Chemical composition of essential oil and antioxidant activity of leaves and stems of *Phlomis lurestanica*

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**ABSTRACT**

Essential oils from leaves and stems of *Phlomis lurestanica* at the flowering stage were extracted by hydrodistillation. The essential oils were analyzed using Gas Chromatography and Gas Chromatography-Mass Spectrometry. Twenty-eight and twenty-five compounds were identified in the stems and leaves, respectively. The main compounds of stems were α-pinene (12.40%), γ-cadinene (10.92%), and γ-elemene (6.46%). Hexadecane (8.97%), 2-dodecenal (6.57%), and heptadecane (6.32%) were the major constituents in the essential oils of the leaves. The methanol extracts of stems and leaves were evaluated for their antioxidant properties using 2,2-diphenyl-1-picrylhydrazyl and β-carotene/linoleic acid tests. In the 2,2-diphenyl-1-picrylhydrazyl assay, the leaves (IC$_{50}$ = 1168.9 µg/ml) have stronger antioxidant activity than the stems (IC$_{50}$ = 1563.6 µg/ml). The extracts of the leaves and stems showed remarkable antioxidant activity in β-carotene/linoleic acid assay (96.2% and 95%, respectively). The total phenol and flavonoid contents of the species were determined using Folin-Ciocalteu and AlCl$_3$ assays, respectively. The phenol and flavonoid contents of the leaves (301.0 µg/ml and 45.2 µg/ml, respectively) were observed more than the stems (172.3 µg/mg and 18.8 µg/mg, respectively).

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**Introduction**

Aromatic plants are considered of great interest for their flavors and medicinal properties, along with human consumption, animal foodstuff, and ornamental uses; thus, they are specially suitable for multifunctional sustainable crop models. A large number of these aromatic species belong to the Lamiaceae family, whose center of differentiation is located in the Mediterranean area.[1]

Essential oils are complex mixtures, composed of terpenoid hydrocarbons, oxygenated terpenes, and sesquiterpenes. They originate from plant secondary metabolism and are responsible for their characteristic aroma. The various applications of essential oils account for the great interest in their study. These applications may be found in the cosmetic industry, as ingredients of components of medicines and as antibacterials/antimicrobials, and in aromatherapy.[2]

Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells, for example, it may cause cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Many natural molecules, especially those produced in the plant kingdom, have at least one benzene ring with a hydroxyl functional group in their skeleton. These compounds are collectively known as phenolic compounds and due to their hydrogen or single electron donating potentials, they usually play important roles in the antioxidant activity of the plant extracts.[3]

The genus *Phlomis* consists of about 100 species in the world. According to the last report, this genus has 17 species in the flora of Iran, of which 10 are endemic.[4] *Phlomis lurestanica* Jamzad

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(Lamiaceae) is a herbaceous plant, perennial, height 20–40 cm, and its habitat is in Southwest Iran (Lorestan province). Many different glycoside compounds have been identified in *Phlomis*, for example, diterpenoids, iridoids, phenylpropanoids, phenylethanoids, and flavonoids. Some species of *Phlomis* have important activities, including antiinflammatory, immunosuppressive, antimutagenic, antinociceptive, antifibriel, free radical scavenging, antiallergic, antimalaria, and antimicrobial effects.

The main components in the essential oils of *Phlomis olivieri* were β-caryophyllene (25.7%) and germacrene-D (19.5%), while germacrene-D (17.2%) and γ-elemene (15.4%) were the major constituents in *Phlomis persica*. Chu et al. reported geranial (16.5%), linalool (13.3%), cis-geraniol (7.4%), β-myrcene (6.2%), 1,8-cineol (5.3%), 4-terpineol (5.1%), and myristicin (5%) as the major compounds in the aerial parts of *P. umbrosa* in the flowering stage. The major compounds of *P. chimera* were β-caryophyllene (31.6%), α-pinene (11.0%), and germacrene D.

The chemical composition of the essential oils and antioxidant properties of *P. lurestanica* extracts have not been previously reported. Therefore, the aim of the present study was to evaluate the oil composition and antioxidant properties of its methanol extracts.

**Materials and methods**

**Plant material**

*P. lurestanica* were collected at the flowering stage from Kohdasht located at west of Lorestan province (Iran) and dried in the shade for a month at room temperature.

**Essential oils extraction**

The essential oils were extracted by hydrodistillation using Clevenger type apparatus. Distillation process was done for 3 h. The obtained essential oils were stored in the freezer at −20°C until analysis.

**Analysis of essential oils**

Flame ionization detector-gas chromatography (FID-GC) was performed using a Hewlett-Packard 6890 with HP-5 capillary column (phenyl methyl siloxane, 25 m × 0.25 mm i.d., 0.25 µm film thickness); carrier gas, He; split ratio, 1:25, and FID. Temperature program: 60°C (2 min) rising to 240°C at 4°C/min; injector temperature, 250°C; detector temperature, 260°C. GC-MS was performed using Hewlett-Packard 6859 with quadrupole detector, on a HP-5 column (GC), operating at 70 eV ionization energy, using the same temperature program, and carrier gas as mentioned earlier. Retention indices were calculated by using retention times of *n*-alkanes that were injected after the oils at the same chromatographic conditions according to Van Den Dool’s method. Identification of the components was done by comparing their mass spectra with those of internal Wiley Gas chromatography-Mass Spectrometry (GC-MS) spectral library, or with published mass spectra and those described by Adams.

**Preparation of methanol extracts**

The samples (10 g) were extracted separately with 100 mL of 100% methanol for 72 h at room temperature. The extracts were separated from solids by filtering using Whatman No. 1 filter paper. The remaining residue was reextracted thrice and the extracts were pooled pulled. The solvent was removed under vacuum at 45°C, using a rotary vacuum evaporator (IKA RV 06-ML1B 230V, Germany) and stored at −4°C until used for further analyses (Methanol extraction yields of leaves and stems were 22.02% and 45.38% W/W, respectively).
**Antioxidant activity**

**DPPH assay**
In this method, 50 μL of the different concentrations of the extracts was mixed with methanol solution (1 mL) containing DPPH radicals (0.004%, w/v). After 30 min, the absorbance of the specimens was measured at 517 nm using a microplate reader (Bio Tek, U.S.A). The inhibition of free radicals was measured using the following equation:

\[ I\% = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100 \]

where \(A_{\text{blank}}\) is the absorbance of the control reaction (including all reagents, except the defined concentration of the given extract) and \(A_{\text{sample}}\) is the absorbance of the test. The IC\(_{50}\) represents the concentration of the extracts that causes 50% inhibition of the radical.\(^{[12]}\)

**β-carotene/linoleic acid assay**
In this method, the antioxidative potential of the extracts was measured by plotting decolorization of the β-carotene/linoleic acid assay. To prepare the β-carotene/linoleic acid solution, 0.5 mg β-carotene was mixed with 1 mL chloroform, and then 25 μL linoleic and 200 mg Tween-40 were added. The chloroform was completely evaporated. In the next stage, 100 mL oxygen-saturated distilled water was added and the container was vigorously shaken. Then, 2500 μL reaction mixture and 350 μL of the obtained extracts (500 μg/mL) were added to the test tube. In zero time and after 2 h incubation at 50°C, the absorbance of the specimens were measured at 470 nm using a microplate reader (Bio Tek, USA). The antioxidative capacity of the extract was compared with positive tests. All the tests were carried out in triplicate. The activity was expressed as inhibition percentage using the following equation:

\[ \text{AA}\% = (1 - \frac{DR_S}{DR_C}) \times 100 \]

where AA% is the antioxidant activity, \(DR_C\) and \(DR_S\) are the degradation rates of β-carotene in reactant mixture without and with the sample,

\[ DR = \ln \left( \frac{a}{b} \right) \times \frac{1}{t} \]

where \(a = \) initial absorbance at 0 min, \(b = \) absorbance at 120 min, and \(t = 120.\(^{[13]}\)

**Phenolic compounds**
Phenolic compounds were determined according to the method of Amiri\(^{[14]}\) with some modifications. Briefly, 100 μL of the sample (2 mg/mL) was mixed with 1500 μL of Folin-Ciocalteu reagent (diluted ten-fold) and 1 mL distilled water was added; after 1 min, 1500 μL of a solution of 20% sodium carbonate was added and the mixture was kept in the dark at room temperature, then absorbance was measured at 760 nm. The same procedure was repeated for all standard Gallic acid solutions and the concentration of the phenolic compounds was calculated accordingly and the standard curve was obtained using the following equation:

Absorbance: 0.001 Gallic acid (μg/ml) + 0.111 (\(r^2 = 0.994\)).

**Flavonoid compounds**
Here, 500 μL sample (2 mg/mL) was mixed with 1500 μL methanol, 100 μL of 10% aluminum trichloride, 100 μL of potassium acetate 1 M, and 2.8 mL of distilled water. After 10 min at room temperature, the absorbance was determined at 420 nm. The same procedure was repeated for all standard quercetin solutions and the concentration of flavonoid compounds was calculated accordingly and the standard curve was obtained using the following equation\(^{[15]}\):

Absorbance: 0.0091 quercetin (μg/ml) + 0.0206 (\(r^2 = 0.995\)).
**Statistical analysis**

Experimental results were represented as mean ± standard error (SE) of three parallel measurements and analyzed by the Minitab software. Differences between means were determined using Tukey’s and student’s-t-test.

**Results and discussion**

**Chemical composition of essential oils**

The results of the essential oils analysis are shown in Table 1. According to these results, hexadecane, 2-dodecnenal, and heptadecane were the main components found in the leaves and α-pinene, γ-cadinene, and γ-elemene, were the main components found in the stems. Table 2 shows the major compounds in some species of Phlomis genus. Based on the results of Table 2, α-pinene as the main monoterpane hydrocarbons in *P. lurestanica* oil is also the major compound of essential oils in some *Phlomis* species such as *P. herba-venti* subsp. pungens (7.3%), *P. anisodontia* subsp. occidentalis in vegetative stage (6.8%), *P. leucophracta* (19.2%), *P. chimera* (11%), and *P. bourgaei* (5.65%). Other major compounds of *P. lurestanica* were not found in other *Phlomis* species as the main constituent.

The volatile oil constituents in leaves and stems were divided into terpenoids and aliphatic compounds. Terpenoids are the most important components of *P. lurestanica* oil and have commercial uses in the pharmaceutical industry, makeup material, production of vitamins (A, D, and E), etc.

Based on previous studies, *Phlomis* species can be divided into four chemotypes: (1) the first chemotype is rich in sesquiterpene: in this group, germacrene-D and β-caryophyllene are the two main components; (2) the second chemotype is rich in monoterpane and sesquiterpene: the main components of this class are α-pinene, limonene, linalool, germacrene-D, and β-caryophyllene; (3) fatty acids, aliphatic compounds, and alcohol (diterpenoid alcohol, fatty acid alcohol) constitute the main components of the third chemotype: this group contains a high percentage of hexadecanoic acid, trans-phytol, and 9,12,15-octadecatetraen-1-ol; (4) the last chemotype is rich in terpene, fatty acids, aliphatic compounds, and alcohol (diterpenoid alcohol, fatty acid alcohol) as its main constituents: this mixed group contains hexadecanoic acid, α-pinene, and germacrene-D as major fatty acid, monoterpene, and sesquiterpene, respectively. Concerning the aforementioned classification, the results of the present study showed that *P. lurestanica* can be placed in groups 2 and 3 according to stems and leaves oil analyses, respectively.

**Antioxidant activity**

Since the amount of essential oil was little, antioxidant activity was investigated just on methanolic extracts.

**DPPH assay**

Figure 1 and Table 3 shows the antioxidant activity of leaves and stems in comparison with butylated hydroxytoluene (BHT) using DPPH assays. IC$_{50}$ values show that all data in this method were statistically significant ($p < 0.05$) and the leaves have stronger antioxidant activity than the stems, but lower than the BHT (BHT> leaves> stems). In recent years, the possible toxicity of synthetic antioxidants like BHT and butylated hydroxyanisole (BHA) has been considered. Thus, the potential antioxidants of plants are suitable to protect against various diseases induced by free radicals.

The leaves have higher antioxidant activity when compared with the stems using DPPH method; this result showed that the type of organ can be effective on the antioxidant activity of the plant. This shows that the leaves have a stronger hydrogen donating capacity than the stems. Zhang and Wang$^{24}$ reported the IC$_{50}$ of *P. umbrosa* and *P.megalantha* to be 20.2 and 15.7 µg/ml, respectively; also, the IC$_{50}$ in *P. caucasica*, *P. lanceolata*, and *P. aucheri* was reported to be 0.1 mg/mL.$^{15}$

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**Table 1**

| Chemical Constituents | Leaves (%) | Stems (%) |
|-----------------------|------------|-----------|
| Hexadecane            | 7.3        |           |
| 2-Dodecnenal          | 19.2       |           |
| Heptadecane           | 5.65       |           |
| α-Pinene              | 11%        |           |
| γ-Cadinene            | 6.8%       |           |
| γ-Elemene             | 7.3%       |           |

**Table 2**

| Chemical Constituents | Leaves (%) | Stems (%) |
|-----------------------|------------|-----------|
| Germacrene-D          | 19.2       |           |
| γ-Cadinene            | 6.8%       |           |
| Germacrene-D          | 7.3%       |           |
| γ-Elemene             | 5.65%      |           |

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$^{17}$ Concerning the aforementioned classification, the results of the present study showed that *P. lurestanica* can be placed in groups 2 and 3 according to stems and leaves oil analyses, respectively.
The methanolic extracts of the leaves and stems showed high antioxidant activity in \( \beta \)-carotene/linoleic acid assay. The inhibition percentage of \( \beta \)-carotene degradation of BHT, leaves, and stem were not statistically significant (\( p > 0.05 \)) (Figure 2 and Table 3).

In \( \beta \)-carotene/linoleic acid assay, stems and leaves extracts both showed high antioxidant activity. But the percent antioxidant activity of leaves was more compared to that of stems. The antioxidant activity in \( P. \) umbrosa 88.6% and \( P. \) megalantha 87.8% have been previously reported.\[^{24} \] In \( P. \) bourgaei, the antioxidant activity was measured in three concentrations (0.4, 1.0, and 2.0), such that

| Compound Name | RI (STD) | Area% |
|---------------|---------|-------|
| Hexanal       | 800     | 1.00  |
| 2-Hexanal     | 854     | 0.81  |
| n-Hexanol     | 867     | 1.40  |
| Heptanal      | 899     | 0.33  |
| -Thujenea     | 931     | 0.33  |
| pinene-\( \alpha \) | 939 | 12.40 |
| Decane        | 999     | 0.52  |
| Limonene      | 1031    | 0.56  |
| Linalool      | 1098    | 5.10  |
| n-Nonanal     | 1102    | -     |
| Nonanal       | 1102    | 1.12  |
| Nonanal-\( \alpha \) | 1171 | 0.81 |
| Dodecane      | 1199    | 1.92  |
| n-Decanal     | 1204    | -     |
| Decanal       | 1204    | 1.04  |
| Geraniol      | 1255    | 0.48  |
| Decanol       | 1272    | 0.85  |
| Carvacrol     | 12.98   | -     |
| Tridecane     | 1299    | 0.690 |
| -Cubebenea    | 1351    | -     |
| Cubebene      | 1351    | 6.15  |
| -Copaenea     | 1376    | 1.48  |
| Copacene      | 1376    | -     |
| Elemene-\( \gamma \) | 1433 | 6.46 |
| Geranyl acetate | 1453 | 0.73 |
| 2-Dodecenal   | 1464    | -     |
| -Selenea      | 1494    | -     |
| Pentadecane   | 1500    | 1.52  |
| Cadine-\( \gamma \) | 1505 | 10.92 |
| Cadine-\( \delta \) | 1524 | 0.88 |
| Hexyl benzoate| 1576    | -     |
| Hexadecimal   | 1600    | 4.94  |
| Heptadecane   | 1700    | 5.06  |
| Dodecanal     | 1707    | -     |
| Benzyl benzoate| 1759 | -    |
| Aniline       | 1793    | 5.46  |
| Octadecane    | 1800    | 2.86  |
| Nonadecane    | 1900    | 4.21  |
| Eicosane      | 2000    | 2.85  |
| Terpenoids    | 44.08   | 19.57 |
| Monoterpenes  | 13.21   | 3.56  |
| Oxygenated     | 5.06    | 3.56  |
| Sesquiterpenes | 25.81 | 12.42 |
| Aliphatics    | 33.31   | 41.95 |
| Alcohols      | 3.50    | 1.09  |
| Aldehydes     | 4.26    | 12.73 |
| Alkanes       | 24.82   | 28.13 |
| Esters        | 0.73    | -     |
| Other         | 5.46    | 5.5   |
| Total         | 82.85   | 67.02 |
Table 2. The major compounds of essential oil of some species of *Phlomis*.

| Species                     | Main compounds                                                                 | References |
|-----------------------------|-------------------------------------------------------------------------------|------------|
| *P. armeniaca*              | Germacrene D (24.1%), n-hexadecanoic acid (21.8%), and hexahydrofarnesyl acetone (13.7%) | [16]       |
| *P. nissolii*               | Germacrene D (15.1%), β-caryophyllene (12.7%), hexahydrofarnesyl acetone (11.9%), and linalool (11.3%) | [16]       |
| *P. pungens Var pungens*    | n-hexadecanoic acid (68.1%) and germacrene D (7.2%)                           | [16]       |
| *Phlomis herba-venti*       | Germacrene D (11.7%), terpinolene (9.1%), and α-pinene (7.3%)                 | [17]       |
| *P. anisodonta* subsp. occidentalis* vegetative stage | Germacrene-D (14.3%), bicyclogermacrene (12.4%), and α-pinene (6.8%) | [18]       |
| *P. anisodonta* subsp. occidentalis* flowering stage | Germacrene-D (52.6%), β-caryophyllene (15.9%), caryophyllene oxide (4.2%), and bicyclogermacrene (3.4%) | [18]       |
| *P. anisodonta* subsp. occidentalis* fruiting stage | Germacrene-D (27.9%), bicyclogermacrene (17.6%), caryophyllene oxide (14.7%), and β-caryophyllene (11.3%) | [18]       |
| *P. linears*                | β-caryophyllene (24.2%), germacrene D (22.3%), and caryophyllene oxide (9.2%) | [19]       |
| *P. bourgaei*               | β-caryophyllene (37.37%), (Z)-β-farnesene (15.88%), germacrene D (10.97%), and α-pinene (5.65) | [20]       |
| *P. bovei*                  | Germacrene D (21.45%), β-caryophyllene (7.05%), and β-bournonene (2.96%)       | [21]       |
| *P. oliveri*                | Germacrene D (66.1%), β-selinene (5.1%), β- caryophyllene (4.2%)              | [6]        |
| *P. umbrosa*                | Geranial (16.5%), linalool (13.3%), cis-geraniol (7.4%)                       | [8]        |
| *P. leucophracta*           | β-caryophyllene (20.2%), α-pinene (19.2%), and limonene (11.0%)               | [9]        |
| *P. chimera*                | β-caryophyllene (31.6%), α -pinene (11.0%), and germacrene D (6.1%)           | [9]        |
| *P. grandiflora*            | Germacrene D (45.4%), β-caryophyllene (22.8%), and bicyclogermacrene (4.9%)   | [9]        |
| *P. ferruginea*             | β-caryophyllene (15.6%), hexadecanoic acid (12.8%), and germacrene D (8.9%)   | [22]       |
| *P. lurestanica* (leaves)   | Hexadecane (8.97%), 2-dodecenal (6.75%), and heptadecane (6.32%)              |            |
| *P. lurestanica* (stems)    | α-pinene(12.40%), γ-cadinene (10.92%), and γ-Elemene (7.46%)                  |            |

Table 3. Antioxidant activity, phenol and flavonoid contents of leaves and stems of *Phlomis lurestanica*.

| Antioxidant activity | DPPH (IC<sub>50</sub> (µg/ml)) | %β-carotene linoleic acid | Phenol (µg GAE/mg extract) | Flavonoid (µg QE/mg extract) |
|----------------------|---------------------------------|---------------------------|----------------------------|-----------------------------|
| Leaves               | 1168.9 ± 30.1<sup>b</sup>       | 96.2 ± 1.1<sup>a</sup>    | 301.0 ± 0.3<sup>a</sup>    | 45.2 ± 2.4<sup>a</sup>     |
| Stems               | 1563.6 ± 12.2<sup>a</sup>      | 95.0 ± 1.2<sup>a</sup>    | 172.3 ± 2.9<sup>b</sup>    | 18.8 ± 0.6<sup>b</sup>     |
| BHT                 | 42.3 ± 0.9<sup>c</sup>         | 97.3 ± 1.3<sup>c</sup>    |                            |                            |

Same letters indicate are not statistically significant ± standard error (n = 3) (p > 0.05 for antioxidant activity and p > 0.01 for phenol and flavonoid contents).

Figure 1. Free radical scavenging of leaves and stems of *Phlomis lurestanica* compared with BHT by DPPH assays. All data in this method were statistically significant ± standard error (n = 3) (p < 0.05).
the activities were 69.28, 80.19, and 82.62%, respectively. Sarikurkcu et al. reported the antioxidant activity of *P. armeniaca* to be 75.63%.

**Total phenolics and flavonoids**

The results from phenol and flavonoid contents are as shown in Figure 3 and Table 3. These results showed that the leaves have more phenol and flavonoid contents in comparison with the stems and the phenol content was more than the flavonoid content. The data are statistically significant (*p* < 0.01).

Lipid oxidation is a major cause of food quality deterioration and generation of off odors and off flavors, decreasing shelflife, altering texture and color, and decreasing the nutritional value of food. The results of phenol and flavonoid contents showed that leaves have more phenol and flavonoids content in comparison with the stems.

Karamian et al. reported the amount of phenol in *P. caucasica*, *P. lanceolata*, and *P. aucheri* to be 7.65, 719, and 6.34 (mg GAE/g extract), respectively. Also, the amount of flavonoid for these three species...
was reported to be 4.97, 4.11, and 1.99 (mg QE/g extract), respectively. Zhang and Wang\textsuperscript{[24]} showed that the phenol contents of \textit{P. umbrosa} and \textit{P. megalantha} were 39.43 and 55.20 (mg GAE/g extract), respectively; also, the flavonoid contents were reported to be 7.12 and 35.91 (mg EE/g extract), respectively.

Naturally-occurring antioxidant compounds are flavonoids, phenolic acids, lignans, terpenes, tocopherols, phospholipids, and polyfunctional organic acids, amongst others as reported by Shahidi and Ambigaipalan.\textsuperscript{[23]} Phenolic and flavonoid compounds are well known for their antioxidant properties. Moreover, these compounds have some important activities, such as antiallergenic, antiarterogenic, antioxidant, anti-inflammatory, antimicrobial, antithrombotic, cardioprotective, and vasodilatory effects.\textsuperscript{[26]} According to the results of this research, in DPPH assays, the leaves have more antioxidant activity than the stems; this is confirmed by the high phenol and flavonoid contents in the leaves. The stronger antioxidant activities in the \(\beta\)-carotene/linoleic acid assay can be related to a lot of non polar compounds in the extracts.

\textbf{Conclusion}

Results of the present study showed that type of organ can affect the main compounds of essential oils, it can also influence the antioxidant activity, phenol and flavonoid contents. Based on the fact that \textit{P. lurestanica} has medicinal and nutritional applications in West of Iran and since the results of this study showed that the antioxidant activity of the leaves are higher and better than stems therefore the outcomes can be useful in better and further application and use of this plant.

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