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Research Article

Chemical Analysis and Toxicity Screening of *Phlomis olivieri* Benth. and *Phlomis persica* Boiss. Essential Oils

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ABSTRACT

Background: *Phlomis olivieri* Benth. and *Phlomis persica* Boiss. (Lamiaceae) are two medicinal species endemic to Iran. In the present study, we investigated the chemical compositions and general toxicity potentials of the essential oils obtained from the aerial parts of these two *Phlomis* species. ***Method:*** The essential oils of the plants were extracted using hydrodistillation method. CG and CG-MS were applied to analyze the chemical constituents of the essential oils and brine shrimp lethality test (BSLT) was used for the evaluation of general toxicity effects of the essential oils. ***Results:*** A total of 46 compounds were identified in the plants essential oils, among them β-caryophyllene (25.7%) and germacrene D (19.5) in *P. olivieri* and germacrene D (17.2%) and γ-elemene (15.4%) in *P. persica* were the main compounds. The essential oils of *P. olivieri* and *P. persica* exhibited a moderate toxicity activity (LD_{50}: 24.2 ± 0.5 and 41.6 ± 0.8 µg/ml, respectively) in brine shrimp lethality test compared to podophyllotoxin (LD_{50}: 2.8 ± 0.3 µg/ml). ***Conclusion:*** The results of present study report essential oils of *P. olivieri* and *P. persica* as two sesquiterpene rich oils with the moderate toxic activity. The chemical constituents of the analyzed essential oils were also found to be different from those reported from other regions of Iran, which may be due to the existence of possible different chemotypes between the populations of these two species.

Introduction

The genus *Phlomis* L. from Lamiaceae family includes about 113 perennial herbs or shrubs distributed in Asia, Europe and Africa.[1,2] Some of the *Phlomis* species have been reported for their traditional uses as analgesic, diuretic, tonic, anti-diarrheic agents and to treat various conditions such as gastric ulcer, diabetes, inflammation, hemorrhoids and wounds.[3] In Flora of Iran, this genus is represented by 19 species, including *Phlomis olivieri* Benth. and *Phlomis persica* Boiss.[4]

Previous *in vivo* and *in vitro* studies demonstrated antioxidant, antimicrobial and antinociceptive activity of *P. olivieri*, as well as antioxidant, cytoprotective, antinociceptive and antidiabetic effects of *P. persica*.[5-10] A review on the literature revealed that these two species have also subjected to the various phytochemical investigations.[11-18] In 2006, Sarkhail *et al*. reported the isolation and structure elucidation of one flavone glycoside; chrysoeriol-7-O-β-D-glucoside and one phenylethanoid glycoside; verbascoside from *P. olivieri* aerial parts, together with chrysoeriol-7-O-β-D-glucoside, chrysoeriol-7-O-β-D-(3"-E-*p*-coumaroyl) glucoside and one iridoid glycoside; lamiide, from *P. persica* aerial parts (11). One dimethoxy flavonoid, namely 6,7-dimethoxy-5-hdroxy flavanone has been also reported from the leaves of *P. olivieri*.[12] Furthermore, there are a number of reports related to essential oils composition of these two species from different regions of Iran.[13-18] In the present study we investigate chemical composition and general toxicity potentials of the essential oils of *P. olivieri* and *P. persica aerial* parts, collected from the Borujen (Chaharmahalo-Bakhtiari province) and Nahavand (Hamedan province) regions, respectively. To our knowledge this is the first report on chemical constituents and general toxicity activity of the essential oil of these two species from the mentioned regions in west of Iran.

Material and Methods

Plant material

The flowered aerial parts of *P. olivieri* and *P. persica* were collected in July 2014 from the Gandoman region (Borujen County, Chaharmahalo-Bakhtiari province, west of Iran) and Sarabe-Gian region (Nahavand County, Hamedan province, west of Iran), respectively.

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The plant specimens were verified by botanist M. Aghaahmadi (Isfahan University, Isfahan, Iran).

Essential oil extraction

The air-dried and grounded plants (300 g each) were individually subjected to essential oil extraction for 3.5 h using hydrodistillation method by a Clevenger-type apparatus. The obtained essential oils were then dehydrated over anhydrous sodium sulfate and stored at 4 °C until analysis.

GC and GC-MS analysis

Essential oils of *P. olivieri* and *P. persica* were analyzed using a HP-6890 gas chromatograph (HP-5MS column with 60m × 0.25mm i.d. and 0.25µm film thickness) coupled with a mass detector (HP-6973). The flow rate of carrier gas (Helium) was 1 ml/min. The oven temperature was initiated at 60 °C for 30 min and was then gradually raised at a rate of 5 °C per minute to 250 °C. The injection temperature was 250 °C and the essential oil sample (1 µl) was injected with a split ratio of 1:90. The MS spectra were acquired by electron ionization at 70 eV. The Kovats retention indices (KI) were calculated for all identified compounds using a homologous series of *n*-alkanes injected in equal conditions to the essential oil samples. Identification of the constituents was based on soft ware matching with the Wiley 7n.L online library and also by direct comparison of the retention indices and MS fragmentation patterns with those reported for standard compounds.[19] The essential oils were also analyzed using an HP-6890 gas chromatograph equipped with a FID detector to get relative percentages of the identified compounds. The FID detector temperature was 290 °C and the operation was conducted under the same conditions as described for GC-MS analysis.

Brine shrimp lethality test

General toxicity potentials of the essential oils were evaluated in the brine shrimp lethality test (BSLT) as described by Mojarrab *et al.* with slight modifications.[20] Brine shrimps cysts (*Artemia salina* L.) were hatched in sterile artificial seawater (prepared using sea salt 38 g/l and adjusted to pH 9 using Na_2CO_3) under constant aeration for 48 hours at 30 °C. Essential oils (50 mg) were dissolved in DMSO (250 µl) and tween 80 (one drop) and diluted with freshly prepared artificial sea water to obtain solutions with 1000, 700, 500, 300, 100, 10 and 2 µg/ml concentrations in a series of tubes containing about 20 active nauplii in each. The tubes were placed in a water bath at 30 °C for 24 hours under light, and the surviving nauplii were then counted to obtain the concentration causing 50% lethality (LC_{50} value). Podophyllotoxin, a known cytotoxic aryltetralin lignan, was also applied as positive control. The assay was performed tree times and LC_{50} value was reported as Mean ± SD.

Results and Discussion

Essential oils composition

The hydrodistillation of the aerial parts of *P. olivieri* and *P. persica* yielded 0.2 and 0.1% (V/W) pale yellowish oils, respectively.

Thirty compounds, representing ۹۷.۵% of the total oil, were identified as a result of GC and GC-MS analyses of *P. olivieri* essential oil, among them β-caryophyllene (25.7%) germacrene D (19.5), (E)-β-farnesene (9.4) and α-pinene (9.0) were the main compounds (Table 1). The results also indicated that sesquiterpene hydrocarbones (65.3%) were the main group of constituents in *P. olivieri* oil. A review of previous studies on chemical compositions of this species from different regions of Iran revealed that germacrene D with relative percentages of 28.1, 26.4, 66.1 and 48.0-58.0%, in the plants collected from Damavand, Taleghan, Chalus and Kojour regions, respectively and hexahydrofarnesyl acetone (13.3%) in essential oil of the plant collected from Semirom region have been reported as the main compounds of *P. olivieri* oils (Table 2), whereas in the present study β-caryophyllene (25.7%) was characterized as the main compound of the essential oil of this species aerial parts collected from Borujen region (west of Iran).[13-17] β-caryophyllene were found at levels of 0-16.0% in previous studies on *P. olivieri* essential oils.[13-17]

This bioactive sesquiterpene (β-caryophyllene) has been also identified as the main compound of some *Phlomis* species, *P. russeliana* (22.6%), *P. chimerae* (31.6%), *P. leucophracta* (20.2%) and *P. linearis* (24.2%), and has been considered for its wide biological activities such as anti-inflammatory, gastric cytoprotection, antispasmodic, local anaesthetic and hepatoprotective effects.[21-28]

GC and GC-MS analyses of *P. persica* essential oil resulted in identification of twenty-eight compounds, accounting for 94.1% of the total essential oil. The results demonstrated that the essential oil was rich in sesquiterpene hydrocarbons (36.3%) with germacrene D (17.2%) and γ-elemene (15.4%) as the main compounds followed by bicyclogermacrene (10.8%) and spathulenol (8.8%). As shown in Table 2, there are differences between the main components of the analyzed *P. persica* essential oil and two respective reports on essential oil of this species.[15-18] Sarkhail *et al.* (2004) reported (E)-β-farnesene (21.7%), germacrene D (32.5%), germacrene B (6.1 %) and β-caryophyllene (5.1%) as the main compounds of the essential oil of *P. persia* aerial part collected from Bojnourd region (northeast of Iran).[18] In another study on *P. persia* from Taleghan region (north of Iran), germacrene D (38.2%), bicyclogermacrene (16.3%) and α-pinene (13.3%) were characterized as the main constituents of its aerial part essential oil.[15]

Regarding to the wide distribution of *P. olivieri* and *P. persica* in Iran, climatic and geographic factors could be considered as the main responsible of observed differences in essential oil constituents of various populations of these species.[29]