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Original article

# Coumaroyl flavone glycosides and cinammic acid derivatives from the aerial parts of *Phlomis bruguieri* Desf.

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## Abstract

**Background and objectives:** *Phlomis bruguieri* Desf. (Lamiaceae) is a perennial herbaceous plant distributed in Iran, Turkey and Iraq. Despite medicinal potentials of this species, the current knowledge on its phytochemical constituents is limited. The aim of the present study was to investigate the phytochemical constituents of the essential oil and various extracts of this species. **Methods:** Essential oils of the plant aerial parts were extracted by hydrodistillation and steam distillation methods and analysed using GC and GC/MS. Column chromatography with silica gel (normal and reversed phases) and Sephadex LH-20 were also used for the isolation of compounds from various extracts obtained from *P. bruguieri* aerial parts. The structures of isolated compounds were established by 1D and 2D NMR techniques. **Results:** By GC and GC/MS analysis, germacrene D (29.8%), apiole (20.7%) and myristicin (16.63%) were identified as the main compounds of hydrodistilled oil. Apiole (53.20%) and myristicin (34.87%) were also detected as the main compounds of the oil extracted by steam distillation method. Phytochemical analysis of the plant extracts resulted in the isolation and structural elucidation of β-sitosterol (**1**), *p*-coumaric acid methyl ester (**2**), chrysoeriol 7-O-(3''-(*E*)-*p*-coumaroyl)-β-D-glucopyranoside (**3**), chrysoeriol 7-O-(3'',6''-di-O-(*E*)-*p*-coumaroyl)-β-D glucopyranoside (**4**), chrysoeriol 7-O-β-D-glucopyranoside (**5**), chlorogenic acid (**6**) and verbascoside (**7**). **Conclusion:** the results of the present study introduce steam distilled oil of *P. bruguieri* as a new source of apiole and myristicin. Moreover, identification of coumaroyl flavone glycosides and cinammic acid derivatives from the aerial parts of this species highlighted the species as a good candidate for further biological and pharmacological studies.

**Keywords:** essential oil, flavonoid, Lamiaceae, phenylpropanoid, *Phlomis bruguieri* Desf.

## Introduction

*Phlomis bruguieri* Desf. from Lamiaceae family is one of nineteen *Phlomis* species represented in flora of Iran [1]. This species grows as a perennial herbaceous plant with the height of 20-50 cm in Iran, Turkey and Iraq [1]. Previous biological studies have reported *P. bruguieri* as a plant with antioxidant, antibacterial and α-amylase inhibitory activities [2-4]. The methanol extract of this species has been found as the most effective extract in stabilizing sunflower oil

 
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among some selected *Phlomis* and *Stachys* species [2]. It has also been reported that the methanol extract of *P. bruguieri* showed a dose dependent antibacterial activity towards a set of bacteria especially *Streptococcus sanguis* (MIC 10 mg/mL) and *Staphylococcus aureus* (MIC 1.0 mg/mL) [3]. In another study, ethyl acetate extract of *P. bruguieri* with $IC_{50}$ value of 1.9 µg/mL was found as the most active extract in inhibition of α-amylase enzyme, among fourteen extract obtained from ten different Lamiaceae taxa [4]. HPLC-PAD-MS analysis of *P. bruguieri* aerial parts revealed the presence of four phenylethanoid glycosides; verbascoside, isoverbascoside, leucosceptoside A and martynoside in its methanol extract [5]. Furthermore, there are two reports on essential oil composition of this species from north and north-west of Iran [6,7]. Literature review, however, has shown the presence of other chemical groups of constituents such as triterpenoids, diterpene glycosides, flavonoids, iridoids, caffeic acid derivatives *etc.* in *Phlomis* genus which have not yet been reported from *P. bruguieri* [8]. Therefore, to evaluate more medicinal potential of *P. bruguieri*, we investigated phytochemical constituents of the extracts and essential oils of this plant collected from west of Iran.

## Methods

*Plant material*

The flowering aerial parts of *Phlomis bruguieri* Desf. were collected from "Sarab-e gian" region located in Nahavand, Hamadan (west of Iran) in July 2014. A voucher specimen (No. 6911-TEH) was deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

*Preparation of the essential oil*

The shade-dried and ground aerial parts were subjected to essential oil extraction using two different methods, hydrodistillation and steam distillation methods. In each method, the procedure was done on 150 g plant sample for 3 h. Essential oil extraction was repeated two times for each method and the pale yellowish oils were individually dried on anhydrous sodium sulphate and kept at 4 °C until analysis.

*Essential oils analysis*

The essential oils were analysed on a HP 6890 gas chromatograph equipped with HP- 5MS column (30 m×0.25 mm id, 0.25 µm), connected to HP 5973 mass detector (70 eV, 290 °C) under the following conditions; carrier gas: helium (0.8 mL/min), temperature program: 50 °C to 240 °C at 3 °C/ min, then 240 °C to 300 °C at 15 °C/min, injector temperature: 290 °C, injection volume: 1 µL, split ratio: 1:90. The retention indices (RI) of the compounds were calculated using a homologous series of *n*-alkanes injected in conditions equal to the sample. Identification of the compounds was carried out based on computer matching with the Wiley 7n.L library, as well as by comparison of KIs and fragmentation pattern of the mass spectra with those published for standard compounds [9]. For quantitative purposes, the essential oils were also analysed by GC-FID with the same conditions described above for GC/MS. Amounts of compounds (real % area) were expressed as mean for each method of essential oil extraction.

*Extraction*

About 1 kg of shade-dried and comminuted aerial parts (1 kg) was macerated successively with *n*-hexane, chloroform, ethyl acetate and 70% methanol (each 5×5 L), at the room temperature. The obtained extracts were then concentrated using a rotary evaporator at 40 °C.

*Isolation and purification of the compounds*

Five grams of the *n*-hexane extract was moved to a silica gel (230-400 mesh, Merck, Germany) column and eluted with a gradient mixture *n*-hexane-EtOAc (10:0 to 5:5) to get six fractions (H1-H6). Silica gel column chromatography of the fraction H4 (0.7 g) using *n*-hexane-EtOAc (8:2) yielded a white powder which was identified as compound **1** (28 mg). Compound **2**

(13 mg) was isolated from the chloroform extract (2.1 g) on a Silica gel column eluted with $CHCl_3$-EtOAc (10:0 to 5:5). A portion of ethyl acetate extract (2.5 g) was divided to five fractions (E1-E5) on a RP-18 (230-400 mesh, Fluka, Switzerland) column using MeOH-$H_2O$ (7:3). Silica gel chromatography of the fraction E3 (0.6 g) using $CHCl_3$-MeOH (9:1) resulted in isolation of compounds **3** (17 mg), **4** (52 mg) and **5** (31 mg). A portion of hydroalcoholic extract (5 g) was moved to a Sephadex LH-20 (Fluka, Switzerland) and eluted with MeOH to achieve six fractions (M1-M6). Compounds **6** (23 mg) and **7** (38 mg) were obtained from the fractions M3 (0.7 g) and M5 (1.1 g), respectively on Sephadex LH-20 columns using MeOH-$H_2O$ (8:2) as the eluent.

In all steps, column chromatographies were monitored by thin layer chromatography (TLC) (pre-coated silica gel $GF_{254}$ plates, Merck, Germany), and fractions giving similar spots under 254 and 366 nm UV wavelengths or after spraying anisaldehyde/sulphuric acid reagent were combined. The structures of the purified compounds were elucidated by $^1$H-NMR, $^{13}$C-NMR, HMBC, and HSQC spectral analysis (Bruker Avance 400 DRX, 400 MHz for $^1$H and 100 MHz for $^{13}$C), as well as by comparing with data published in the literature.

## Results and Discussion

Hydrodistillation and steam distillation of the aerial parts of *P. bruguieri* resulted in the extraction of pale yellowish oils with the yields of 0.1 and 0.13 % (V/W), respectively. Twenty eight compounds representing 97.76% of the total oil were characterized as a result of GC and GC/MS analysis of the essential oil obtained by hydrodistillation method (table 1). Among the identified compounds oxygenated sesquiterpenes (47.13%) and phenylpropanoids (38.40%) were the main groups of constituents and germacrene D (29.8%), apiole (20.7%), myristicin (16.63%) and bicyclogermacrene (6.72%) were the main compounds. The results of GC and GC/MS analysis of the essential oil extracted by steam distillation method revealed that this oil was rich in phenylpropanoid derivatives (92.15%), mainly apiole (53.20%) and myristicin (34.87%).

Comparison of these two methods suggested the considerable ability of steam distillation method for approximately selective extraction of phenylpropanoids present in the essential oil of *P. bruguieri*. Two previous studies on essential oil constituents of this species, however, reported different phytochemical profiles [6,7].

**Table 1.** Composition of the essential oils of *Phlomis bruguieri* extracted by hydrodistillation (HD) and steam distillation (SD) methods

| No. | Compounds[a] | | Real % Area | |
|---|---|---|---|---|
| | | RI[b] | HD[c] | SD[d] |
| 1 | α-Pinene | 935 | 0.69 | 0.47 |
| 2 | β-Pinene | 978 | 0.28 | - |
| 3 | 1-Octen-3-ol | 980 | 0.17 | - |
| 4 | α-Phellandrene | 1007 | 0.16 | - |
| 5 | Limonene | 1024 | 0.17 | - |
| 6 | γ-Terpinene | 1056 | 0.39 | - |
| 7 | Terpinen-4-ol | 1178 | 0.23 | - |
| 8 | Myrtenal | 1196 | 0.98 | 0.55 |
| 9 | α-Ylangene | 1375 | 0.26 | - |
| 10 | α-Copaene | 1378 | 0.53 | - |
| 11 | β-Bourbonene | 1392 | 1.01 | - |
| 12 | β-Elemene | 1395 | 0.92 | - |
| 13 | (E)-Caryophyllene | 1421 | 0.97 | - |
| 14 | (E)-β-farnesene | 1457 | 1.52 | - |
| 15 | α-Amorphene | 1485 | 0.62 | - |
| 16 | Germacrene D | 1491 | 29.80 | 3.54 |
| 17 | Bicyclogermacrene | 1506 | 6.72 | 0.68 |
| 18 | δ-Cadinene | 1514 | 0.88 | - |
| 19 | Myristicin | 1521 | 16.63 | 34.87 |
| 20 | Elemicin | 1561 | 1.07 | 4.08 |
| 21 | Spathulenol | 1583 | 1.93 | - |
| 22 | Viridiflorol | 1598 | 3.16 | 0.16 |
| 23 | α-Cadinol | 1659 | 0.74 | - |
| 24 | Apiole | 1684 | 20.70 | 53.20 |
| 25 | Myristic acid | 1778 | 0.42 | - |
| 26 | Hexahydrofarnesyl acetone | 1886 | 2.82 | - |
| 27 | Hexadecanoic acid | 1889 | 3.54 | - |
| 28 | Phytol | 1948 | 0.45 | - |
| | Monoterpene hydrocarbons | | 1.69 | 0.47 |
| | Oxygenated monoterpenes | | 1.21 | 0.55 |
| | Sesquiterpene hydrocarbons | | 47.13 | 4.22 |
| | Oxygenated sesquiterpenes | | 1.93 | 0.44 |
| | Phenylpropanoids | | 38.40 | 92.15 |
| | Diterpenes | | 0.45 | - |
| | Non-terpenes | | 6.95 | - |
| | Total identified | | 97.76 | 97.83 |

[a] Identified compounds listed in order of elution from HP-5MS column; [b] Retention indices to $C_8$-$C_{30}$ *n*-alkanes on HP-5MS column; [c] hydrodistillation; [d] steam distillation.

Essential oil analysis of *P. bruguieri* aerial parts from northwest of Iran resulted in

characterization of germacrene D (60.5%), γ-elemene (16.5%), germacrene B (7.1%) and bicyclogermacrene (4.1%) as the main compounds [7]. Germacrene D (23.6%), 4-hydroxy-4-methyl-2-pentanone (15.0%), α-pinene (6.8%) and β-caryophyllene (6.7%) have also been identified as the main compounds of the essential oil of this plant aerial parts collected from north of Iran [6]. Beside possible existence of chemotypes within different population of *P. bruguieri*, mentioned variations may be raised from some extrinsic factors such as geographic and climate conditions, stresses caused by drought, insects or microorganisms, plant harvesting time, as well as drying and storage conditions [10].

Phytochemical analysis of the extracts obtained from *P. bruguieri* (extraction yields: 3.2, 0.5, 2.1 and 24.4% (W/W) for *n*-hexane, chloroform, ethyl acetate and hydroalcoholic extracts, respectively) resulted in isolation of β-sitosterol (**1**) from *n*-hexane extract, *p*-coumaric acid methyl ester (**2**) from chloroform extract, chrysoeriol 7-O-(3"-(*E*)-*p*-coumaroyl)-β-D-glucopyranoside (**3**), chrysoeriol 7-O-(3",6"-di-O-(*E*)-*p*-coumaroyl)-β-D-glucopyranoside (**4**), chrysoeriol 7-O-β-D-glucopyranoside (**5**) from ethyl acetate extract, together with chlorogenic acid (**6**) and verbascoside (acteoside) (**7**) from hydroalcoholic extract (figure 1) [11-17].

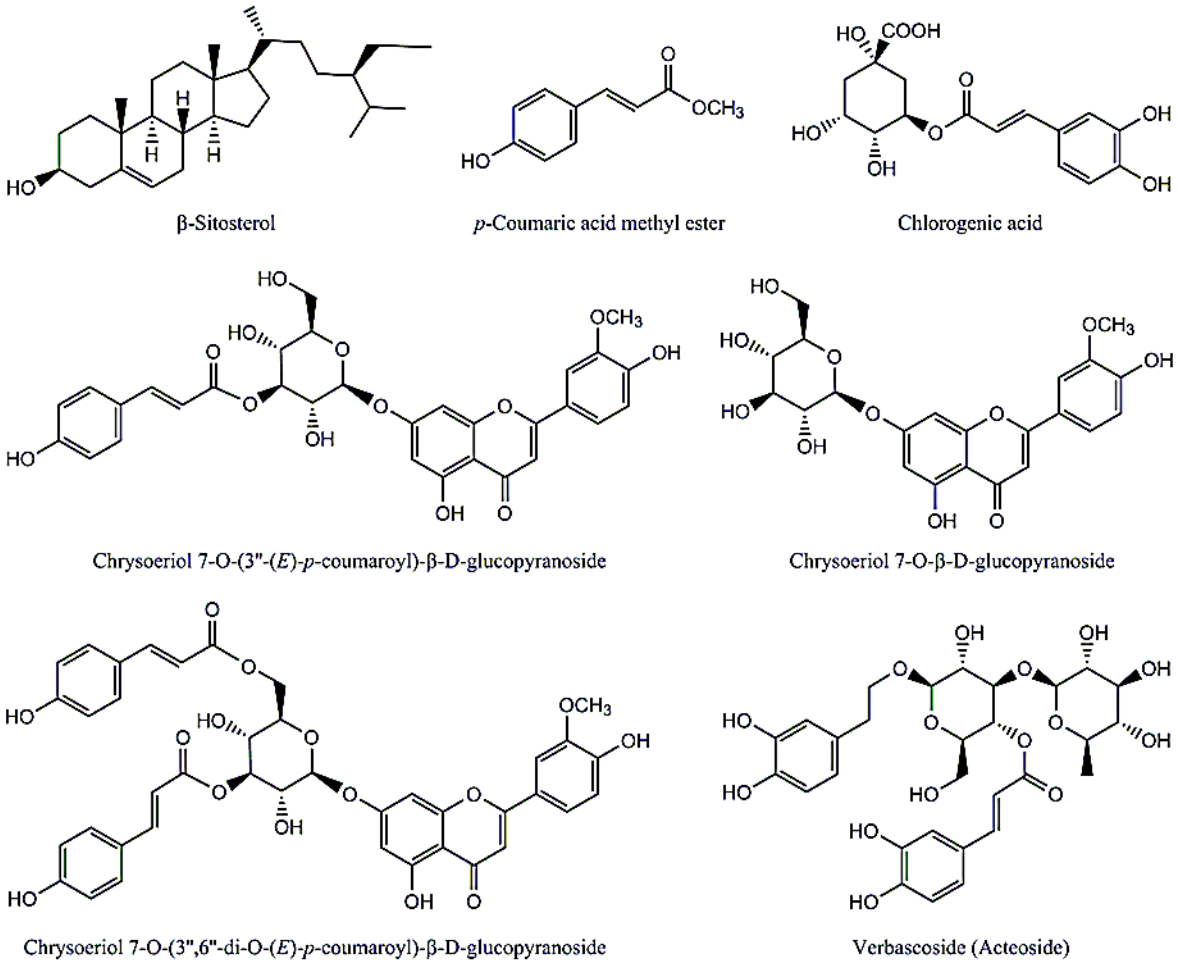


**Figure 1.** Structures of the isolated compounds (1-7) from the aerial parts of *Phlomis bruguieri*

Among the isolated constituents, compounds **1**-**6** are reported from the aerial parts of *P. bruguieri* for the first time. Based on our literature review this is also the first report on isolation of chrysoeriol 7-O-(3",6"-di-*O*-(*E*)-*p*-coumaroyl)-β-D-glucopyranoside (**4**) from the genus *Phlomis*. This compound is a rare chrysoeriol coumaroyl glycoside which was isolated from the methanolic extract of the aerial parts of *Marrubium velutinum* Sm. for the first time [14].
Two other isolated flavonoids (**3**, **5**), along with chlorogenic acid (**6**) and verbascoside (**7**) have been reported from various *Phlomis* species [8]. Among the isolated compounds chlorogenic acid (**6**) and verbascoside (**7**) with the known potent antioxidant activity may be involved in previously documented antioxidant properties of *P. bruguieri* [18-20]. Chrysoeriol has been found as the most active compound with $IC_{50}$ value of 1.27 mM in bioassay-guided isolation of α-amylase inhibitory principles from the aerial parts of *Salvia virgata* Jacq. [21]. Therefore, chrysoeriol derivatives identified in ethyl acetate extract of this species (**3**-**5**) may be involved in appearance of strong α-amylase inhibitory effects of *P. bruguieri* ($IC_{50}$ 1.9 μg/mL), previously reported by Safamansuri *et al*. [4].
In conclusion, the results of the present study introduced the steam distilled oil of *P. bruguieri* as a new source of apiole and myristicin. This study also suggested chrysoeriol glucoside and its coumaroyl derivatives as appropriate candidates for evaluation of their α-amylase inhibitory activity in order to develop new antidiabetic agents.

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**Declaration of interest**
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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