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Original article

Phytochemical constituents, antioxidant activity and toxicity potential of *Phlomis olivieri* Benth.

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Abstract

Background and objectives: *Phlomis olivieri* Benth. (Lamiaceae) is a medicinal plant widely distributed in Iran. In the present study, we have investigated the phytochemical constituents, antioxidant activity and general toxicity potential of the aerial parts of this species. **Methods:** Silica gel (normal and reversed phases) and Sephadex LH-20 column chromatographies were used for isolation of compounds from methanol-soluble portion (MSP) of the total extract obtained from *P. olivieri* aerial parts. The structures of isolated compounds were elucidated using ^{1}H-NMR, ^{13}C-NMR and UV spectral analyses. Antioxidant activity and general toxicity potential of MSP were also evaluated in DPPH free radical-scavenging assay and brine shrimp lethality test (BSLT), respectively. **Results:** One caffeoylquinic acid derivative, chlorogenic acid (**1**), one iridoid glycoside, ipolamiide (**2**), two phenylethanoid glycosides, phlinoside C (**3**) and verbascoside (**5**), along with two flavonoids, isoquercetin (**4**) and naringenin (**6**) were isolated and identified from MSP. The MSP exhibited considerable antioxidant activity in DPPH method (IC_{50}; 50.4 ± 4.6 µg/mL), compared to BHT (IC_{50}; 18.7 ± 2.1 µg/mL), without any toxic effect in BSLT at the highest tested dose (1000 µg/mL). **Conclusion:** the results of the present study introduce *P. olivieri* as a medicinal plant with valuable biological and pharmacological potentials.

Keywords: brine shrimp lethality test, chromatography, DPPH, Lamiaceae, *Phlomis olivieri* Benth.

Introduction

Phlomis olivieri Benth. belonging to the Lamiaceae family, is a perennial herbaceous plant distributed in south-western Asia [1]. In Iranian Traditional Medicine, the leaves of this species have been mentioned useful for alleviation of pains and its aerial parts have also been used as carminative [2,3].

So far, a number of biological and phytochemical studies have been conducted on the various extracts obtained from the aerial parts of *P. olivieri* [4-13]. In 2003, Sarkhail *et al*. reported significant antinociceptive effects from the total extract of *P. olivieri* aerial parts at the dose of 150 mg/kg in visceral writhing test model in mice [4]. The methanol extract of the aerial parts has shown to possess a concentration-dependent antibacterial activity against *Staphylococcus aureus*, *Streptococcus sanguis*, *Escherichia coli*,

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Pseudomonas aeruginosa and *Klebsiella pneumoniae*, as well as antioxidant effect when used in sunflower oil [5,6]. Two flavonoid derivatives including chrysoeriol-7-O-β-D-glucopyranoside and 6,7-dimethoxy-5-hydroxy flavanone, together with one phenylethanoid glycoside, verbascoside have been isolated from the aerial parts of *P. olivieri* during previous phytochemical investigations [7,8]. Moreover, there are some reports on essential oil compositions of this species indicating to the presence of sesquiterpene hydrocarbones (mainly germacrene D), as the main group of its chemical constituents [9-13].

In the present study, phytochemical constituents of *P. olivieri* aerial parts were investigated and its antioxidant and general toxicity potentials were evaluated in DPPH method and brine shrimp lethality test, respectively.

Experimental

General procedures

^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Bruker Avance DRX 500 spectrometer. UV spectra were recorded on a CECIL 7250 spectrophotometer in methanol and after the addition of shift reagents.

Silica gel (230-400 mesh, Merck), RP-C18 (230-400 mesh, Fluka, Switzerland) and Sephadex LH-20 (Fluka, Switzerland) were used as solid phases for column chromatographies. Pre-coated Silica gel GF_{254} sheets (Merck, Germany) were applied for the thin layer chromatography (TLC) and the spots were monitored under UV (254 and 366 nm) and by spraying anisaldehyde/H_2SO_4 reagent. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and *Artemia salina* eggs were obtained from Sigma-Aldrich (Germany) and Ocean nutrition (Belgium) companies, respectively. Other chemicals and all of the used solvents were also purchased from Merck chemical company.

Plant material

The flowering aerial parts of *P. olivieri* were gathered in July 2013 from the southern slopes of Mishu-dagh Mountains, East-Azerbaijan province, Northwest of Iran. The plant specimen was then authenticated by botanist Dr. M. Aghaahmadi from University of Isfahan, Isfahan, Iran.

Extraction

The shade-dried aerial parts (0.8 kg) were powdered and macerated with methanol (6×4 L) at room temperature. The obtained total methanol extract was concentrated using a rotary evaporator at 40 °C and dried completely by a freeze dryer. The freeze dried extract was then defatted by eluting with enough volumes of petroleum ether and chloroform, respectively. Finally, the residual methanol-soluble portion (MSP) was subjected to phytochemical and biological studies.

Isolation and purification of the compounds

Thirty five grams of the MSP was moved to a Sephadex LH-20 column and eluted with MeOH-H_2O (9:1) to get three fractions (A-C). Reversed-phase (C_{18}) column chromatography of the fraction B (10 g) with a gradient mixture of ACN-H_2O (0.5:9.5-2:8) yielded six fractions (B1-B6). Compound **1** (23 mg) was isolated from the fraction B1 (1.2 g) on a Sephadex LH-20 column (MeOH-H_2O, 8:2) and its impurities were removed over a RP-18 column (ACN-H_2O, 1:9). Faction B2 (169 mg) was subjected to RP-18 column chromatography with ACN-H_2O (0.2:9.8-1:9) to get four fractions (B2a-B2d). Compound **2** (25 mg) was obtained from the fraction B2c (43 mg) over the Sephadex LH-20 column eluted with MeOH-H_2O (8:2). Fraction B3 (215 mg) was eluted on a Sephadex LH-20 column with MeOH-H_2O (8:2) to get compound **3** (17 mg). RP-18 column chromatography of the fraction B4 (720 mg) with ACN-H_2O (1:9-2:8) resulted in five fractions (B4a-B4e). Compounds **4** (21 mg) and **5** (36 mg) were isolated from the fractions B4b and B4d, respectively, over the Silica gel columns with EtOAc-CH_3COOH-HCOOH-H_2O (36:1:1:2.4) as the eluent. Silica gel column chromatography of the fraction B6 (276 mg) with $CHCl_3$-EtOAc (7:3-3:7) resulted