fruit extract of *R. sempervirens* expressed the highest activity (IC50=1.2 mg mL-1), while water fresh fruit extract of *R. canina* exhibited the lowest AChE inhibitory potential. In comparison with galantamine, a well-known potent inhibitor of AChE but with adverse effects, all extracts manifested weaker activity.

The results obtained indicate that *R. sempervirens* has good potential for application in treatment of Alzheimer's disease and support further investigation of this poorly investigated species. Additionally, the optimal experimental conditions for successful determination of natural products inhibitory potential towards AChE are defined in this study.

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PW-155

Anti-neuroinflammatory compounds from *Athyrium yokoscense*

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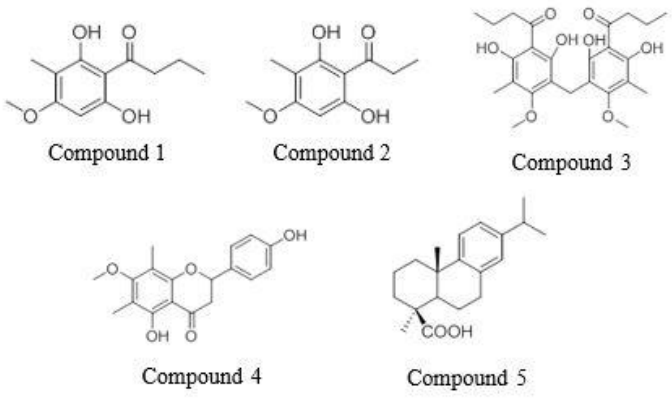
Ferns have been traditionally used for food and medicine in east Asia. Various biological effects of ferns have been reported, such as acetylcholinesterase inhibition, anticancer and antioxidative activity[1-3]. In our search for anti-neuroinflammatory ferns, methanol extract of *Athyrium yokoscense* showed anti-neuroinflammatory activity on BV2 cells. We isolated five compounds, aspidinol B (**1**), aspidinol P (**2**), bismethylene(aspidinol) (**3**), angophorol (**4**) and dehydroabietic acid (5) from the methanol extract of *A. yokoscense* through bioactivity-guided isolation. All of these compounds were isolated from *A. yokoscense* for the first time. The anti-inflammatory activities of each compound were evaluated by inhibition of lipopolysaccharide (LPS)-induced nitric oxide (NO) production on BV2 murine microglial cells. Compound **1** and **2**significantly inhibited NO production at a concentration-dependent manner, with IC50 values of 30 and 50 μM, respectively. In western blot assay for COX-2 and iNOS, Compound **1** and **2** suppressed LPS-stimulated COX-2 expression and Compound 1 also downregulated iNOS expression in BV2 cells.



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PW-156

Sesquiterpene lactones from *Cynara cornigera*: acetyl cholinesterase inhibition and *in silico* ligand docking

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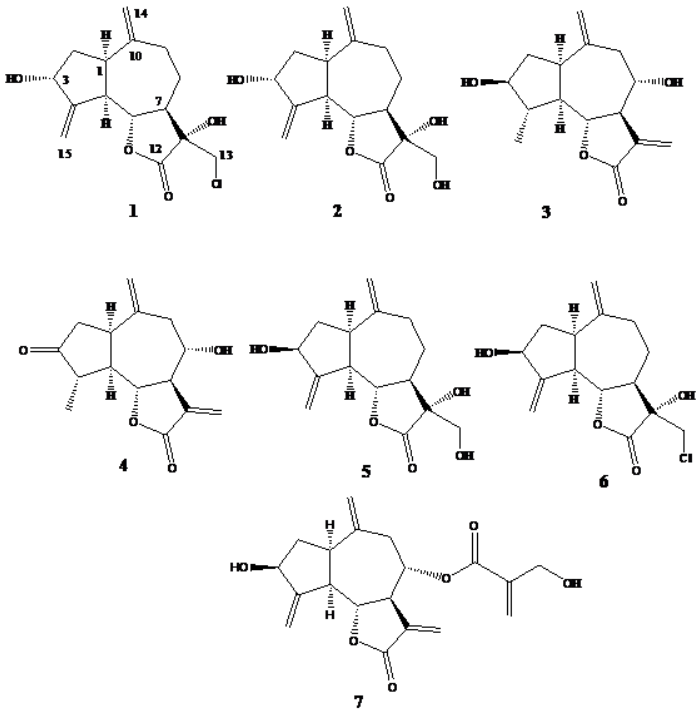
Artichoke (*Cynara cornigera*), a thistle-like perennial in the Asteraceae family is native to the Mediterranean region, northwestern Africa, and the Canary Islands. While the pleasant albeit bitter taste of leaves and flowers is attributed to the sesquiterpene lactones, cynaropicrin and cynarin, a comprehensive phytochemical investigation has yet to be reported. In this study an aqueous methanol plant extract has afforded seven sesquiterpene lactones including: a new halogenated metabolite (**1**) a naturally isolated compound sibthorpine (**2**) and five metabolites isolated for the first time from *C. cornigera*. Structures were established by spectroscopic methods, including HREIMS, ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC NMR experiments as well as x-ray analysis. Isolated bioactive nutrients were analyzed for antioxidant and metal chelating activity. Compound 1 exhibited potent metal chelating activity as well as high antioxidant capacity. Moreover select compounds were effective as acetyl cholinesterase (AChE) inhibitors presenting the possibility for such compounds to be examined for anti-neurodegenerative activity. A computational pharmacophore elucidation and docking study was performed to estimate the pharmacophoric features and binding conformation of isolated compounds in the AChE active site.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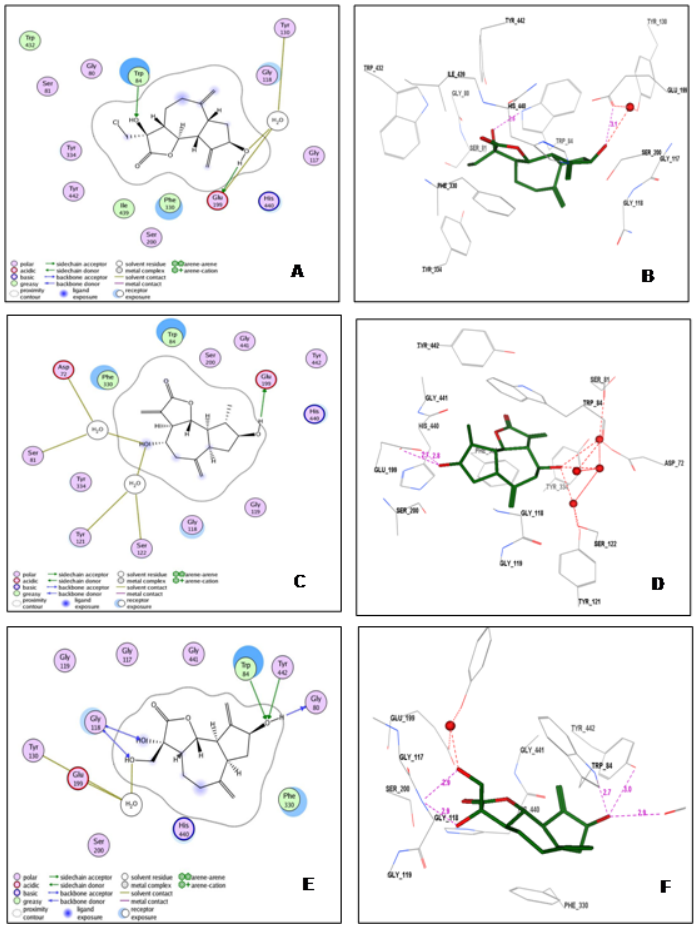
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PW-157

THC exerts neuroprotective effect in glutamate affected murine primary mesencephalic cultures and neuroblastoma N18TG2 cells

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Glutamate is an excitatory neurotransmitter widely distributed in the brain. However, over-accumulation of glutamate results in neurotoxicity, which contributes to neuronal degeneration in neurodegenerative diseases, e.g. Parkinson disease. Glutamate toxicity is mediated by excitotoxicity and oxidative stress. Tetrahydrocannabinol (THC) from *Cannabis sativa*has been discussed as a neuroprotective agent in several in vitro and in vivo models of brain injury. However, the mechanisms by which THC exhibits neuroprotective properties are not completely understood. In this study, we studied neuroprotective effects of THC in in primary murine mesencephalic cultures and in CB1 receptor containing N18TG2 cells. Glutamate (30 μM in primary cell cultures and 30 mM in cell lines) was administered for 48 h with or without concomitant THC treatment (0.1 to 10 μM). THC protected dopaminergic neurons and other cell types of primary dissociated cultures as well as N18TG2 cells from glutamate-induced neurotoxicity. Moreover, THC significantly counteracted glutamate-induced mitochondrial membrane depolarization and apoptosis in both models. SR141716A, a CB1 receptor antagonist, concentration-dependently blocked the protective effect of THC in primary mesencephalic cultures but had no effect in N18TG2 cells. A slightly recovery of glutathione levels in N18TG2 cells co-treated with THC was also observed. In conclusion, THC exerts antiapoptotic and antioxidant properties and further restores mitochondrial membrane potential via a mechanism not exclusively dependent on CB1 receptor. It provides considerable neuroprotection in both models therewith strengthening the hypothesis that THC may be a candidate to slowing down the degenerative processes in PD and other neurodegenerative diseases.

PW-158

Withania somnifera leaf extract delivery as a nanoparticle protect the glioma cells from oxidative damage

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Withania somnifera (L)is a medicinal plant used to treat stress and neurological disorders. Withanolides are the major compounds responsible for pharmacological activities of this species. Some issues like poor water solubility, poor permeability and bioavailability restricts their therapeutic efficacy. To overcome this, some approaches have been envisaged like nanoparticle drug delivery.

*I*n this work, we have developed and characterized a *Withania somnifera* leaf Extract (WSE) encapsulated in PCL and MPEG-PCL nanoparticles, prepared by solvent displacement method. HPLC–DAD analysis was performed and confirmed the presence of similar bioactive compounds in WSE and in the nanoparticles formed: withanolide-A, withanolide-B, withaferin-A, and 12-deoxy-withastramonolide. The MPEG-PCL nanoformulation showed higher entrapment efficacy (73%) than PCL nanoparticles (59%). Nanoparticles were physically characterised by laser doppler anemometry, transmission electron microscopy and X-Ray diffraction. The results confirmed that size of PCL-WSE and MPEG-PCL-WSE range between 210-240 nm and 30-70 nm, respectively, being in spherical shape.

In vitro release behavior of WSE loaded PCL and MPEG-PCL nanoparticles showed features of a controlled release pattern. Cellular studies with U251 giloma cells exhibited a high cellular uptake of WSE-PCL and namely WSE-MPEG-PCL nanoparticles.

WSE, WSE-PCL and WSE-MPEG-PCL nanoparticles significantly protected neuronal cells (U251) against oxidative damage induced by t-BHP. Moreover, both WSE- PCL and WSE-MPEG-PCL nanoparticles showed better protective effect then free WSE. WSE-MPEG-PCL nanoparticles showed the highest neuroprotection effect (95% for 10 µg/ml, EC_{50}1µg/ml). Together, our results indicated that MPEG-PCL-WSE might be an efficient way for WSE brain delivery.

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PW-159

Do Valerian, Melissa and Passion flower and their combination have an anxiolytic effect? Preclinical evidence

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Melissae folium (M), Passiflorae herba (P) and Valerianae radix (V), are well established in the treatment of tenseness, restlessness and irritability, with difficulty in falling asleep. The primary pharmacological effect is more likely to be anxiolytic and not sedative like in benzodiazepines and barbituric acid derivatives.

To test this, three hydroethanolic extracts and their combination STW 32* (P 40%, V 20%, M 40% of fluid extract) were investigated for their influence on exploratory behaviour (vigilance, rearing and locomotory activity), anxiolytic action in the elevated plus maze (EPM), social interaction test, and anti-depressive action in the tail suspension test, all in NMRI mice. Extracts were applied by oral gavage in aqueous solution with 1 % methyl cellulose 60-70 prior to the tests, in up to 4 doses between 30 and 1040 mg/kg b.w..

The EPM test uncovered significant effects: Diazepam (1 mg/kg) increased the number of entries and the duration of stays ($p \leq 0.05$). P, 176 and 352 mg/kg b.w., was likewise anxiolytic, as well as V, 1040 mg/kg b.w. ($p \leq 0.05$), which also significantly lowered the duration of the stays. STW 32, 30 mg/kg b.w., significantly increased the numbers of entries and their duration, as well as the higher doses of 120 and 240 mg/kg b.w.. In the social interaction test, for P, M, V, and the combination, the lowest significantly active doses were 88, 704, 520, and 30 mg/kg b.w., with significant effects of the combination also at the higher doses tested (60, 120 and 240 mg/kg b.w.). Therefore, in the models of elevated plus maze and social interaction, the lowest active dose of the combination is far lower than of the combination partners tested, so that a synergistic effect is plausible.

In conclusion, data point to a synergistic action of the combination. They suggest, that the sleep inducing effects of the herbal extracts and their combination, STW 32, are due to an anxiolytic effect.

*Phytonoctu

PW-160

Ginkgo-specific acylated flavonol glycosides modulate neurotransmitter and improve motor coordination in rats

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We previously observed that sub-chronic treatment (14 days) with two Ginkgo-specific acylated flavonols (3-*O*-(2''-*O*-(6'''-*O*-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)-quercetin [Q] and 3-*O*-(2''-*O*-(6'''-*O*-(p-hydroxy-trans-cinnamoyl)-β-D-glucosyl)-α-L-rhamnosyl)-kaempferol [K]) increases dopamine and acetylcholine levels in the rat medial prefrontal cortex (PFC) and it was speculated that these two flavonoids contribute to the dopamine enhancing properties of the special *Ginkgo biloba* extract EGb 761® [1]. Depending

on the brain region, the pharmacological effects of dopamine release are different. Beside emotional and cognitive functions in the PFC, dopamine is the main neurotransmitter in the nigrostriatal pathway and plays a significant role in the control of motor function. Thus, it was hypothesized that these two flavonoids may also improve motoric dysfunction by modulation of dopaminergic neurotransmission. We therefore, evaluated the influence of these flavonoids on motor coordination in the rotarod test using spontaneously-hypertensive rats (SHR). This rat strain displays insufficiencies in fine motor skills and is used as a rodent model of attention deficit hyperactivity disorder (ADHD). Following oral administration, both flavonoids dose-dependently increased the time animals stayed on the rotating rod before falling off (see table).

Dose (mg/kg, p.o.)	Q Time on rotarod (sec)	K Time on rotarod (sec)
0	62 ± 90	124 ± 120
3	112 ± 111	110 ± 108
10	163 ± 94 *	202 ± 95
30	224 ± 84 #	290 ± 22 #

$p<0.05$ vs vehicle group; # $p<0.01$ vs vehicle group

The results suggest that these flavonoids as well as EGb 761® may be useful in the therapy of coordination disorders such as ADHD, Parkinson's disease or presbyvertigo.

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PW-161

Supercritical extraction of main alkaloids from *Galanthus elwesii*

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Supercritical fluid extraction (SFE) can be used for a wide range of chemical components, including alkaloids. Using pure CO_2 is not sufficient for the extraction of polar components, adding a small amount of polar modifier increases the effectiveness of the method [1]. In this study, the effect of carbon-dioxide density with the use of methanol as modifier, basic plant material treatment and temperature were evaluated from *Galanthus elwesii* L., an ornamental member of the Amaryllidaceae family. Galanthamine is used in the treatment of Alzheimer's disease as an inhibitor of acetyl-cholinesterase, while lycorine's antitumor activity has been demonstrated both in vivo and in vitro [2].

Quantitative determination of alkaloids was performed by HPLC. Samples were taken at temperatures 40 °C, 50 °C and 60 °C, pressures of 100 bar, 200 bar and 300 bar were used

with methanol modifier (Jasco Laboratory SFE system). The highest quantity of galanthamine was obtained at 40 °C, 100 bar, the highest quantity of lycorine was found at 60 °C, 300 bar. Galanthamine: lycorine ratio changes at 60 °C, 300 bar. At low CO_2 density the extraction ratio of galanthamine is higher than at high density where the yield does not differ from each other compared to the density value change. For galanthamine, the best extraction value is at low, for lycorine at high density.

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PW-162

Root cultures of *Canscora decussata* as a potential source of acetylcholinesterase inhibitors

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Root extracts of *Canscora decussata* Schult. are traditionally used as a brain tonic and in the treatment of nervous debilities, including convulsions, epilepsy, insanity and memory loss [1]. Natural resources of this plant have become limited due to unscrupulous harvesting and urbanization. Therefore, root cultures were established using leaf explants of *in vitro* grown *C. decussata* plants [2]. Highest root biomass (2.582±0.167 g/flask fresh weight) was observed in Gamborg B5 medium supplemented with indole-3-butyric acid (1 mg/l) when incubated at 25±2 ºC in dark. Root growth kinetics were studied and total phenolic and flavonoid contents were determined. Root extracts exhibited various antioxidant capacities. Ethanolic extract showed highest acetylcholinesterase (AChE) inhibitory activity (IC_{50}, 23.79±0.09 µg/ml). As AChE inhibitors are currently employed in the treatment of Alzheimer's disease, *C. decussata* root cultures can serve as a potential source of novel AChE inhibitors [3].

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PW-163

A new isoflavonoid glycoside and other constituents from *Tilia amurensis* with anti-neuroinflammatory activity

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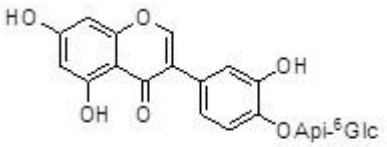
Tilia amuresnsis Rupr. is a tree traditionally used in Korean medicine. Its leaves have been used as tea for health purposes [1]. As part of our ongoing search for bioactive constituents of natural Korean medicinal resources, we found in a preliminary study that the MeOH extract from the trunks of *T. amurensis* showed an inhibitory effect on nitric oxide (NO) production in an activated murine microglial cell line. A bioassay-guided fractionation and chemical investigation of the MeOH extract resulted in the isolation and identification of a new isoflavonoid glycoside, orobol 4'-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**1**) and 16 known compounds (**2**-**17**). The structure of the new compound was determined by spectroscopic methods, namely 1D and 2D NMR techniques, HRMS, and chemical methods. Anti-neuroinflammatory activities of the isolated compounds were determined by measuring NO levels in the medium using murine microglia BV-2 cells. Among them, twelve compounds, including compound **1** (most active with an IC_{50} value of 23.42 μM), inhibited NO production in lipopolysaccharide-stimulated BV-2. Moreover, compounds **1**-**4** showed moderate anti-proliferative activities against the SK-MEL-2 cell line with IC_{50} values ranging from 12.31 to 19.67 μM.



1

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PW-164

Analysis of antidepressant properties of some *Papaver* species by *in vivo* and *in vitro* methods

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The genus *Papaver* is represented by 37 species in Turkish flora [1]. Some species have been used for the treatment of several diseases such as inflammation, diarrhea, and sleep disorders, as well as used as anti-depressant in some regions of Anatolia [2-4]. Six *Papaver* species *(P. lacerum, P. syriacum, P. macrostomum, P. glaucum, P. rhoeas* and *P. commutatum*) were collected from different localities of Turkey. Methanolic extracts prepared from the aerial parts of the plants were first tested for the presence of alkaloids by using classical Dragendorff's reagent and TLC analysis. Methanolic extracts were then subjected to in vivo analysis for their antidepressant activity [5]. *P. lacerum* and *P. syriacum* extracts which may contain higher amount of alkaloids, at doses of 100 mg/kg p.o significantly antagonized the ptosis and motor depression ($p<0.001$) induced by tetrabenazine and also shortened the immobility time in the forced swimming test ($p<0.01$). Imipramine was used as positive control in both tests. In order to understand the mechanism of the antidepressant activity extracts will be subjected to SH-SY5Y cell lines and BDNF expression levels will be evaluated [6].

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PW-165

Effect of *Cannabis sativa* extract on oxidative stress and brain damage induced by toluene in rats

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We investigated the effect of treatment with *Cannabis sativa* extract on the development of oxidative stress and brain damage induced by toluene injection in rats. The extract of *Cannabis sativa* was obtained from the dried flowering tops and leaves of the plant by chloroform treatment. Δ^9-tetrahydrocannabinol (Δ^9-THC) content of the extract (10%) was quantified using gas chromatography–mass spectrometry (GC-MS). The doses of cannabis extract were expressed as Δ^9-THC content of 10 or 20 mg/kg. Toluene (2.6 ml/kg) was intraperitoneally administrered alone or in combination with the *cannabis*extract (10 or 20 mg/kg, subcutaneously) daily for 6 days. The brain content of malondialdehyde, reduced glutathione and nitric oxide as well as the activity of paraoxonase (PON1), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in brain homogenates were determined. Histopathology and caspase immunohistochemistry were also performed. Results showed that, compared to controls, toluene resulted in increased oxidative stress in brain. Malondialdehyde and nitric oxide concentrations were markedly raised along with decreased reduced glutathione. Toluene also inhibited PON1, AChE and BChE activities. The biochemical changes induced by toluene were alleviated to great extent by cannabis administration. Cannabis, however, offered little effect on brain damage caused by the solvent. The results indicate that treatment with cannabis reduces oxidative stress but not neuronal damage induced by tolunene in rats.

Other

PW-166

Levels of phenolic polycyclic aromatic hydrocarbons in Koreans' urine by LC-MS/MS

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Medicinal plants are widely used throughout the world. About 80% of the world's population relies on plant-derived medicines for their primary healthcare [1]. Also some of them are willingly used for culinary purposes or as raw materials for pharmaceutical products, cosmetics and herbal medicinal products. However, the chemistry and efficacy of many of these plants are relatively unknown [2]. Levels of polycyclic aromatic hydrocarbons (PAHs)

are generally low in fresh plants, but in grown in close proximity to urban pollution sources, levels of PAHs might be higher [2]. PAHs are products of the incomplete combustion of organic compounds, and man is exposed to PAHs by smoking, his diet and environmental pollution. Several PAHs are classified as carcinogens. Because of their global occurrence in food and the environment, they are of toxicological and public concern.

Urine samples for this study were collected from 1028 Korean adults. Urine samples were prepared by enzymatic hydrolysis and solid-phase extraction with Sep-pak cartridges. The analysis of PAHs was validated with blank urine by liquid chromatography tandem mass spectrometry; we used blank urine because all the urine samples some metabolites contains of PAHs. The linearity was very satisfying for 17 PAH compounds, with a coefficient of correlation (r^2) higher than 0.99. The limit of detections was 0.01 to 0.08 ng/mL, the accuracy was 80% to 120%, and the precision was lower than 20%. Detected PAH metabolites from 1028 adult urine samples included (mean ± SD) 1-naphthol (8.56±20.16 ng/mL), 2-naphthol (8.77±17.46 ng/mL), 2-OH-fluorene (0.81±1.39 ng/mL), 3-OH-fluorene (0.23±0.45 ng/mL) and 1-OH-IP (0.23±0.60 ng/mL), respectively.

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PW-167

Integrated skin, liver, and serum metabolomics on the influence of green tea in ultraviolet B-induced photoaging mice model

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Our previous study revealed green tea administration was effective in reducing the wrinkles and numerous skin metabolites levels altered by UVB radiation [1]. Along with this, this study investigated the effects of green tea on liver and serum using metabolomics approach under the similar experimental condition to explain integrated effects on UVB exposure and green tea intake in photoaging model. Mice exposed to UVB radiation and green tea diet (GTD) showed significant reduction of wrinkle formation. Although no significant macroscopic changes were observed in both UVB irradiated (UVB) and GTD groups compared to normal group in liver, biochemical parameters such as malondialdehyde, TNF-α, triglyceride levels were decreased and total cholesterol levels were increased by GTD. According to PLS-DA models derived from skin, liver, and serum metabolite profiling datas, each groups were clearly discriminated. GTD attenuated UVB-induced alterations in most skin metabolites, especially ascorbic acid showed most remarkable change same as previous research. Most of serum metabolites alteration in UVB group including amino acids, organic compounds, fatty acids, lipids, nucleosides, and carbohydrates were attenuated by GTD likewise skin metabolites. However, hepatic metabolites, especially amino acids, bile acids and lysophospholipids, highly affected by green tea itself other than the effects of UVB radiation as indicating that GTD had a direct influence on several hepatic metabolism. Of them, the increase of bile acids by GTD in serum may closely related with bile acid synthesis in liver [2]. Overall, our results suggest

that integrated metabolomics approach for determining regulatory metabolites in various biological samples could help to understand the relationship between phenotype and metabolites alteration in general.

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PW-168

Renal and hepatic toxicity of methomyl and ameliorative effects of green tea (*Camilla sinensis*) administration

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This study was conducted to test the toxicity of the carbamate insecticide (methomyl) and to evaluate the ameliorative effect of green tea (Camilla sinensis) administration. Male albino rats (Rattus norvegicus) were used as test animal model organism. A total of 30 rats were divided into 6 groups and assigned as: G1(control), G2 (green tea in drinking water at a rate of 6 g/ 300 ml), G3 (methomyl at a dose of its corresponding ADI; 0.025 mg/kg b.w.), G4 (methomyl at a dose of 10X- ADI; 0.25 mg/kg b.w.), G5 (methomyl ADI + green tea), and G6 (methomyl 10X- ADI + green tea). Methomyl was given daily by gavages. Compared to control results, the body weights in G2 had recorded slight decrease, while that of G5 & G6 recorded high significant decrease.The two later groups showed significant increase of liver and spleen weights, and decrease of kidney, heart and testes weights. In control group, ALT and AST were 25.0 and 63.06 U/ml, respectively, while G4 were 74.61 and 75.53 U/ml, respectively. Lipid peroxidation (LPO) as MDA was highly elevated in methomyl treatments (G3 &G4), while SOD was strongly decreased in these treatments. Based on MDA results, the "Ameliorative Index; AI" for green tea in conjunction with methomyl, equaled to 1.2 and 1.1 for the low and high doses, respectively. In the case of SOD, the AI reached 0.74 and 0.7, respectively. These results revealed the ameliorative effect of green tea against methomyl intoxication. Also, the other measured biochemical parameters (e.g., ALT, AST, ALP, TAC, Creatinine, Urea, BuChE) were normalized due to administration of green tea when given with with the insecticide. In conclusion, green tea achieved some body weight decrease. However, it alleviated the oxidative stress induced by methomyl.

PW-169

Anti-inflammatory activity of the fruits from some cultivars of *Rubus idaeus* and *Rubus occidentalis*

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Berry fruits are a source of biologically active compounds, especially anthocyanins and other polyphenols like flavonoids and ellagitannins. These groups of secondary metabolites possess strong antioxidant activity, which implies an anti-inflammatory effect of medicinal plants containing them. Moreover, the aglycone cyanidin revealed the strongest antioxidant and COX inhibition properties compared to other anthocyanins. In the traditional medicine of eastern Europe raspberries are used in the treatment of cold and flu-like infections [1]. The aim of the study was to compare anti-inflammatory activity of fruits from 4 cultivars of *Rubus idaeus* L. (‘Poranna Rosa’, ‘Polesie’, ‘Ljulin’, ‘Veten’) , and 1 cultivar of *Rubus occidentalis* L. (‘Litacz’) (*Rosaceae*), differentiated in the content of ellagitannins, free ellagic acid and anthocyanins. The extract from black raspberries revealed the strongest inhibition of paw edema at a dose of 500 mg/kg (48.6% inhibition) at 1h of the experiment, which was stronger compared to the standard, indomethacin (39.6%). Moreover, after 5h of the experiment, an increase of edema inhibition was observed for all ‘Litacz’ extract doses, with the highest at 1000 mg/kg (54.6%). The highest anti-inflammatory activity of black raspberries was connected with their strongest antioxidant activity determined in the DPPH and phosphomolybdenium assays (EC50 43.81 μg/ml, 170.86 mg/g, respectively). Basing on Spearman’s correlation, the anti-inflammatory activity of the black raspberries was directly related to the total amount of phenolics (0.94) and anthocyanins (0.98) ($p < 0.01$). The high value of edema inhibition of the extract at 1000 mg/g after 5h of the experiment, may suggest a contribution in the anti-inflammatory activity a free aglycone - cyanidin, as a product of slow hydrolysis of cyanidin glycosides. Di and tri-glycosides of cyanidin dominate in the complex of *R. occidentalis*anthocyanins.

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PW-170

Leaf structure, metabolic profiling and evaluation of the antimicrobial activity of six mediterranean medicinal plants

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It is well known that plants develop specific anatomical features and accumulate various secondary metabolites. Those metabolites are part of a major strategy that protects plants from biotic/abiotic stress and contribute to color, scent, flavor etc. However many of them have several application in human health.

In this study the anatomical features, the phytochemical profiling and screening for antimicrobial activity were performed in six medicinal plants found in Greece; *Ficus carica, Phillyrea latifolia, Pistacia lentiscus, Euphorbia characias, Globularia alypum* and *Ricinus communis*. Leaves were investigated using Transmission (TEM) and Scanning Electron Microscopy (SEM). Histochemical tests were performed at fresh and fixed tissue. Leaves were extracted and the metabolic profiling was recorded by GC-MS and LC-HRMS and analyzed using in-house and commercial libraries. The antibacterial activity of all extracts was firstly tested by Agar Disc Diffusion method against one Gram-positive (*Staphylococcus aureus* ATCC29213), one Gram-negative (*Escherichia coli* ATCC25922), and two clinical strains of *Candida albicans*. Active extracts were then tested against 26 clinical strains (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *S. aureus* and *Streptococcus pneumoniae*).

The epidermal, mesophyll cells and the glandular trichomnes of most examined plants showed accumulation of osmiophillic metabolites. Histochemical treatments defined their subcellular localization. SEM micrographs revealed interesting features of trichomnes (glandular and non-glandular). Laticifers were observed at *F.carica* and *E.characias*. In the phytochemical study phenolic compounds, steroids, alkaloids, flavonoids, and terpens were detected. Yet, from all the above plant extracts, only those of *P. lentiscus* had significant activity against *S. aureus*, *P. aeruginosa*, *S. pneumoniae*, and *K. pneumoniae*.

Acknowledgment: This work was supported by IKY - State Scholarship Foundation, Athens, Greece.

PW-171

Optimization of bioactive phenolic compounds extraction by modified supercritical fluid.

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Extraction by supercritical fluid is an environmentally friendly method by comparison with the conventional liquid/liquid or solid/liquid extraction. Moreover the physicochemical properties of supercritical CO_2 (viscosity, diffusivity and surface tension) [1], with addition of polar co-solvents, favors the extraction of phenolic compounds [2, 3].

Supercritical fluid extraction of bioactive phenolic compounds such as caffeic acids derivatives identified by dereplication with LC-DAD-MS² in a halophytic Asteracae, was tested. 100% CO_2 and CO_2 mixed with co-solvent were evaluated. Two temperatures were tested and a kinetic study of the extraction was made. Total extraction yields were measured and the major dicaffeoylquinic acids content was analyzed by HPLC-DAD-DEDL.

Presence of 10% ethanol in CO_2 is not enough to extract polar compounds of interest; the addition of 20% water in ethanol provides a strong enhancement of the extraction of dicaffeoylquinic acids. Moreover, in these conditions, it is not necessary to increase temperature to extract phenolic compounds. Kinetic study shows that in 1h, 80% of final extract mass is obtained.

The results show that supercritical modified fluids (CO_2/EtOH/water) are very interesting for finding an alternative to the organic solvents used in the fields of cosmetics and food.

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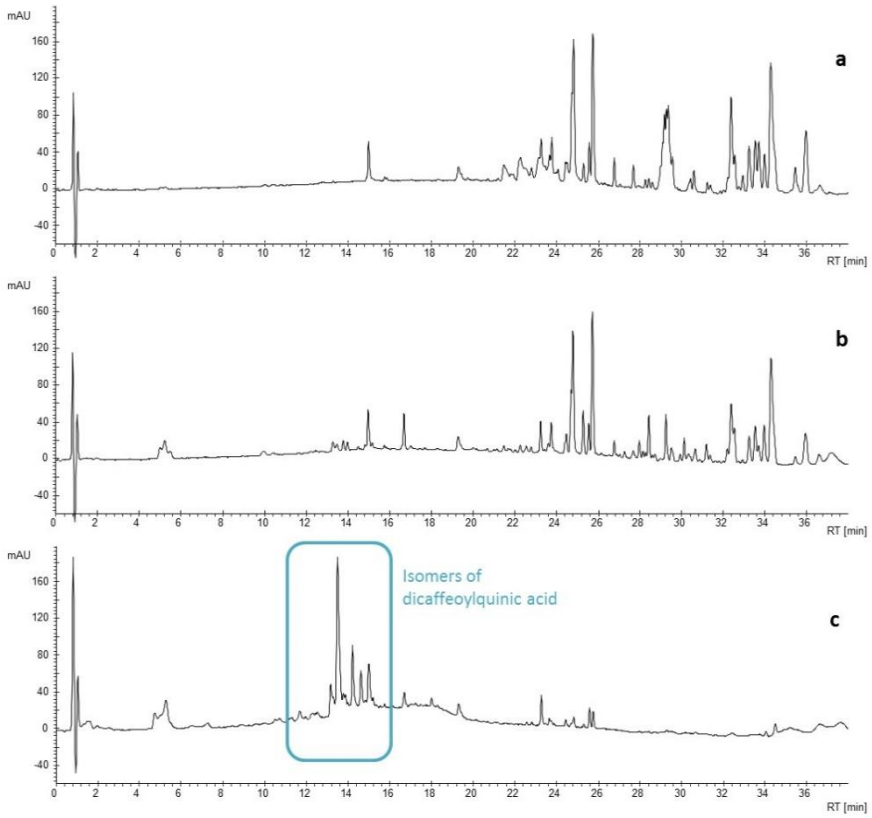

RP-HPLC chromatographic profiles of halophytic Asteraceae extracts obtained by SFE with different conditions (198nm)
a- 100% CO_2
b- 90% CO_2 + 10% EtOH
c- 90% CO_2 + 10% EtOH/H_2O (80/20) (v:v)

PW-172

Adverse effects of pesticide on weanling female rats: protective role of a grape seed extract

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The protective effects of a grape seed extract against cyhalethrin-induced oxidative stress, lipid peroxidation and liver and kidney damage in weanling female rats was studied. Weanling female rats were orally administered cyhalethrin (Cyh) at a dose equal 1/10 LD_{50} for 28 consecutive days. Two Cyh groups (6 rats each) received extract at doses of 100 and 200 mg kg-1 body weight throughout the duration of the study. Three additional groups served as extract-treated and as control groups. Administration of Cyh resulted in a significant increase in lipid peroxidation and alterations in antioxidant enzymes, e.g. superoxide dismutase (SOD) and catalase (CAT),glutathion-s-transferase (GST). Also, significant increase was recorded in serum marker enzymes, e.g. aminotransferases (AST and ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), and increase the levels of urea and creatinine. On the other hand, Cyh caused significant decrease in levels of total protein and albumin, and caused histopathological alterations in liver and kidneys. Co-administration of grape seed extract to Cyh-intoxicated rats, restored most of these biochemical parameters to within normal levels, especially at the hig dose of extract. Administration of grape seed extract to Cyh-treated rats resulted in overall improvement of liver and kidney function in weanling female rats.

PW-173

Effect of fertilization and arbuscular mycorrhizal fungi on active substances of marjoram

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The aim of this study was to examine and to compare the effect of arbuscular mycorrhizal fungi (AMF) inoculation and artificial fertilization (NPK) on the growth and content of active substances of marjoram (*Origanum majorana* L.). The soil of the experimental field without the addition of commercial AMF and of NPK served as a control. In the 1st treatment NKP, in the 2nd treatment commercially available AMF inoculum (INOQ) and in the 3rd treatment NKP and AMF mixture were added to the soil of the experimental field. The efficiency of the mycorrhization was verified by monitoring the fungal root colonization of the plants. Essential oil composition and the main polyphenolic compounds were identified and quantified in the flowering shoots of marjoram harvested at the end of the vegetation period using GC-MS and HPLC-ESI-qTOFMS respectively.

The presence of 30 essential oil components was determined among which terpinen-4-ol, *cis*-sabinene hydrate, *trans*-sabinene hydrate, linalyl acetate, *trans*-sbinene hydrate acetate and *p*-cimol were the major ones representing the 70-78% of the total content. Major polyphenols were identified as rosmarinic acid, lithospermic acid, apigenin-di-*C*-hexoside, luteolin-glucuronide, apigenin-glucoronide.

We found that the rate of the colonized roots was doubled for inoculated plants, whereas it was halved for fertilized plants compared to control. The root system of the control group was colonized by the native AMF found naturally in the soil of the experimental field. The results indicate that the colonization with native AMF increased the essential oil production, and the content of phenolic acids compared to other treatments. Interestingly, NPK treatment significantly decreased the content of phenolic acids. There was no significant difference in the yield of biomass among the treatments.

Authors thank OTKA (PD105750) for the financial support.

PW-174

Genetic diversity of wild *Rhodiola rosea* populations in Central-Europe revealed with ssr markers

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Rhodiola rosea L. (*Crassulaceae*), commonly known as golden root or roseroot is a traditional adaptogen plant of the cool climates in the northern hemisphere. This species is highly variable both in morphological and phytochemical traits. Genetic structure and relationships of 16 populations from the high mountains of Europe have been characterized with the use of microsatellite markers.

Altogether 266 individuals from sixteen populations located in the Pyrenees, Alps, Carpathians and from North-Scandinavia were studied. Out of the 13 markers only 6 turned out to be informative in this study. The primer pairs for these six SSR loci produced 68 fragments. The number of alleles per locus ranged from 9 to 17. Mean expected heterozygosity (He) was 0.73, ranging from 0.51 to 0.74 in the populations.

A dendrogram of the genetic relationships revealed that populations from different mountains clustered together without any correlation with the geographic distribution of the populations. Principal co-ordinate analysis showed that all individuals are grouped together, which confirmed that diversity within and among the populations were almost equivalent. Interestingly, a population from the Italian Dolomites is even more distant within this group than the Norwegian samples. AMOVA showed that the vast majority of the molecular variance is attributed to within population variability (85%) while only 11% was among populations variation, and 4% among regions variation. This much less differentiation observed between the Eastern Alpine and Carpathian populations supports the existence of a former common glacial refugia and a historical relationship between the two regions.

PW-175

Development of Industrial Scale Centrifugal Partition Chromatography

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Centrifugal Partition Chromatography (CPC) is a special chromatographic technique where both stationary and mobile phase are liquid, and the stationary phase is immobilized by a strong centrifugal force. The main advantage is the cost-effectiveness, since it does not need any expensive and bulky solid stationary phase, and both quantity and quality requirements for solvents are less decreased contrast to standard liquid chromatographic techniques. CPC consists of series connected network of extraction cells, which operates as elemental extractors, and the efficiency is guaranteed by the cascade [1]. CPC instruments vary on scale from 50 ml to 25 liter, however all advantages are realized on bigger scale use in industry. Up

to date there were no available instruments that could be used for purification on industrial scale.

Our team started development two years ago with the flow-simulation of the elementary extraction cells. By determining the drawbacks of the current extraction cells available, realized in dead-volume and back-mixing, we were able to develop a more optimal extraction cell, leading more efficient separation and outdating all current research [2].

All current studies show that our cells are way more efficient. We managed to do pH-zone separations of fatty acids with only 16 cells instead of 3200, at 1/5 pressure drop and 20x flow rate, unprecedent in the literature. We also managed to separate API intermediers at 0.3 l/g solvent consumption instead of 3.0 l/g. Our instrument is scalable up to 10 l/min of flow rate bringing an unbelievable industrial chromatographic performance.

Our research is past two prototypes and by the time of the conference we are expected to see the first ready-for-production industrial CPC. According to our expectations CPC can outperform 80% of current prep-LC separations in the next 10 years.

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PW-176

Exploitation of agricultural by-products for the recovery of bioactive compounds with applications in cosmetic industry.

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The agricultural, food and forestry industries produce large amounts of by- products that are considered wastes. However, recent studies have shown that these by-products contain bioactive compounds with several applications in industry.

In the present study, we investigated by-products derived from the wood industry of *P. nigra, P. heldreichii, E. globulus*and *J. phoenicea* and the food-juice industry of *P. granatum* and *P. persica.* Totally, 20 (H2O, EtOH & H2O/EtOH) extracts and 4 hydrosols were investigated initially using HPLC and LC-HRMS for identification of their major compounds. The bark from Pinus species showed high level of phenolic constituents (catechin, epicatechin, taxifolin, phenolic acids), while the bark from Eucalyptus was rich in total phenols, polymeric proanthocyanidins and ellagitannins. The major constituents of peach were flavonoids (luteolin, prunin, taxifolin), triterpenes, and phenolic acids. Pomegranate peel consisted of punicalagins, anthocyanidins, gallic acid and several flavonoids.

The samples were evaluated for their antioxidant activity using DPPH and ABTS assays and for their whitening activity by the tyrosinase-inhibitory method. The extracts were also

analyzed for their total phenolic content according to the Folin−Ciocalteu procedure and their total flavonoid content using AlCl3 colorimetric assay.

It is noteworthy that the extracts of *P. granatum* and *E. globulus* showed significant phenolic (up to 103.9 and 103.2 mg GAE/g, respectively) and flavonoid (up to 29.3 and 15.7 mg EQC/g) content with strong antioxidant activity (up to 81% and 71% inhibition of DPPH, up to 100% and 99.8 inhibition of ABTS, and up to 44.6 and 48.6 % inhibition of tyrosinase at 300 μg/ml, respectively). Thus those agricultural by-products could serve as potential cost effective source of bioactive extracts, with applications in cosmeceutical industry.

Acknowledgment: This work has been financially supported by Greek GSRT (1440-BET-2013-AGROSMETICS project).

PW-177

Influence of methanol leaf extracts of *Hilleria latifolia* and *Laportea ovalifolia* on in vitro activity of selected antibiotics

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Antibiotic resistance has become a major health and economic problem worldwide since it leads to treatment failure, complications and eventually death [1]. This has led to the search for newer and effective antimicrobial agents as well as resistance modifying agents. Our aim was to investigate the influence of methanol leaf extracts of *Hilleria latifolia* (Lam.) H. Walt. (HLML) and *Laportea ovalifolia* (Schumach.) Chew (LOML) on the activity of some selected antibiotics.

Minimum inhibitory concentrations (MIC) of the test antibiotics (alone), extracts only and test antibiotics in the presence of a sub-inhibitory concentration (5 mg/mL) of the extracts against selected typed strains of bacteria were determined using micro-dilution [2]. HLML and LOML exhibited antibacterial activity with MIC of 50 to 100 mg/mL against the test organisms. HLML potentiated the activity of amoxicillin (2 to 8-fold) against test organisms except *Klebsiella pneumoniae*and *Streptococcus pyogenes*, whereas LOML potentiated the activity of amoxicillin (2-fold) against *Escherichia coli* and*Staphylococcus aureus*. In addition, HLML increased the activity of ampicillin (2 and 4-fold) against *E. coli* and *Salmonella typhi* respectively, whiles LOML caused a 2 to16-fold reduction in the activity of ampicillin. Both HLML and LOML reduced the activity of erythromycin and ciprofloxacin by 2 to 16 folds (Table 1). HLML and LOML potentiated the antimicrobial activity of amoxicillin and ampicillin against some of the test organisms. The activity of erythromycin and ciprofloxacin reduced in the presence of both extracts.

Table 1: MIC of test antibiotics and the test antibiotics in the presence of sub-inhibitory concentration of HLML and LOML

Antibiotics		MIC (µg/mL)						
		Test micro-organisms						
		E. coli	*B. subtilis*	*S. typhi*	*K. pneumoniea*	*S. aureus*	*S. pyogens*	*P. aeruginosa*
	Amox	256	128	128	128	256	256	128
Amoxicillin	HLML	32	32	64	128	32	>1024	64
	LOML	128	128	256	256	128	>1024	128
	Ampi	128	32	128	128	64	64	64
Ampicillin	HLML	64	32	32	128	64	>1024	64
	LOML	128	128	128	256	64	>1024	>1024
	Eryt	32	256	64	128	64	64	128
Erythromycin	HLML	512	1024	1024	1024	1024	512	512
	LOML	512	512	512	1024	512	1024	1024
	Cipr	2	2	4	4	4	4	4
Ciprofloxacin	HLML	16	16	16	8	8	8	16
	LOML	16	32	32	32	32	16	32

Amox- amoxicillin, Ampi- ampicillin, Eryt-erythromycin and Cipr- ciprofloxacin. Sub-inhibitory concentration (5 mg/mL) of HLML and LOML

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PW-178

Investigation of lysozyme from the latices of *Euphorbia coerulescens* and *Euphorbia fortissima*

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Lysozyme is an enzyme from the group of hydrolases classified in the enzyme classification group EC 3.2.1.17. It was discovered by A. Fleming in 1922 as an element of the innate immune system and serves as a defense against Gram-positive and Gram-negative bacteria. It acts as a mucolytic and is present in many body fluids such as sweat, saliva and nasal secretions, as well as hen egg white, milk and expressed vegetables [1, 2]. It is known that latices of the plant family Euphorbiaceae Juss. show lysozyme activity [3]. In this study, latices of *Euphorbia coerulescens* Haw. and *Euphorbia fortissima* L.C.Leach were tested by MALDI-TOF-MS in terms of their sequence coverage with hen egg white lysozyme (HEWL). For this purpose relevant samples were separated by SDS-PAGE electrophoresis; the relevant protein bands were excised and processed using tryptic-in-gel-digestion. As a positive control, HEWL

was treated like the latex samples. The results show that the 33 kDa bands in the samples of *E. fortissima* have a sequence coverage with HEWL of 36.7% and from *E. coerulescens* of 23,8%. Results with a sequence accordance greater than 20% and at least two major peptides detected are to be regarded as a significant positive [4]. By that, it can be concluded, that lysozyme is present in latices of *Euphorbiaceae* and seems to be a highly conserved protein comparable with isoenzymes in animals.

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PW-179

Determination of in vitro photoprotection on human skin fibroblasts and keratinocytes after UVA and UVB radiation by aqueous and ethanolic extracts from *Stellaria media* herb

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There is a growing need for substances exhibiting antioxidant activity, which may protect the skin against UV-induced cellular damage and photoageing. *Stellaria media* (Linn.) Vill. (Caryophylaceae) is common weed used as food and medicinal plant. Extracts are applied topically onto the skin, to treat dermatological diseases. Because of that, and the presence of polyphenols in the herb, we evaluated the protective effect of water and ethanol extracts (most commonly used in medicine) derived from the *Stellaria media* herb on skin fibroblasts and keratinocytes against UVA and UVB radiation.

Cells were pre-incubated with tested extracts (25-200 μg/ml) and then irradiated with UVA (25 and 50 J/cm^2) or UVB (250 and 500 mJ/cm^2). Intracellular ROS production, proliferative activity, lactate dehydrogenase release and apoptosis after UV-irradiation were measured.

The results showed that the aqueous extracts from Stellaria herb decreased intracellular ROS production in a concentration-dependent manner. However, extract only moderately increased cell proliferation inhibited by UV and decreased cytotoxicity . Moreover, aqueous extract decreased the number of cells in late apoptosis (annexin V-FITC and propidium iodide positive). In contrary, ethanolic extract increased UV-induced cytotoxicity, despite of the similar antioxidative activity as aqueous extract.

Our results show, that aqueous extract of *Stellaria media* may be effective as photoprotector, because of ROS generation inhibiting activity, and partially explain the use of extract in skin diseases,such as sunburns.

PW-180

Stereochemical study of natural products by electronic circular dichroism

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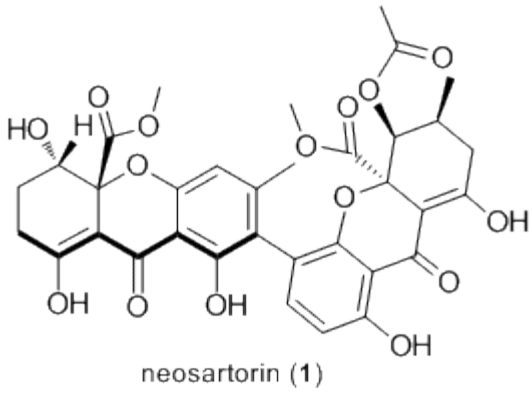
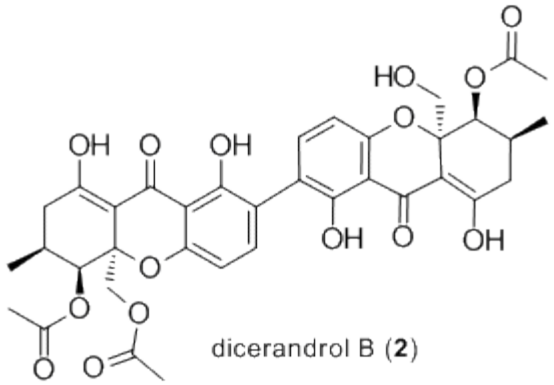
The axial chirality of biaryl natural products with restricted rotation along the biaryl axis or the preferred helicity of biaryls with low rotational energy barrier is a stereochemical feature, which can affect the bioactivity of xanthone biaryl natural products profoundly. The applicability of electronic circular dichroism (ECD) method supported with TDDFT-ECD calculation was demonstrated on the stereochemical study of biaryl xanthone natural products such as the axially chiral neosartorin (**1**) [1] and dicerandrol B (**2**) [2] having free rotation along the biaryl axis.

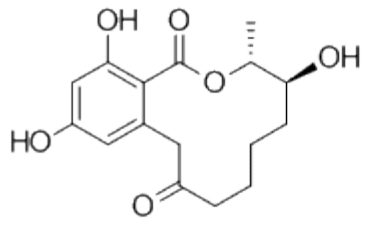
The absolute configuration of the chirality centers and preferred solution conformations were determined by TDDFT-ECD calculations for conformationally flexible natural products such as 13-hydroxy-dihydroresorcylide (**3**) [3] and brocaeloid A (**4**) [4], and for the bridged O,N-heterocycle varioxepine A (**5**) [5].



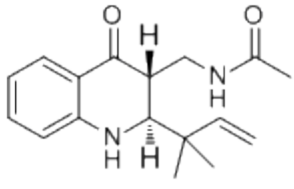
neosartorin (**1**)



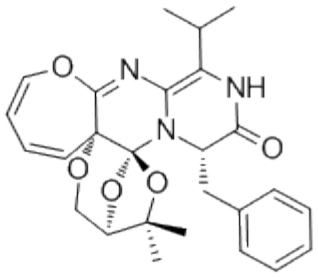
dicerandrol B (**2**)



13-hydroxydihydro-resorcylide (**3**)



brocaeloid A (**4**)



varioxepine A (**5**)

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PW-181

Taxol purification with Centrifugal Partition Chromatography

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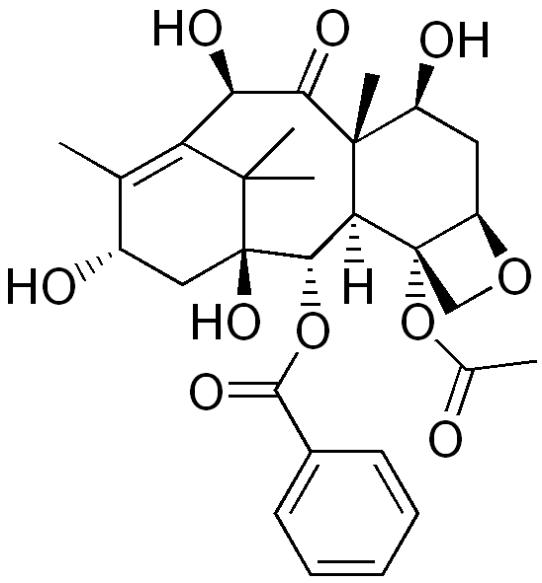
Taxols are anti-cancer chemotherapy drugs. 10-Deacetyl Baccatin III (10-DAB III) is an important intermediate for both Paclitaxel and Docetaxel, two of the best anti-cancer drugs ever invented. 10-DAB III is one of the several taxols, which can be easily extracted from Yew Tree leaves (Taxus baccata), so it became an important starting raw material in the semi-synthetic manufacturing of these drugs, because of its particularly high concentration in Yew tree leaves.



The centrifugal partition chromatography (CPC) is a typical preparative chromatography technique, where both phases (mobile and stationary) are liquids, so both phases can be optimized for different separation methods. As no silica gel needed, and the used amount of solvent is lower the operational costs significantly decrease. The liquid static phase is immobilized by a strong centrifugal field, so high flowrate of mobile phase can be used, which means fast separation. Several references can be found in literature about successful taxol purification by CPC technique [1].

In the course of our work we measured 10-DAB III samples by centrifugal partition chromatography. By the separation of main component and impurities we wanted to create highly purified product. First, we tested several solvent systems, than we determined partition coefficient of taxol components by high performance liquid chromatography (HPLC). Next step was the optimization of CPC separation method with suitable partition coefficient solvent systems. Selectivity of all compounds are over 1,25. Finally, with repeated CPC purifications we collected purified fractions, which were measured by HPLC. CPC separations showed good result, fraction analysis has shown over 99% purity with 95% recovery possible. Further we were able to develop a CPC method, which is scalable to industrial levels, and is able to provide 10-DAB III with specified purity at high yields. On the whole, we determined a quick and low-cost method for 10-DAB III purification.

It is very important to find a green chemsitry technique which cause less ecological damage, since it is necessary to take 3000 Yew trees to produce only 1 kilogram of mentioned drugs [2]. CPC technique has several economic and environmental advantages such as low production cost from the reuse of solvents, consistent purity and yield, high sample loading (2,0 gramm of injection to the 250 ml column), and quick separation (one cycle takes around 1.5 hours) against other techniques, like MPLC and HPLC purification techniques.

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[2] Malik S, Cusidó RM, Mirjalili MH, Moyano E, Palazón J, Bonfill M: Production of the anticancer drug Taxol in Taxus baccata suspension cultures: A review. Process Biochemisrty 2011; 46: 23-34

PW-182

Molecular and cultural characterization of a new licorice cultivar

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Licorice has been used as a source of medicine and a food material in East-Asia. Recently, demand for licorice increased in market due to a growing interest in health. Thus we developed a new licorice variety, 'Won-gam' in Korean through hybridization between *G. uralensis* and *G. glabra*. We studied on its cultural and molecular characterization. In the results of SSR analysis derived from EST sequences of *Glycyrrhiza* spp., Won-gam was differentiated from other *Glycyrrhiza*species including its parent species, *G. uralensis* and *G. glabra,* and was

identified as a hybrid. Two parental *Glycyrrhiza*species and Won-gam was independently differentiated in sequence analysis of ITS region, and the paternal species, *G. glabra* was differentiated in sequence analysis of *rbc*L and *trn*L-F region in chloroplast DNA. Won-gam showed a higher ingredient content of glycyrrhizin (3.5%) and yield (3.59 ton/ha) than *G. uralensis.*

[1] Sudo H, Seki H, Sakurai N, Suzuki H, Shibata D, Toyoda A, Totoki Y, Sakaki Y, Lida O, Shibata T, Kojoma M, Muranaka T, Saito K. Expressed sequence tags from rhizomes of Glycyrrhiza uralensis. Plant Biotechnology. 2009; 26: 105-107

[2] Lim JM, Ahn YS, Park CG, Park CB, Cho JH. Authentication of traded medicinal herb, Glycyrrhiza spp.(Licorice), based on nrDNA-ITS2 sequence analysis. Korean J. Intl. agri. 2012; 24(4):435-443

PW-183

Extraction and separation of pinosylvins by Centrifugal Partition Chromatography

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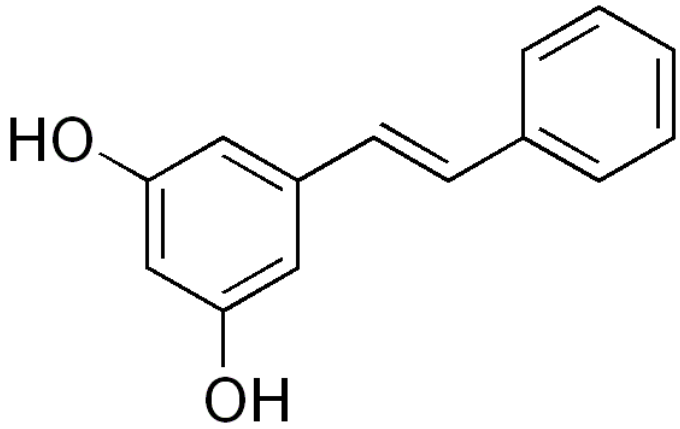
Pinosylvins are stilbenoid toxins available in the heartwood and knots of Pinaceae, protecting them from microbiological attacks [1]. Extraction of pinosylvins can be easily realized from chopped knots, a less expensive byproduct of wood production, by well-known extraction techniques using non-chlorinated solvents. However chromatographic separation is needed to separation pinosylvin from methyl-ethers and other impurities. Our goal of research was to find an economic method for production of pure pinosylvin for further studies, alternative to former flash chromatographic techniques [2].



Centrifugal Partition Chromatography Chromatography (CPC) is a special chromatographic technique where both stationary and mobile phase are liquid, and the stationary phase is immobilized by a strong centrifugal force. The main advantage is the cost-effectiveness, since it does not need any expensive and bulky solid stationary phase, and both quantity and quality

requirements for solvents are less decreased contrast to standard liquid chromatographic techniques.

We screened various combination of non-chlorinated solvents for a scalable and economic separation of pinosylvins by CPC. A few suitable systems, were partition coefficients and selectivity were in a good range were screened on an Armen SCPC-250 laboratory scale CPC. The most suitable systems were scaled up in 20x size to a RotaChrom pilot CPC, to check possibility of later industrial production.

It was found that combinations of n-pentane, n-hexane, cyclohexane, n-heptane as alkane, acetone, isopropanol as intermediate solvent and water, as other combinations, provide suitable systems for scalable production. The exact system choice depends only on solvent availability, price, ICH residual solvent regulation, and other industrial parameters.

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PW-184

Effects of fermented *Asterina pectinifera* using *Cordyceps militaris* mycelia on antioxidant

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Cordyceps militaris is a species of fungus in the family Clavicipitaceae, and the type species of the genus *Cordyceps*. The medicinal mushroom (*Cordyceps* species) is an abundant source of useful natural products with various biological activities.*Asterina pectinifera* (A. pectinifera) is a species of starfish in the family *Asterinidae.* It is found in the northern Pacific Ocean along the coasts of Korea, Japan, China and Russia. It is used as a model organism in developmental biology. The advantages it has for this purpose are that it is common, easy to collect, and easy to maintain in the laboratory. In this study, *A. pectinifera* was fermented with *Cordyceps militaris* (*C. militaris*) mycelia at solid–state. The various radical scavenging activities of the extracts from fermented *A. pectinifera* (APCM) were evaluated by electron spin resonance (ESR). The antioxidant activities of the extracts of APCM were also determined based on the ferric reducing antioxidant power (FRAP), 2, 2'-azinobis-(3-ethybenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity. The free radical scavenging activity and the antioxidative effects of APCM extracts were higher than *A. pectinifera* or *C. militaris* mycelia alone. These results indicate that APCM extracts have different chemical ingredients from the *A. pectinifera* and might provide beneficial antioxidant activity. The APCM extracts could be suitable as an antioxidant in the food industry.

PW-185

Anatomical characteristics and tissue localization of various flavonoid subclasses in *Cotinus coggygria*stem and leaf cross-sections

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Leaves, twigs and wood of *Cotinus coggygria* (Anacardiaceae) are employed in European and Chinese traditional medicine to treat skin and mucosal injuries, fever, diarrhea, emesis and to enhance appetite. While being a popular medicinal plant, ancient tinctorial species and common ornamental shrub, its anatomical characteristics and tissue localization of major groups of active compounds have not been studied yet, to the best of our knowledge. Freehand cross sections of the stem at different developmental stages, leaf lamina and petiole were performed. Tissue types were studied using conventional dyes (toluidine blue; congo red and malachite green). Folin-Ciocalteu reagent, shift reagents (ammonia) and Naturstoffreagenz A were used to complement light microscopy and autofluorescence observations of flavonoid localization. Microscopes of type Optika XDS-3FL inverted fluorescence, and Olympus BH-2 for observation in visible light were employed. *C. coggygria*stem has a typical secondary structure. The bark contains resin ducts placed in the phloem; in younger twigs they are limited by outer sclerenchyma arches. Vascular cambium generates distinct annual rings, with a porous structure. The vessel frequency is of 100-200 mm2, and their perforations are simple. Medullar rays are narrow (1-2 cells). Chalcone and aurone derivatives are present in inner wood rings aged at least 2 years, in earlywood parts. Leaves display epidermis with anomocytic stomata on the lower surface. A hypodermis is present on the adaxial side. The midrib contains four main vascular bundles and cambium. The phloem encloses resin secretory cavities. The center of the midrib is occupied by parenchyma. Palisade parenchyma is two cells deep, and spongy mesophyll is situated towards the abaxial side. Reducing flavonoids are present in leaf mesophyll.

Acknowledgement: This work was supported by grant UEFISCDI, PN II, CT-397/30.06.2014, contract nr. 789/30.06.2014.

PW-186

Alpha-cypermethrin induced lipid peroxidation, oxidative damage in the liver and kidney of male rat: the antioxidant and protective effect of *Cedrelopsis grevei* essential oil

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Liver is playing the essential role in metabolism and detoxification of pesticides. Due to these functions, hepatotoxicity continues to be among the main threats to public health, and they remain problems throughout the world. Therefore, we evaluated the antioxidant and protective effect of of *Cedrelopsis grevei* essential oil (EO) against pesticide induced liver and kidney damage in male rat. The protective activity was studied by observing the effect of *C. grevei* EO on alpha-cypermethrin induced lipid peroxidation, oxidative stress and hepato-renal toxicity in rat. EO was analyzed by gas chromatography-mass spectrometry (GC-MS) and the antioxidant activity was studied. The reducing power of the EO and their ability to scavenge

free radicals were evaluated using two antioxidant assay systems i.e. 2,2-diphenyl-1-picrylhydrazyl (DPPH), and [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid (ABTS). The results obtained showed that *C grevei* EO exhibited significant antioxidant activity. Results indicate the ability of EO to protect liver and kidney against pesticides-induced toxicity, which might be correlated to their radical scavenging potential. Histopathological examination of liver and kidney of rat administrated EO at 300 mg/kg/day showed marked improvement in histological structure. In view of the data of the present study, it can deduce that alpha-cypermethrin caused oxidative damage and liver and kidney dysfunction in male rat. The administration of *C. grevei* EO is useful, easy, and economical to protect humans against pesticide toxicity. The results presented here can be considered as the first information on the protective effect and antioxidant properties of *C. grevei* EO.

N.B.: Reducing power: the reducing power reflects the electron-donating capacity of bioactive compounds is associated with antioxidant activity.

PW-187

Polar γ-oryzanol, a new class of γ-oryzanol in RBO: Identification and monitoring of qualitative and quantitave alternations throughout the rice milling process.

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Rice Bran Oil (RBO) is cooking oil obtained from rice bran, a by-product of the rice milling process. In contrast to other vegetable oils, RBO contains high amount of γ-oryzanol (OZ). OZ has been reported to display health benefits including antidiabetic, antihyperlipidemic and anti-inflammatory effects. In the context of our studies of edible oils, RBO has been investigated and the identification of a new group of OZ hydroxylated derivatives called pollar γ-oryzanol (p-OZ) was recently reported.

The present work aims at characterizing of both OZ types using HPLC-DAD and UHPLC-HRMS/MS techniques in diverse samples. Specifically, different cultivars (Gladio, Ronaldo) and rice preparations (regular, parboiled) were qualitatively and quantitatively investigated in the 3 whitening steps of rice production. Initially, different extraction methods were assayed. Ultrasonic extraction using EtOAc was the optimum being specific for both OZ and p-OZ with yields up to 25% (w/w). Additionally, a targeted HPLC-UV method was developed for the separation and quantification thereof. 12 different samples were analyzed and measured concentrations were in the range of 1 mg/g and 10 mg/g for OZ and p-OZ respectively. Highest concentrations of OZ and p-OZ were found in the extracts from the first whitening step. In Gladio parboiled OZ and p-OZ levels were generally maintained during the process in contrast to other preparations. Furthermore, the same samples were studied using UHPLC-APCI(±)-HRMS/MS methods and selected extracts have been subjected to hydrostatic CCC for fractionation and purification. Both initial extracts and derived fractions were analyzed for further identification of minor constituents. This is the first time that detailed information regarding the p-OZ content in different RBOs is reported as well as the thorough characterization of its constituents. Moreover, purification thereof is ongoing as well as evaluation of p-OZ and OZ biological properties.

PW-188

Sage (*Salvia officinalis*) syrup alleviates liver enzymes and kidney functions induced by the carbamate pesticide- methomyl

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This study determined the effect of [common sage (*Salvia officinalis* L.) syrup] in improving the intoxication of methomyl on liver enzymes, oxidative stress enzymes and kidney functions. Thirty male albino rats weighing 105± 15g were segregated into 6 groups and designed as: G_1 (control); G_2 (sage syrup; 6g/300 ml); G_3 [mehtomyl at ADI(acceptable daily intake]= 0.03mg/kg/day); G_4 (methomyl at 1/100 LD_{50}= 0.24 mg/kgBW); G_5 (sage+ methomyl at ADI); G_6 (sage+ mehtomyl at 1/100 LD_{50}). Sage syrup was given in drinking water, and methomyl was administrated orally by gavages for 28 days. At the end of the experiment, rats were sacrificed and internal organs as well as blood samples were collected. The mean body weight of control was 287.4 g at the end of exposure time which was not differed significantly in G_3 and G_4 groups. Sage syrup group (G_2) showed an increase in body weight which was significant, and G_6 showed a highly significant increase. weights of liver and testes in G_6 were improved and were comparable to those of control group G_1.The high elevation of ALP (442.2 IU/L), ALT (74.6 IU/L) and AST (75.53 IU/L) induced by methomyl at G_4, was considerably lowered by administration of sage syrup in G_6. The ameliorative index (AI) of sage for ALP and ALT equalled 0.9 and 1.1, respectively. The percent of change in MDA (malondialdehyde), TAC (total antioxidant capacity) and SOD (superoxide dismutase) between G_4 (methomyl in high dose) and G_1 (control) were 69.3, 80.2 and 45.0%, respectively. while the percent of change in the same parameters between G_6 (sage +methomyl in high dose) and control group was 5.51, 19.0 and 26.7%, respectively. Such results provide an evidence that sage syrup improved the effect of methomyl intoxication.

PW-189

Involvement of PKA, but not Epac, in flavonoid accumulation in cell cultures of *Hypericum androsaemum*

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Hypericum androsaemum L. is a perennial herb found in damp or shady places throughout Europe. Leaf infusions have been used traditionally for its diuretic, cholagogue, and hepatoprotective properties.

Cell cultures established from hypocotyl-derived callus of *H. androsaemum* were reported [1] to accumulate small amounts of flavonoids, with the highest levels occurring on the 14th day of the growth cycle.

Later on [2], it was found that treatment of 11-day-old cultures for 72h with 15 mM $CaCl_2$ or 5 μM calcium ionophore A23187 induced a substantial increase in the accumulation of

flavonoids and in the activity of phenylalanine ammonia-lyase (PAL, a key enzyme of phenolic metabolism).

Because Ca^{2+} and cAMP are known to interact with each other and influence several metabolic processes, similar experiments were conducted using 100 µM db-cAMP, a membrane-permeable cAMP analog. The treatment with this cAMP modulator also induced a marked increase in both PAL activity and flavonoid content of cells measured on day 14.

To understand the mechanism by which cAMP exert these effects, cell cultures were then treated with either 50 µM Sp-cAMPS, an activator of cAMP-dependent protein kinase A (PKA), or 50 µM 8-pCPT-2'-O-Me-cAMP, an activator of exchange protein directly activated by cAMP (Epac). Addition of the PKA agonist to control cultures mimicked the Ca^{2+}-induced enhancement of flavonoid accumulation and PAL activity, whereas the Epac activator had no significant effect on the levels of these parameters.

Taken together, these results suggest that PKA, not Epac, plays a role in regulating flavonoid accumulation in *H. androsaemum* cell cultures.

[1] Paranhos A. Effect of calcium on enzyme activities and phenolic accumulation in *Hypericum androsaemum* cell cultures. Planta Med 2006; 72: 1060-1061.

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PW-190

Investigation of complex protein patterns of latex proteins of various *Euphorbiaceae* Juss. by two dimensional gel electrophoresis

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For analysis of proteins, polyacrylamide gel electrophoresis is a widely used technique, to separate proteins according to their electrophoretic mobility.Two dimensional gel electrophoresis (2-DE), described by O´Farrel, represents one of the most appropriate techniques for separation of complex protein patterns, and combines IEF in the first dimension, so proteins are separated according to pI and separation due to the moleclar weight in the second dimension. Due to the capability a lot of possibilities were established for plant proteomic analysis.

However, to obtain high quality resolution of proteins in proteomic analysis, sample preparation is important for optimal results. Plant tissue is often rich in compounds that interfere with 2-DE e.g. phenolic compounds and natural rubber. Most of the difficulties in plant proteomics are associated with co- extraction of non- protein components, which affect separation of proteins and causes to protein loss.

In our investigation we explored an approach to separate proteins from latices of genus *Euphorbia* L. in particular by 2-DE, to elucidate the complex composition of containing proteins. Different protein patterns were analyzed, and compared by software analysis based

on the corresponded number of spots. Comparative analysis exhibited different protein patterns and allowed the conclusion of the occurence of individual protein patterns of different species.

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[3] Wahler D. Proteomic analysis of latex from the rubber- producing plant *Taraxacum brevicorniculatum*. Proteomics 2012; 12: 901- 905plant Taraxacum brevicorniculatum. Proteomics 2012; 12: 901- 905

PW-191

Effects of pre-chilling, incubation temperature and light on seed germination of *Saponaria officinalis*

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Saponaria officinalis L. has been used as a soft cleaning agent and medicinal plant. Understanding germination patterns of seeds of S. officinalis is required for domestication. We examined seed germination behaviors in *S. officinalis* by pre-treatment with chilling (4 ℃), incubation at two constant (20 ℃ and 25 ℃) and an alternating (20 ℃/30 ℃) temperatures, and incubation in dark and light/dark (8h/16h) conditions. Although the seeds had a seed viability of 96% determined by tetrazolium test, it showed 0.5~3% of the germination rate at 20℃ and 25 ℃ in 28 days after incubation. When incubated at 20℃/30℃, seeds showed that 75% of the germination rate and 10.2 of the number of days to germination. Pre-chilling promoted seed germination to 85% and 97% in 20 ℃- and 20 ℃/30 ℃-incubation, respectively. The number of days to germination intensively decreased to 7.7 at 20℃/30℃. Any positive effect of pre-chilling on seed germination was not found at 25 ℃-incubation. Light seemed to have negative effect on seed germination of *S. officinalis* as the germination rate strikingly decreased to 2.5% in 20 ℃- and 75% in 20 ℃/30 ℃-incubation under light/dark condition despite pre-chilling. These results indicate that pre-chilling and alternating temperature accelerate seed germination of *S. officinalis* and keeping dark during incubation is more effective.

PW-192

Levels of markers for smoking and oxidative stress in the urine of Korean adults

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Reactive oxygen species (ROS) can attack lipid, protein and nucleic acid simultaneously in the living cells. In nuclear and mitochondrial DNA, 8-hydroxydeoxyguanosine (8-OHdG) is the most frequently detected [1]. Oxidative stress is free radical-mediated damage caused by excess levels of ROS. Increased oxidative stress is associated with increased DNA damage, obesity, carcinogenicity, coronary heart disease and various chronic diseases. Smoking increases oxidative DNA damage by ~50%. In this study, we performed biomonitoring for cotinine and 8-OHdG in 1000 Korean adults' urine by developed and validated method.

We developed simultaneous determination method of cotinine as marker of smoking and 8-OHdG as oxidative stress marker. 8-OHdG and cotinine in human urine were determined by LC-MS/MS (API 4000) techniques, after pretreatment by solid-phase extraction.

Linearity of calibration curve of target compounds were sufficient for analysis of these compounds at ppb level, with the coefficient of determination (r2) higher than 0.999. The accuracies was 93 to 105% and precision was lower than 10% RSD. Very strong positive correlation was found in the result of cross validation results of cotinine and 8-OHdG, performed by two laboratories. Cotinine and 8-OHdG were detected from all of the urine samples. The mean±SD of 8-OHdG and cotinine were 4.46±7.7, 292.8±638.5 ng/mL, respectively. We found statistically significant positive correlation between urinary 8-OHdG and cotinine concentration ($p<0.001$). The urinary concentrations of 8-OHdG were varied from general characteristics and life styles of subjects.

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PW-193

Alcohol-based natural deep eutectic solvents (NADES) as green solvents for extraction of mangostins from*Garcinia mangostana* pericarp

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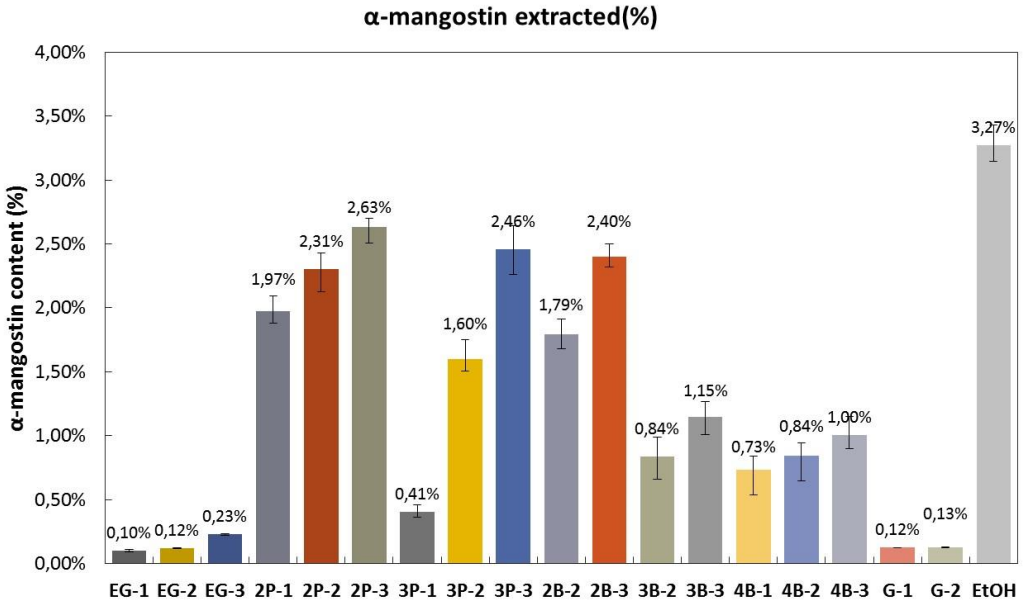

Natural deep eutectic solvents (NADES) are emerging as green solvents for extraction of bioactive compounds from plants [1]. They are usually non-toxic, environmentally friendly, and cost less than ionic liquids. Initially, NADES consisting of choline chloride and various hydrogen-bond donors or HBDs (1,2-propanediol, glycerol, citric acid, d-(+)-glucose) were screened as solvents for extraction of α-mangostin from the pericarp of mangosteen (*Garcinia mangostana L*). As the highest extraction yield was obtained using 1,2-propanediol, polyalcohols were further investigated as HBD compounds. The highest extraction yield of 2.6 wt-% was obtained using choline chloride and 1,2- propanediol eutectic mixture in 1:3 molar ratio after shaking at 30 °C for 4 h, comparable to the extraction yield of 3.3 wt-% obtained using ethanol as the organic solvent. LCMS analysis showed that both extracts contained mangostins, quantitatively in the order of α-mangostin>β-mangostin>γ-mangostin. Evaluation of the extraction yield data showed that polyalcohols containing two adjacent hydroxyl groups and a non-polar alkyl group gave better extraction yields. The polarity of each NADES was determined using Nile Red as the solvatochromic probe, however, we found no significant correlation between the polarity of the solvent and the extraction yield data. In conclusion, the excellent properties of NADES with alcohol-based HBD and choline chloride show their potential as green solvents for extraction of bioactive compounds. The mixture code in the picture signifies the HBDs tested: ethylene glycol (EG), 1,2-propanediol (2P), 1,3-propanediol (3P), 1,2-butanediol (2B), 1,3-butanediol (3B), 1,4-butanediol (4B), glycerol (G), and ethanol (EtOH); the last digit signifies the HBD to choline chloride molar ratio.

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PW-194

Effects of *Melissa officinalis* hydromethanolic extract on DNA damage induced by bleomycin in normal human dermal fibroblasts

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Natural plant antioxidants can protect cells against oxidative damage caused by various agents or pathological conditions.*Melissa officinalis* L., lemon balm, is a valuable aromatic and medicinal plant used in the food, pharmaceutical and cosmetic industries [1]. In this study, a hydromethanolic extract (MOE) obtained from lemon balm leaves was investigated for its ability to protect against DNA damage induced by bleomycin (BLM) in normal human dermal fibroblasts (NHDF). The chemical analysis of MOE was carried out using RP-HPLC-DAD. DNA damage was monitored by the comet and cytokinesis-block micronucleus assays. An increase in the comet parameters (tail moment, olive tail moment, %DNA in tail) was noticed following exposure of NHDF to MOE (25, 100 and 200 mg/mL) as well as to BLM (10 mg/mL). The treatment with MOE (200 mg/mL) produced the most pronounced effect: tail moment increased from 2.21±0.17 to 56.63±1.59, olive tail moment from 3.79±0.17 to 45.93±0.94 and %DNA in tail from 7.65±0.35 to 52.18±0.98. The exposure of NHDF to MOE (25 and 100 mg/mL) after preincubation with BLM resulted in an insignificant decrease of comet attributes. Conversely, MOE (200 mg/mL) exhibited a potentiating effect; thus, %DNA in tail in BLM-treated cells was 46.79±1.27 whereas in BLM+MOE 200-treated group it increased to 61.47±1.97. At 200 mg/mL, MOE caused an increase in the micronuclei frequency (18.56%) compared with the control. MOE did not protect against BLM-induced DNA damage. Moreover, MOE itself exhibited, in a concentration-dependent manner, some degree of genotoxicity.

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Pharmacokinetics of phytochemicals

PW-195

Plasma levels of rosiridin after oral administration of a *Rhodiola rosea* extracts in rats

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Extracts from *Rhodiola rosea* roots and rhizomes (RRE) are used therapeutically as adaptogens to mitigate stress-associated fatigue, attention deficiency, asthenia and anxiety [1]. To date, rosavins and salidroside have mostly been discussed as bioactive constituents. Another main component, rosiridin, a monoterpene, has until now received only limited attention. Although pharmacological activity of rosiridin (e.g. inhibition of MAO) has been described in vitro, data on oral bioavailability are not available. Therefore, in the present study we comparatively evaluated the plasma pharmacokinetics of the main constituents in rats after oral administration of RRE.

Eight rats were treated with an RRE (28 mg/kg p.o.) containing rosavins (5.5%), salidroside (1.4%), rosin (0.8%), and rosiridin (5%). EDTA-plasma was collected before and at defined time points after administration. The plasma concentration of the main ingredients was quantified after solid phase extraction by HPLC-MRM-mass spectrometry.

All quantified components of the RRE could be detected and displayed distinct Cmax-values at 15 to 30 min after application: rosavins (25.5 ng/ml), salidroside (50.5 ng/ml), rosin (298.3 ng/ml), and rosiridin (501.5 ng/ml). Interestingly, rosiridin was found at the highest concentration in plasma although it is present in RRE in a concentration comparable to that of rosavins. Further pharmacological research is now ongoing to prove whether this compound contributes to the health benefits of Rhodiola rosea.

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PW-196

Comparative study of natural and synthetic linalool isolated from Ginger (*Zingiber officinale*) using photochemical reactions

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Ginger (*Zingiber officinale* Roscoe) is very important plant for its medical properties from ancient time and used as spicy herb all over the world. This study was designed to examine the chemical composition of the hydrodistillated-essential oils and various solvent crude extracts (n-hexane, chloroform and ethanol) of fresh ginger rhizomes as well. 38 chemical compounds of different concentration were isolated and identified by gas chromatography-mass spectroscopy in the essential oils as citral (11.4%), curcumene (10.67%), zingiberene (28.6%), and linalool (15.0%) which represent the major chemical compounds. However, some other minor chemical constituents were also identified from the essential oil. Based on its chemical complexity comfirmed by TLC, the hexane crude extract was selected for GC-MS analysis,

and the results revealed the presence of several volatile compounds such as terpineol (13.96%), farnesol, 6-gingerol (14.3%) and linalool (11.4%) as main components. Further investigation on the structure elucidation of the selected bioactive compound (linalool) using IR, GC-MS and NMR techniques compared to authenticated linalool were studied. Linalool was subjected to purification using preparative and column chromatography. Then linalool has been epoxidized using m-chloroperbenzoic acid (mcpba) at 0 °C (thermal reaction) and the results revealed the presence of two cyclic oxygenated products which are 2,2,6-trimethyl-6-vinyle-tetrahydropyran-3-ol and 2-(5-methyl-5-vinyl-tetrahydro-furan-2-yl) as a stereospecific product. Another photo-epoxidation was carried out on linalool in the presence of florescent lamps and H_2O_2 and the results revealed the presence of the same above mentioned two cyclic oxygenated products and a new photo-epoxide compound named 2,8-trimethyl-6-oxinyl-tetrahydro-pyran-3-ol. Oxidation process is enhanced by irradiation and results in the formation of epoxide derivatives, which may be subjects of biological investigations.

PW-197

Effect of *Calliandra portoricensis* extract on the pharmacokinetics of glibenclamide in rats

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Calliandra portoricensis (Jacq.) Benth (Fam. Leguminosae), is a West African plant used traditionally for its medicinal values e.g. as analgesic and anti-inflammatory. It is widely used with orthodox medicines such as glibenclamide, a potent oral sulfonylurea antidiabetic agent. This study aims at evaluating the disposition of the pharmacokinetics of glibenclamide with or without *C. portoricensis (*CP) in rats.

Twenty rats of both sexes weighing 150±15g and allowed to acclimatize for 2 weeks were used for the study. All the animals received a single oral dose of 0.6 mg/kg body weight of glibenclamide, and blood samples (0.1 ml) were withdrawn from each rat by retro-orbital puncture at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 and 48 hrs to determine the level of glibenclamide in the plasma at each time using a validated HPLC method with a UV Detector at λ=253 nm . After 2 weeks they received 500 mg/kg of CP orally for 5 consecutive days and on the 5^{th} day were treated with the same dose of glibenclamide orally 15 minutes post administration of CP and the level of glibenclamide in the plasma determined as earlier stated.

No significant ($p \geq 0.05$) change in any pharmacokinetic parameter (elimination half-life, t½ and C_{max}, (T_{max}), volume of distribution (Vd) and clearance (CL)) was observed compared to the control. The non-significant change observed in Cmax and Tmax could be an indication of reduced rate of drug absorption which was demonstrated by the reduction in AUC. Drug elimination found to be slower after co-administration of glibenclamide; CP non- significantly decreased t½ but significantly increased Vd.

PW-198

Comparison of biotransformation efficiency to produce Compound K according to the different types of Pectinase

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The root of *Panax ginseng* C.A. Meyer is one of the most popular traditional herbal medicines in Asia region. Major active phytochemicals are ginsenosides, which have been reported to show various biological activities such as anti-fatigue, anti-obesity, anti-cancer and anti-viruses. It is thought that these activities are performed by the minor ginsenosides (F1, F2, Rg3, Rh1, Rh2, compound Y, compound Mc, and Compound K) metabolized by human intestinal microflora [1]. The metabolites as deglycosylated ginsenosides are more readily absorbed into the blood stream and function as active compounds. The metabolites could be produced via hydrolysis of the sugar moieties from the major ginsenosides using acid hydrolytic, heating, microbial, and enzymetic transformation techniques. Among these methods, the enzymatic method has been known as the most efficient preparation by its high specificity, yield, and productivity [2]. Thus, we decided to find the most appropriate type of Pectinase which is not only efficient tool for produce compound K but also industrially available. Three different enzymes, Pectinase 441L, Sumizyme AC and Plantase TCL, were performed with several various pH and temperature to optimize conditions. As a result, Plantase TCL proved as the most efficient catabolism mediator of ginsenosides to compound K. The yield of compound K from red ginseng extract was 47.147 mg/g. In addition, the optimal conditions were determined to be as follows: pH 4, 60℃ and incubation for 2 days.

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PW-199

Study of the time-dependent uptake of mistletoe lectin by cultured tumor cells

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In cancer therapy phytopreparations of *Viscum album* L. (Santalaceae) are used as an alternative treatment [1]. The main ingredients of the aqueous extracts are the mistletoe lectins

which belong to the ribosome-inactivating proteins type II [2]. In this work, the intracellular uptake of these lectins by cultured tumor cells was analysed in order to determine its time-dependency. Furthermore, we analysed the uptake in relation to the mistletoe triterpenes, mainly oleanolic acid.

The uptake of mistletoe lectin by THP 1-, HL 60-, HOS 143B- and Ewing TC 71-cells were determined after 30, 60 and 120 minutes by an enzyme linked immunosorbent assay (Sandwich-ELISA) which quantifies the a-chain of the hololectin. The isolated lectin and the aqueous extract, with or without added mistletoe triterpenes, were analysed. After 120 minutes, an intracellular uptake of 20% was reached in all cell lines by aqueous extract with added triterpenes. Furthermore, the HOS 143B-cells - unlike the other models - pick up mistletoe lectin only with concomitant mistletoe triterpenes. The uptake in THP 1-, HL 60- and Ewing TC 71-cells was time-dependent. The addition of triterpenes did not influence the process. Interestingly, the uptake of mistletoe lectin by HOS 143B-cells could only be measured after addition of triterpenes. The reason of this special behavior is still not known and needs further investigations.

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PW-200

Principal component analysis of metabolites in urine from rats treated with procyanidin A2 and two oligomeric procyanidin fractions from cranberry

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Cranberry *(Vaccinium macrocarpon)* fruits are widely used for the prevention and treatment of urinary tract infections. The mode of action is usually explained with an inhibition of bacterial adherence to the urothelium due to the content of oligomeric procyanidins (OPC) with A-type bonding. However, oral bioavailability of OPC is limited and it is unlikely that they reach the urinary tract as such. Instead, it is assumed that metabolites like procyanidin dimer A2 (PC-A2) or further degraded compounds contribute to the biological effect. Therefore, changes of the urinary metabonom may be helpful for identifying relevant marker substances or even metabolites.

Each four rats were treated orally with vehicle, pure PC-A2 or two OPC fractions with different degrees of polymerization (DP), respectively. Urine samples were collected during defined intervals over a period of 48 h. Besides quantification of PC-A2, all further detected signals were characterized by PES-HPLC-MS2. More than 100 signals were identified by mass (m/z), retention time, and area, and were subjected to group comparison by principal component analysis (PCA).

PC-A2 was not bioavailable (LLOQ: 5 ng/mL) when applied in form of the pure compound (50 mg/kg) or an enriched extract (500 mg/kg) containing mainly OPC with a DP about >6.

Interestingly, PC-A2 was detected at a concentration close to LLOQ (6.7 ± 4.8 ng/ml) when given as an extract (500 mg/kg) containing mainly OPC with a low DP (about <6) including approx. 5% of this dimer. PCA revealed that urine samples from the four different groups clearly separated into distinct clusters applying OPLS-DA classification (SIMCA P version 13).

The results indicate that PC-A2 has a negligible oral bioavailability. While OPC with a higher DP are obviously not metabolized into PC-A2, it cannot be excluded that OPC with a lower DP are metabolized to PC-A2. Thus, the changes in the urinary metabonom observed by PCA are obviously due to other constituents.

PW-201

Absorption of lycopsamine from a comfrey ointment through human skin

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In the last three decades more than 350 different hepatotoxic pyrrolizidine alkaloids (PAs) have been identified in several plant species. The application of Symphiti radix is restricted in several countries due to its PA content. In medicines, the daily alkaloid quantity and duration of treatment is limited even in case of topical application. Due to the confirmed good absorption of PAs from the gastrointestinal tract the prohibition of oral use is rational, however the limitation of external application is not supported by relevant scientific data. To date such absorption experiments were not carried out on human skin which would justify the maintenance of restriction related to the application.

The aim of our work was to develop and validate a HPLC-MS/MS method for the quantitative determination of a major PA (lycopsamine) of Symphytum officinale and to carry out pharmacokinetic studies on the absorption of lycopsamine from a traditional Symphytum product through a synthetic membrane and human skin. Pharmacokinetic investigations were carried out on vertical Franz diffusion cell and the lycopsamine content of the samples were quantitate by the validated HPLC-MS/MS method.

Our results show that the amount of absorbed lycopsamine on supporting Porafil membrane in function of time changes between 0.07% and 0.72% through the analysis time (24 h). On human skin, the maximal absorption was lower (0.07-0.22%). These results are in good agreement with the results of an experiment made in rats (0.1-0.4) [1], and reassure the relative safety of the analyzed preparation.

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PW-202

Nanocochleates: innovative nanocarriers to enhance oral bioavailability of andrographolide

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Nanocochleates are cylindrical microstructures that consist of a series of lipid bilayers. They have a unique multilayered structure consisting of solid, lipid bilayer sheet rolled up in a spiral or in stacked sheets, with little or no internal aqueous space [1]. They are stable phospholipid-cation precipitates composed of simple, naturally occurring materials such as lecithin, phosphatidylserine, phosphatidylcoline, phosphatidic acid and calcium ion. Andrographolide (AG) is the main active ingredient of *Andrographis paniculata* (Burm.f.) Nees, which is used in Chinese medicine against inflammation, internal fever and upper respiratory tract infection and diarrhea due to bacteria and virus [2]. Aim of our work was to develop andrographolide loaded nanocochleates in order to improve its dissolution and bioavailability. Unilamellar vesicles were prepared according to the film hydratation method from P90G (lipid), AG and cholesterol following sonication and ultracentifugation until obtaining nanoliposomes suspension. Nanocochleates were prepared by trapping method. A solution of $CaCl_2$ (0.1 M) was added dropwise into preformed nanoliposomes under magnetic stirring at room temperature. Transmission electron microscopy exhibited nanocochleates as tubular rod structures with an average diameter of 132.2±3 nm, a polydispersity of 0.19±0.02, and a zeta potential of -15.9±1.1 mV. The average drug-entrapment efficiency was 95.6%±0.54. The gastrointestinal absorption of AG-loaded nanocochleates was simulated *in vitro* by PAMPA assay using HPLC-DAD analysis. Cochleates acted as delayed release delivery vehicles.

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PW-203

Polyphenols profiles of fermented and non fermented *Cassia obtusifolia* leaves and their antifungal activity against *Madurella mycetomatis*

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Cassia obtusifolia (Caesalpiniaceae) is a wild African plant found in wastelands in the rainy season. Its leaves are usually fermented by people from the eastern part of Chad and western Sudan [1].

C. obtusifolia leaves of both intact and fermented forms were dried, ground and extracted with 80% ethanol. The concentrated extract was fractionated sequentially using petroleum ether, chloroform, and ethyl acetate. The extracts of fermented and non-fermented and their respective fractions were screened for their activity against *Madurella mycetomatis*, the causative agent of mycetoma infection, using a microtitre plate-based assay developed by our group incorporating resazurin as an indicator of measuring cell growth [2].

All tested extracts of both the fermented and non-fermented and their respective fractions exhibited consistently significant activity against *M. mycetomatis* with MIC of 39.1 µg/mL. Interestingly, however, that analysis by TLC employing Natural Products Reagent (NPR), RP-HPLC and RP-HPLC-DAD coupled with ESI tandem mass spectrometry revealed significant qualitative and quantitative differences of the polyphenols profiles of the fermented and non-fermented leaves of *C. obtusifolia*.

It was clearly seen that the effect of fermentation resulted in a remarkable chemical transformation of the flavones present in the non-fermented leaves of *C. obtusifolia* into phenolic acids following fermentation as has been clearly demonstrated by both TLC [3] and RP-HPLC-DAD coupled with ESI tandem mass spectrometry.

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PW-204

Phytochemical investigations of Polygoni avicularis herba. Quantification of phenolic compounds with UHPLC-CAD method.

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Polygonum aviculare L. also known as common knotgrass is an annual weed spread all over the world in the temperate regions. Recent studies showed that flavonol glucuronides are major constituents of this medicinal plant material. There is no comprehensive analytical procedure for the standardization of Polygoni avicularis herba available on the European market.

The aim of study was to develop a method for the proper standardization of Polygoni avicularis herba and to evaluate variability in chemical composition composition among commercial samples and samples from wild harvesting defined as*Polygonum aviculare s. l.*

A UHPLC-ESI(+)-MS method using method using Kinetex XB-C18 column was developed for the qualitative screening of nine independent samples of *Polygonum aviculare* herb. The UHPLC-CAD method was developed and validated for the quantification of the major compounds in water extract using quercetin-3-O-glucuronide as a standard.

Twenty-five major constituents, mostly flavonoids, were detected and characterized. Among them three new natural products were tentatively identified as myricetin-3- *O*-(2" or 3"- *O*-acetyl-glucuronide), mearsetin-3-*O*-(2" or 3"-*O*-acetyl-glucuronide) and kaempferide-3-*O*-(2" or 3"-*O*-acetyl-glucuronide). Twelve compounds were quantified using a developed UHPLC-CAD method. In all nine samples flavonol glucuronides were confirmed as major constituents. The total flavonoid content was estimated for all samples and varied from 0.70 to 2.20%.

The developed procedure may be used for the standardization of common knotgrass. The results indicate that the method described in the pharmacopoeial monograph used for the authentication and standardization of *Polygonum aviculare* herb should be improved.

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PW-205

Analysis of Amaryllidaceae alkaloids in *Galanthus krasnovii* by GC-MS

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Galanthus L. species (Amaryllidaceae) elaborate Amaryllidaceae alkaloids which were shown to possess several pharmacological and biological activities. Therefore, they have been intensively studied for their content of alkaloids [1]. Gas Chromatography-Mass Spectroscopy (GC-MS) is a valuable tool for the detection, identification and quantification of alkaloids in

Amaryllidaceae plants [2,3]. In the present study, the alkaloidal profiles of the aerial parts and bulbs of*Galanthus krasnovii* Khokhr., collected from north-eastern Turkey (Kafkasor, Artvin) were investigated by GC-MS. As a result of GC-MS analysis, totally 13 alkaloids were determined. Of the known alkaloids, 11-hydroxyvittatine, anhydrolycorine, and 11,12-didehydroanhydrolycorine were found to be the main alkaloids. Also, an interesting finding of our study was the identification of an indole alkaloid, 1-acetylbetacarboline in the aerial parts of *Galanthus krasnovii*. This alkaloid was previously reported from *Galanthus rizehensis* [4].

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PW-206

Phenolic content and antioxidant activity of *Nepeta parviflora* Bieb. species from wild populations in Republic of Moldova

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The genus *Nepeta* includes species that are wildly distributed, several having a special conservation status. Many therapeutic properties of *Nepeta* species are due to the phenolic and terpenic compounds, mainly essential oils.

Within the three *Nepeta* species in the spontaneous flora of Republic of Moldova (*N. cataria* L., *N. pannonica* L. and *N. parviflora* Bieb), our study focused on *N. parviflora*, species threatened with extinction in the local flora. Our study aimed to assess the total phenolic content and antioxidant activity of *N. parviflora* methanolic extracts. In this respect, samples were harvested before the flowering stage and at full flowering, from Buceag natural reservation.

The qualitative phytochemical study was carried out by thin layer chromatography (TLC), and the quantification of total phenolic content by Folin-Ciocalteu assay. The antioxidant activity was tested by DPPH and ABTS assays.

The TLC analysis revealed the presence of the phenolic acids (chlorogenic, caffeic, rosmarinic acids) and flavonoids (luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside). Folin-Ciocalteu assay showed a higher phenolic content in the samples harvested at full flowering (104.31±2.27 mg gallic ac. equiv./g dry extract) compared with the samples harvested before the flowering stage (68.19 ± 1.49 mg/g d.e.). The antioxidant activity was in correlation with the total phenolic content, the samples harvested at full flowering having a higher antioxidant capacity (EC_{50} values of 40.7±0.3 µg/mL in DPPH assay and 13.2±0.1 µg/mL in ABTS assay) compared with the samples harvested before the flowering stage (EC_{50} values of 50.7±0.4 in DPPH assay and ±0.4 in ABTS assay). The species is rich in phenolic compounds with antioxidant activity, thus development of cultures having potential both for its capitalization and conservation.

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PW-207

UHPLC-QTOF-MS as a valuable tool for the identification of novel faradiol fatty acid diesters of*Calendula officinalis* flowers

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Calendula officinalis L. (marigold) flower extracts are recommended for the treatment of minor inflammation of the skin and wounds [1].Triterpenes from the 20-taraxastene type, especially faradiol seem to be crucial for the anti-inflammatory potential [2-3]. Besides monols triterpene diols and triols are present in the form of their C3 lauric, myristic and palmitic acid monoesters. Here we report on the isolation of a mixture of novel faradiol fatty acid diesters (20-taraxastene 3β,16β-diester). 1D and 2D NMR analysis revealed the triterpene skeleton and the esterification position. However, only after UHPLC-QTOF-MS analysis the fatty acids could be identified and assigned to the respective triterpene. This method allowed identification of intact $[M+H^+]^+$ molecular ion peaks of faradioldimyristate (863.7354 *m/z*), faradioldipalmitate (919.8302 *m/z*) and faradiolmyristate, palmitate (891.7633 *m/z*). Moreover, this technique is also suitable for the separation and identification of the arnitriol A 3- and the lupane-3β,16β,20-triol 3-fatty acid monoesters which is here reported for *Calendula officinalis* L. flowers for the first time.

Acknowledgments: We are grateful to S.Ferlaino, Department for Pharmaceutical and Medical Chemistry, University of Freiburg, Germany for measuring the NMR spectra and to Dr. Junghanns for providing us with *Calendula* flowers.

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PW-208

Optimization and validation of an analytical RP-HPLC method for the analysis of glucosinolates in*Nasturtium officinale*

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Nasturtium officinale R. Br. (watercress) is a plant that belongs to the Brassicaceae and is growing mainly in Europe and Asia. The plant contains a considerable amount of vitamins, minerals and secondary metabolites and is used in food and for its medicinal properties. These are mainly attributed to the glucosinolates which are precursors of bioactive compounds such as the isothiocyanates. Glucosinolates are sulphur containing secondary metabolites, containing a β-D-thioglucose and an aglucone. The main glucosinolate in *Nasturtium officinale* R. Br. is gluconasturtiin. Since the quality of a food supplement of *Nasturtium officinale* R. Br. depends on the content of its glucosinolates and isothiocyanates, a quantitative method was developed to analyse the glucosinolates. An existing method for the determination of gluconasturtiin [1,2] was optimized by changing the volume of the extraction solvent, extraction time and number of extraction steps. Sinigrinemonohydrate was used as internal standard. The method was validated conform the ICH guidelines [3] on the validation of analytical methods. The standard curve of sinigrinemonohydrate was linear in the concentration range of 33.2-166.2 µg/mL. The mean concentration of gluconasturtiin was 8.5 mg/g lyophilized watercress. The precision of the method with respect to time (3 days) and concentration (3 concentration levels) was respectively 9.74% and 8.96%, although relatively high, it was still accepted because of the complexity of the method.

[1] International standardization organization. Rapeseed – determination of glucosinolates content – part 1: Method using high-performance liquid chromatography 9167-1. Genève; 1992

[2] Heyerick A. Waterkers (Nasturtium officinale): onderzoek naar de invloed van procesparameters op de inhoud aan bioactieve bestanddelen. University of Ghent, report IWT-KMO portefeuille; 2010:1-27.

[3] ICH, Text on validation of analytical procedures – ICH Harmonised Tripartite Guideline, 1994.

PW-209

Analytical method with ICP-MS for heavy metals as an unintended hazardous material in commercial pharmaceuticals

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One major safety issue that has been evident for several years relates to the possible adulteration of traditional medicines with harmful ingredients such as the heavy metals lead, mercury and arsenic. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic. Detection of toxic heavy metals that exceeded Singapore's legal limits in 42 Chinese proprietary medicines was reported. Unintended contamination of heavy metals may be introduced in the finished drug from raw materials and in the process of production and it may be very low level. We developed an analytical method for 9 heavy metals as an unintended hazardous material in commercial pharmaceuticals.

A total of 105 commercial pharmaceuticals were collected, and divided into 5 kinds of formulations, tablet, solution, capsule, cream, patches. All pharmaceutical samples (0.02 ~ 0.10 g) were prepared by acid digestion with microwave (Mars-X, Agilent) after homogenization. Nine heavy metals (As, Cd, Co, Hg, Mo, Pb, Sb, Se, V) were determined in the pretreated samples with inductively coupled plasma-mass spectrometry (ICP-MS) in the CCT mode.

We tried several pretreatment methods according to the drug formulations and compared the data by the methods The linearity obtained was very satisfying for analyzing heavy metals, with a coefficient of determination (R^2) higher than 0.99. The limits of detection were 1~10 ng/g sample. The accuracies were 80% to 120% and precisions were lower than 20% RSD. Some heavy metals were identified in a few pharmaceuticals and the concentrations of heavy metals were varied depending on the formulation and efficacy of samples. Further investigation is necessary to identify source and reduce the unintended heavy metals in pharmaceuticals.

PW-210

Analysis of ingenol and its conjugates in some species of the *Euphorbia* genus by ultra-high performance liquid chromatography-tandem mass spectrometry using isotope dilution method

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Ingenol is a chemically and biologically important plant-derived diterpenoid compound, first isolated in 1968 from the seed oil of a leafy shrub *Croton tiglium* belonging to the Euphorbiaceae plant family. Ingenol is the key precursor for the synthesis of ingenol mebutate (angelate), used to treat actinic keratosis. Although, its total synthesis was published lately, isolation from natural raw material is still used for its industrial scale production. Therefore, a rich source of ingenol seems to be of great economic importance. We have developed an ultra-high performance liquid chromatography-tandem mass spectrometry method for screening of ingenol in 38 species of the Euphorbia genus. The highest ingenol concentration (547 $mg.kg^{-1}$ of dry weight) was found in the lower leafless stems of *E. myrsinites*. Moreover, three ingenol conjugates (two known: 3-*O*-deca(2'*Z*,4'*E*)-dienoyl ingenol, 20-*O*-acetyl-3-*O*-deca (2'*Z*, 4'*E*)-dienoyl ingenol and one new: 3-*O*-octa (2'*Z*,4'*E*)-dienoyl ingenol) were successfully identified in *E. myrsinites* by means of accurate mass determination.

PW-211

A metabolomics study for the quality control of Black seed oil (*Nigella sativa*) based on Gas Chromatography-Mass Spectrometry

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Black cumin seed oil (BCSO) is the cold-pressed oil obtained from the seeds of *Nigella sativa*, Ranunculaceae. *Nigella sativa* is widely cultivated throughout southern Europe and the middle east for culinary and medicinal purposes. Recently, attention has been focused on BCSO as an edible oil with high nutritive value and role in human health attributed to high content in unsaturated fatty acids including linoleic and oleic acids among other components [1]. BSCO is available as a dietary supplement and marketed worldwide, which prompted to study the quality differences in the oil metabolome globally and conclude an association with geographic distribution. A total of 28 samples of marketed BSCO were collected from Egypt, Libya, Saudi Arabia, Ethiopia, Syria and Europe. The oils were analyzed using GC-MS carried out on a RTX-5MS column using temperature programming from 45 to 300° at 5°/min rate and fatty acid composition was determined following the reported protocol [1]. A total of 125 of oil components were identified using Kovat's index and comparison of mass spectra to NIST Mass Spectral Library. The normalised GC peak areas were measured, expressed as percentages and 14 common components were used in the Principal Component Analysis (PCA) data set.

The loading plot revealed the major peaks: linoleic acid (LA 0%-33.5%), oleic acid-methyl ester (OAM 7.3%-49.6%) and oleic acid (OA 0%-12.9%) as main chemical markers contributing to the segregation. The PCA score plot showed that most Egyptian samples were segregated above the line demarcating PC2 showing high concentrations in all three components while European, Ethiopian and Syrian samples were clearly segregated below the PC2 line with much lower values.

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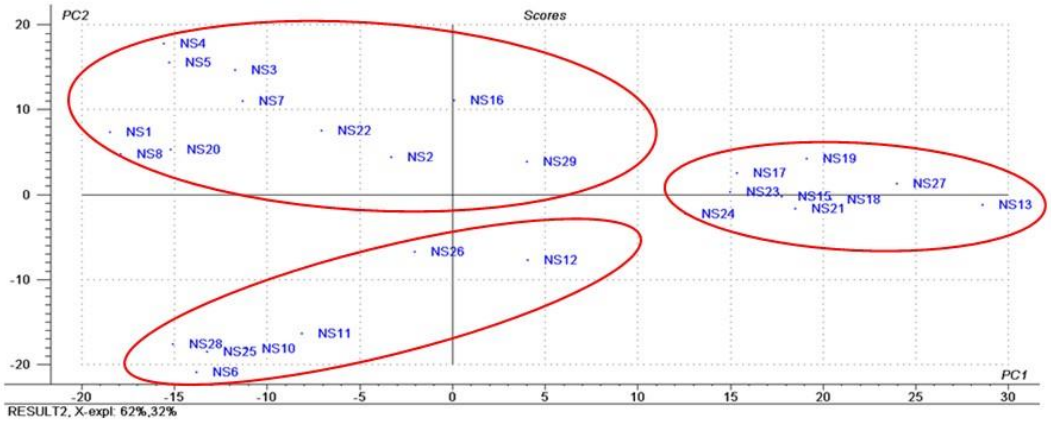
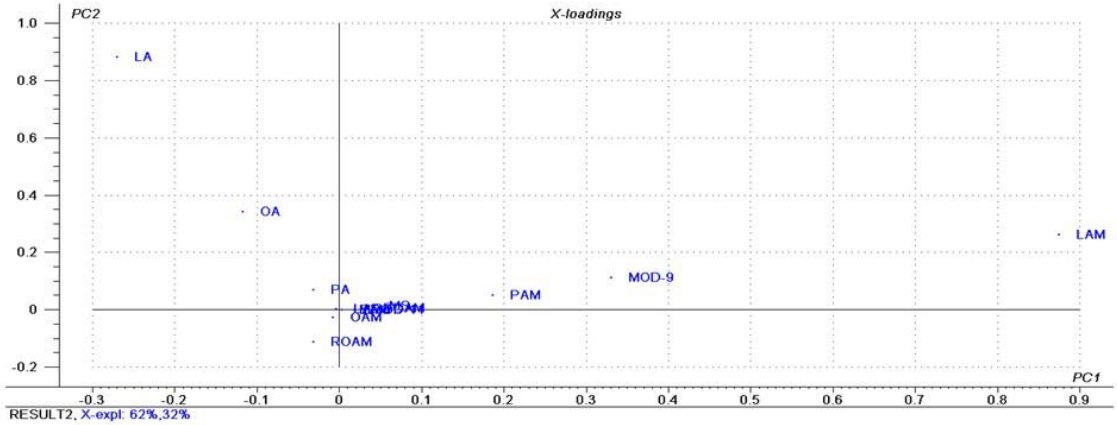


PCA score plot showing segregation of 28 samples of black cumin seeds oil



Loading plot showing major chemical markers

PW-212

Stability study of Meliloti herb extract: coumarin and flavonoid content

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Yellow sweet clover - *Melilotus officinalis* (L) Pallas, Fabaceae is a medicinal plant traditionally used in the phytopreparations for the treatment of the chronic venous insufficiency. Coumarin heterosides are the most important ingredients of this drug and have been regarded responsible for the mentioned activity. The flavonoids contribute to the overall efficacy. The aim of our study was to monitor stability of these ingredients within the extract which has been known as constituent of the herbal dietary supplement used in the treatment of venous insufficiency placed on market in Balkan countries. In Meliloti extract, the content of coumarin and flavonoids were determined according to accepted tests for monitoring stability during the period of one year. Plant material was collected during the flowering period on the mountain Cer, Serbia, at the end of June 2013. Ethanol 67% v/v was used as a solvent. The ratio of drug-extract was 1:5. Samples were kept in Stability chamber Rumed®- Germany (temperature 25° C; humidity 60%) for the period of the investigation. For the coumarin content determination, HPLC Pharmacopoeia method was applied [1]. The flavonoid content was determined by Markham method, with rutin used as standard. The evaluation of the active substances content was performed after 3, 6 and 12 months of storage. The start of examination, revealed that the cconcentration of coumarin was 0.074%; flavonoid content was determined to be 0.035%. Our investigation confirmed that the coumarin and flavonoid content in the extract was constant during the one year. The differences of the determined values were in the acceptable range of ±1%.

[1] European Pharmacopoeia 7.0

PW-213

Active Component Composition and Efficacy Variability in North American Ginseng

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North American Ginseng has a history of use by indigenous peoples of North America and has been harvested for export from the wild in Canada since the 1700's as well as grown commercially under artificial shade since the 1930s. As it is a protected species in Canada, there is not a wild source of the plant available for commercial use and the commercial crop consists largely of collections of producer-maintained land races. It is grown mostly on the sandy loam soils of southern Canada and surveys of the root product from that area since 2007

have shown wide variation in root composition and the efficacy of immune-stimulatory and anti-oxidant activities in in vitro assays. As this crop is valued for its neutracetical properties, the high degree of variability is of concern for producers, natural health practitioners and consumers. The long reproductive cycle of ginseng has been prohibitive to the development of genetically superior and predictable-performance cultivars and the genetic basis of this unimproved crop is believed to be the primary reason for the variability in the root product. A clonal propagation protocol was developed and used to produce lines from "heritage" seed collections and clonal lines have been grown in the field since 2009. These clonal lines show potential for superior cultivar development. Ginsenosides are the widely recognised active component in ginseng and six forms make up the majority of the ginsenoside content; this content has been observed to vary from 1% to 11% in mature commercial root. Evaluation of the anti-oxidant activity using an ORAC assay adjusted to Gallic Acid Equivalents revealed a 4-fold range difference in individual lines grown under commercial field conditions and assessment of the immune-stimulatory response in clonal lines also showed a 9-fold range difference of activity. Assessment of 1- to 6-year old clonal lines confirmed that there is a strong genetic component responsible for the observed variability.

PW-214

Discrimination of Zi Cao species based on genomic analysis, TLC and HPLC

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Plants used in traditional Chinese medicine (TCM) represent a great source of novel lead compounds due to its long history and tradition. However, the exact identification of the plant material sometimes remains a problem because of trivial and local names or wrong identification. Since plants differ in their chemical composition this mix-up can lead to serious health problems when applied. Roots of the genera *Onosma*, *Arnebia* and *Lithospermum* are traditionally known as Zicao. On markets they are sometimes distinguished by prefixes such as Ruan Zi Cao (*Arnebia euchroma*), Dian Zi Cao (*Onosma paniculata*) or Ying Zi Cao (*Lithospermum erythrorhizon*). But even this distinction does not protect from mistakes. The main active principles in Zicao species are shikonin and alkannin derivatives. The exact content and composition varies within the different species. In this study, we investigated 16 samples from various places and identified them using genomic analysis. Moreover, we tried to find a TLC and/or HPLC method to identify them phytochemically. 13 of the samples were sold as Arnebia euchroma and three as Lithospermum erythrorhizon. After genomic analysis, we identified 5 samples as *Arnebia euchroma*, one as *Onosma paniculata* and one as *Lithospermum erythrorhizon*. The rest of samples could not be identified by DNA analysis. Moreover, we did not succeed to distinguish the different species unambiguously by using HPLC. However, we were able to develop a method to distinguish the species by TLC on the basis of several blue fluorescent zones.

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PW-215

Effect of water supply on the accumulation levels of phenolic compounds, essential oil properties and antioxidant activity of *Thymus vulgaris*

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Thyme is a widely cultivated medicinal plant adapted to sunny and dry habitats. Essential oil properties of Thymi herba can be quite different, depending both on the growing site and chemotaxon. Among phenolic compounds, rosmarinic acid is the most relevant, however, the importance of phenolic acids and flavonoids is also well documented. In our studies we were aimed at evaluating the effects of water deficit on different thyme taxa ('Varico 3' and essential oil chemotypes of TV17- thymol, TV115- geraniol, TV143- alpha-terpineol), with special respect to its impact on the accumulation levels of active compounds. A pot experiment was conducted among controlled conditions in 2014, where soil water capacity (SWC%) values were kept at 40% (S: stress condition) and 70 % (C: control condition), respectively. Three harvest periods were included when vegetative shoots were sampled for detecting the levels of essential oils (EO: ml/100 g DW) and their compounds (GC %), rosmarinic acid (RA: mg/g by HPLC), flavonoids (FL: %, Ph. Hg. 8), total phenolics (TPC: mg/g GSE), and antioxidant capacity (AC: mg/g ASE by FRAP assay). According to our results, the content and composition of active substances were highly affected by water supply, while sampling time and genotype were also influencing factors. In general, higher levels of RA, TPC and AC have been detected under stress conditions. However, negative effects of water deficiency were observed when measuring the amounts of EO and FL. Significant differences were found among taxa involved, concerning drought stress tolerance: the lowest differences between S and C plants were measured at TV 17. During our experiment, increasing levels of RA and TPC were found, while EO content decreased. Among essential oil compounds, percentages of geraniol and thymol were generally higher in C plants, while alpha-terpineol % changed in an opposite direction.

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PW-216

Comparison of ICP-MS and direct mercury analyzer for the analysis of mercury in pharmaceuticals

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Mercury (Hg) is a harmful heavy metal and non-essential element. Also Hg has bioaccumulating characteristics and is rated as one of the most hazardous heavy metals. It seems very toxic at only trace amount. But Hg is found at various foods and cosmetics as part

of an ingredient by chance. Unintended contamination of Hg may be introduced in the products from raw materials and in the process of production and it may be trace level. This study compared the determination method for mercury by instruments (Hydra-ⅡC and ICP-MS).

One method for mercury determination was by the Hydra-ⅡC direct mercury analyzer (Teledyne Leeman Labs, Hudson, NH, USA). The Hydra-ⅡC have fully automated operation and pre-treatment is unnecessary. The other was by inductively coupled plasma-mass spectrometry (ICP-MS) in the CCT mode after microwave digestion in closed pressurized vessel. Each method was validated for limit of detection, linearity, accuracy and precision before comparison.

The linearity of analyzing mercury obtained by two methods were very satisfied, with a coefficient of determination (r^2) higher than 0.99. The limit of detections was 0.2 ng/g sample for Hydra-ⅡC and 2.0 ng/g for ICP-MS. The accuracies for both methods were 80% ~ 120% and precisions were lower than 20% relative standard deviation. The concentrations of mercury in samples detected by two methods were comparable.

PW-217

Microscopic control of Rhizoma Curcumae Longae using multivariate analysis

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Rhizomae Curcumae Longae is the dried rhizome of *Curcuma longa* L. (Zingiberaceae), commonly known as turmeric. This herbal material has a long history of traditional use for culinary purposes as a spice and as a food colorant. In Algeria, turmeric is known in Arabic as «Kurkuma», and used in culinary and in traditional medicine [1]. This study was aimed at establishing the microscopic identification of different commercial samples and developing parameters for discriminating turmeric powders. Fifteen samples from different origins were analyzed, and each experiment was performed in triplicate. Statistical techniques were used to analyze the partition of the structure observations.

The homogeneity or the variability was investigated by multivariate test. Multiple Correspondence Analysis (MCA) was also applied in the course of the experiments, using SPSS Statistics 17.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA, 2008). In consequence, eleven discriminating structure features were identified. The most diagnostic features are yellow clumps of gelatinized starch, covering trichome, starch granules, vessels, cork, and fibers. The results showed that microscopic observation of powder samples of rhizoma Curcumae longae could be grouped according the presence of non-glandular trichome, and calcium oxalate crystals clusters. Moreover, it appeared that the presence of calcium oxalate cluster is a discriminated parameter of the group II and differentiated between subgroups. Indeed, this criterion is rarely observed and is present only in three observations of the subgroup II-1. These findings revealed that microscopic analysis, coupled with statistical methods, could provide a simple platform for medicinal plant identification, particularly for the diagnostic authentication of commercial samples.

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PW-218

The effect of harvest time on essential oil content and composition of holy basil (*Ocimum sanctum*)

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Holy basil (*Ocimum sanctum*) is one of the most important medicinal plant belongs to Lamiaceae family. Holy basil is a popular home remedy for many ailments such as bronchitis, liver diseases, catarrhal fever, lumbago, hiccough, gastric disorders, genitourinary disorders and skin diseases [1]. In order to evaluate the effect of harvest time on essential oil content and composition of holy basil, an experiment was conducted at research farm of Department of Horticultural science, Shahid Chamran University of Ahvaz based on randomized complete block design, with three treatments and three replications. The treatments were harvest times; first, second and third harvests. Plants were harvested at flowering stage in May, August and November for first, second and third harvests, respectively. Plants were dried in shade place and room temperature. Essential oils were extracted by Clevenger apparatus with three hours distillation time. The oils were analyzed by gas chromatograph and gas chromatograph equipped to mass spectrometry. There was not significantly difference in essential oil content between three harvests. Main essential oil components at three harvests were alpha pinene (1.21-1.40%), beta phelanderene (0.75-1.18%), beta pinene (1.77-2.57%), 1, 8 cineol (18.40-24.79%), ocimene (3.43-5.24%), alpha terpinyl (0.99-1.36%), methyl chavicol (11.18-12.05%), chavicol (0.79-1.10%), eugenol (30.29-37.85%), methyl eugenol (0.9-1.06%), caryophylene (0.86-1.03%), beta farensine (1.34-1.68%), bisabolene (5.77-7.73%) and alpha bisabolene (4.23-4.60%). The highest eugenol (37.85 %) was obtained at second harvest. The lowest value of eugenol was observed at first harvest. According to result on essential oil content and composition, three harvests of holy basil are recommended.

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PW-219

Optimization of extraction method and evaluation of minimum content of sesquiterpene lactones in*Aucklandia lappa* roots

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Aucklandia lappa Decne. is indigenous in India, Pakistan and China. It is widely used in traditional ancient medicines of China, Ayurveda and Tibet. Its active constituents are terpenes but also anthraquinones, alkaloids and flavonoids which possess antifungal, antimicrobial,

anthelmintic, antitumor, antiulcer, immunostimulant, antiinflammatory and antihepatotoxic properties [1]. Aim of this study was to optimize the extraction method of the roots of *A. lappa* and to evaluate the minimum content of costunolide and of the sum of costunolide and dehydrocostus lactone. As a starting point the Monograph of the Chinese Pharmacopoeia was used [2]. 14 different extraction methods were evaluated. Maceration time, shaking time, the type of sonication and the duration were investigated. The best extraction method was 1 hour of shaking plus 30 minutes of sonication bath, using 100% methanol. ^{1}H-NMR analysis was performed directly on the powered herbal drug using deuterated DMSO to extract the constituents. Characteristic signals of both sesquiterpenes were not present in the extracted herbal drug. The optimized method is able to extract from 82.2% to 97% of sesquiterpens lactones saving 23 hours. The minimum content of costunolide and dehydrocostus lactone in 7 different samples was calculated. Five samples contained more than 1.8% of the sum of constituents, according to Chinese Pharmacopoeia limits. A high instability of sesquiterpenes was found after powdering the herbal drug with a loss of about 20% of active compounds after 15-20 days. It is strongly suggested to use fresh herbal drug powder to avoid errors in the quantification of constituents.

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PW-220

Identification and quantification of an sntibacterial flavonoid from the complex extract prepared from six species of medicinal plants

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Bacterial diseases have been severe problems in aquaculture. The wide and frequent use of antibiotics in aquaculture has resulted in the development of antibiotic resistance and problems in food safety [1]. In our on-going research to discover natural products with antimicrobial properties concerning fish bacterial pathogens, we previously found that the complex extract prepared from 6 species of medical plants, seeds of *Benincasa hispida* C., peels of *Citrus unshiu* M., flowers of*Lonicera japonica* T., leaves of *Perilla frutescens* B., roots of *Scutellaria baicalensis* G. and *Sophora flavescens* A. has antibacterial, antiparasitic and antifungal activities against fish pathogens. In this study, we identified an antibacterial compound from the complex extract through bioactivity-guided isolations and quantified it using HPLC/UV analysis. Antibacterial activity-guided fractionation for the complex extract yielded the active methylene chloride (MC) fraction with minimal inhibitory concentrations (MICs) of 1 mg/ml against *Edwardsiella tarda* and *Streptococcus iniae* in microdilution method. From further isolations for the MC fraction, an active compound, baicalein was obtained with MICs of 0.125 and 0.25 mg/ml against *E. tarda* and *S. iniae*, respectively. The content of baicalein was found to be 9.8% of the active MC fraction. The method developed allowed the limits of detection and quantification of 0.757 and 2.295 μg/ml, respectively. The calibration curve showed good linearity (r >0.9993) within the test range. Intra- and interday precisions were good with RSD < 6.52%. The average recovery was 105.36%. These results suggested that the active MC

fraction of the complex extract may be used as an antimicrobial alternative for fish bacterial diseases and the HPLC method can be used for the quality control.

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PW-221

Comparative assessment of polyphenolic content and antioxidant capacity of *Arnica montana* samples differentiated on organ types from wild populations in the Romanian Eastern Carpathians

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Arnica montana L. flowerheads are mainly used for therapeutic purposes. In the last years a high interest in using the whole plant was observed, both for human and veterinary uses. Considering the conservation status of the species, the use of the whole plant is possible only through development of cultures. Although the anti-inflammatory activity of arnica extract is mainly associated with the specific action of sesquiterpen-lactones, the phenolic compounds have an important role due to their antioxidant activity.

The aim of our study was the comparative assessment, on organ type (flowerheads, leaves, root and rhizome, respectively) of the total phenolic content (Folin-Ciocalteu method), and antioxidant activity of the methanolic extracts. In vitro antioxidant activity was investigated by DPPH and ABTS assays; caffeic acid was used as positive control. We report for the first time the antioxidant activity for the whole plant harvested from the Romanian Eastern Carpathians.

The highest phenolic content was found in the leaf extract (154.42±1.65 mg gallic acid Equivalents (GAE)/g), followed by the root (140.73±0.74 mg GAE/g) and flower extracts (130.43±1.59 mg GAE/g). All extracts showed important antioxidant activities. However, reducing power of the leaf extract (0.776±0.005 at 5 mg/mL) was higher than that of the other *Arnica*extracts (0.682±0.007 and 0.643±0.005 for the root and flower extracts). At 5 mg/mL, leaf extract displayed DPPH (90.63±0.22 %) and ABTS (97.26±0.08 %) quenching capacities similar to those of the standard - caffeic acid (DPPH scavenging activity: 97.69±0.03 % and ABTS scavenging activity: 100.34±0.03 %).

Along with flowers, leaves and underground parts of the plant (root and rhizome) are important sources of phenolic compounds with significant antioxidant activity.

Acknowledgement: The work was sustained through the program Partnership in Priority Areas – PNII, implemented with the support of MEN – UEFISCDI, Romania, project No. 74/2014.

PW-222

Comparison of HPLC-UV and NMR methodologies for the quantification of Silymarin complex in*Silybum marianum* fruit extracts

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Silybum marianum (L.) Gaertn. (Asteraceae) is one of the most investigated plant extracts with known mechanism of action. Milk thistle preparations have been used to treat a variety of ailments, particularly against liver damage. The beneficial properties of *S. marianum* are ascribed to silymarin, a mixture of (at least) six flavonolignans and one flavonoid, used in clinical research as well as in dietary supplements. The separation of individual compounds from the complex mixture of regioisomers remains a challenging task. This study presents two validated HPLC-DAD and NMR methods, according to the ICH guidelines, for the simultaneous determination and quantification of six bioactive compounds in *S. marianum*extracts.

A HPLC method was developed for determination of flavonolignans incorporating rapid separation with highly sensitive UV detection. The method, beside the analysis, was used for their quantification in the fruit extracts. As *Silybum*flavonolignans are difficult to obtain as fully characterized pure compounds, the development of a non-targeted approach by quantitative 1H NMR represented an attractive alternative to conventional chromatographic analysis. Besides quantification, qNMR provides valuable structural information, requires simple sample preparation and reasonably short measuring times, especially with contemporary NMR instrumentation. Silybins and isosilybins exhibit near-identical 1H NMR spectra, which led us to quantify them in pairs. Measurement of two different types of extracts showed comparable results in composition between the two different techniques.

For more than five decades, silybins and isosilybins have presented a wealth of challenging interdisciplinary research problems. The successful implementation of the current work could comprise an established approach for future investigations, giving new insight into the quantification of bioactive compounds of other plant species containing complex mixtures of isomers.

PW-223

Ionic liquid-assisted extraction as a sample preparation technique for HPLC determination of biologically active alkaloid galantamine in *Leucojum aestivum* (Summer snowflake)

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Galantamine is a biologically active alkaloid used for the treatment of mild to moderate Alzheimer's disease and various other memory impairments, in particular those of vascular origin.

Leucojum aestivum L. (Amaryllidaceae), commonly named as Summer snowflake, is a plant species widely cultivated as an ornamental species, but it is also the main source for the industrial production of galantamine. Therefore a fast and exhaustive method for determination

of this alkaloid in the plant material is desired. The current protocol consists of sequential extractions with acidic (H_2SO_4) aqueous solution and its implementation takes more than 15 h.

In order to improve the extraction step we studied a series of hydrophilic 1-alkyl-3-methylimidazolium-based ionic liquids (ILs) as additives instead of H_2SO_4 in the extraction of galantamine from plant material of *L. aestivum*. The extractions were carried out both under ultrasonic and conventional heating conditions and the extraction efficiency was monitored by HPLC analysis. The influence of the anion, alkyl chain length in the imidazolium ion, IL concentration, extraction time, particle size and solid-liquid ratio on the extraction efficiency was comprehensively investigated. As a result, optimal conditions for quantitative extraction of galantamine with 5% aqueous solution of 1-butyl-3-methylimidazolium chloride {[C_4C_1im]Cl} were found. The system under study was shown to provide same extraction efficiency in comparison with the conventional method, but with significant reduction in extraction time (from 15 h to 1 h). The data obtained resulted in the development of an analytical procedure for determination of galantamine in plant material of *L. aestivum*. This could be of a great importance from an industrial standpoint due to the faster and safer nature of the proposed method.

Acknowledgement: The financial support of the National Science Fund of Bulgaria (project DFNI T02/23) is greatly acknowledge by the authors.

PW-224

Antioxidant activity of methanolic extracts of *Lamium album* and *Lamium maculatum* species from wild populations in the Romanian Eastern Carpathians

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Lamium genus includes species which, due to their medicinal properties, were studied for specific classes of biologically active compounds, mainly iridoids, terpenic compounds and polyphenols. *Lamium* plants are used in official and folk medicine as antiproliferative, antioxidant, antiinflammatory agents, for treatment of skin and respiratory disorders.

The aim of this study was to assess the phenolic content of *L. album* L. and *L. maculatum* L. methanolic extracts, and their antioxidant capacity. The phytochemical analysis was performed by means of thin layer chromatography (TLC - qualitative analysis) and spectrophotometry (quantitative analysis). The antioxidant activity was determined by two complementary test systems: DPPH and ABTS assays.

The plant material consisted of *L. album* and *L. maculatum* herba samples harvested from the Romanian Eastern Carpathians, in 2013 and 2014. For the antioxidant assays, there were selected several samples harvested in 2014 from the same site.

The TLC fingerprint showed the presence of phenolic acids (chlorogenic, caffeic acids) and flavonoids (rutin). The spectrophotometric analysis highlighted a higher phenolic content in *L. maculatum* samples (105.86 mg Gallic acid equiv./g dry extract), when compared with *L. album* samples (72.63 mg/g d.e.). *L. maculatum* extract showed a higher antioxidant activity

than *L. album* extract in both antioxidant assays. For the DPPH assay the EC_{50} (μg/mL) values were 32.3±0.1 for*L. maculatum* extract and 63.5±0.7 for *L. album* extract, while in the ABTS assay EC_{50} (μg/mL) values were 13.2±0.1 for*L. maculatum* extract and 19.9 ± 0.5 for *L. album* extract.

The antioxidant activity of the tested extracts is due mainly to the phenolic content. Thus, the tested *Lamium* species show potential for the development of natural products with therapeutic use.

Acknowledgement: The work was sustained from the Bilateral Collaboration Project RO-MD (694/2013) and the project PN 09-360401 (BIODIV) financed by ANCSI, Romania.

PW-225

One single standard substance for the simultaneous determination of seven coffee polyphenols in green coffee bean extracts

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Coffee polyphenols [1] in green coffee bean extracts (GCBE) have many healthy benefits. These polyphenols called chlorogenic acids included 3-CQA, 4-CQA, 5-CQA, 5-FQA, 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA. Due to the limited availability of reference standards, multi-components analysis is not easy. In this work, the quantitative analysis of multi-components by single reference standard was proposed to simultaneously determine the contents of seven polyphenols in GCBEs by HPLC with Phenomenex Luna C18 column. A gradient elution system was developed for the analysis. The mobile phases consist of A (acetonitrile/water 25:75) and B (acetonitrile/water 10:90). The limit of quantification of 5-CQA was 5 μg. 5-CQA was used as the single standard to determine the content of seven components in GCBEs with relative response factors obtained in this work. Figure 1 shows the chromatograms of standard and sample solutions. The contents of seven polyphenols in different samples collected from the markets were determined with the single standard, 5-CQA. The results were listed in Table 1. In this work, using an easily available single component contained in the GCBEs as reference standard to determine multiple components was a practical option.

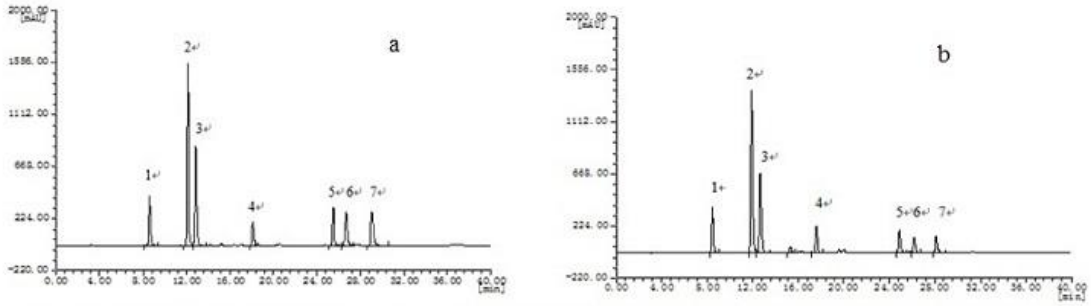

Fig 1. Representative HPLC Chromatograms. a. Standard solution and b. Sample solution of GCBE under the experimental conditions. 1: 3-CQA. 2: 5-CQA. 3: 4-CQA; 4: 5-FQA. 5: 3,4-diCQA. 6: 3,5-diCQA. 7: 4,5-diCQA.

Table 1. Contents (in percentage) of seven chlorogenic acids in samples of green coffee bean extracts collected in the markets

Compound	1	2	3	4	5	6	7
3-CQA	5.25	6.17	3.66	6.07	2.56	5.12	3.13
5-CQA	18.56	24.24	25.91	27.56	20.67	21.12	25.44
4-CQA	6.67	6.92	6.38	7.96	4.26	10.01	6.53
5-FQA	3.95	4.33	5.87	3.17	5.46	3.29	3.99
3,4-diCQA	2.56	1.92	2.87	1.31	3.52	2.36	2.21
3,5-diCQA	1.92	1.19	2.11	0.82	3.02	1.80	2.28
4,5-diCQA	2.11	1.36	2.47	0.90	3.07	1.94	2.74

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PW-226

Optimization of an UHPLC method for flavonoids from *Hypericum* species

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The broad spectrum of pharmacological activities of Herba Hyperici preparations is determined by the potential additive effect, synergism or even possible antagonism of this multicompound system.

The *in silico* assisted development of an analytical chromatographic method for acquisition of a well separated fingerprint of flavonoids, suitable for bioprocess control of conventionally and biotechnologically derived plant material of *Hypericum* species is presented here. Drylab®

software is based on modelling of physicochemical phenomena in an LC system and supports efficient method development by experimental design models. *H. perforatum*, as well as species with indigenously high (*H. richeri*, *H. rumeliacum*) and lacking hypericin production (*H. calycinum*) were selected. Separation was performed on ACQUITY UPLC (Waters) system with PDA Detector. DryLab® software (Molnár Institute) was used to model and predict experiments for the optimization of UHPLC conditions establishing an appropriate method for separation of flavonoid compounds. The fingerprint and the identification of the well described lead flavonoids rutin and hyperoside for the quantification of flavonoids as a sum parameter are suitable for comparison of *Hypericum* samples from different accessions or bioprocess conditions. It was confirmed that the predicted chromatogram matched the peak order in the fingerprint analysis of a real sample from *H. calycinum* extract. The analysis of the samples with the *in-silico* optimized method revealed that *ex situ* sample of *H. perforatum* has content of around 9-11 ug/100ug of total flavonoids calculated as hyperoside of all species tested. Among the different *in vitro* samples, the flavonoid content varied in the order of magnitude of 2-7 ug/100ug. It was demonstrated that the *in silico* assisted optimized UHPLC method is suitable for bioprocess control of flavonoids in *in vitro* and *ex situ* biomass.

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PW-227

A first step in the quest for the active constituents in *Filipendula ulmaria* (meadowsweet): comprehensive secondary metabolite identification by liquid chromatography-quadrupole orbitrap mass spectrometry

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Many natural products are pro-drugs that are metabolized and activated after oral administration. Nevertheless this aspect is usually overlooked when searching for new lead compounds for therapeutic agents. This study is part of a project that aims to identify new leads by fast metabolic profiling of plant extracts before and after treatment with a human gastro-intestinal metabolization model. Pharmacological and chromatographic / phytochemical data of extracts and metabolized extracts will be analyzed in a metabolomics approach in order to characterize the active constituents.

In this project *Filipendula ulmaria* (meadowsweet) has been selected as case study for the characterization of new leads for anti-inflammatory drugs. Previous studies have shown that meadowsweet extracts contain phenolic constituents such as flavonoids (glycosides and aglycons), tannins and salicyl derivatives, as well as a limited number of non-phenolic constituents (e.g. triterpenes, carotenoids). In view of the phenolic nature of the main reported constituents, extensive metabolisation after oral intake before absorption can be expected. Salicylic acid, the *in vivo* metabolite of salicylic alcohol derivatives has been described as being responsible for part of the pharmacological activity, but there is increasing evidence that other constituents contribute as well.

The current study presents a comprehensive identification of secondary metabolites in meadowsweet by liquid chromatography-photodiode array detection-accurate mass mass spectrometry as a first step in the search for its active constituents. Several sample preparation protocols were compared to optimize a comprehensive extraction method that covers the full range of constituents. Next to salicyl derivatives, many other compounds were detected in meadowsweet that may contribute to its health benefits: a large variety of phenolic derivatives were tentatively identified, many of which have not been identified in meadowsweet before.

PW-228

Content of free anthraquinone aglyca in anthranoid-containing herbal drugs/laxatives of the European Pharmacopoeia (Ph. Eur.)

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The monographs of the Ph. Eur. for laxatives drugs undergo a modernization of the determination of the hydroxyanthracene glycosides to HPLC techniques whereby a determination/limitation of aglyca is actually discussed for Sennae folium and fructus, due to the postulated mutagenic and genotoxic effects of the aglyca, especially aloe-emodin [1,2]. In regard to a limitation for aglyca the consideration of the contents in all applied laxative drugs would be reasonable. However, there are no reliable and comparable data available for the content of free aglyca.

The aim of the this study was to provide reliable comparison on the distribution and content of the aglyca aloe-emodin, chrysophanol, emodin, rhein and physcion in different batches of Aloe capensis, Frangulae cortex, Rhei radix, Rhamni purshiani cortex and Sennae folium and fructus measured by HPLC.

The drugs were extracted with acetonitrile/$NaHCO_3$ aq. and diluted with acidified water. A Nucleodur 100-3 C18 column was used as stationary phase. The mobile phase consists of acidified water and acetonitrile/methanol in a gradient. The detection was at 435 nm.

Rhei radix has the highest amount of nearly all measured aglyca, the separation is shown in figure 1. Results of several samples in regard to the variations will be presented on the poster.

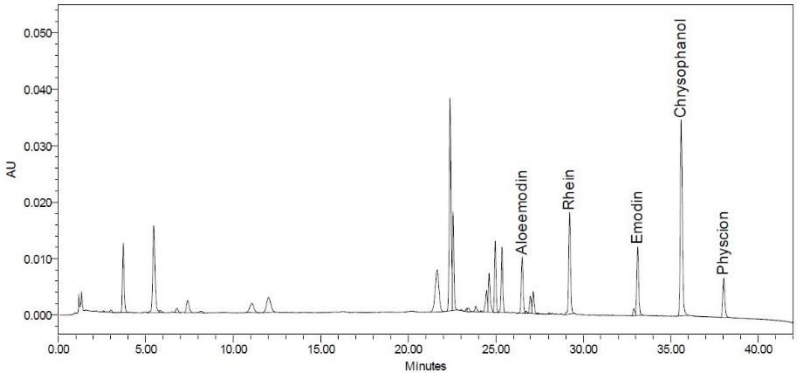

Fig. 1: HPLC Chromatogram from a Rhei radix sample

Considering a limitation of aloe-emodin, the herbal drugs of Sennae showed the lowest content whereas Aloe capensis and Rhei radix contain much higher amounts and therefore should be considered in the limit setting process. The method we developed is simple, precise and a helpful tool to support the discussion, if the analyses of aglyca in anthranoid-containing laxatives will become necessary.

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PW-229

Investigation of metabolite differences during fruit development of hot pepper (*Capsicum annuum* L. cv. CM334) using GC-TOF-MS and UHPLC-LTQ-IT-MS/MS

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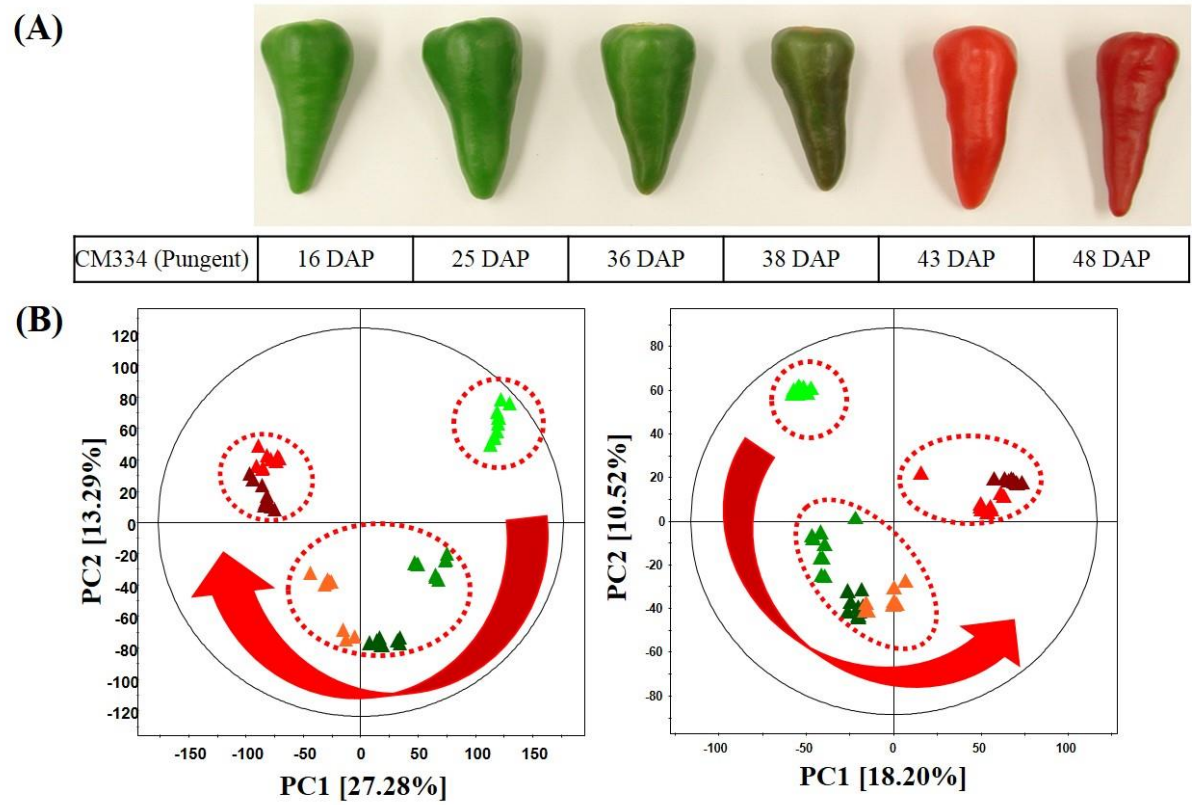
This study investigated non-targeted metabolite profiling of hot peppers [*Capsicum annuum* L. cv. CM334 (Criollo de Morelos 334)] using by GC-TOF-MS, UHPLC-LTQ-IT-MS/MS, and multivariate statistical analysis. The hot peppers were harvested at six different days after pollination (DAP): 16, 25, 36, 38, 43, and 48 day. In PCA score plots of GC-TOF-MS and UHPLC-LTQ-IT-MS/MS, each day showed clear distributions and clustered into three groups (16 DAP, 25-38 DAP, and 43-48 DAP). Alternations of various primary and secondary metabolites including amino acids, organic acids, sugars and flavonoids levels were observed according to pepper development. Most of primary metabolites were increased from 16 to 25, but secondary metabolites were decreased. And in 38 DAP, most of primary and secondary metabolites were decreased until 43 DAP. These primary metabolites have principal roles associated with photosynthesis, respiration, and growth and development of plants [1]. The levels of shikimic acid and phenylalanine, which are the precursors of phenylpropanoid pathway, were gradually decreased or increased, respectively. But capsaicin and dihydrocapsaicin levels showed opposite patterns. Previously, genome sequence of identical hot pepper has been reported [2]. The expression levels of capsaicin biosynthesis related genes including PaAL, C4H, 4CL, COMT, AMT, and CS were decreased gradually developmental stage dependent manner, which may closely related with metabolite levels [3]. From our results, we revealed that MS-based metabolite profiling approach is useful tool to understanding the metabolites involved in hot pepper developmental process.



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[3] Perucka I, Materska M. Innovative Food Sci & Emerging Tech 2001; 2.3: 189-192.

PW-230

Antibacterial, antioxidant activities and phytochemical analysis of methanolic extract from the leaves of*Ludwigia grandiflora* spp invasive

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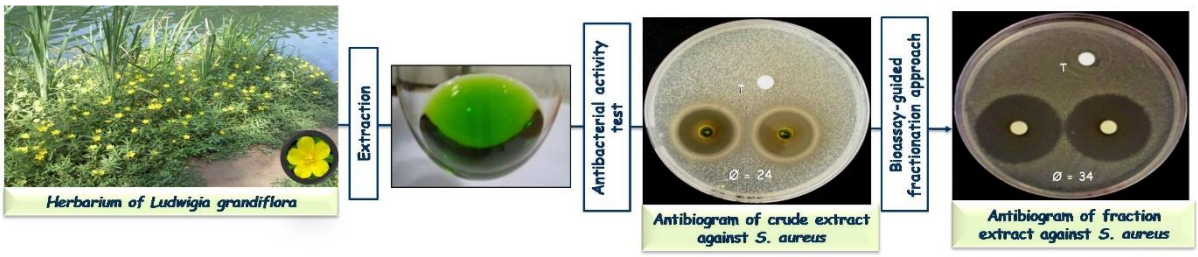
Introduced from South America in the 19th century as an ornamental aquatic plant, *Ludwigia* has since invaded almost every continent. *L. grandiflora* is widespread and invasive in France, forming dense aquatic stands, which cause major ecological and economic problems and reduce local biodiversity. The eradication of *L. grandiflora* is either very difficult or even impossible. The purpose of this study was the valorization of potential antibacterial and antioxidant compounds in the leaf extract of *L. grandiflora* using a bioassay-guided fractionation approach. Antibacterial activity was assessed using the disc diffusion and

microdilution methods against 14 strains of Gram-positive and Gram-negative bacteria. Crude methanolic leaf extract showed strong activity against most of the tested strains, sometimes being more active than common antibiotics. Maximum antibacterial activity was measured against two pathogenic bacteria, *Staphylococcus aureus* and *Salmonella enterica*. The bioassay guided fractionation led to the isolation of several fractions more active than the crude extract; antibacterial activity of fraction (A) was 52% higher than that of the crude extract against *S. aureus*.

The antioxidant activity was evaluated by the scavenging effect on DPPH (2,2-diphenyl-1-picrylhydrazyl) and was expressed as EC_{50} (µg/ml), the extract dose required to cause a 50% inhibition. The fraction (A) exhibited a higher antioxidant activity (EC_{50}=6 µg/ml), as compared to the crude extract (EC_{50}=26.3 µg/ml) and the Trolox (EC_{50}=15.9 µg/ml), which was used as reference standard. RP-HPLC analysis showed that myricetin was the major phenolic compound. However, there was insufficient time to determine the active molecules.

This innovative study, which allowed the submission of a patent, suggests that *Ludwigia* may be considered as an interesting source of antibacterial and antioxidant compounds for therapeutic uses and for the cosmetic and nutraceutical industries.



PW-231

Oxygen radical absorption capacity of *Lippia origanoides* (thymol and phellandrene chemotypes) and *Turnera diffusa* essential oils and extracts

Elena Stashenko, Yuri Córdoba, Jairo R. Martínez

Research Center for Biomolecules, CENIVAM, Universidad Industrial de Santander, Bucaramanga, Colombia

Damiana *(Turnera diffusa*) and Mountain oregano (*Lippia origanoides*) are shrubs that grow wild in semi-arid terrains of Central and northern South America. Three *L. origanoides* chemotypes have been found, characterized by their different essential oil main constituents (thymol, carvacrol and phellandrene). GC-MS analysis of essential oils (EO) and extracts (obtained with supercitical CO_2) from stems and leaves of *T. diffusa* and *L. origanoides* (thymol and phellandrene chemotypes) showed that the secondary metabolites common to these aromatic plants were: α-pinene (0.5-1.8%), limonene (0.2-37.8%), linalool (0.2-1.1%), trans-β-caryophyllene (1.0-26.4%), α-humulene (4-2.4%), caryophyllene oxide (3-7.2%), and germacrene D (0.05-37.68%). Dehydrofuquinone (52.3%) and drima-7,9-diene (4.4%) were the main components of damiana oil. The oxygen radical absorption capacity (ORAC) of a *L. origanoides* (phellandrene chemotype) extract obtained with supercritical CO_2 showed a remarkably high value (4130±29 micromol Trolox/g substance), attributed to its high pinocembrin content. Lower ORAC values were obtained for *L. origanoides* (thymol chemotype) EO (3710±10 micromol Trolox/g sample), followed by *L. origanoides*

(phellandrene chemotype) EO (1260±29 micromol Trolox/g sample) and damiana EO (813±9 micromol Trolox/g sample). EO were obtained by microwave radiation-assisted hydrodistillation. Extracts were obtained with CO_2 at 400 bar, 50 g/min and 10% ethanol in a Thar SFE-2000-2-FMC50 system; extract fractions were collected at 40 and 80 bar. The separation and identification of secondary metabolites was performed on an Agilent Technologies GC 6890 with 5973 mass selective detector (EI, 70 eV). Chromatographic columns DB-5 and DB-WAX (60m x 0.25mm, ID x 0.25 mm) were used. The ORAC assay was performed in a Turner Biosystems multiplate reader. Trolox® was used as a reference standard for these measurements.

PW-232

Advances in profiling of natural products by triple detection techniques combined with super critical CO_2mobile phases and Sub-2µm stationary phases

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Convergence chromatography (CC) is a separation technique that utilizes both super and sub-critical carbon dioxide (CO_2) and sub-2 µm stationary phases to achieve unique selectivity, low solvent usage and high efficiencies. The use of supercritical fluid as a mobile phase provides higher diffusivity and lower viscosity than liquid mobile phases, thereby providing higher throughput and chromatographic efficiencies as compared to liquid chromatography.

The analysis of natural products has typically been challenging, not only because of the complex matrices but also the numerous components with varying physical and chemical properties. To address these difficulties, multiple detectors are typically used during a single analysis whereas each detection technique is based on a different physical or chemical property of the molecule. For example, mass detectors and PDA are commonly combined to obtain both mass and UV-spectral information. Evaporative light scattering, a more universal technique, addresses compounds without ultraviolet absorbance (no chromophore) and poor ionization by MS. The combination of these three detection techniques allows for analysis of a wide range of compounds.

In this presentation, we will investigate the analysis of a number of natural products using triple detection in combination with CC. Identification and quantitation of compounds will also be illustrated. Guidance combining triple detection with CC will be provided based on the observations attained throughout the analysis. This approach when combined with sub-2 µm column chemistries will allow for the detection of compounds with a wide range of physical and chemical properties.

PW-233

Flower metabolomics: volatile compound profile, antioxidant capacity and LC-MS-TOF identification of flavonoids in various tropical flowers

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Volatile organic compounds in *Brownea macrophylla*, *Petrea volubilis* and *Hibiscus spp*. flowers were sampled in vitro and in vivo with HS-SPME and analyzed by GC-MS. Flavonoids present in flower extracts, were identified with LC-MS and their antioxidant capacity was evaluated with the ORAC assay. LC-MS data obtained in positive ionization mode from extracts were used to generate possible molecular formulas that satisfied the accurate masses. Fragmentation and mass errors were used as selection criteria for structure assignment. Pelargonidin-3,5-glucoside, pelargonidin-3-glucoside, 8-p-hydroxybenzyl and peonidin were identified in the *B. macrophylla* extract. Pelargonidin glucuronide, apigenin 7-glucuronide methyl ester, cyanidin-3-*O*-glucuronide, pelargonidin-3-*O*-(6-acetyl-glucoside), and delphinidin-3-glucoside were found in the *P. volubilis* flower extract. Cyanidin-3-glucoside, cyanidin-3,5-glucoside, delphinidin-3-glucoside, and peonidin-3-arabinoside were identified in the *Hibiscus spp.* extract. ORAC antioxidant capacity values of 3460, 670 and (1200-4035) micromol Trolox/g of substance, were measured for the *B. macrophylla*, *P. volubilis* and *Hibiscus spp*. extracts, respectively. These were higher than the values of 500 and 480 micromol Trolox/g of substance observed for the reference antioxidants, BHT and α-tocopherol, respectively. *B. macrophylla*, *P. volubilis* and *Hibiscus spp*. flowers were obtained from experimental plots at the CENIVAM research center. The plant material was freeze-dried (Virtis Advance Plus). Solid-liquid extraction with acidified (1% HCl) ethanol-water (3:1, v/v) afforded extracts which were roto-evaporated. An LC-MS with electro-spray ionization (LC-ESI-TOF, 1200-6210 Agilent Technologies, Palo Alto, CA, USA) was used for extract analysis. The ORAC assay was implemented in a 96-well microplate reader (Turner Biosystems Inc., Modulus Microplate Reader II).

PW-234

Rapid chemical analysis and antiprotozoal effect of the solvent extracts and the essential oil of *Artemisia indica*

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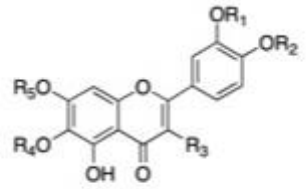
Artemisia indica is used as antipyretic in malarial fevers during malaria outbreaks in India [1]. We selected this plant because reports concerning the presence of artemisinin is contradictory, the content of methoxyflavonoids that potentiate the antimalarial efficacy of artemisinin has remained unstudied and the essential oil of the plant from different regions shows great chemical variations. Solvent extracts [petroleum ether, *n*-hexane, dichloromethane, acetone, MeOH or EtOH (96, 80 or 60% v/v), and hot water] of *A. indica* leaves originated from the West Bengal region (India) were assessed by HPLC-DAD and HPLC-MS for the content of artemisinin and the characteristic *Artemisia* methoxyflavonoids, eupatin, casticin, chrysoplenetin, cirsilineol, chrysosphenol-D and artemetin. None of the extracts contained artemisinin or the methoxyflavonoids chrysosphenol-D and artemetin, while all extracts contained chrysoplenetin. Eupatin, casticin and cirsilineol were found in all extracts except for the p. ether, n-hexane and hot water infusion. The acetone and EtOH extracts contained the highest levels of polymethoxyflavonoids (1.15-1.17%), whereas the infusion was devoid of them. The essential oil of the plant was obtained by hydrodistillation and analyzed by GC and GC-MS simultaneously. Of the 92 compounds detected in the oil, camphor (13.0%) and caryophyllene oxide (10.87%) were the major components. All solvent extracts and the volatile oil showed in vitro antimalarial activity (1.8-20 μg/mL). Except for the infusion, all extracts were also active against other parasitic protozoa (*Trypanosoma b. rhodesiense*, *T. cruzi*, *Leishmania donovani*). This is the first study investigating both artemisinin and polymethoxyflavonoid content and detailed in vitro antiprotozoal potential of *A. indica* extracts and the essential oil.



Eupatin	R_1=H, R_2=Me, R_3=OH, R_4=R_5= Me
Casticin	R_1=H, R_2=Me, R_3=OMe, R_4=R_5=Me
Chrysoplenetin	R_1=Me, R_2=H, R_3=OMe, R_4=R_5=Me
Cirsilineol	R_1=Me, R_2=R_3=H, R_4=R_5=Me
Chrysosphenol-D	R_1=R_2=H, R_3=OMe, R_4=R_5=Me
Artemetin	R_1=R_2=Me, R_3=OMe, R_4=R_5=Me

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PW-235

Comparative study of *Arnica montana* root and rhizome samples from Eastern Carpathians of Romania

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NIRDBS Bucharest/ "Stejarul" Biological Research Center, Piatra Neamt, Romania

In phytotherapy mainly the flower heads of *A. montana* L. are used, but use of rhizome and roots has also been reported in human and veterinary medicine [1]. Arnicae radix is listed in the European Council Regulation No. 2377/90, Annex II for veterinary purposes. Previous phytochemical studies on the underground parts of the plant focused mainly on the essential oil, while few data have been reported for the phenolic compounds constituents.

Use of Arnica roots and rhizome is only economically reasonable through development of cultures. Our study aims to evaluate the underground parts (roots and rhizome – average sample) wild populations of A montana with respect to biomass and content of phenolic compounds. The assessments focused on 6 wild populations in the Romanian Eastern Carpathians. Phytochemical assessments were performed by TLC and Folin-Ciocalteu method.

Over the three years of the experiment (2012 - 2014), the assessment of the biomass production showed substantial variations in dry substance (11.60-40.57 g d.w. for 25 plants) and of the loss on drying (56.71- 70.69% of fresh weight). The TLC fingerprint revealed the presence of isochlorogenic acids. A total content in phenolic compounds of 1.11-2.72 g caffeic ac. equiv./ 100 g d.w. was found. Based on these data plant material will be selected for the development of cultures using autochthonous plant material.

Acknowledgement: The work was sustained through the program Partnership in Priority Areas – PNII, implemented with the support of MEN – UEFISCDI, Romania, project 74/2014.

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PW-236

Determinatin of proscillaridin in *Drimia maritima* from two provinces of Iran

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Drimia maritima (L.) is an endemic plant of Iran that have been used for varies disease such as dropsy, asthma and cancer since ancient time [1]. Bufadienolides are the main constituents of this plant. Proscillaridin is one of the bufadienolides that possess important biological activities such as antitumor, and T- cell suppressive effects [2-3]. This compound is determined in *D. maritima* grows in two distinct regions of Iran. Powder of dried bulbs (0.25 g) was extracted with methanol (10 ml) via ultrasonic method (60min, 40 °C, three times). A validated high performance liquid chromatography (HPLC) method was approved for prosicllaridin determination in our previous study [4]. The chromatographic separation was carried out on a reversed phase ACE C18 with eluting at flow rate of 1 ml/min using a gradient with methanol-water for 50 min. Proscillaridin amount of *D. maritima* from Fars and Hamedan provinces determined as 4.1 and 1.5 (µg/ ml) respectively. So utilization of samples from Fars provinces may be more beneficial for preparing herbal formulations consisting proscillaridin.

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PW-237

A chemometric approach to the quality control of turmeric (*Curcuma longa*) by high performance liquid chromatography

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Curcuminoids, are the principal natural yellow pigments found in the turmeric rhizomes (*Curcuma longa* L. Zingiberaceae). Among them, curcumin (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) represent the main chemical components responsible for the bioactivity [1]. The presence of a simple and precise method based on the curcuminoids content that can be used for the quality control of turmeric has not been developed. Therefore, in this study, we developed a method for the analysis of turmeric samples collected from Algeria (AC) and Egypt (EC) using HPLC technique. The methanol extracts of 30 samples were analyzed in triplicates on an RP-18 column using methanol: acetic acid: acetonitrile as a mobile phase and a photo diode array detector. The curcuminoids were characterized by co-chromatography and their relative peak areas were calculated. Different pattern recognition procedures, including principal component (PCA) and hierarchical cluster (HCA) analyses were applied using the matrix built on the relative peak areas of 90 chromatograms. The score plot of total curcuminoids showed that the samples were discriminated according to the different percentage of total curcuminoids and that the main discriminating marker was DMC. Interestingly, the samples were subsequently segregated according to their geographical location, where the total curcuminoids in the Egyptian samples ranged between 92 - 96%, whereas the Algerian samples ranged between 58 and 97%. In HCA, the resulting dendogram classified the samples into two main clusters, based on the location confirming the results of PCA. This study demonstrates that the combination of fingerprints and PCA may serve as an efficient and simple method for the quality control of tumeric rhizomes.

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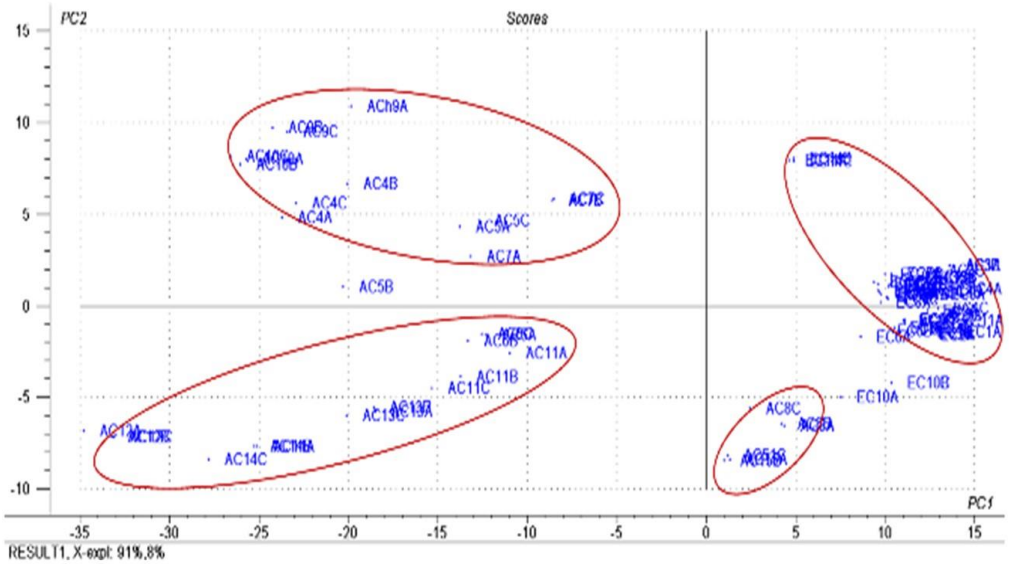

PCA score plot of relative peak areas of total curcuminoids

PW-238

Principal component analysis of production batches of the Ginkgo biloba extract EGb 761® over a period of 10 years on the basis of routine quality control parameters

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Quality control of herbal extracts as drug substances in medicinal products requires the application of a battery of analytical parameters to guarantee safe use and reproducible therapeutic efficacy. In this respect, quality of EGb 761® is regularly controlled by verification of the content of most important ingredients which contribute to efficacy (e.g. terpene lactones, flavone glycosides) and compliance with limits of unwanted compounds (e.g. ginkgolic acids). Additionally, general parameters like ash, color and particle size are analyzed (altogether 18 parameters).

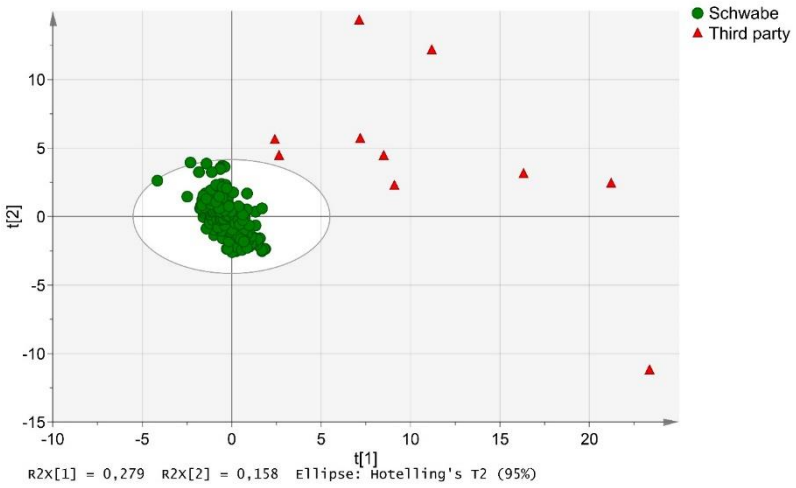
Principal component analysis is a helpful tool to structure and illustrate large sets of data by converting numerous statistical variables in a reduced number of combinations. The calculated scores (principal components) represent the main variations or deviations of the data. Illustration through projection into 2D or 3D-representations is an easily interpretable way to confirm group specific properties.

The full set of routine quality control parameters of 379 production batches from the last ten years as well as data determined for 10 quantified extracts from other manufacturers were imported into SIMCA 14 and analyzed with univariate scaling for principal components (Fig.).

Applying Hotelling's T2 statistic 99% of the Schwabe extracts (spheres) clustered within the 95% confidence interval, whereas all analyzed extracts from third party origin were outside this area (triangles).

The data demonstrate an exceptionally high consistency of extract composition over an extended period of time as basis for reproducible pharmacological activity, therapeutic efficacy and tolerability of EGb 761®.

Fig.: Score scatter plot of *Ginkgo* extracts



PW-239

Role of thin layer chromatography in detection of antibacterial activity of essential oils

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The appearance of multidrug resistant bacteria and growing antibiotic resistance increased the significance of natural drugs against infections. Results of the previous *in vitro* studies focusing on the antimicrobial activity of essential oils (EOs) are very different, sometimes their reliability is questionable. Thin layer chromatography-direct bioautography (TLC-DB) belonging to the effect-directed analysis (EDA) provides information for the biologically active compounds even in a multiple matrices [1]. The aim of our study was the chemical characterization of different EOs (citronella, chamomile, clove, cinnamon bark, thyme and tea tree) using GC-MS and the investigation of their antibacterial activity by TLC-DB. EOs were isolated by water-steam distillation or obtained from a Hungarian drug store chain.

Furthermore, semi-quantitative densitometric evaluation of EOs and their main components was done by CAMAG VideoScan program (Muttenz, Switzerland). Citronellal (36.2%), *α*-bisabolol (62.9%), eugenol (88.6%), *trans*-cinnamic aldehyde (74.0%), thymol (46.3%) and terpinene-4-ol (44.0%) was the main component of the EO of citronella, chamomile, clove, cinnamon bark, thyme and tea tree, respectively. Clove, cinnamon bark and thyme EOs were the most active oils against*Staphylococcus aureus, S. epidermidis*, MRSA and *Escherichia coli* in 30 mg/ml concentration (equivalent to 0.15 mg of pure oil). After TLC separation the antibacterial activity of citronellal, *α*-bisabolol, eugenol and thymol could be demonstrated. Citronellal (0.01 mg) showed the highest activity. TLC-DB bioassay allows a rapid identification of the antibacterial compound in a complex mixture, e.g. in EOs.

Acknowledgements: This work was supported by OTKA PD 104660 grant (Hungarian Scientific Research Fund).

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PW-240

Phytochemical fingerprints combined with bioautographic assays for possible multiple enzyme inhibiting activities of *Verbascum* (Scrophulariaceae) and *Ajuga* (Lamiaceae) plant extractives.

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The genera *Verbascum* and *Ajuga* with multiple traditional therapeutical uses are rich in phenolic compounds with potential anti-inflammatory, antioxidant and immunomodulatory properties [1].The aim of this study was to screen the phytochemical and biological potential of the species *V. nigrum* L. and *A. chia* Schreb. for prevention and treatment of nonmelanoma skin cancer as part of a joint research project on *multitarget* activity of plant extractives [2].

Extraction of the aerial plant parts of *A. chia* and *V. nigrum* was done with methanol, followed by fractionation by RP-C18 flash chromatography with step gradients. HPTLC fingerprints were carried out for flavonoids, iridoids and phenolic acids on CAMAG equipment linked with bioautographic enzyme inhibition assays applying Acetylcholinesterase (AChE;EC No.3.1.1.7) and Lipase (EC No.3.1.1.3). UPLC-ESI-MS(Waters) was used for phytochemical characterization of extracts and fractions.

Preliminary bioautographic results show that both crude extracts contain potential AChE and lipase inhibitors. *A. chia*, not fully characterized yet, contains with high probability luteoline-7-*O*-glucoside, apigenin and verbascoside, along with phytochemicals with a caffeoylquinic acid moiety, a quercetin glycoside based on MS data of the methanolic extracts. For *V. nigrum*, the presence of verbascoside, harpagoside, a quercetin-glucuronide and possible aucubin

derivatives are identified. Detailed data on UPLC-MS, HPTLC phytochemical and antioxidant fingerprints and bioautographic assays of the fractions will be presented.

Acknowledgement: Sciex-HMSch CRUS Switzerland No.13.218 & N. Ciocirlan and V. Ghendov for plant material identification (Botanical Garden of Academy of Sciences of Moldova).

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PW-241

Metabolic profiling of Greek propolis samples and evaluation of their antioxidant, antimutagenic and anti-ageing properties

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Propolis, a resinous material collected by honeybees *(Apis melifera L.)* from various plant sources, is rich in phenolics which are recognized widely as strong antioxidants. Since the composition of propolis depends on its origin (vegetation of the area, geographical origin), the intensity of antioxidant activity varies, affecting moreover their anti-ageing properties.

In this project, several propolis samples from different regions of Greece were collected and their secondary metabolites were obtained using sequential PLE extraction with n-heptane and methanol. The metabolic profiling of these preparations was investigated using NMR and HPTLC techniques, as well as the total phenolic and total flavonoid content was determined by Folin-Ciocalteau and AlCl3 colorimetric methods, respectively. For the toxicity profiling, the viability of human cells (A375 and HaCat) treated with propolis extracts was determined by MTT and SRB assays.

The HPTLC and NMR analysis revealed high variability in the phytochemical profile of the methanolic extracts with three major groups to be observed, depending on the presence of flavonoids such as chrysin, galangin, pinocembrin etc. Moreover, various other phenolic derivatives such as 3-O-caffeoyl allyl esters were also identified. The most promising extracts, which possessed the higher phenolic content and antioxidant activity, were forwarded for the

evaluation of their antimutagenic activity using the Comet assay and anti-ageing properties by exposing reconstituted human skin to UV radiation.

The metabolic analysis revealed a great differentiation between propolis samples, both in the chromatographic profile as well as to the quantity of the components. Greek propolis fingerprinting by such methodology is described for the first time, providing a useful tool, which enables their origin discrimination and in accordance with the biological results can act as an UVB protector-indicator for their use in cosmeceutical industry.

PW-242

Possible adulterations of *Ginkgo biloba* food supplements

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Products containing the standardized *Ginkgo biloba* L. (Ginkgoaceae) leaves extract, EGb 761, are used for the "improvement of cognitive impairment and of quality of life in mild dementia" (EMA-HMPC). The herbal drug Ginkgonis folium is used for the treatment of peripheral circulation disorders. Ginkgonis extractum siccum raffinatum et quantificatum (Ph. Eur. 8) shall contain 22%-24% flavonoids, and 5.4%-6.6% terpene lactones (ginkgolides, bilobalide). Next to mass products (medicines), some food supplements also contain the EGb 761 extract. The aim of this work was to prove whether food supplements possess pharmaceutical quality, and if they even obey the European Pharmacopoeia criteria. The experimental part is focused on the quantification of two groups of secondary metabolites in 3 mass product medicines (positive control), and in 11 food supplements containing EGb 761 or Ginkgonis folium. Flavonoids were quantified with and without aglycone hydrolysis using HPLC-UV. Terpene lactones (diterpenes – ginkgolides A, B, C, and the pentanorditerpene bilobalide) were quantified using LC-MS/MS. Microscopic analysis was included as well. The medicine mass products contained 29.06%-32.04% of flavonoids, whereas the food supplements just 0.2 to 39.79%, expressed as acylglycoside (Mr 756.7). The medicine mass products contained 5.22%–5.63 % ginkgolides, and 4.52%-6.59% bilobalide, i.e. they meet the Ph. Eur. 8 requirements. Dietary supplements contained 0.00 to 29.66 % ginkgolides, and 0.00%-13.44% bilobalide. One dietary supplement did not contain any *Ginkgo*, the presence of other herbal drugs was confirmed microscopically. Our results suggest that many *Ginkgo* food supplements are not of pharmaceutical quality, neither do they satisfy the requirements for medicines. Only two of 11 dietary supplements met the Ph. Eur. 8 requirements (*lege artis*), some used an adulterated extract, and two did not contain any *Ginkgo* herbal drug or extract.

PW-243

Antioxidant activity and total flavonoids content in variable phyto-stem cells extracts obtained by high-pressure homogenization method and assigned for use in biocosmetics

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Antioxidant activity (AO) of with DPPH (μmol Trolox, TE) and total flavonoid content (TFC) with $AlCl_3$ (μg catechins CAE eq/mL) was determined for six commercially available callus culture extracts (CCE) from paper mulberry PM (*Brussonetia kazinoki* (family Moraceae)), grape stems GS (*Vitis vinifera* (Vitaceae)), magnolsi MA (*Magnolia sieboldii*(Magnoliaceae)), green tea GT(*Camelia sinensis* (Theaceae)), white ginseng WKG (*Panax ginseng* (Araliaceae)) and hydroponically cultivated ginseng HPG (*Panax ginseng* (Araliaceae)) and containing butylene glycol (3.0%), phenoxyethanol (0.9), ethylhexylglycerin (0.1) as preservatives. Highest TFC was presented by the WKG extracts (mean 2.8 μg CAE eq/mL). Not significantly different TFC in case of GT (2.2), HPG (2.1), MA (2.0) and decreased for the GS (1.9) and PM (1.5) was shown. Scavenging of DPPH radicals was highest for the GT extracts (mean 30.30 μmol TE) and not significantly different from WKG (29.99), HPG (29.80) and ME (29.71) but significantly lower than GS (28.82) and PM (27.66) extracts. These results for WKG and HPG extracts was quite parallel with previously reported value of the 29.10 μmol TE for 1.0% CCE of white *P. ginseng* roots. However, values from 5 to 20 mmol TE were reported for 0.001% CCE of *Leontopodium alpinum* (Asteraceae) flowers and 190 nmol TE for the 0.025% CCE from *Euterpe oleracea* (Arecaceae) fruits. Relationship between AO and TFC was not observed for studied CCE. However, high molecular antioxidant compounds with a high proton donor ability could indicate an increased steric inaccessibility to the stable nitrogen radical of DPPH thus contributes to inadequate results of this test [1]. Studied here CCE have DPPH based AO comparable with the 0.15-0.20% water solution of small molecule plant antioxidants as quinic (27.65 μmol TE), ascorbic (27.60), cinnamic (27.33), caffeic (28.84) or chlorogenic (28.60) acid.

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PW-244

Comparative analysis of total phenolic content and biological potential of *Urtica dioica* L. and *Urtica kioviensis* extracts

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Urtica dioica L. (stinging nettle) have been used as a leaf vegetable and in a traditional medicine primarily in the treatment of rheumatod arthritis and as a diuretic [1]. Eventhough belonging to the same genus and having a lot of similarities with the stinging nettle, biological potential of *U. kioviensis* Rogow. have never been assesed before. Therefore, the aim of this work is to compare the phenolic content, antioxidant and anti-acetylcholinesterase activity of

a widely known *U. dioica* and poorly investigated *U. kioviensis*. The 80 % methanolics extracts were made from herb and root for both species. Total phenolic contend was determined with FC reagent, antioxidant capacity in the test of superoxide anion (SOA) radical neutralisation and anti-acetylcholinesterase activity by modified Ellman's method [2]. *U. kioviensis* herb extract had the twice higher phenolic content and expressed considerably higher capacity to neutralise SOA radicals (IC_{50}=2.60 μg/ml) compared to the stinging nettle herb extract (IC_{50}=155.4 μg/ml). Both root and herb extract of *U. kioviensis* were better inhibitors of the acetylcholinesterase with the inhibition of 92% and 77%, respectfully, compared with the 61% and 60% inhibition by *U. dioica* herb and root extracts. Form the obtained results it can be concluded that the *U. kioviensis* has a greater biological potential than its related species *U. dioica* and therefore, it should be incorporated in a human consumption.

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PW-245

Development of an HPTLC-method for identification of Scrophulariae radix (Xuanshen) and quantification of the two main iridoids, harpagide and harpagoside

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Herbal drugs originating from Traditional Chinese Medicine (TCM) are becoming more popular in Europe. Challenges related to clear definitions, identity, purity, adulteration/falsification prompted the German Pharmacopeia and the Commission of the PhEur to develop and establish quality monographs for the most important TCM herbal drugs commonly used in Europe. Clear identification- and validated assay methods should be the most important part of any new monograph [1,2].

Scrophulariae radix (Xuanshen, Ch P 2010) is the dried root of *Scrophularia ningpoenis* Hemsl.,[3] used in TCM for the treatment of fever, swelling, etc. The iridoids harpagide and harpagoside as well as phenylpropanoid glycosides were described as the most abundant secondary metabolites [4] According to the Ch P 2010 monograph, harpagide and harpagoside were chosen as reference compounds for the development of an identification method based on a new simplified extraction protocol and HPTLC fingerprinting. The use of DCM/EtOH/water (70:45:6.5) as mobile phase and detection with anisaldehyde reagent guarantee the stability of harpagide and harpagoside on the HPTLC plate and afford a clear separation of both iridoids from each other as well as fingerprints appropriate for the

characterization of Scrophulariae radix. For quantification of harpagide and harpagoside a densitometric HPTLC assay was also developed using the same mobile phase and comparing quantification with and without derivatization reagent.

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PW-246

Determination of kratom (*Mitragyna speciosa)* alkaloids in kratom cocktail using anti-mitragynine monoclonal antibody -based ELISA

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Mitragynine is an exclusive indole alkaloid which found in leaves of kratom or *Mitragyna speciosa* (Roxb.) Korth. (Rubiaceae). This plant has been traditionally used to combat fatigue by chewing the fresh leaves or brewing tea. In the recent years, the kratom cocktail has been abuse in the teenagers and become a major concern in Thailand and Malaysia. It typically comprised of kratom leaves boiling in water and mixed with other substances such as cola drink, cough syrup, tranquilizer and even mosquito coil. Therefore, a simple and reliable analytical method is needed for the qualitative and/or quantitative analysis of the active compound, mitragynine, in the cocktail. We previously reported the establishment of monoclonal antibody (mAb) against mitragynine and developed an indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) for determination of mitragynine in kratom leaves samples. In the present study, we applied the developed ic-ELISA using anti-mitragynine mAb to quantify mitragynine in kratom cocktail. The compounds which might be adulterated in cocktail were tested for the cross-reactivity. The benzodiazepines including alprazolam, diazepam and lorazepam and the mosquito coil showed the moderate cross-reactivity as 44%, 40%, 15% and 31%, respectively. In order to reduce the matrix interference, the cocktails were pretreated by liquid extraction using chloroform under basis condition. The recovery of mitragynine was 73% when compare with untreated cocktail. However, the ic-ELISA showed the results of total alkaloids contents (mitragynine, paynantheine and speciogynine) in kratom cocktail when compare with HPLC method.

PW-247

Yarrow herb (*Achillea millefolium*) oil extracts: Qualitative and quantitative analyses of major phenolic compounds and assessment of antioxidant activity

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The aim of this study was to investigate chemical composition, in terms of qualitative and quantitative analyses of major phenolic compounds evaluated using high performance liquid chromatography and also antioxidative potential, assessed by means of ferric reducing antioxidant power (FRAP) test of four oil extracts (OE) (with the final drug: extract ratio of 1:5) of yarrow herb using two oils and two preparation protocols. Method 1: dry yarrow herb was macerated in 96% ethanol (for 24 hours at room temperature), followed by the addition of olive oil (E-O) or sunflower oil (E-S) and heating on the water bath until ethanol evaporation (4 hours at 45 °C). Method 2: dry yarrow herb was macerated in olive oil (O) or sunflower oil (S) on a water bath for 4 hours at 45 °C. Major phenolic compounds identified in all the tested samples were flavones (luteolin and apigenin) present in all OE, while the flavonol (rutin) and hydrocinnamic acid derivative (chlorogenic acid) were detected only in the sample E-S. In addition, this sample also revealed the highest luteolin and apigenin content (0.0167 and 0.0124 mg/g, respectively) compared to O (0.0031 and 0.0025 mg/g, respectively), S (0.0038 and 0.0022 mg/g, respectively) and E-O (0.0101 and 0.0086 mg/g, respectively). FRAP test indicated the best reducing power in the sample O (0.0016 mmol Fe^{2+}/g), followed by E-S, S and E-O (0.0013, 0.007 and 0.0006 mmol Fe2+/g, respectively) singling out this OE of Yarrow herb prepared with olive oil and by method 2 as the sample with the best antioxidant potential and at the same time suggesting other constituents, aside the identified phenolic compounds, to play an additional role in the exerted activity.

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PW-248

Metabolic profiling of Greek honey samples and evaluation of their antioxidant activity

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Physicochemical properties and organoleptic features of honey vary according to its floral origin, geographical and seasonal conditions. However the content of secondary metabolites possessing significant biological properties plays an important role in consumers' demand. In this study, we investigate the metabolic profile of several honey samples from different regions of Greece using GCMS, HPTLC and NMR techniques. Initially the floral sources of honeys were certified with pollen analysis. An extraction protocol based on the use of macroporus resin XAD-4 was developed to obtain fractions enriched in the components of interest such as phenolic compounds. Therefore, resin was stirring with aqueous extracts of honeys, followed by filtration for removal of sugars and the recovery of phenolics using methanol. The enriched extracts were evaluated for their chemical content (Total Phenolic Content, Total Flavonoid Content) and antioxidant properties (DPPH and ABTS methods). For the evaluation of their toxicity MTT and SRB assays were used. The extracts obtained from thyme honeys showed considerable phenolic content and significant antioxidant activity. The most promising honeys and enriched fractions were forwarded for the evaluation of their anti-mutagenic and anti-ageing activity.

HPTLC metabolic profiling and multivariate data analysis of the NMR spectra revealed a high variability in the content of the honey extracts. It is noteworthy that there are remarkable similarities between the samples derived from the same plant source, such as the honey produced by thyme and Chestnut tree, despite their different geographical origin. Indeed, the head space analysis based on solid-phase microextraction (SPME) technique revealed the presence of phenylacetaldehyde, thymoquinone, carvacrol, thymol, benzaldehyde and decanal as the main volatile constituents in the majority of thyme honey samples. However, there is a difficulty in categorizing multiflora honey samples.

PW-249

Immunochromatographic assay for mulberroside A detection using polyclonal antibody against mulberroside A

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Mulberroside A is a major active compound from root barks of *Morus alba* L. (Moraceae). It is widely employed as an active ingredient in cosmetic products due to its anti-tyrosinase and anti-oxidant activities [1]. In order to verify the present of mulberroside A in large number of samples, a rapid and simple assay system is required to apply as small quantities utilization method. Previously, we reported enzyme-linked immunosorbent assay (ELISA) using highly specific mulberroside A polyclonal antibody for determination of mulberroside A in plant samples [2]. In this study, an immunochromatographic strip test was developed by using anti-mulberroside A polyclonal antibody. The qualitative assay was based on competitive immunoassay in which the detector reagent consisted of anti-mulberroside A polyclonal antibody colored with colloidal gold particles. The capture reagent was mulberroside A ovalbumin conjugate immobilized on the test strip membrane. The sample containing mulberroside A and the detector reagent were incubated together with immobilized capture reagent on nitrocellulose membrane. The detection limit for the strip test was 2 μg/ml. The developed immunochromatographic strip test was applied to determine mulberroside A in plants, medical preparations and cosmetic samples.

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PW-250

Development of an HPLC method for the characterization and quantification of cardenolides in*Strophanthus kombé* extracts

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Strophanthus kombé Oliv. is a climbing plant, which may be found mainly in Central Africa. Its seeds contain cardenolides exhibiting cardioactive properties. Hence, *Strophanthus* extracts and isolated cardenolides have been used for treating heart diseases. Thin-layer (TLC) and high performance liquid chromatography (HPLC) are commonly used as analytical tools for quality control [1,2]. However, these methods have not been updated taking into account novel analytical developments. Latest reports in this context refer to the separation of a known K-strophanthin mixture and characterization of individual compounds by HPLC-MS/MS [3]. Thus, a *Strophanthus* seed extract was used for method development in the present study. The

method was optimized using an RP-HPLC system coupled with DAD (220 nm) and MS^n. For this purpose, a C18 column (100x2.1 mm, 3.5 µm) with a gradient of water-acetonitrile acidifying both eluents with formic acid was evaluated. Separation of more than 20 cardenolides was achieved within 37 minutes. Individual cardenolides were characterized by MS^n experiments. Subsequently, the method was transferred to an UHPLC C18 system (50x2.1 mm, 1.9 µm) using Chromeleon™ UHPLC Method Speed-Up Wizard allowing reduction of the separation time to 10 minutes. Separation was equivalent to that of the HPLC method. UHPLC method was validated according to ICH guidelines, thus, allowing quantitation of individual compounds. The developed method is straightforward and applicable for the quality control of raw material from *S. kombé* and pharmaceutical preparations produced therefrom.

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PW-251

HPLC analysis of phenolic compounds, antioxidant and thrombolytic activities of *Origanum vulgare* using various solvent extracts

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Origanum vulgare is traditionally used as food and medicinal plant. The aim of this study was to examine the HPLC chemical composition, total phenolic (TP) and total flavonoid (TF) contents and to evaluate the antioxidant and anticoagulant activities of methanol, acetone and dichloromethane extracts of *O. vulgare*. HPLC analysis of methanol, acetone and dichloromethane extracts revealed the presence of benzoic acid (2290.80, 294.35 and 228.11 mg/100g, dw) and coumaric acid (232.48, 26.70 and 23.33 mg/100g, dw) as the major phenolic compounds, respectively. Considerable amounts of TP and TF were detected and the highest content of TP (75.3 mg/g, dw) and TF (3.06 mg/g, dw) was detected in the methanol extract. All the extracts exhibited moderate to strong antioxidant activity in terms of DPPH radical, reducing power and ABTS radical. The methanolic extract was found to inhibit coagulation process *in vitro* and significantly prolonged prothrombin time in a dose-dependent manner. This study suggests that the antioxidant and anticoagulant activities of *O. vulgare* which may be due to the presence of different phenolic compounds in the extracts. Extra studies are recommended to elucidate the active components of *O. vulgare* which may be useful in the development of new and effective antioxidant and anticoagulant agents.

PW-252

X-ray fluorescence spectroscopic determination of heavy metal and trace element concentrations of*Origanum sipyleum* from Turkey

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Origanum species have been used as medicinal tea or food additives and for the production of its essential oil. *Origanum sipyleum* L., native to western Anatolia, is widely used as spice and against gastrointestinal disorders and cough [1,2]. Plants assimilate the elements dissolved in water through roots. In addition to purity, safety and efficacy assesments, a part of quality control studies on medicinal plant is the quantification of heavy metals. The aim of this work was to determine the heavy metal and trace element compositions in aerial parts of *Origanum sipyleum* and its water extract prepared by 2 % infusion. In the present work, in addition to quality assurance, for understanding its medicinal and nutritive value, the presence and the quantity of heavy metals and trace elements in powdered plant material and water extract of *O. sipyleum* were determined by X-ray fluorescence (XRF) spectroscopy. The major elements such as K, Ca and Na, known as macronutrients, are detected in 11990, 10490 and 970 ppm in powdered drug and 8910, 2991 and 810 ppm in water extract, respectively. Toxic elements such as Pb, Cd and As were in low concentrations in powdered drug and its water extract. Zn and Cu were found to be accumulated in water extract of *O. sipyleum* in 2832 ppm and 879 ppm concentrations respectively. Fe was determined as 74.3 ppm and 44.2 ppm for powdered drug and water extract.

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PW-253

UV spectroscopy and chemometrics: A simple approach to herbal quality control

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Recently, the application of multivariate analyses to spectroscopic and chromatographic data has been widely used in the quality control of herbal medicine [1]. In this sense, the work presented describes a simple technique developed using UV spectroscopy together with chemometric techniques of hierarchical cluster analysis (HCA), principal component analysis (PCA), and soft independent modeling of class analogy (SIMCA) in herbal authentication. This model was successfully used to authenticate *Thymus vulgaris* and discriminate it from *Thymus*, *Satureja*, *Origanum*, *Plectranthus* and *Eriocephalus*species, all traded in the Egyptian market as different types of thyme [2]. The model was also applied to authenticate 25 samples of monofloral Yemeni Sidr honey; one of the most expensive honey types worldwide and

extensively adulterated [3]. In the former case, the model was constructed using UV spectroscopic data from methanolic extracts of 30 samples of*T. vulgaris*. PCA and HCA discriminated 20 samples of different botanical origins while 12 samples of commercial thyme were segregated into thyme or non-thyme using SIMCA. In case of the Sidr honey study, HCA and PCA achieved segregation of the genuine Sidr samples from the lower priced local polyfloral and non-Sidr samples. The SIMCA model was used to identify genuine Sidr honey samples as well as detect admixture with lower priced polyfloral honey by detection limits >10%.

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PW-254

Annual variation of the essential oil production of *Artemisia asiatica*

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Artemisia asiatica Nakai (Asteraceae) is an Asian traditional medicinal plant. It has been used for the treatment of inflammation, cancer, microbial infections. The biological activities of this species can partly be attributed to its essential oil content. Our study aims to evaluate the essential oil production (phytomass, essential oil content, composition and production) of *A. asiatica*, taking into account both of the annual and seasonal variations of the plant.

Plant material was collected from *A. asiatica* population, growing in the experimental field of the Centre for Ecological Research, Vácrátót. Sampling was done bi-weekly from the middle of April to the end of the vegetation period in three successive years (to the middle of September in 2012). Lengths, fresh and dry weights of the leaf and stem of shoots were recorded. Essential oil was obtained by hydro-distillation from the fresh plant material according to the prescription of Ph.Hg.VIII. Its content was calculated both on fresh and dry weights. The oil composition was determined by using GC/FID (HP 5890 series II, HP-5 column) and GC/MS (Finnigen GCQ, DB-5MS column). Authentic samples, Kovats indices and data base figures were used for identification.

Plants have reached the full height in June-July, together with the leaf and stem weights of the shoots. From the second part of August a quick decrease in the fresh weight could be observed.

The essential oil content (0.03–0.72 fresh wt. %) showed a similar tendency but the maxima appeared earlier, in May and June respectively. The maximum yield of essential oil could also be measured in June. Twenty-seven components were regularly determined in the essential oil samples. Twenty mono- and seven sesquiterpenes were identified. Artemisia- and yomigi alcohols, 1,8-cineol, terpinen-4-ol, α- and γ-terpinene, β-caryophyllene, germacrene D were the main components. During the vegetation period the composition of the essential oil has not varied significantly.

PW-255

Optimization of extraction conditions of acorn pollen for tyrosinase inhibition using response surface methodology

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Bee pollen is a complex of flower pollen and nectar, collected by honey bees. It is rich in phenolic compounds including flavonoids, and has been used as a functional products such as dietary supplement. In our present study, the extract of acorn bee pollen inhibited tyrosinase activity, a key enzyme in melanin synthesis. For development as products, extraction procedure is indispensable and extraction conditions greatly affect the biological activity and chemical composition. Therefore, optimization of extraction conditions for maximum tyrosinase inhibition was determined using response surface methodology of Box-Behnken design (BBD) with three-level-three-factor such as extraction solvent (50, 75 and 100% EtOAc in MeOH), extraction time (19, 31 and 43 h) and extraction temperature (10, 30 and 50 ℃). Regression analysis showed a good fit of the experimental data with F-value of 52.16 and p-value of 0.001 and showed the importance of extraction solvent for maximum tyrosinase inhibition with p-value of 0.001. The optimal condition was obtained as EtOAC concentration, 66.8%, extraction time, 19.0 h, and extraction temperature 10.0 °C with 65.6 % tyrosinase inhibition. Further analysis of flavonoid content and tyrosinase activity in the extract prepared from different extraction condition in response surface methodology suggested the positive correlation of flavonoid content and tyrosinase inhibition with R2 of 0.176. Taken together acorn pollen is a promising candidate for decrease in skin hyperpigmentation and food browning. In addition, optimized extraction condition for tyrosinase inhibition will provide useful information for the development of acorn bee pollen as functional products.

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PW-256

Metabolite profiling of minor constituents in *Salicornia gaudichaudiana* by countercurrent chromatography and ESI-MS detection

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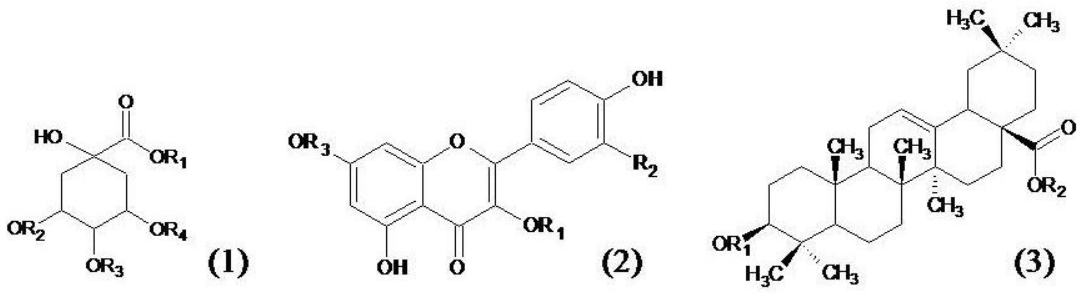
Salicornia gaudichaudiana (Chenopodiaceae) is a halophyte plant growing in high-level salt soils [1]. In Brazil, plant material is used in food preparations as *green salt* for people suffering of high blood pressure, kidney and heart diseases. Therefore, the nutritional and chemical properties with the concomitant profile of metabolites are of high interest. The major compounds of *S. gaudichaudiana* had been studied by preparative countercurrent chromatography (CCC) combined with off-line injections of fractions in the sequence of recovery to an ESI-MS device [2]. In this work, minor constituents of *S. gaudichaudiana* were investigated using the mentioned method combination. This resulted in the rapid recognition of, at least, 60 target compounds which were identified or partly characterized by molecular weights and ESI-MS/MS fragmentation based on previously isolated compounds[1,2]. The existing profile of natural products could be divided in four main groups (Figure): (**1**) chlorogenic acid derivatives, with one, two and three attached caffeoyl-, feruloyl- or dihydro-caffeoyl units, esterificated as methyl quinate or not; (**2**) flavonoid derivatives, including kaempferol, quercetin and ishorametin aglycones with up to three sugar units; (**3**) triterpenoid saponins, including calenduloside E differing in the number of sugar units and sugar substituents; and (**4**) other phenolics, including benzoic and cinnamic acids derivatives. Besides common substituents, losses of *m/z* 36 and 63 were frequent and could indicate de presence of chlorinated and sulfonated derivatives, respectively.

Figure. Main groups of natural compounds in *S. gaudichaudiana* EtOAc extract and its possible position of substitution



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PW-257

Metabolic and biological prospecting of *Aphyllocladus spartioides*

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Aphyllocladus spartioides known as "tojra tola" is a native shrub distributed in southern Bolivia and the northwest of Argentina, being used medicinally by indigenous people for the treatment of digestive infections and to treat rheumatic diseases. In order to improve the knowledge on its metabolite profile and biological properties, two different leaf extracts (hydroethanolic and decoction) were studied. 18 phenolic compounds were determined by HPLC-DAD. The phenolic content of the hydroethanolic extract was higher, isorhamnetin-3-*O*-glucoside being the main constituent. 8 organic acids were identified by HPLC-UV, malic acid was the compound present in the highest amount in both extracts. The chemical profile of essential oil of *A. spartioides* leaves was also characterized by HS-SPME/GC-FID, and 67 volatiles were identified. *δ*-Cadinene was the most abundant constituent. The inhibitory effects against cholinesterases, the antioxidant potential (DPPH•, $O_2^{\bullet-}$ and •NO) and the antibacterial activity were checked by *in vitro* assays. Decoction was the most active against the first two radicals (IC_{50}=79 and 28 μg/mL, respectively). Thet hydroethanolic extract proved to be the most active on nitric oxide (IC_{50}=206 μg/mL), acetylcholinesterase (IC_{50}=926 μg/mL), butyrylcholinesterase (IC_{50}=917 μg/mL) and α-glucosidase (IC_{50}=181 μg/mL). Additionally, antibacterial activity was also investigated here against a set of pathogenic bacteria. The bioactivities observed may be due, at least partially, to the presence of organic and phenolic acids, flavonoids and volatiles determined in this work. Furthermore, *A. spartioides* can be used in food industry as food additive, or can be applied in cosmetic and pharmaceutical industries providing protections against several diseases.

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PW-258

Development and validation of a method for standardization of infusions of *Herniaria hirsuta*

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Herniaria hirsuta L. is traditionally used in Morocco to treat kidney stones, and shows cholesterol lowering effects and activity against gallstones [1,2]. An HPLC-DAD method for the quantification of flavonoids and saponins, the main compounds, was developed and validated according to the ICH guidelines in order to standardize the extract. Several analytical columns and mobile phases were tested. For each solvent system, the gradient was optimized.

The extraction solvent composition and the sonication time were evaluated. Sonication for 20 minutes with 50% (v/v) methanol gave the best extraction results. A gradient, starting with 5% acetonitrile (ACN) + 0.05% formic acid (FA) going to 100% ACN + 0.05% FA, using the Apollo C18 column, gave the best resolution. The flavonoïd content was expressed as rutin and the saponin content as hederacoside C. The calibration model appeared to be linear. Though for most of the flavonoids and for the saponins, there is a significant effect of the factor day and/or factor concentration, the precision of the method is acceptable taking into account the complexity of the analysis. The $RSD_{I(Tc)}$ for the determination of flavonoïds is smaller than 3% and values for saponins ranged from 3.13% to 14.54%. Saponins are detected at a wavelength of 210 nm causing higher variation in results, especially when the concentration is low, which is the case for one of them. Therefore only the total content of saponins can be determined with an acceptable $RSD_{I(Tc)}$ (3.13%). The method can be considered as accurate for flavonoids (mean recovery 99.18%). For the saponins the mean recovery is 109.20%, and because this falls out of the generally accepted limits (97% to 103%), it should be mentioned with every result obtained with this method.

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PW-259

Effect of the growing location on herb yield and active substances of *Melissa officinalis* and *Thymus vulgaris*

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Lemon balm and thyme are important and popular MAP species widely utilized and processed all over the world. In the practise, the production of these species is going on under diverse ecological conditions. The goal of our investigation was studying the effect of growing location, as complex environment on these species.

In 2014 open field plots were established in Budapest (BP) and Poznan (PZ) characterised by different soil and weather conditions. Five cultivars of lemon balm (’Lorelei’, ’Lemona’, ’Soroksári’, ’Quedlinburger Niederliegende’ and ’Gold Leaf’) and four cultivars of thyme (’Sloneczko’, French Summer’, ’Varico 3’ and ’Standard Winter’) were tested. Herb yield, essential oil content, total phenolics, total flavonoid and rosmarinic acid contents were investigated.

Significant differences of herb mass of lemon balm were found: 291 and 107 g/plant in PZ and BP, respectively. Similarly, shoot bimass of thyme was double in PZ than in BP (105 and 53 g/plant, respectively). In PZ, total phenolic content was higher by 10% (lemon balm) and 20% (thyme), flavonoid content elevated by 57% (lemon balm) and 27% (thyme) compared to BP.

Only essential oil content of lemon balm proved to be higher in BP than in PZ (0.185 and 0.105%, respectively). That of thyme, however, was more favourable in PZ (+27%).

Besides, differences among cultivars were detected. In lemon balm 'Soroksári' responded strongly, while 'Gold leaf' changed least. In thyme, for each characteristics different cultivar proved to be most sensitive: essential oil content of 'Varico 3', flavonoid content of 'Standard Winter', and phenolic content of 'Sloneczko' showed largest differences.

Data show, that the quality of the drugs of these typical Mediterranean species may be excellent also at Northern locations. Although accumulation of phenolics might be the result of stress conditions, the higher biomass in our case contradicts to this assumption.

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PW-260

Integrated HPTLC-based methodology for the tracing of bioactive compounds in herbal extracts by employing multivariate chemometrics. The case study of anti-tyrosinase agents from *Morus alba*

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The contribution of naturally derived products for drug discovery purposes is undisputed. The implementation of high-throughput screening (HTS) technologies has allowed the rapid and effective investigation of the chemical composition and pharmacological properties of a large number of plant extracts against several biological targets. The scope of this study was the discovery of plant derived tyrosinase inhibitors utilizing a HTS approach and the introduction of an integrated methodology for the rapid identification of bioactive compounds during bioassay-guided procedures.

An extended extract library encompassing 3600 extracts from approximately 150 families was generated from plants originated from six regions with intense biodiversity and subsequently was screened for tyrosinase inhibition. Chemometric tools supported the development of a novel integrated HPTLC-based procedure for the tracing and targeted isolation of bioactive compounds in active extracts. Fractions resulted from CPC separation of *Morus alba* extract, the most prominent agent identified during anti-hyperpigmentation screening, were assayed for tyrosinase inhibition potential and analyzed with HPTLC. Multivariate data analysis tools enabled the tracing of compounds that contributed to the appearance of a tyrosinase inhibitory effect in active fractions. Two methodologies were developed for the generation of the dataset; one based on chromatogram binning and the second based on manual peak picking. Targeted isolation of compounds indicated to contribute best to the anti-hyperpigmentation activity was performed and IC_{50} values were estimated. Both methodologies were found capable to trace the components (e.g. oxyresveratrol, *trans*-dihydromorin, 2,4,3'-trihydroxydihydrostilbene) that exhibit the highest bioactivity in the mixture. All steps of the experimental procedure implemented techniques that afford essential key elements for application in HTS procedures for drug discovery purposes.

PW-261

Ionic liquid micellar extraction for quantitative determination of sesquiterpenic acids in Valerianae Radix

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A series of hydrophilic 1-alkyl-3-methylimidazolium, tetraalkylammonium and pyrrolidinium-based ionic liquids (ILs) were investigated as extractants for the sample preparation and quantitative determination of valerenic and acetoxyvalerenic acids in Valerianae radix. The extraction outcome was monitored by means of HPLC and the results were compared with these obtained according to the well established methods such as European Pharmacopoeia 8 method and Soxhlet extraction (total extraction), both conducted with methanol as a solvent. The extractions were carried out both at room temperature and conventional heating conditions, and the influence of the anion (chloride, bromide, acetate, trifluoroacetate, thiocyanate, dicyanamide, tricyanomethanide, acesulfamate and saccharinate), type of cation, alkyl chain length in the imidazolium ion, concentration, temperature, extraction time, particle size of root, and solid-liquid ratio was investigated.

The results obtained showed that the extraction yield is strongly dependent on the cation type. The best outcome was obtained with ILs composed of cations capable to form micelles in aqueous solution, e.g. 1-decyl-3-methylimidazolium chloride {[$C_{10}C_1$im]Cl}. Further, the extraction was found to be highly dependent on the IL concentration with best outcome (same with the reference methods) at concentrations slightly higher than IL CMC. As a result, an improved protocol for quantification of sesquiterpenic acids in root of *Valeriana officinalis* L. (Caprifoliaceae) was developed. Compared to the conventional procedures, it ensures same extraction yield but excludes the toxic and flammable organic solvents, the latter being of a great importance from safety point of view.

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PW-262

LC-HRMS based chemical profiling of *Opuntia ficus indica* and assessment of its antioxidant, whitening, protective activity and toxicity

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Opuntia ficus indica is an important food source with high nutritional value that contains also several bioactive secondary metabolites. In continuation of our research we have performed a comparative study on the chemical content and the biological activity of different tissues

(cladode, fruit flesh, flower, fruit peel, and seed). Each of the plant materials was extracted with different solvents and analyzed by HPLC and then by LC-HRMS to compare relative concentrations and identify main phytochemicals. Then all extracts have been assessed for their antioxidant activity against DPPH and ABTS radicals, and for their potential skin whitening proprieties and toxicity.

The aqueous extracts of cladodes and flowers have been shown to be a particularly rich source of phenolic acids and flavonoids, respectively. On the other hand, the fruit flesh aqueous extract showed a high indicaxanthin content. In addition, cladode, flower and peel extracts demonstrated high radical scavenging activities against the ABTS radical cation. However, a poor scavenging effect on DPPH radical was observed. Most extracts showed also whitening activity by the tyrosinase assay.

Furthermore, the potential protective and cytotoxic effects of aqueous extracts have been evaluated *in vitro*. Cell viability was determined using the MTT assay and none of the extracts showed cytotoxicity neither in HepG2 cell line nor in Hela cells. The genotoxic potential was studied with the Comet assay and none of the tested extracts exhibited genotoxic activity (DNA damage) in HepG2 cells. Furthermore, some of the extracts exhibit protective activity against DNA damage induced by hydrogen peroxide in HepG2 cells.

Therefore, the absence of any cytotoxic effect of the extracts in combination to the protective, antioxidant and the promising skin-whitening effects also lay the foundation for a wider use of this plant in the cosmetic industry.

PW-263

An accurate experimental design for the characterization and quantitation of *Antrodia cinnamomea*triterpenoids with RSM, qNMR and HPLC-Tandem MS: A tough case to crack

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Antrodia cinnamomea (AC) is an Asian treasured medicinal mushroom, which has attracted attention due to the recent reports on its potency in targeting several serious ailments including cancer and liver diseases. Among different AC components, triterpenoids are considered the most therapeutically attractive constituents due their cytotoxic and anti-inflammatory activities. In the current investigation, we proposed a mathematical and statistical extraction protocol for evaluating the concentrations of the total ergostane and lanostane triterpenoid derivatives, from the ethanolic extract of the wild fruiting bodies of AC (EEAC) by using response surface methodology (RSM) and quantitative NMR (qNMR). The optimum response

surface model illustrated that the variations of the studied response variables reached more than 90% indicating the accuracy of the developed model in explaining the variability of responses. On the other hand, the quantification of EEAC total triterpenoids was performed by comparing the HPLC-tandem MS results with those of the qNMR results. The accuracy of the used techniques was also evaluated. The experimental design of EEAC optimum extraction procedure obtained using RSM and qNMR allowed the accurate characterization and quantitation of *Antrodia cinnamomea* triterpenoids.

PW-264

Effects of harvest period and distillation method on the chemical composition and anti-microbial activity of the essential oil of *Cyperus scariosus*

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The essential oil of *Cyperus scariosus* R. Br. is gaining importance in the realm of fragrances due to its unique earthy-woody aroma. This paper details the monthly variations of the essential oil composition from October to February. Two distillation methods, wet steam distillation and dry steam distillation were used for the extraction of the essential oils. Cyperene, one of the major constituents varied significantly between 12-36% of the essential oil while β-cyperione composition varied between 6-23%. Anti-microbial studies against bacteria including *Escherichia coli* and *Staphylococcus aureus* revealed significant antimicrobial activites for samples from all seasons. Moreover, principal component analysis revealed correlations between the essential oil yield and composition.

PW-265

Effect of traffic pollution on physiological parameters, phenolic profile and antioxidant activity of *Rosa canina*

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Road traffic emits a cocktail of pollutants that can influence the vegetation and plant diversity in neighboring areas. However, the effects of pollutants on the physiological parameters and production of plant secondary metabolites are still little explored. In this study, we compared the physiological parameters in leaves of dog rose (*Rosa canina* L.) at two locations – degraded site near the high frequency road (DS) and preserved meadow site isolated from traffic and direct urban impact (MS). Also, we compared the content of 45 selected phenolic compounds and antioxidant activity of leaves extract of *R. canina* samples collected at these two locations. Photosynthetic rate (the basic parameter of bioproduction), transpiration rate, stomatal conductance and substomatal CO_2 concentration were measured in leaves of intact plants. The concentration of macroelements was determined in leaves (nitrogen by Kjeldahl's method, phosphorus by spectrophotemetric method, potassium by flame photometry). Phenolics were quantified by LC-MS-MS [1]. The assessment of antioxidant activity was done by several

assays (FRAP, DPPH, NO, $OH^{\bullet}$ assays and ability to inhibit lipid peroxidation). The higher transpiration rate was observed at MS (1.67 *vs*. 1.46 mmol H2O $m^{-2} \cdot s^{-1}$ at DS), indicating more stabile water regime (6.22 *vs*. 5.76 μmol CO_2 $mmol^{-1}$ H2O at DS), corresponding to higher photosynthetic activity (9.60 *vs*. 8.43 μmol CO_2 m^{-2} s^{-1}) and higher bioproduction in plants at unpolluted location. The concentration of P was at the lower limit (0.09%) in plants at DS. There was no significant difference in content of 45 phenolics and antioxidant activity between two investigated sites.

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PW-266

HPTLC-ESI-MS and HPTLC-fluorescence methods for identification and quantification of darutoside in *Sigesbeckia orientalis* leaves extracts

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Sigesbeckia orientalis L. (Asteraceae) is a small shrub native to India and widely distributed in tropical and temperate parts in the world. In some countries, this herb is an important traditional medicine to treat skin disorders. In Malagasy Pharmacopeia, the leaves are used externally as protective covering for wounds and burns to stimulate wound healing. We confirmed the real interest of this plant for cosmetic application by the development of an extract of leaves of *Sigesbeckia orientalis* really efficient for sensitive skins. Our phytochemical investigations showed that diterpenoids are one of the main groups of secondary metabolites. Thus, we developed a convenient and reliable analysis method to assess the quality control of the leaves and darutoside was selected as marker. RP-18 HPLC with UV detection at 210 nm was often used to identify terpenoids. Nevertheless, the methodology is limited in its ability to separate all components of interest in hydroethanolic extracts of *Sigesbeckia* due to the presence of a resin-gum (mix of oligosaccharides and terpenoids). The aim of our study was to develop a quantitative analysis of darutoside in leaves extracts by HPTLC-fluorescence. The separation was performed on Si60 HPTLC plates. The mobile phase was chloroform/methanol/water in the ratio 65/25/4 (v/v/v). For revelation, the plates were sprayed with primuline reagent and then scanned in the fluorescence mode in a TLC scanner at 366 nm. Darutoside was previously identified by ESI-MS with TLC interface. We applied our method for quantification of darutoside on our leaves samples collected in Madagascar. We observed that the amount of darutoside is variable depending on the date of harvest. April to June is the best period to harvest the leaves because darutoside reaches up to 1.5% of dry extract. This present study described for the first time the quantification of darutoside in *Sigesbeckia orientalis* leaves extracts by HPTLC-fluorescence method.

PW-267

Evaluation of intraspecific variability of biologically active compounds of *Melissa officinalis*

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Melissa officinalis L. has been used in the traditional medicine for therapeutic properties [1]. Intraspecific chemical variability of five lemon balm cultivars (Gold Leaf, Lemona, Lorelei, Soroksári and Quedlinburger Niederliegende) were compared concerning essential oil content, total phenolic content, total flavonoid content, rosmarinic acid content and antioxidant activity [2]. It was established the investigated genotypes represent different chemical qualities and there is a considerable intraspecific diversity in the mentioned characteristics. Samples of Lemona presented the highest essential oil content (0.298 mg/g DW) while Soroksári showed the lowest (0.067 mg/g DW). Total phenolic content of extracts ranged between 359 mg GAE/g DW ('Lorelei') and 426 mg GAE g/DW ('Gold leaf'). However, Lorelei showed outranging levels of total flavonoid content (0.948 mg QE/g DW) exceeding the value of the lowest one in Quedlinburger Niederlegende (0.570 mgQE/g DW). Rosmarinic acid being the highest in Lorelei (3.01 mg/g DW) and the lowest in Lemona (2.42 mg/g DW). The antioxidant capacity of the samples indicated the highest activity (309.1/mgASE/g DW) for Soroksári and 258.9 / mgASE/gDW for Lemona. Our study showed that proper definition of biological raw material is necessary for obtaining pharmaceutical products of standard quality.

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PW-268

Milk thistle in Wilson's disease: what is the pledge of safety?

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Silybum marianum (milk thistle) has been used for centuries as a herbal medicine for the treatment of liver diseases. Its purified active fraction, silymarin, is applied in treatment of liver diseases of toxic and viral origin in the modern therapy. The plant and its products are applied in the treatment of patients suffering from Wilson's disease as well with the aim of protecting liver from the consequences of copper accumulation.

Based on the available data we hypothesized that products prepared from milk thistle may include copper in significant amount. Preparations with high copper content may be real danger for patients. Our aim was to examine this hypothesis and measure the copper content of different products by ICP-MS.

The copper content of the daily doses of the examined preparations ranged between 0.01-114.18 μg. For patients suffering in Wilson's disease copper-free diet is recommended and milk thistle preparations containing a high concentration of copper significantly increase the copper load and therefore can be regarded as undesirable during the treatment. The copper contents of samples containing silymarin, the purified flavonolignan complex of milk thistle fruits, were rather low (0.01-3.51 μg in the daily dose). However, products based on *Silybum* extracts other than of silymarin or ground plant material contained copper at concentrations which were magnitudes higher than silymarin (36.04 and 12.04-96.50 μg in the daily dose, respectively). These results emphasize that the preparations of the copper-concentrating milk thistle may greatly vary in copper content. The method of extraction has major impact on the copper content therefore in the treatment of Wilson's disease, products containing silymarin should be preferred.

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PW-269

Atractylosides, chlorogenic acids and antioxidant capacity of raw Arabica green coffee beans (AGCB) used by regular and spontaneous dieters to supports body weight loss and slimming

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Using DPPH, ABTS and Folin-Ciocalteu procedures [1] the antioxidant capacity was characterized of fresh infusions of the raw AGCB distributed in Poland and originating from Central and South America. Next, UHPLC-TOF-MS/MS analyses of all infusions and MeOH extracts of raw AGCB were made using a Waters ACQUITY UPLC TabMode system interfaced with a Bruker microOTOF-Q mass spectrometer with an ESI source operating in negative mode at 150 °C and 4.5kV with N_2 nebulisation at 1.2 bar and a dry gas flow at 6 mL/min. An Agilent Poroshell 120EC-C18 column (2.1x150, 2.7μm) was used operating at 0.6 mL/min flow rate with a mobile phase consisted of a linear gradient of 0.1% formic acid in water (A) and 0.1% formic acid in AcN (B), mixed by increasing eluate B in the range 5-90% from 0-20 min and followed by a split of column effluent flow 3:2 before the ESI ion source was used. MSMS spectra were analyzed with a Bruker Compass Data Analyzer v.4. The atractyligenin derivatives, i.e. 2-*O*-beta-D-glucopyranosyl-carboxyatractyligenin (m/z 525) (**1**), 2-*O*-(2'-O-isovaleryl-beta-D-glucopyranosyl)-carboxyatractyligenin (m/z 608) (**2**) and 3'-*O*-beta-D-glucopyranosyl-2'-*O*-isovaleryl-2-beta-(2-desoxy-carboxyatractyligenin)-beta-D-glucopyranoside (m/z 771) (**3**), were identified in the MeOH extracts of the raw but not milled AGCB from Colombia. Only compound 1 with a high inhibitory activity of adenine nucleotide translocase (ANT) [2] was confirmed by MSMS in the infusions of this AGCB sample. This indicates that some atractylosides in raw AGCB were hydrolyzed by hydrothermal treatment [3]. Compounds **1** and **3** with, respectively, significant and non-significant ANT inhibition were recently detected in the AGCB [2], but glucoside **2** was identified here for the first time.

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PW-270

Qualitative and quantitative analysis of polyphenolic compounds from clone plants of *Actaea racemosa* L. by high-performance thin layer chromatography and reversed-phase high-performance liquid chromatography

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Black Cohosh [*Actaea racemosa* L. (Ranunculaceae)] is widely used in the treatment of climacteric disorders. In the last years the market for medicinal products from its rhizome extracts grew heavily. This led in an increasing demand for the plant material, whereby the natural population is compromised and adulteration with other *Actaea* species takes place [1,2]. Thus, especially in order to achieve reproducible quality, efficacy and safety of the medicinal products, manufacturers started to clone and cultivate Black Cohosh [3]. However, homogeneity of those plant materials regarding their containing compounds has never been investigated.

Therefore we phytochemically characterized the rhizomes from 40 clone plants and compared the data to plants from different origins. We developed and validated a reversed-phase high-performance liquid chromatography method with UV detection to determine the polyphenolic constituents such as fukinolic acid and cimicifugic acids, whose identity was ensured by mass spectrometric experiments.

The variation of the compounds concentration we investigated for the clone plants, was slightly higher than the variation of the method itself (RSD<3,0 %). The data shows that in reference to plants from different origins the plant material derived by cloning and cultivation is remarkable homogeneous regarding the mentioned compounds.

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PW-271

Benefits of using mass detection for analysis of non-chromophoric compounds

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The development of chromatographic methods for natural products and pharmaceuticals can be challenging for many reasons. These challenges can include poor or no UV absorbance, or similar UV spectra among components. For components that exhibit poor or no UV absorbance, practitioners typically explore alternative modes of detection such as FLR and evaporative light scattering (ELS). In this work, we will present a method for analyzing the non-UV absorbing active pharmaceutical ingredient memantine HCl and its associated metabolites. We will use a compact mass detector to efficiently track these components during the method development. We will demonstrate the linearity, reproducibility, and specificity achievable with mass detection.

Components of the sample were identified and tracked by mass detection over the method development runs. For final UPLC method, we selected low pH (125 mM formic acid in water), CORTECS C18+ column at 45 °C, and gradient of 5-90% with acetonitrile over 5 minutes. Flow rate and injection volume were set to 0.6 mL/min and 1.0 µL, respectively.

Memantine HCl and its associated metabolites do not absorb in UV as they lack chromophore but ionize well and are detectable by MS. To demonstrate that MS detection is suitable for analysis of these compounds, we tested the developed method for linearity, reproducibility, and specificity. Linearity show good correlation between peak responses and concentrations with correlation coefficient (r^2) greater than 0.999 for each component. System suitability results of 5 replicate injections were evaluated and compared with the USP specifications. The developed UPLC method was applied for memantine HCl assay in the tablet formulation.

In summary, mass detection enabled accurate identification and tracking of non-UV absorbing components during the method development process. Reproducibility and linearity of the developed UPLC/MS method was excellent.

PW-272

Variation of antioxidant activity and polyphenol content in safflower (*Carthamus tinctorious)* germplasm collection

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Safflower originated from Egypt and Afghanistan is an annual herb which usually used as the medicinal crop in Asian countries. Safflower seeds, rich in unsaturated fatty acid and α-linoleic, have been used for the promotion of bone formation, clinical treatment of osteoporosis and rheumatism in Korea. In order to identify the genetic resources with high antioxidant activity, we investigated the ABTS, DPPH and polyphenol content in safflower germplasm. A

total of 100 safflower accessions which had collected from two sites adjacent to the northern area of the Saudi Arabian peninsula were obtained from National Biodiversity Center of South Korea. Safflower seed extracts showed wide variation in ABTS antioxidant activity ranging from 34.5±0.70 to 156.5±3.06 μg trolox mg^{-1} dw. The antioxidant activity of DPPH was in the range of 1.6±0.070 to 14.1±0.23 μg ABC mg^{-1} dw. Total polyphenol content ranged from 14.2±0.41 to 81.6±1.56 μg GAE mg^{-1} dw. ABTS, DPPH antioxidant activity and polyphenol content were not different according to the collection sites. However, ABTS values showed a significant positive correlation (r=0.95) with DPPH antioxidant activity. Among the accession, K185841, K185863 and K185879 which showed high content of total polyphenol and antioxidant activity were recommended as a potential sources of safflower breeding. This study will provide the valuable information for safflower breeder in developing and producing functional materials.

PW-273

Comprehensive evaluation of the quality of *Platycodi Radix* with reference standard extract

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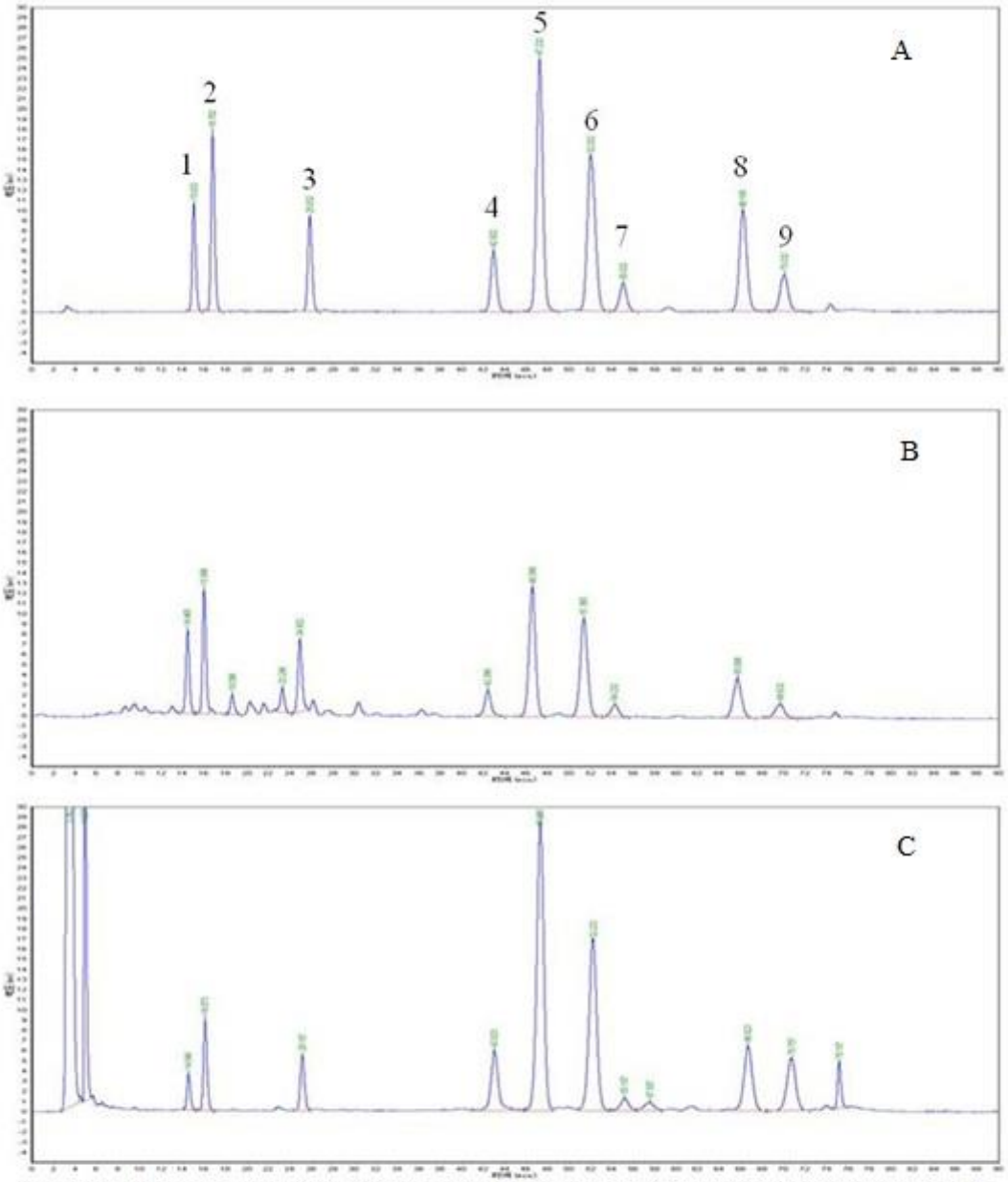
Reference standard extract (RSE), a new type of the reference substance, typically consisted of main components from raw material [1]. *Platycodi Radix* mainly contains platycosides [2]. In this study, a new quality control method employed RSE to comprehensively evaluate quality of herbal medicine was proposed. Through preparation and standardization of the platycosides RSE in which the content of total platycosides was more than 95 %, the quality of *Platycodi Radix* was evaluated as follows:

Section 1: Identification. Five main platycosides from *Platycodi Radix* were clearly identified by TLC with R_{fs} of 0.21, 0.30, 0.40, 0.52 and 0.65. Nine platycosides were identified by characteristic HPLC chromatogram with relative retention time (RRT) as 0.32, 0.36, 0.55, 0.91, 1.00, 1.10, 1.17, 1.40 and 1.48 (Fig.1).

Section 2: Quantitative determination of the platycosides with HPLC-ELSD. Contents of nine platycosides in *Platycodi Radix* were analyzed using standardized RSE obtained in this work. The results showed that contents of the nine platycosides were ranging from 0.02 to 0.7 %, which was also confirmed by conventional method using nine reference standards.

RSE could provide more comprehensive chemical information to assess the quality of complex characteristics of herbal medicine, compared with conventional quality-assessment methods, and also relive the stress of searching for pure reference standards.

Fig.1 Representative HPLC-ELSD chromatogram of *Platycodi Radix*



A: standard compounds; B: reference standard extract; C: sample; 1: deapioplatycoside E; 2: platycoside E; 3: platycodin D_3; 4: deapioplatycodin D; 5: platycodin D; 6: polygalacin D; 7: 3′′-*O*-acetylplatycodin D; 8: 2″-*O*-acetylplatycodin D_2; 9: 2″-*O*-acetylplatycodin D

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PW-274

Quantitative Determination of Protopine in *Hypecoum procumbens* subsp. *atropunctatum*

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The genus *Hypecoum* L. (Papaveraceae) is represented by five species and two subspecies in the flora of Turkey [1]. Protopine, the main isoquinoline alkaloid found in *Hypecoum* species, has been shown to possess important biological activities [2]. In the present study, a reversed-phase HPLC-DAD method has been used for the quantitative determination of protopine in the aerial parts of *Hypecoum procumbens* L. subsp. *atropunctatum* Å. E. Dahl collected from four different localities in Western Turkey [3]. Standard sample of protopine was previously isolated in our laboratory and authenticated by spectral analysis. Quantitative determination was carried out by the external standard method based on peak areas. The linearity of the method was studied by injecting six known concentrations of protopine in the range of 15-120 μg mL^{-1}. The calibration curve for protopine was determined as y=22.15862x+4.16731. Validation studies were also carried out to show that the method is specific, accurate and precise. Limit of detection and limit of quantification were established at a signal-to-noise ratio (S/N) of 3 and 10, respectively. In the four specimens, the content of protopine ranged between 0.0604-0.1361%.

Acknowledgements: This study was financially supported by Ege University Research Fund (13/ECZ/001).

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PW-275

Thin-layer chromatographic method optimization of a *Piper guineense* extract: comparison with published methods.

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Piper guineense Schumach is a medicinal plant that contains piperine as its major bioactive compound. Piperine alkaloid has antidepressant like properties [1]. The analysis of piperine is usually performed by thin layer chromatography (TLC), a method widely used in the analysis of bioactive compounds for drug discovery. However, the TLC method used for the separation of *P. guineense* extracts lacks optimization of the mobile phase and the effects of the developing chamber on the separation. The aim of the study was to develop an optimal TLC method for the separation of *P. guineense* extracts.

In the present study, a TLC method was optimized for the analysis of *P. guineense* extracts using solvents of various solvent strength (ST) and solvent selectivity (PS) values according to Nyiredy [2]. The mobile phase composition was systematically tested using various proportions of solvents differing in ST and PS under the same experimental conditions. In addition, the effects of developing chamber were tested using three types of unsaturated chamber conditions: horizontal chamber in sandwich configuration, horizontal chamber in non-sandwich configuration and twin trough chamber and vertical development. During the study a TLC method was developed and the best mobile phase composition giving good resolution of bands was found to be ethyl acetate: toluene (PS 4-6 at ST 3.2; corresponding to 40:60 % v/v). The tested developing chamber conditions did not affect the TLC separation efficacy.

The optimized TLC method resulted in improved separation when compared with the published method on the extracts of this species [3]. This present study provides the improved choice of mobile phase for the analysis of *Piper guineense*extracts.

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PW-276

Antileukemic lanostanoids from *Poria cocos*

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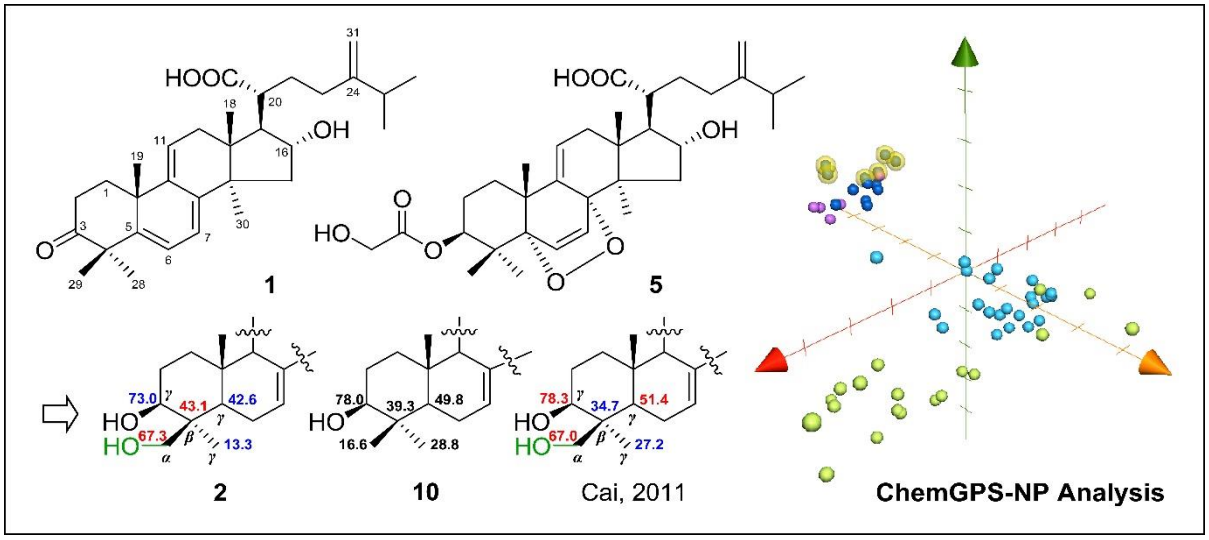
Seven new lanostanoids, isolated from the sclerotia of *Poria cocos*, were elucidated to be (20*ξ*)-16*α*-hydroxy-3-oxo-24-methyllanosta-5,7,9(11),24(31)-tetraen-21-oic acid (**1**), (20*ξ*)-3*β*,16*α*,29-trihydroxy-24-methyllanosta-7,9(11),24(31)-trien-21-oic acid (**2**), (20*ξ*)-3*β*,16*α*,30-trihydroxy-24-methyllanosta-7,9(11),24(31)-trien-21-oic acid (**3**), (20*ξ*)-3*β*-acetyloxy-16*α*,24*α*-dihydroxy-lanosta-7,9(11),25-trien-21-oic acid (**4**), (20*ξ*)-5*α*,8*α*-epidioxy-3-*O*-hydroxyacetoxy-3*β*,16*α*-dihydroxy-24-methyllanosta-6,9(11),24(31)-trien-21-oic acid (**5**), (20*ξ*)-3*β*,16*α*-dihydroxy-7-oxo-24-methyllanosta-8,24(31)-dien-21-oic acid (**6**) and (20*ξ*)-3*α*,16*α*-dihydroxy-7-oxo-24-methyllanosta-8,24(31)-dien-21-oic acid (**7**), based on the extensive spectroscopic analyses. The antileukemic activity of the new compounds (except **3** and **4**), along with the fifteen known lanostane-type triterpenoids, was evaluated against four leukemic cell lines (Molt 4, CCRF-CEM, HL 60 and K562). Dehydropachymic acid (**9**), dehydroeburicoic acid (**12**), pachymic acid (**14**) and lanosta-7,9(11),24-trien-21-oic acid (**20**) exhibited cytotoxic effect on CCRF-CEM cancer cell line with IC_{50} values of 1.43, 2.96, 2.61 and 5.96 μg/mL, respectively. Both dehydropachymic acid (**9**) and dehydroeburicoic acid (**12**) showed cytotoxicity against Molt 4 (IC_{50} 7.26 and 6.67 μg/mL) and HL 60 (IC_{50} 3.84 and 2.79 μg/mL) leukemic cell lines. ChemGPS-NP analysis on the active lanostanoids from *P. cocos* suggested that targets other than topoisomerases may be involved in the cytotoxic effect.



PW-277

Vasorelaxant studies on stilbenoids and phenanthrene derivatives from *Brasiliorchis porphyrostele* Rchb.f. (Orchidaceae)

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Seven compounds were isolated from the South American orchid *Brasiliorchis porphyrostele* (formerly known as*Maxillaria porphyrostele* [1]) and identified as 1,2,6,7-tetrahydroxy-4-methoxyphenanthrene (**1**), 9,10-dihydro-2,7-dihydroxy-4,6-dimethoxyphenanthrene (**2**), 3,4'-dihydroxy-5,5'-dimethoxydihydrostilbene (**3**), 3,4'-dihydroxy-3',5'-dimethoxydihydrostilbene (**4**), shikimic acid, *p*-hydroxybenzenepropanoic acid and euphorbol; compound **1** has not been described previously. As similar structures are recognized as spasmolytic or vasorelaxing agents [2,3], as well as having been shown to be selectively cytotoxic [4], the aim of the present investigation was to assess firstly their vasoactivity and secondly their cytotoxicity in various cancer cell lines. Preliminary results indicate that compounds **2**, **3**, and **4** possessed vasorelaxing activity on *in vitro* rat aorta rings pre-contracted with either phenylephrine or high K^+. However, compound **1**was inactive. Furthermore, compound **4** inhibited, in a concentration-dependent manner (IC_{50} = 20.2 µM), Ba^{2+} currents through L-type Ca^{2+} channels of rat tail artery myocytes. Compounds **1** and **2** were tested at 10 µM against several leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines. The compounds did not meet activity criteria in the one-dose NCI59 cell test for further testing. In conclusion, *B. porphyrostele* may represent a source of vasoactive agents potentially useful for the treatment of vascular diseases such as hypertension. Further studies are needed, however, to clarify their mechanism of action.

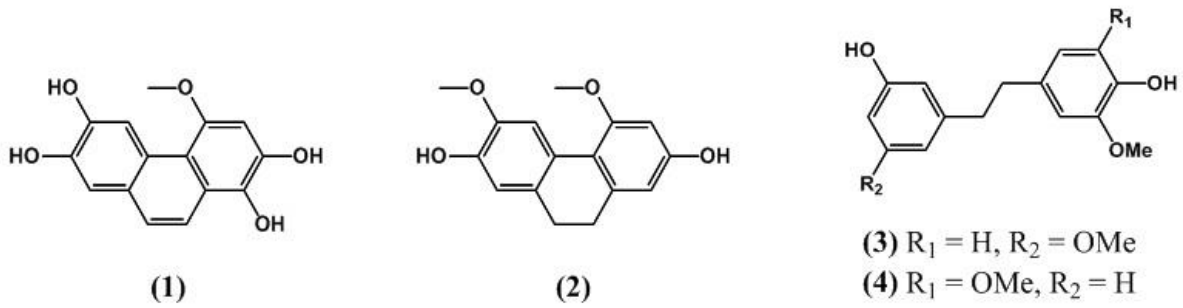

Figure 1 Compounds isolated from *B. porphyrostele*

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PW-278

Discovery of natural products potentially active against myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is a genetically inherited muscle disorder that is characterised by progressive muscle wasting and weakening, cataracts, and cardiac conduction defects. At present there is no cure or effective treatment for this disabling disease. In this context, a collection of 70 pure compounds and 2100 extracts from different plants and fungal strains were screened with a novel DM1-based biochemical assay for their ability to inhibit the formation of the pathogenic complex formed between (CUG)n-RNA and the splicing-factor muscleblind-like 1 (MBNL1). As a result, eight extracts from different plant species were found to be active (≥50% inhibition at 100 µg/ml). Active constituents were tracked using HPLC-based activity profiling, an approach which combines bioactivity data, structural information from online HPLC-UV-MS and offline microprobe NMR analyses, and database searches. Methylenetanshinquinone and 1,2-dihydrotanshinquinone were found to be the most active compounds in *Salvia miltiorrhiza*. The β-carboline alkaloid harmine was responsible for the activity of *Peganum harmala*, and the iridoid-glycoside auroside was identified as the active constituent in *Lamium album*. The HPLC profiles suggested the presence of tannins in the remaining five active extracts. Retesting of these extracts after tannin removal by filtration over polyamide confirmed the nonspecific interaction of the original extracts with the protein-based screen. In addition, the protoberberine alkaloid berberine was identified as a potent hit from the library of pure compounds. Overall, this study identified several small molecules of

natural origin which are promising hit compounds in (CUG)n-MBNL1 complex inhibition. In a secondary cellular assay some of the identified small molecules partially reversed the splicing defects associated with DM1. Detailed secondary *in vitro* and *in vivo* investigations on these compounds are ongoing.

PW-279

Phytochemical and pharmacological investigation of *Kickxia ramosissima*

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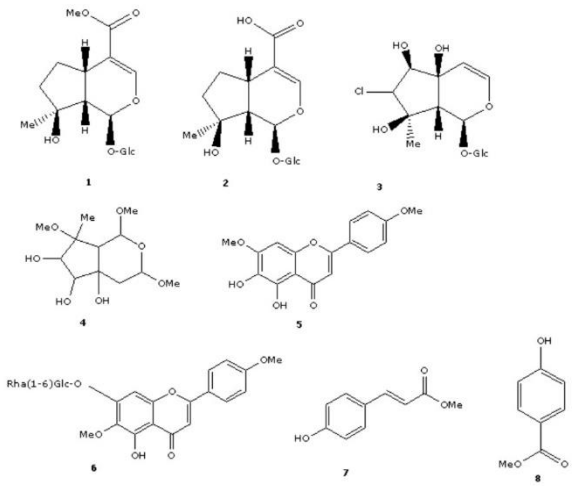
Kickxia ramosissima (Wall.) Janch.(Scrophulariaceae) is a small herb that is highly appreciated as a traditional medicine in the Indian subcontinent [1-2]. The scientific data reporting its constituents are poor and therefore the present investigation was undertaken to discover the main constituents. A double maceration was performed at room temperature with methanol 90%, followed by liquid-liquid partition with various solvents. Each fraction was then tested for claimed biological activities including antibacterial, antifungal, cytotoxic and antiglycation assays. The microorganisms used were *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Microsporum canis* and MRC-5 cells as a cytotoxicity control. The n-hexane fraction showed notable antibacterial (IC_{50} 8 µg/mL) and antifungal (IC_{50} 24.40µg/mL) activity. All other fractions were considered as inactive (IC_{50}>64 µg/mL).The ethyl acetate fraction showed the highest anti-glycation (Advanced Glycation Endproducts, AGEs) activity (IC_{50} 87.14 µg/mL), followed by the *n*-butanol (IC_{50} 144.62 µg/mL), methanol 90% (IC_{50} 167.16µg/mL) and chloroform (IC_{50} 175µg/mL) fractions. In order to provide adequate phytochemical information all extracts were further fractionated using repetitive flash chromatography and analysed by TLC and HPLC-DAD. For the isolation of major compounds, a semi preparative HPLC(RP)-DAD-MS system was used. Subsequently NMR and mass spectra were recorded to elucidate the structure of the isolated compounds, which could be identified as iridoids (**1-4**) flavonoids (**5-6**),p-hydroxy-coumaric acid methyl ester (**7**) and p-hydroxy-benzoic acid methyl ester (**8**). Compound **4** was a new iridoid. Biological evaluation of isolated compounds is in progress.



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PW-280

Synthesis and SAR of ecdysteroid derivatives as adjuvant anticancer agents

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Ecdysteroids are known as regulators of moulting and reproduction in arthropods, and they can also be found in many plants as defensive agents against herbivores [1]. Our research group has recently reported their adjuvant antitumor activity in combination with various chemotherapeutics on several cancer cell lines, by studying fourteen 20,22-, seventeen 2,3;20,22- and two 2,3-dioxolane derivatives semi-synthesized from 20-hydroxyecdysone (20E) [2-4].

Previously obtained SAR data turned our attention to the selective dioxolane-formation at the 2,3-diol. The more reactive 20,22-diol was protected by phenylboronic acid, and subsequent reactions followed by de-protection yielded 4 new derivatives. In order to obtain corresponding analogues of poststerone (Ps), a known in vivo metabolite of 20E, oxidative side chain cleavage was applied on 20E at the gram scale [5]. Further six new dioxolanes of

Ps were obtained in consecutive reactions with various aldehydes and ketones, such as acetone, propanal, butanal, pentanal and methyl-isobutyl-

All compounds were tested for their anticancer activity in combination with doxorubicin on a multi-drug resistant mouse lymphoma cell line expressing the human ABCB1 transporter. Poststerone 2,3-dioxolanes possessed very low intrinsic cytotoxicity but could greatly potentiate the cytotoxic activity of doxorubicin. By means of SAR of altogether 43 semi-synthetic ecdysteroids, the new derivatives were found promising leads against multi-drug resistant cancer.

Acknowledgement: Szeged Foundation for Cancer Research.

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PW-281

"Ligand fishing" in chemical space reveals new potential leishmanicidals

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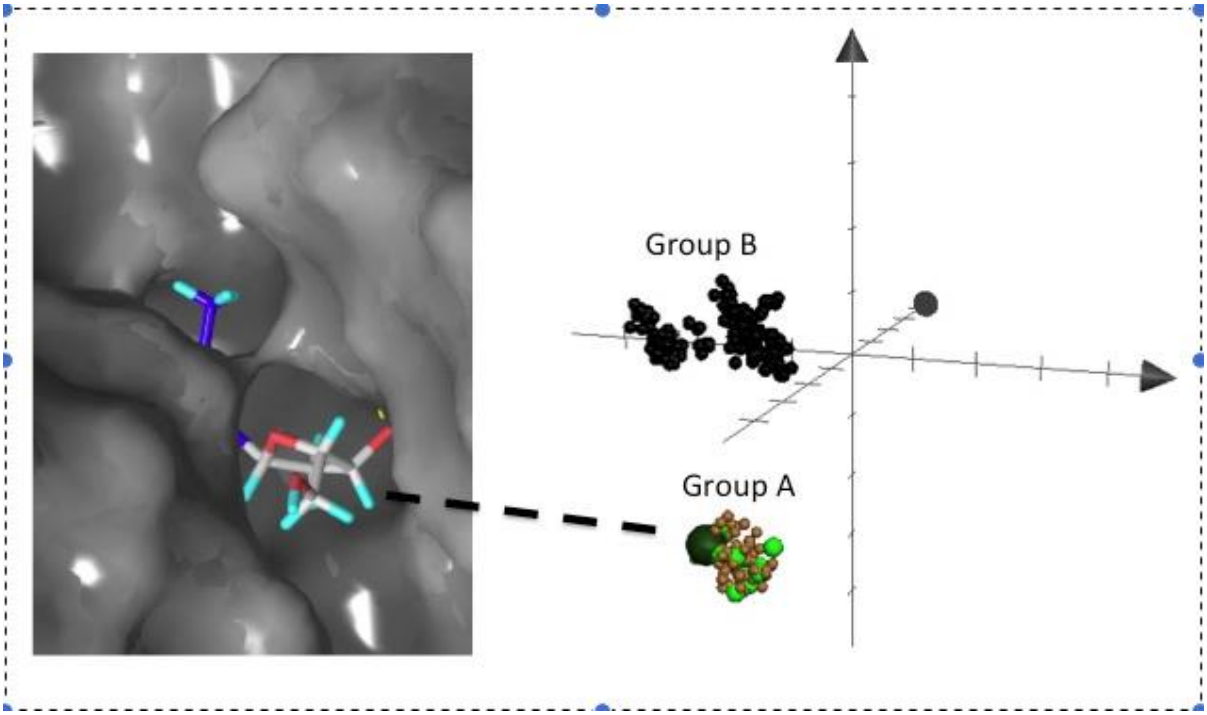
Pteridine reductase 1 (PTR1) is suggested to be a potential drug target in *Leishmania* parasites, because it is predicted to be essential for the pathogen's survival and it appears to lack human homologues [1].The aim of this study is to elucidate if "ligand fishing" in chemical space using ChemGPS-NP can be used to find new potential PTR1-inhibitors of natural origin. PTR1 in complex with 7,8-dihydrobiopterin (DHB), was obtained from the Protein Data Bank. Two sets of compounds, A and B, of natural origin were retrieved using ChemGPS-NP. ChemGPS-NP positions compounds in ch emical space according to their physical-chemical properties [2,3]. Group A included natural compounds that are positioned near DHB. Group B included natural compounds positioned far from all ligands in all crystalized structures of PTR1. The inhibitory effects of the compounds in group A and B, on PTR1 were assessed by predicting their affinity towards the enzyme using molecular docking. Thirteen of the 78 compounds in Group A were predicted to bind with a higher affinity than DHB to PTR1, and nine of these, interacted with the binding pocket of PTR1 in other ways than known ligands. None of the 191 compounds in Group B, were predicted to bind to PTR1 with the same or higher affinity than DHB. Hence, "ligand fishing" in chemical space using DHB as bait can be a successful path for finding new potential PTR1 inhibitors of natural origin.



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PW-282

A phytochemical and biological study of *Juncus maritimus*, an extremophile plant from Tunisia

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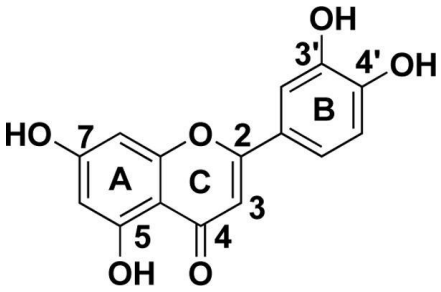
[3] *UDSL, INSERM U995, Faculty of Pharmacy, University of Lille (Lille 2), LILLE, France*
[4] *Inserm U1019, CNRS UMR8204, Center for Infection and Immunity of Lille (CIIL), Institut Pasteur Lille, University of Lille (Lille 2), LILLE, France*

In many regions of Tunisia, plants are often subjected to severe environmental conditions that influence the production of some secondary metabolites involved in stress defence mechanism.

Some of them are phenolic compounds known for their biological activities.These plants can be promising sources of potential drug leads [1].

In this context, 8 extremophile plants have been collected in different areas in Tunisia. Crude methanolic extracts of different parts of these plants have been prepared, and then evaluated for their antiradical, antimicrobial (on 36 strains Gram + and Gram -) and antiviral activities (hepatitis C). Two plants showed the most interesting activities, *Limonium virgatum*Fourr. and *Juncus maritimus* Lam. The extract of *J. maritimus* rhizomes demonstrated a moderate antiradical activity (IC_{50}= 45.23 ± 2.38 µg/mL) and a specific antibacterial activity against *Streptococcus dysgalactiae* and *S. pyrogenes* (MIC = 39 µg/mL). In addition, this extract showed the highest activity against hepatitis C virus (relative infection < 10% at 50 µg/mL).

Bioactivity-directed fractionation of the *J. maritimus* rhizomes extract showed that the ethyl acetate partition exhibited the highest antiradical activity while the methylene chloride partition was most likely responsible for antibacterial and antiviral activities. The major compound of the ethyl acetate partition was isolated using Centrifugal Partition Chromatography (CPC). It is luteolin, a common flavone known for its antiradical activity.



The two major compounds of the methylene chloride partition were isolated by CPC followed by semi-preparative HPLC. According to preliminary NMR and mass analyses, these natural products are phenanthrene derivatives.

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PW-283

Neuroprotective effects of xylopic acid on lipopolysaccharide-induced neuroinflammation

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Oxidative stress and neuroinflammation are implicated in several central nervous system (CNS) disorders. Xylopic acid has CNS effects including anti-neuropathic pain [1], anxiolytic and antidepressant effects [2] while other kaurene diterpenes have protective effect against MPP^+-induced neuronal death [3]. This study evaluates a possible neuroprotective effect of xylopic acid to help explain its myriad CNS effects. 8-week old mice received either xylopic acid (3, 10 or 30 mg/kg), fluoxetine (3, 10 or 30 mg/kg) or distilled water 10 ml/kg for 14 days.

Neuroinflammation was then induced by intraperitoneal injection of 830 µg/kg lipopolysaccharide (LPS) [4]. 24 h post LPS injection, sucrose preference test, forced swim and social interaction tests were performed to assess neurologic functions. Mice brain were remove 48 h after LPS injection for antioxidant enzymes assay and staining for degenerating neurons. Brain derived neurotropic factor was also measured using ELISA. Xylopic acid attenuated LPS-induced depressive-like symptoms by reducing immobility, increasing sucrose preference and enhancing social interaction ($F_{3, 35}$=56.14, P< 0.001). Oxidizing enzyme myeloperoxidase was significantly ($F_{7, 32}$=7.251, P< 0.001) reduced while antioxidant enzymes superoxide dismutase and catalase activity were elevated along with increased glutathione levels. Lipid peroxidation was also ameliorated as indicated by reduced TBARS in xylopic acid-treated mice. Xylopic acid potently (EC_{50}=1.72±1.65, Emax=93.92±12.18) increased brain derived neurotropic factor as well as reduced neurodegeneration. These results indicate neuroprotective effects of xylopic acid which may contribute to its myriad beneficial CNS effects.

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PW-284

Stimulatory and depressant-like effects of the crude alkaloids of *Picralima nitida*

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Picralima nitida (Stapf) T.Durand & H.Durand (Apocynaceae) locally known in Ghana as Akuamma (Asante-Twi) is widely used in West Africa for various medicinal purposes including infections and pain [1]. The rich alkaloidal nature of the plant accounts for majority of its pharmacological actions [2-3]. Although a number of scholarly studies exist on the plant (eg. analgesia, anti-inflammatory) [3], its in-depth effect on the CNS has not been explored. The aim of our research wa to investigate the stimulatory and depressant effect of the crude alkaloids of *P. nitida*. Powdered seeds were de-fatted in petroleum ether, cold macerated in 10% v/v HCl, basified with 36% v/v NH_3 and solvent-solvent extracted with $CHCl_3$ to obtain the crude alkaloids (PNE). The effect of PNE (30 to 3000 $mgKg^{-1}$ *p.o.*) on behavioural and physiological functions was assessed using Irwin's model in male ICR mice [4]. The sleep effect was further investigated in pentobarbitone interaction test [5]. Male ICR mice were treated with PNE (100 to 1000 $mgKg^{-1}$ *p.o.*) and sleep induced with sodium pentobarbitone (50 $mgKg^{-1}$ *i.p.*), first after an hour and in another experiment, 30 min after pretreatment with PNE to assess the onset of drug action. In both cases, mice were observed for latency and duration of sleep. **Results:** Initially observed CNS stimulating effect of PNE marked by

hyperactivity progressed steadily into depressive action. PNE significantly enhanced sleeping effects ($P<0.001$, $F_{5,32}=13.86$) in a dose-dependent manner indicating a depressant-like effect Figure 1. LD_{50} of PNE was approximately 3000 $mgKg^{-1}$. PNE acts biphasically; an initial temporal CNS stimulation and a sustained sleeping effect.

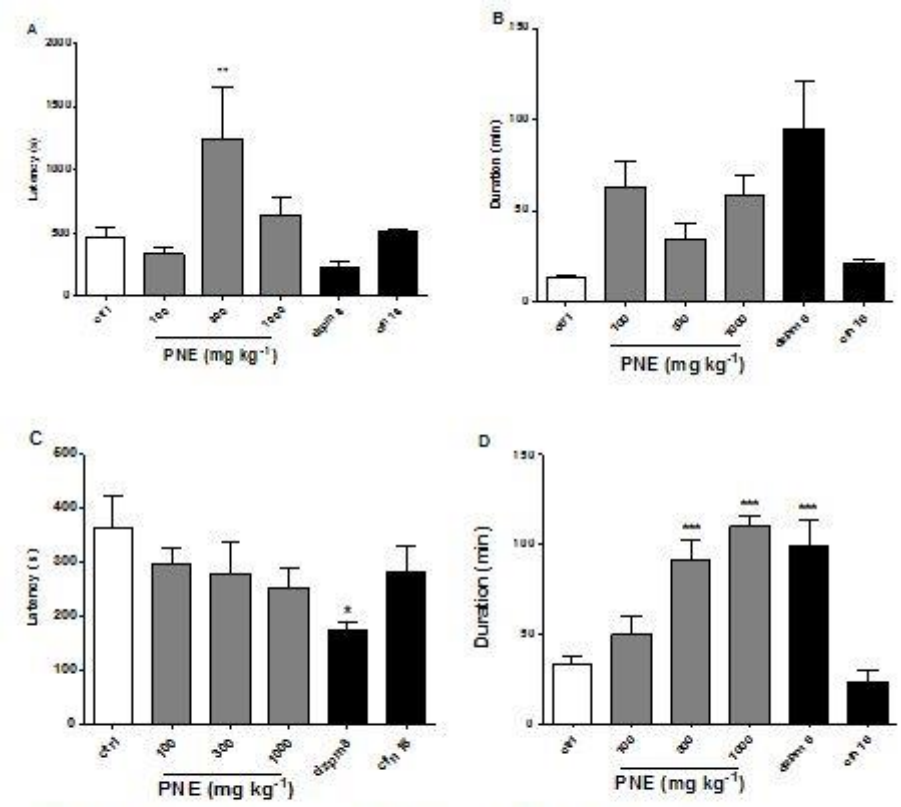

Figure 1: **Effect of PNE, diazepam and caffeine in pentobarbitone interaction test**

A= Latency to sleep (1 hour) **B**= Duration of sleep (1 hour)

C= Latency to sleep (30 minutes) **D**= Duration of sleep (30 minutes)

Data presented as group mean ± SEM ($n=6$); $*P<0.05$, $**P<0.01$, $***P<0.001$; compared to vehicle treated group, $P>0.05$ = not significant (One-way ANOVA followed by Newman-Keuls' test).

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PW-285

Lepidotols and lepidotins: new phenylcoumarins from *Mesua lepidota* as promising inhibitors of endothelial immune responses and dysfunction

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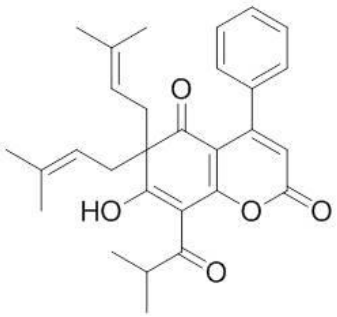
During organ transplantation, graft endothelium is the first barrier encountered by immune cells of the recipient. Endothelial cells surface presents inflammatory and immune proteins which are over-expressed after activation by pro-inflammatory cytokines, Damage Associated Molecular Patterns (DAMPs) or Advanced Glycation End Products (AGEs) [1]. Among natural products, several polyprenylated polyphenols have shown anti-inflammatory, immunomodulatory and anti-AGEs properties [2-3]. Such secondary metabolites are biosynthesized by *Calophyllaceae* species such as *Calophyllum* or *Mesua*species. In order to identify natural products able to prevent endothelial dysfunction, a dereplication analysis was conducted on various extracts from *Calophyllum* and *Mesua* species native to Malaysia. It appeared that the fruits of *Mesua lepidota*T. Anderson are a rich source of original phenylcoumarins named as lepidotols and lepidotins. The main compound, lepidotol A, was evaluated for its anti-inflammatory, immunomodulatory and anti-AGEs potential. Beside a potent inhibitory effect of the VCAM-1, class II HLA and HLA-E induced surface-expressions on human endothelial cells (52 %, 97 % and 66 %, respectively), lepidotol A exhibited an inhibition of AGEs formation five to thirty times higher than aminoguanidine (positive control). These results are consistent with the marked pharmacological activities of prenylated aromatic metabolites [4], and highlight a new approach to discover protective compounds against graft rejection.



Lepidotol A

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PW-286

Plant derived natural products as novel fusion inhibitors against *Herpes simplex* virus type 1 (HSV-1)

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Herpesvirus infection and spread can be specifically blocked by preventing the fusion between the virion and the host cell membrane. The core fusion machinery of HSV-1 consists of the glycoproteins gD, gH, gL and gB. While gD mediates the interaction with various host cell receptors, gB executes the fusion of viral with cellular membrane after activation by a reaction cascade between the glycoproteins.

Based on this mechanism a virus-free *in vitro* screening assay was developed for direct identification of antiviral compounds with fusion-inhibiting capability. Vero cells are transfected with gD, gH, gL and gB (effector-cells) and seeded on untransfected Vero cells (target-cells). The formation of syncytia and thereby the amount of fusion is visualized through mCherry-labelled gB. To quantify fusion activity, effector cells transfer a transactivator into the target cells, which in turn switches on a reporter gene, e.g. luciferase [1]. The use of Tet-On 3G as transactivator reduced cytotoxicity, widened the measureable window and allowed selective induction of reporter gene expression.

Docosanol (5 mg/ml), a known entry inhibitor of enveloped viruses, and α-gB-2c, a neutralising, gB-specific monoclonal antibody, served as positive controls. Aescin from *Aesculus hippocastanum* was identified as a potent fusion inhibitor against HSV-1. Two different batches, characterized in detail by LC-MS, showed IC_{50} between 5 and 10 µM, depending on incubation time and serum concentration in the cultivation media. Aescin reduces also HSV-1 plaque formation. Within a broader screening of saponins as fusion inhibitors we identified 2 active oleanan glycosides: hederacoside C (IC_{50} about 200 µM) from *Hedera helix*, and esculentoside A (IC_{50} about 150 µM) from *Phytolacca esculenta*.

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PW-287

A polyphenol enriched fraction of rose oil distillation water inhibits proliferation in HaCaT cells and induces apoptosis

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Water steam distillation of rose flowers (*Rosa damascena*) separates the essential oil from the polyphenol containing rose oil distillation waste water (RODW). While the essential oil represents the desired liquid for the cosmetic industry, the polyphenol containing RODW is in the center of our interest. Recently, a strategy was developed to separate RODW into a polyphenol depleted water fraction and a polyphenol enriched fraction [RF20-(SP-207)]. Polyphenols are known to have a wide spectrum of biochemical and pharmacological effects. In the present study, it was of interest to investigate possible antiproliferative effects of RF20-(SP-207) and fractions thereof F(I)-(IV) in immortalized human keratinocytes (HaCaT). The BrdU cell proliferation assay was used to measure cell proliferation. Cell migration was elucidated by time lapse microscopy. The data demonstrated that from all tested fractions only F(IV) revealed a dose dependent antiproliferative effect which is comparable to RF20-(SP-207) (IC50 of approx. 10 µg/mL). This effect is similar to both positive controls LY294002 (PI3K-inhibitor, 30 % inhibition) and NVP-BEZ235 (dual PI3K/mTOR-inhibitor, 30 % inhibition) and clearly exceeds the anti-proliferative action of quercetin (approx. 20 % inhibition). Time lapse microscopy revealed that cell migration was dramatically decreased under influence of RF20-(SP-207) and F(IV). This effect was comparable to LY294002 and NVP-BEZ235. Fluorescence microscopy images confirm the qualitative increase of apoptosis under influence of RF20-(SP-207) and (IV).

PW-288

Preparation and analysis of nanocarriers for brain delivery of neuroprotective andrographolide

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Andrographolide (AG) is a major diterpenoid of *Andrographis paniculata* (Burm. f.) Nees, the clinical utility of which has been demonstrated in the treatment of inflammation-related neurodegenerative disorders [1]. Low bioavailability and poor water solubility limit the further development of the compound.To overcome these limitations AG was loaded into albumin based nanoparticles (HSA NPs) and polyethylcyanoacrylate nanoparticles (PECA NPs). NPs were prepared by coacervation using thermal cross-linking, and by emulsion-polymerization, respectively. Both NPs appeared as spherically shaped with an average diameter of 255,4 ± 8,9 nm, a polydispersity (PD) of 0,19 ± 0,02, and a zeta potential of -4,77 ± 0,18 mV for PECA NPs. HSA NPs showed a mean diameter of 202.15 ± 6.15 nm, with a PD of 0.17 ± 0.01, and

a zeta potential of -10.20 ± 0.15 mV. The average drug-entrapment efficiency (EE) and loading capacity (LC) were 94,6 ± 0,41% and 13,2 ± 0,36%, respectively, for PECA NPs, and 98.21 ± 0.01% and 8.50 ± 0.01 % for HSA NPs.The ability of free AG and AG-loaded NPs to cross the blood-brain barrier (BBB) was evaluated with two *in vitro* BBB models based on human hCMEC/D3 and murine bEnd5 endothelial cells. For that purpose, a quantitative LC-MS/MS method for AG in Ringer HEPES buffer, in the range of 10-2000 ng/mL, and with forskolin as internal standard was developed and validated according to FDA/EMA guidelines [2-3]. Apparent permeability coefficients (Papp) in apical-to-basolateral (A-B) direction across cell monolayers cultured on 24-well format will be discussed.

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PW-289

Effects of urolithins on prostate cancer cells and activity of antiandrogens

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Herbal products are popular among cancer patients as elements of complementary and alternative medicine [1]. Some clinical studies provide evidence for positive effects of administration of ellagitannin rich preparations in prostate cancer. Urolithins, gut microbiota metabolites of ellagitannins, are readily absorbed from gastrointestinal tract [2]. In this study, we examined the effects of urolithin A, B and C (concentration 10-40 μM; 72h) on LNCaP and DU-145 prostate cells proliferation and interaction between urolithins and androgen receptor antagonists, bicalutamide and 2-hydroxyflutamide, used in prostate cancer therapy. Cell proliferation was determined by DNA-Hoechst 33285 stain complexes fluorescence intensity measurement in cell lysates. Cells were also double stained with Annexin V-FITC/propidium iodide and tested for apoptosis by flow cytometry. All tested urolithins dose-dependently inhibited prostate cells proliferation ($p<0.05$). Urolithin A was the most active against LNCaP cells, while urolithin C showed the greatest anti-proliferative effect in DU145 cells. Both urolithin A and B dose-dependently induced apoptosis in LNCaP cells. Urolithin A and 2-hydroxyflutamide additively inhibited LNCaP cells proliferation (combination index CI=1). Combinations of urolithin B and C with 2-hydroxyflutamide and urolithin A, B, and C with bicalutamide exhibited antagonism. These results suggest that ellagitannin rich products may be used in prostate cancer chemoprevention, but should be very carefully used during antiandrogen prostate cancer therapy.

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