Volatile Constituents from Different Parts of Three Lamiacea Herbs from Iran

Shiva Masoudi

Department of Chemistry, Central Tehran Branch, Islamic Azad University, Tehran, Iran.

Abstract

The essential oils obtained by hydrodistillation from the stem, leaf and flower of *Phlomis aucheri* Boiss., which is endemic to Iran, stem, leaf and root of *Teucrium polium* L. and solvent free microwave extraction oil from leaf of *Ajuga chamaecistus* Ging. Subsp chamaecistus were analyzed by GC and GC/MS. Germacrene D (11.10%, 28.31% and 21.06%) was the main constituent in the stem, leaf, and flower oils of *P. aucheri*, respectively. The other main component in the stem oil of the plant was (E) - anethole (24.58%) and in the flower oil was β- caryophyllene (15.93%). All three oils were rich in regard to sesquiterpenes. The main components in the stem, leaf and root of *T. polium* were α- muurolol (25.02%, 20.03% and 19.53%), α- cadinol (15.72%, 8.11% and 13.01%) and β-cayophyllene (10.86%, 10.11% and 10.64%) respectively. All three oils were rich in regard to sesquiterpenes. The major components in the leaf oil of *A.chamaecistus* were (z)-β-ocimene (12.11%) and germacrene D (10.11%). The oil of the plant was rich in regard to both monoterpenes and sesquiterpenes.

Keywords: *Phlomis aucheri*; *Teucrium polium*; *Ajuga chamaecistus*; Lamiacea; Essential oil; Hydrodistillation; Microwave extraction.

Introduction

The genus *Phlomis* is comprised of about 100 species, 17 of them are described in the flora of Iran, among which 10 are endemic (1, 2).

Some species of *Phlomis* are used in folk medicine as stimulants, tonics, diuretics and for the treatment of ulcers and haemorrhoids (3-5). There are reports indicating various activities such as anti inflammatory, antinociceptive (4), antifibrel (6), antiallergic (7), antimalaria (8) and antimicrobial effects (9-11), for some species of this plant.

Chemical studies on some *Phlomis* species have resulted different classes of glycosides containing diterpenoids (6), iridoids (11-13), phenyl propanoids (13), phenyl ethanoids (3, 5, 9), and flavonoids (14, 15).

The genus *Teucrium* comprises about 340 species, 12 are described in the flora of Iran, among which three are endemic: *Tmelisoides* Boiss et Hausskn ex Boiss., *T. macrum* Boiss et Hausskn and *T. persicum* Boiss. (1, 2).

*Teucrium polium* is a perennial shrub, 20-50 cm high, distributed widely in the dry and stony hills and deserts of almost all Mediterranean countries, South western Asia, Europe and North Africa.

In the traditional Iranian medicine, *T. polium* tea is used to treat oilments such as abdominal pain, indigestion, common colds, and urogenital diseases (16).

Some biological and therapeutic effects have been reported for *T. polium* such as antioxidant (17), anti inflammatory (18), antinociceptive...
(19), anti pyretic (20), antimicrobial (17), hypolipidemic (21), hepatoprotective (22), antigastric ulcer (23), cytotoxic, and apoptotic effects (24).

Several reports on the composition of volatile oils from \textit{T. polium} are found in literature. In the oil from \textit{T. polium} collected in Athens (Greece), \(\beta\)-caryophyllene (17.7\%) was shown to be the major constituent accompanied by \(\delta\)-cadinene (9.3\%), caryophyllene oxide (5.9\%) and \(\alpha\)-cadinol (5.4\%) (25). The oil of \textit{T. polium} subsp. valentinum from Spain, was found to contain \(\alpha\)-pinene (15.8\%) and \(\beta\)-pinene (11.7\%) as major constituents (26). \(\alpha\)-Murolene (8.7\%), \(\alpha\)-cadinol (5.9\%) and \(\delta\)-cadinene (5.1\%) were found to be the major constituents of the essential oil of \textit{T. polium} from northern region of Saudi Arabia (27). The dominant compounds in \textit{T. polium} from Corsica were \(\alpha\)-pinene (28.8\%), \(\beta\)-pinene (7.2\%), and \(p\)-cymene (7.0\%) (28).

The genus \textit{Ajuga} is represented in the flora of Iran by five species in which \textit{Ajuga chamaecistus} has contained several endemic subspecies including \textit{A. chamaecistus} ssp. chamaecistus (1, 2).

Several biological studies have been performed on many species of this genus which have confirmed their ethno pharmacological properties such as hypoglycemic (29), anti inflammatory (30), anabolic, analgesic, antiarthritic, antipyretic,hepatoprotective, antibacterial, antifungal, antioxidant, cardiotonic (31), and antimalarial (32) properties and also their application in the treatment of joint diseases (33).

Many phytochemical studies on \textit{Ajuga} species were performed which led to the isolation of phytoecdysteroids (34, 35), clerodane and neoclerodane di terpenoids (36, 37), iridoids (38) as well as phenylethyl glycosides (39). Previously, we studied the essential oil obtained by hydrodistillation from the aerial parts of \textit{A. chamaecistus} Ging. ssp. chamaecistus, collected from Fasham, 35 km east of Tehran, which contained \(\beta\)-pinene (15.0\%) and linalool (14.5\%) as major constituents (40).

Our study deals with the analysis of the oils from stems, leaves, and flowers of \textit{Phlomis aucheri}, from stems, leaves, and roots from \textit{Teucrium polium} and also from leaves of \textit{Ajuga chamaecistus} growing wild in Iran.

**Experimental**

**Plant materials**

The stems, leaves, and flowers of \textit{Phlomis aucheri}, which is endemic to Iran, and stems, leaves and roots of \textit{Teucrium polium}, were collected from Salehabad area, Province of Ilam, west of Iran, both in July 2013, during the flowering stage. The leaves of \textit{Ajuga chamaecistus} were collected from Mehran, Province of Ilam, in July 2013. Voucher specimens have been deposited at the Herbarium of the Research Instituents of Forests and Rangelands (TARI), Tehran, Iran.

**Isolation of the essential oils**

**Hydrodistillation**

The stems (102.5 g), leaves (84.0 g), and flowers (82.0 g) of \textit{P. aucheri} and also stems (110 g), leaves (80 g), and roots (65 g) of \textit{T. polium} were separately subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. After decanting and drying of the oils over anhydrous sodium sulfate the corresponding yellowish coloured oils were recovered [in the yield of 0.2\%, 0.4\%, 0.3\%, 0.2\%, 0.4\% and 0.1\% (w/w), respectively].

**Solvent-free microwave extraction**

Solvent-free microwave extraction (SFME) of the leaf of \textit{A. chamaecistus} was performed in a Milestone ETHOM 1600 batch reactor, which is a multimode microwave reactor operating at 2455 MHz with a maximum delivered power of 1000 W, variable in 10 W increments. The dimensions of the PTFE coated cavity are 35\times35\times35 cm. During the experiment, time temperature, pressure, and power were controlled using the «easy-WAVE» Software package. Temperature was monitored with the aid of a shielded thermocouple (ATC- 300) inserted directly in to the sample container.

In a typical SFME procedure, 250 g of dry leaves of \textit{A. chamaecistus} were moistened prior to extraction by soaking in water for 1 h, then draining off the excess water.

This step is essential to give the leaves the initial moisture. Moistened leaves were next...
Table 1. Comparative percentage composition of the stem, leaf and flower oils of *Phlomis aucheri*.

| No. | Compounds† | RI* | Stem Oil (%) | Leaf Oil (%) | Flower Oil (%) |
|-----|------------|-----|--------------|--------------|---------------|
| 1   | α-Thujene  | 928 | 0.12         | -            | -             |
| 2   | α-Pinene   | 935 | 3.12         | 4.81         | 0.40          |
| 3   | β-Pinene   | 978 | 0.13         | 0.19         | 0.12          |
| 4   | Myrcene    | 991 | 0.15         | -            | -             |
| 5   | α-Phellandrene | 1003 | 0.18     | -            | -             |
| 6   | Limonene   | 1031 | 0.62      | 0.57         | 0.33          |
| 7   | Fenchone   | 1086 | 1.65      | -            | -             |
| 8   | Linalool   | 1096 | 0.13      | 0.36         | 0.12          |
| 9   | Nonanal    | 1098 | 0.12      | 0.19         | 0.14          |
| 10  | Camphor    | 1143 | 0.47      | 0.21         | -             |
| 11  | Terpin-4-ol| 1176 | 0.15      | 0.22         | -             |
| 12  | α-Terpineol| 1189 | -         | 0.23         | -             |
| 13  | methyl Chavicol | 1195 | 0.47      | -            | -             |
| 14  | endo-Fenchyl acetate | 1220 | 0.23    | -            | -             |
| 15  | exo-Fenchyl acetate | 1232 | 0.61    | -            | -             |
| 16  | p-Anisaldehyde | 1252 | 0.30    | -            | -             |
| 17  | (E)-Anethole | 1283 | 24.58   | -            | -             |
| 18  | Carvacrol  | 1298 | -         | 2.29         | 0.47          |
| 19  | Undecanal  | 1306 | -         | -            | 0.37          |
| 20  | iso-dihydro Carveol acetate | 1325 | -        | -            | 0.26          |
| 21  | - Elemene  | 1339 | 0.32      | 3.68         | 3.93          |
| 22  | Citronellyl acetate | 1352 | 0.18    | -            | -             |
| 23  | Neryl acetate | 1365 | 4.58    | -            | -             |
| 24  | α-Ylangene | 1372 | -         | 0.44         | 0.58          |
| 25  | Butanoic acid- butyl ester | 1373 | -        | 0.43         | -             |
| 26  | α-Copaene  | 1374 | 0.76      | 1.40         | 1.63          |
| 27  | β- Bourbonene | 1384 | 0.55    | 2.69         | 1.05          |
| 28  | β- Cubebene | 1390 | -        | 0.36         | 0.13          |
| 29  | β- Elemene | 1391 | -        | 0.94         | 0.69          |
| 30  | Tetradecane | 1399 | -        | 0.23         | -             |
| 31  | α- Gurjunene | 1409 | -        | -            | 0.27          |
| 32  | β-Caryophyllene | 1418 | 5.58    | 4.96         | 15.93         |
| 33  | β-Gurjunene | 1432 | 0.15    | 1.12         | 0.81          |
| 34  | γ-Elemene  | 1433 | 0.36      | 5.46         | 7.90          |
| 35  | Aromadendrene | 1439 | -        | 0.43         | -             |
| 36  | α-Guaiene  | 1440 | -        | -            | 0.21          |
| 37  | α- Himachalene | 1447 | -        | -            | 0.39          |
| 38  | α-Humulene | 1454 | 0.85      | 2.01         | 3.46          |
| 39  | (E)-β-Farnesene | 1458 | 0.75    | 3.52         | 2.45          |
| 40  | 9-epi-(E)-Caryophyllene | 1465 | 0.78    | 1.13         | 2.90          |
| 41  | Germacrene D | 1480 | 11.10   | 28.31        | 21.06         |
| 42  | β-Selinene | 1485 | -        | 1.16         | 2.76          |
placed in the reactor without any added solvent or water. The essential oil is collected, dried with anhydrous sodium sulphate and stored at 0 °C until used.

| No. | Compounds             | RI   | Stem Oil (%) | Leaf Oil (%) | Flower Oil (%) |
|-----|-----------------------|------|--------------|--------------|----------------|
| 43  | Viridiflorene         | 1493 | 2.38         | -            | -              |
| 44  | Bicyclogermacrene     | 1494 | 6.30         | 8.86         | 7.63           |
| 45  | Germacrene A          | 1501 | 0.63         | -            | -              |
| 46  | γ-Cadinene            | 1511 | -            | -            | 0.18           |
| 47  | 7-epi-α- Selinene     | 1515 | 0.42         | -            | 0.48           |
| 48  | δ-Cadinene            | 1522 | 2.58         | 2.18         | 2.81           |
| 49  | (z)-Nerolidol         | 1530 | 0.77         | -            | -              |
| 50  | Germacrene B          | 1556 | 4.53         | 2.89         | 1.70           |
| 51  | (E)- Nerolidol        | 1564 | 0.60         | -            | 0.26           |
| 52  | Spathulenol           | 1576 | 6.01         | 3.28         | 2.05           |
| 53  | Caryophyllene oxide   | 1580 | -            | 0.36         | 1.33           |
| 54  | Globulol              | 1581 | -            | 0.56         | -              |
| 55  | Viridiflorol          | 1590 | 0.52         | 0.51         | 0.57           |
| 56  | Hexadecane            | 1600 | -            | 0.23         | -              |
| 57  | Humulene epoxide II   | 1604 | -            | -            | 0.34           |
| 58  | Iso spathualenol      | 1637 | -            | 0.41         | 0.35           |
| 59  | epi-α- Muurolol       | 1639 | -            | -            | 0.57           |
| 60  | α- Muurolol           | 1645 | 0.47         | 0.69         | -              |
| 61  | β- Eudesmol           | 1649 | 2.68         | -            | -              |
| 62  | Selin-11-en-4- - ol   | 1652 | -            | 0.26         | -              |
| 63  | α- Cadinol            | 1653 | 1.01         | 0.91         | 0.74           |
| 64  | α- Bisabolol          | 1680 | 0.72         | -            | -              |
| 65  | ( E,E )- Farnesol      | 1720 | 0.70         | -            | -              |
| 66  | 6,10,14- trimethyl 2-Pentadecanone | 1845 | 1.15 | 2.05 | 1.28 |
| 67  | Hexadecanoic acid     | 1973 | 0.35         | -            | -              |
| 68  | Eicosane              | 2000 | 0.77         | -            | -              |
| 69  | Tricosane             | 2300 | -            | 0.51         | 0.88           |

|                          |      | Monoterpene hydrocarbons | Oxygenated monoterpenes | Sesquiterpene hydrocarbons | Oxygenated sesquiterpenes | Other compounds | Total         |
|--------------------------|------|--------------------------|-------------------------|---------------------------|---------------------------|----------------|---------------|
|                           |      | 4.32         | 8.00                   | 38.04                     | 13.48                     | 27.74         | 91.58        |
|                          |      | 5.57         | 3.31                   | 71.54                     | 6.98                      | 3.64          | 91.04        |
|                          |      | 0.85         | 0.85                   | 78.95                     | 6.21                      | 2.67          | 89.53        |

Note: *Compounds listed in order of elution from HP- 5 MS column;*  
*Retention indices to C8 - C24 n-alkanes on HP- 5 MS column.*

Gas chromatography analysis

Gas chromatography analysis was performed on Schimadzu 15A gas chromatograph equipped with a split/splitless injector (25 °C) and a flame
Volatile Constituents from Different Parts

Gas chromatography – mass spectrometry analysis

Analysis was done using a Hewlett-Packard 5973 with a HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 μm). The column temperature was kept at 60 °C for 3 min and then programmed to 220 °C at a rate of 5 °C and kept constant at 220 °C for 5 min. The flow rate of Helium as carrier gas with MS was taken at 70 eV.

The retention indices for all the components were determined according to the Van Den Dool method, using n-alkanes as standard (41, 42). The compounds were identified by (RI, DB5) with those reported in the literature and by comparison of their mass spectra with the Wiley library or with the published mass spectra (43).

Results and Discussion

The composition of the essential oils from stems, leaves, and flowers of *Phlomis aucheri*, stems, leaves and roots of *Teucrium polium* and leaves of *Ajuga chamaecistus*, are listed in Table 1, 2 and 3, respectively, in which the percentage and relative retention indices of components are given.

As it is shown from Table 1, in *P. aucheri* we identified 46 compounds representing 91.58%, 40 constituents representing 91.04% and 40 components representing 89.53% of the stem, leaf and flower oils, respectively. The main component in three oils was germacrene D (11.10%, 28.31% and 21.06%), respectively. Other notable constituents were β-caryophyllene (5.58%, 4.96% and 15.93%) and bicyclogermacrene (6.30%, 8.86% and 7.63%), respectively.

(E)- Anethole (24.58%) was the other main component in the stem oil of the plant and not detected in the leaf and flower oils. As can be seen from the above information, all three oils were rich in regard to sesquiterpenes (51.52%, 78.52% and 85.16%), respectively. The monoterpenic fraction was relatively small, representing only 12.32%, 8.88% and 1.70%, respectively. In the stem oil of *P. aucheri*, considerable percentage of non terpenoid compounds, comparing to other parts of the plant oils, were identified (27.74%). In an earlier study, Javidnia et al. analyzed the essential oil of the aerial parts of *P. aucheri* collected in Fars province. The oil was found to be rich in caryophyllene oxide (33.5%), β-caryophyllene (27.0%) and δ-selinene (9.9%) (44).

Only a few reports on the analysis of essential oils of *Phlomis* species have been published.

Water distilled essential oils from aerial parts of *P. persica* and *P. olivieri*, which are endemic to Iran, have been the subject of our previous studies. The major components of both oils were germacrene D (38.2% and 26.4%) and bicyclogermacrene (16.3% and 12.7%), respectively.

Both oils consisted mainly of sesquiterpene hydrocarbons (45). Also we reported the oil composition of *P. pungens*. The major constituents of the oil of the plant were germacrene D (24.5%), bicyclogermacrene (14.1%), α-pinene (13.5%) and (E)-β-farnesene (13.4%) (46).

Comparison of the present results with those of our previous investigation of oils of the *Phlomis* genus showed that they are also dominated by sesquiterpenes.

Germacrene D has been identified in other species of *Phlomis*, including *P. cancellata* (47), *P. bracteosa* (48), *P. armeniaca* (49), *P. chorassanica* (50), *P. herba-venti* (51), *P. bruguieri* (52, 53), *P. lanceolata*, *P. anisoonata* (53) and *P. linearis* (54).

As it is shown from Table 2, 45 components representing 96.84%, 41 constituents representing 98.48% and 20 compounds representing 88.7% were identified in the oils of stem, leaf and root of *T. polium*, respectively.

The main components in all three oils were α-muurolol (25.02%, 20.03% and 19.53%), α-cadinol (15.72%, 8.11% and 13.01%) and β-caryophyllene (10.86%, 10.11% and 10.64%), respectively.

Other notable compounds were in stem oil;
| No. | Compounds* | RI    | Stem Oil (%) | Leaf Oil(%) | Flower Oil (%) |
|-----|------------|-------|--------------|-------------|----------------|
| 1   | (E)-2- Hexenal | 854   | -            | 0.10        | -              |
| 2   | α- Pinene   | 935   | 0.66         | 2.89        | -              |
| 3   | Camphene    | 950   | t            | 0.16        | -              |
| 4   | β- Pinene   | 978   | 1.70         | 6.65        | -              |
| 5   | Myrcene     | 991   | 0.37         | 1.51        | -              |
| 6   | p- Cymene   | 1024  | 0.30         | t           | -              |
| 7   | Limonene    | 1031  | 0.56         | 3.12        | -              |
| 8   | (E)-β- Ocimene | 1050  | 0.22         | 0.32        | -              |
| 9   | γ-Terpinene | 1062  | -            | 0.13        | -              |
| 10  | Terpinolene | 1086  | t            | 0.18        | -              |
| 11  | Linalool    | 1098  | 0.37         | 0.87        | -              |
| 12  | 1-Octen- 3 yl acetate | 1110 | - | 0.60 | - |
| 13  | trans- Pinocarveol | 1136 | - | 0.91 | - |
| 14  | cis- Verbenol | 1140 | t | 0.69 | - |
| 15  | Pinocarvone | 1160  | -            | 0.47        | -              |
| 16  | Borneol     | 1165  | 0.50         | 0.85        | -              |
| 17  | Terpin-4- ol | 1175 | - | 0.33 | - |
| 18  | Myrtenal    | 1193  | -            | 1.20        | -              |
| 19  | α- Fenchyl acetate | 1220 | 0.26 | - | - |
| 20  | cis- Chrysanthemyl acetate | 1262 | 0.71 | 1.01 | - |
| 21  | Bornyl acetate | 1285 | 0.23 | 0.16 | - |
| 22  | 2- methyl Naphthalene | 1292 | 0.28 | - | - |
| 23  | δ- Elemene  | 1339  | t            | 0.38        | 0.15           |
| 24  | α- Copaene  | 1374  | 0.44         | 0.35        | 0.18           |
| 25  | β- Caryophyllene | 1418 | 10.86 | 10.11 | 10.64 |
| 26  | trans-α- Bergamotene | 1434 | 0.42 | 1.07 | - |
| 27  | Geranyl acetone | 1453 | 0.27 | - | - |
| 28  | α- Humulen   | 1454  | 2.25         | 3.08        | 0.75           |
| 29  | allo- Aromadendrene | 1459 | 0.35 | - | - |
| 30  | Germacrene D | 1480 | 0.84 | 2.77 | 1.23 |
| 31  | Bicyclogermacone | 1494 | 0.98 | 2.02 | - |
| 32  | α- Murolene  | 1497  | 0.30         | -           | -              |
| 33  | γ- Cadinene  | 1513  | 3.78         | 4.56        | 2.06           |
| 34  | δ- Cadinene  | 1522  | 2.79         | 2.96        | 1.55           |
| 35  | α- Calacorene | 1542 | 0.27 | - | - |
| 36  | (z)- Nerolidol | 1534 | - | 7.13 | - |
| 37  | Elemol      | 1549  | 5.53         | 3.17        | 3.50           |
| 38  | Geranyl-n- butyrate | 1560 | 0.58 | - | - |
| 39  | (E)- Nerolidol | 1564 | 2.51 | 2.26 | 2.28 |
| 40  | Spathulenol | 1576  | -            | 2.37        | -              |
| 41  | trans-Sesquisabinene hydrate | 1580 | 0.91 | - | - |
| 42  | Caryophyllene oxide | 1581 | 6.49 | 3.77 | 3.19 |
| 43  | Dodecanoic acid | 1589 | 1.26 | - | - |
| 44  | Guaiol      | 1593  | 0.26         | -           | -              |
| 45  | Tetradecanal | 1611 | 0.62 | - | - |
| 46  | α- Muurolol | 1645  | 25.02        | 20.03       | 19.53          |
caryophyllene oxide (6.49%), elemol (5.53%) and hexadecanoic acid (5.17%), in leaf oil; (z) nerolidol (7.13%) and β-pinene (6.65%), in root oil; hexadecanoic acid (16.37%).

According to these results, the composition of the stem, leaf and root oils of *T. polium* show significant similarity for the concentration of the main components. All three oils were rich in regard to sesquiterpenes (80.89%, 75.58% and 59.96%), respectively.

The monoterpene fraction of the stem and leaf oils was relatively small, representing (6.73% and 21.45%) of the total oils, respectively. In the root oil of the plant we could not find any trace of monoterpene. In the root oil of *T. polium*, considerable percentage of non terpenoid compounds, compared to other parts of the plant oils, were identified (28.74%).

Water distilled oil obtained from the aerial parts of *T. persicum*, which is endemic to Iran, have been the subject of our previous studies. epi-α-Cadinol (23.2%) and α-pinene (17.3%) were the main components among the thirty-one constituents characterized in the oil of *T. persicum* representing 95.9% of the total components detected (55). The oil of *T. gnaphalodes* was characterized by higher amounts or β-caryophyllene (12.1%), sabinene (8.8%) and trans-pinocarveol (7.8%) (53).

As it is shown from the Table 3, in *A. chamaecistus* oil, 68 components, which representing about 92.6% of the total composition, were identified. The leaf oil of *A. chamaecistus* consists of 14 monoterpene hydrocarbons (26.38%), 12 oxygenated monoterpene (16.25%), 17 sesquiterpene hydrocarbons (24.21%), 8 oxygenated sesquiterpenes (11.36%), and 17 non terpenoid compounds (14.40%). The major components of this oil were (z)-β-ocimene (12.11%) and germacrene D (10.11%) followed by spathulenol (6.10%) and bornyl acetate (6.08%).

---

Table 2. Continue.

| No. | Compounds* | RI* | Stem Oil (%) | Leaf Oil(%) | Flower Oil (%) |
|-----|------------|-----|--------------|-------------|---------------|
| 47  | β- Eudesmol | 1649 | -            | -           | 1.47          |
| 48  | α - Cadinol | 1653 | 15.72        | 8.11        | 13.01         |
| 49  | Valerianol  | 1655 | 0.34         | -           | -             |
| 50  | Khasinol    | 1675 | -            | 0.54        | -             |
| 51  | (z, z)-Farnesal | 1713 | 0.48         | 0.53        | 0.42          |
| 52  | Cadina-4,10(15)- dien-3-one | 1740 | 0.35 | 0.37 | - |
| 53  | Tetradecanoic acid | 1771 | 0.67 | - | - |
| 54  | 6.10,14-trimethyl-2-Pentadecanone | 1872 | 0.39 | 0.11 | 0.23 |
| 55  | (z, z)-9,12- Octadecadienoic acid | 1953 | 0.83 | - | 1.06 |
| 56  | (E)-9-Octadecenoic acid | 1958 | - | - | 4.16 |
| 57  | (z)-9,17-Octadecadienal | 1965 | - | - | 1.99 |
| 58  | Hexadecanoic acid | 1973 | 5.17 | 0.64 | 16.37 |
| 59  | Eicosane    | 2000 | -            | -           | 4.93          |

Monoterpene hydrocarbons: 3.81, 14.96, -
Oxygenated monoterpenes: 2.92, 6.49, -
Sesquiterpene hydrocarbons: 23.28, 27.30, 16.56
Oxygenated sesquiterpenes: 57.61, 48.28, 43.40
Other compounds: 9.22, 1.45, 28.74
Total: 96.84, 98.48, 88.7

Note: *Compounds listed in order of elution from HP-5 MScolumn; Retention indices to C8-C24 n-alkanes on HP-5 MS column; *= trace (< 0.1%)
### Table 3. Percentage composition of the leaf oil of *Ajuga chamaecistus*.

| No. | Compounds                        | RI  | (%)   |
|-----|----------------------------------|-----|-------|
| 1   | α- Thujene                       | 928 | 0.55  |
| 2   | α- Pinene                        | 935 | 4.42  |
| 3   | Camphene                         | 950 | 0.23  |
| 4   | Sabinene                         | 976 | 0.16  |
| 5   | β- Pinene                        | 980 | 2.38  |
| 6   | 6- methyl-5- Hepton-2- one       | 983 | 0.34  |
| 7   | 1-Octen-3- ol                    | 986 | 3.89  |
| 8   | Myrcene                          | 991 | 0.77  |
| 9   | α- Phellandrene                  | 1003| 0.37  |
| 10  | p- Cymene                        | 1024| 1.05  |
| 11  | Limonene                         | 1031| 0.77  |
| 12  | (2)-β- Ocimene                   | 1040| 12.11 |
| 13  | (E)-β- Ocimene                   | 1050| 0.56  |
| 14  | γ-Terpinene                      | 1062| 1.58  |
| 15  | 1-Octanol                        | 1070| 0.58  |
| 16  | Terpinolene                      | 1088| 0.46  |
| 17  | Linalool                         | 1096| 5.25  |
| 18  | Nonanal                          | 1098| 0.41  |
| 19  | all- Ocimene                     | 1126| 0.97  |
| 20  | trans- Pinocarveol               | 1137| 0.17  |
| 21  | Camphor                          | 1141| 0.36  |
| 22  | trans-Verbenol                   | 1142| 0.62  |
| 23  | (E)-2- Nonenal                   | 1157| 0.18  |
| 24  | Lavandulol                       | 1164| 0.72  |
| 25  | Terpin-4- ol                     | 1177| 0.43  |
| 26  | α- Terpineol                     | 1189| 0.75  |
| 27  | Geraniol                         | 1253| 0.33  |
| 28  | Bornyl acetate                   | 1285| 6.08  |
| 29  | Lavandulyl acetate               | 1289| 0.73  |
| 30  | Carvacrol                        | 1296| 0.17  |
| 31  | Eugenol                          | 1356| 0.49  |
| 32  | α- Copaene                       | 1376| 0.68  |
| 33  | Geranyl acetate                  | 1381| 0.64  |
| 34  | β- Bourbonene                    | 1383| 0.78  |
| 35  | β- Elemene                       | 1391| 0.40  |
| 36  | (z)- Jasmame                     | 1394| 0.39  |
| 37  | methyl Eugenol                   | 1401| 0.12  |
| 38  | β- 1,7- di- epi Cedrene           | 1410| 0.52  |
| 39  | trans-α- Ambrinol                | 1412| 1.31  |
| 40  | β- Caryophyllene                 | 1418| 1.52  |
| 41  | β- Gurjunene                     | 1432| 0.25  |
| 42  | trans - Bergamotene              | 1436| 2.39  |
| 43  | α - Humulene                     | 1454| 0.62  |
| 44  | ( E )-β- Farnesene               | 1456| 1.54  |
| 45  | γ - Murolene                     | 1476| 0.58  |
| 46  | Germacrene D                     | 1480| 10.11 |
The leaf oil of *A. chamaecistus* was rich in regard to both monoterpenes (42.63%) and sesquiterpenes (35.57%).

The qualitative and quantitative variation between our results and our previous reports (40) for the constituents of the oil from aerial parts may be attributed to the different environment conditions and different methods of extraction of the oils.

The oils of the genus *Ajuga* have been the subject of only a few studies. The oil of *A. chamaecistus* subsp. tomentella contained thymol (34.5%) and exo-fenchol (15.6%) as the major components (56).

The oil of *A. chamaecistus* subsp. scoparia was characterized by higher amounts of *α*-cymene (34.5%), *β*- pinene (18.0%), α-phellandrene (17.8%) and *α*- pinene (15.2%) were major constituents (57).

The major constituents of the aerial parts of *A. chamaepitys* ssp. chamaepitys were *β*-pinene (34.3%) and *α*-pinene (16.1%) (58). The dominant compounds in *A. bombycina* were *β*-pinene (28.2%) and *α*-pinene (18.5%) (59).

**Acknowledgment**

We are grateful to Dr. V. Mozaffarian.
References

(1) Rechinger KH. Phlomis, Teucrium, Ajuga In flora Iranica, Labiatae. No. 150, Rechinger KH and Hedge IC (eds). Akademische Druk and Verlagsanstalt, Graz, Austria (1982) 298, 32, 15.

(2) Mozaffarian V. A Dictionary of Iranian Plant Name, Farhang Moaser. Tehran (1996) 406: 542, 21.

(3) Saracoglu I, Kojima K, Harput US and Oghara Y. A new phenyl ethanol glycoside from Phlomis pungens Willd var. pungens. Chem. Pharm. Bull. (1998) 46: 726-7.

(4) Sarkhail P, Abdollahi M and Shafiee A. Antinociceptive effect of Phlomis Olivieri Benth., Phlomis anisodontia Boiss and Phlomis persica Boiss. Total extracts. Pharmacol. Res. (2003) 48: 263-6.

(5) Kirmizibekmez H, Montoro P, Piacenti S, Pizza C, Doennem A and Calis I. Identification by HPLC-PAD-MS and quantification by HPLC-PAD of phenyl ethanol glycosides of five Phlomis species. Phytochem. Anal. (2005) 16: 1-6.

(6) Katagiri M, Ohtani K, Kasai R, Yamasaki K, Yang CR and Tanaka O. Diterpenoid glycosyl esters from Phlomis samia. J. Nat. Prod. (2008) 71: 494-7.

(7) Shin TY and Lee JK. Effect of Phlomis umbrosa root on mast cell-dependent immediate-type allergic reactions by anal therapy. Immunopharmacol. Immunotoxicol. (2003) 25: 73-85.

(8) Kirmizibekmez H, Calis I, Perozzo R, Brun R, Doennem AA, Linden A, Rueded P and Tasdemir D. Inhibiting activities of the secondary metabolites of Phlomis brunneogaleata against parasitic protozoa and plasmodial enoyl ACP Reductase, a crucial enzyme in fatty acid biosynthesis. Planta Med. (2004) 70: 711-17.

(9) Kyriakopoulou I, Magiatis P, Skaltounis AI, Aliqiannis N, Harvala C and Samioside A. A new phenyl ethanol glycoside with free-radical scavenging and antimicrobial activities from Phlomis samia. J. Nat. Prod. (2001) 64: 1095-7.

(10) Aliqiannis N, Kalpoutzakis E, Kyriakopoulou I, Mitaku S and Chinou IB. Essential oils of Phlomis species growing in Greece. Chemical composition and antimicrobial activity. Flavour Fragr. J. (2004) 19: 320-4.

(11) Camil MS, Mohamed KM, Hassanane HA, Ohtani K, Kasai R and Yamasaki K. Iridoid and magastigmane glycosides from Phlomis aurea. Phytochemistry (2000) 55: 353-7.

(12) Kasai R, Katagiri M, Ohtani K, Yamasaki K, Yang CR and Tanaka O. Iridoid glycosides from Phlomis younghusbandii roots. Phytochemistry (1994) 36: 967-70.

(13) Calis I, Basaran AA, Saracoglu I, Sticher O and Ruedi P. Phlomisides D and E, phenyl propanoid glycosides and iridoids from Phlomis linearis. Phytochemistry (1991) 30: 3075-77.

(14) Bucar F, Ninov S, Ionkova I, Kartign T, Schubert Z and Asenov I. Flavonoids from Phlomis nissolii. Phytochemistry (1998) 48: 573-4.

(15) Tomas F, Nieto JL, Barberan FAT and Ferreres F. Flavonoids from Phlomis lychnitis. Phytochemistry (1986) 25: 1253-4.

(16) Abdollahi M, Kariimpor H and Monsef - Esfaheni HR. Antinociceptive effects of Teucrium polium L. total extract and essential oil in mouse writhing test. Pharm. Res. (2003) 48: 31-5.

(17) Chedia A, Ghazghazi H, Brahim H and Abderrazak M. Secondary metabolite antioxidant and antibacterial activities of Teucrium polium L. methanolic extract. Int. J. Agron. Plan. Prod. (2013) 4: 1790-7.

(18) Tariq M, Ageel AM, al-Yahya MA, Mossa JS and al-Said MS. Anti inflammatory activity of Teucrium polium. Int. J. Tissue React. (1989) 11: 185-8.

(19) Balchnejadmojarad T, Rogni M and Rogni Dehkordi F. Antinociceptive effect of Teucrium polium leaf extract in the diabetic rat formalin test. J. Ethnopharmacol. (2005) 97: 207-10.

(20) Autore G, Capasso F, De Fusco R, Fasulo MP, Lembo M, Mascolo N and Menghini A. Antipyretic and antibacterial activities of Teucrium polium (L.). Pharmacol. Res. Commun. (1984) 16: 21-9.

(21) Rasekh HR, Khoshnoosh-Mansourkhan MJ and Kamalinejad M. Hypolipidemic effects of Teucrium polium in rats. Fitoterapia (2001) 72: 937-9.

(22) Amini R, Nosrat N, YaZdanparast R and Molaei M. Teucrium polium prevention of steatohepatitis in rats. Liver int. (2009) 29: 1216-21.

(23) Meherabani D, Rezaee A, Azarpira N, Fatahi MR, Amini M, Tanideh N, Panjehshahin MR and Saberi-Firouzi M. The healing effects of Teucrium polium in the repair of indomethacin induced gastric ulcer in rats. Saudi Med. J. (2009) 30: 494-9.

(24) Rajabali S. Methanolic extract of Teucrium polium L. potentiates the cytotoxic and apoptotic effects of anticancer drugs of vincristine, vinblastine and doxorubicin against a panel of cancerous cell lines. Exp. Oncol. (2008) 30: 133-8.

(25) Vokou D and Beniere J M. Volatile constituents of Teucrium polium. J. Nat. Prod. (1985) 48: 498-9.

(26) Pere Z-Alonso MJ, Velasco-Negueruela A and Lopez-Saez JA. The essential oils of two Liberian Teucrium species. J. Essent. Oil Res. (1993) 5: 397-402.

(27) Guetat A and Al-Ghamdi FA. Analysis of the essential oil of the Germander (Teucrium polium L.) aerial parts from the northern region of Saudi Arabia. Int. J. Appl. Biol. Pharm. Technol. (2014) 5: 128-35.

(28) Cozzani S, Muselli A, Desjobert JM, Bernardini AF, Tomi F and Casanova J. Chemical composition of essential oil of Teucrium polium subsp. capitatum (L.) from Corsica. Flav. Frag. J. (2005) 20: 436-41.

(29) Hilaly JE and Lyousi B. Hypoglycemcic effect of lyophilized total water extract of Ajuga iva in normal and streptozotocin diabetic rats. J. Ethnophar. (2002)
(30) Gautam R, Jachak SM and Saklani A. Anti-inflammatory effect of Ajuga bracteosa Wall ex Benth. mediated through cyclooxygenase (COX) inhibition J. Ethnopharmacol. (2011) 133: 928-30.

(31) Iradi HZ and Lyoussi B. Ethnopharmacology of the plants of genus Ajuga. Pak. J. Pharm. Sci. (2009) 22: 425-62.

(32) Kuria KAM, Coster S, Muriuki G, Masengo W. Kibwage I, Hoogmartens J and Laekeman GM. Antimalarial activity of Ajuga remota Benth (Labiatae) and Caesalpinia volkensii Harms (Caesalpinioideae): in-vitro confirmation of ethnopharmacological use. J. Ethnopharmacol. (2001) 74: 141-8.

(33) Ono Y, Fukaya Y, Imai S and Yamakuni T. Beneficial effects of Ajuga decumbens on osteoporosis and arthritis. Biol. Pharm. Bull. (2008) 31: 1199-204.

(34) Vanyolos A, Simo A, Toth G, Polgar L, Kele Z, Ilku A, Matyus P and Bathori M. C-29 Ecdysteroids from Ajuga chamaecistus. J. Essent. Oil Res. (2003) 15: 177-8.

(35) Castro A, Coll J, Tandron YA, Pant AK and Mathela N. Chemical constituents of the essential oil of Phlomis persica Boiss. & Hohen. Phlomis anisodontata Boiss and Plomis bruguieri Desf. from Iran. Flavour Fragr. J. (2004) 19: 29-31.

(36) Morteza-Semnani K, Azadbakhsh M and Goodarzi A. The essential oils composition of Phlomis herba-venti L. leaves and flowers of Iranian origin. Flavour Fragr. J. (2006) 18: 154-60.

(37) Van den Dool H and Kratz PD. Generalization of the thermal programed gas-liquid partition chromatography. J. Chromatogr. (1965) 11: 463-71.

(38) Massada Y. In Analyses of Essential Oil by Gas Chromatography Mass Spectrometry. Wiley, New York (1976).

(39) Adams RP. Identification of Essential Oil Components by Gas Chromatography / Quadrupole Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, Illinois (2001).

(40) Javidnia K, Miri R and Soltani M. Essential oil composition of two species of Phlomis L. (Phlomis aucheri Boiss. and Phlomis elliptica (Benth) (Lamiaceae) from Iran. J. Essent. Oil Res. (2010) 22: 314-7.

(41) Khalilzadeh MA, Rustaiyan A, Masoudi S and Tajbakhsh M. Essential oil of Phlomis persica Boiss and Phlomis olivieri Benth. Iran. J. Essent. Oil Res. (2005) 17: 624-625.

(42) Masoudi S, Rustaiyan A, Aberoumand Azar P and Larijani K. Composition of the essential oils of Cyclotrichium straussii (Bornm.) Rech.f. and Phlomis pungens Wild from Iran. J. Essent. Oil Res. (2006) 18: 16-8.

(43) Akhlaghi H and Motavali-zadeh Kahky A. Volatile constituents of Phlomis cancellata Bge., a labiatae herb indigenous in Iran. J. Essent. Oil Bearing Plants. (2010) 13: 650-4.

(44) Joshi RK and Pande C. Chemical composition of the essential oil of Phlomis bracteosa Royal ex Benth. J. Essent. Oil Res. (2010) 22: 297-299.

(45) Kasiri P, Amin G and Shafiee A. Composition of the essential oil of Phlomis armeniaca Willd. from Mediterranean region in Turkey. Asian J. Chem. (2010) 22: 2887-90.

(46) Sarkail P, Amin G and Shafiee A. Composition of the essential oil of Phlomis persica Boiss. and Phlomis chorasanica Bunge from Iran. Flavour Fragr. J. (2001) 14: 538-40.

(47) Morteza-Sammani K and Saeedi M. The essential oil composition of Plomis bruguieri Desf. from Iran. Flavour Fragr. J. (2005) 20: 344-6.

(48) Demirci B, Dadandi MY, Paper DH, Franz C and Baser KHC. Chemical composition of the essential oil of Phlomis linearis Boiss. & Hohen., Phlomis anisodontata Boiss and Plomis bruguieri Desf. from Iran. Flavour Fragr. J. (2005) 20: 327-9.

(49) Morteza-Sammani K and Saeedi M. The essential oil composition of Plomis lanceolata Boiss. & Hohen., Phlomis anisodontata Boiss and Plomis lanceolata Boiss. & Hohen., Phlomis anisodontata Boiss and Plomis lanceolata Boiss. & Hohen. from Iran. Flavour Fragr. J. (2005) 20: 327-9.

(50) Kibwage I, Hoogmartens J and Laekeman GM. Chemical constituents of the essential oil of Phlomis lanceolata Boiss. & Hohen., Phlomis anisodontata Boiss and Plomis bruguieri Desf. from Iran. Flavour Fragr. J. (2005) 20: 327-9.

(51) Masoudi S, Aghajani Z, Rustaiyan A, Feizbakhsh AR and Motavali-zadeh Kahky A. Essential oil composition of Teucrum persicum Boiss., Thymus caucasicus Willd ex Ronniger subsp. grossheimii (Ronniger) Jalas. and Marrubium crassidentis Boiss. Three Labiatae herbs growing wild in Iran. J. Essent. Oil Res. (2009) 21: 5-7.

(52) Shamsh Ardekan MR, Khanavi M, Taheri P, Samadi N, Safaripour E and Salimpour F. The essential oil composition of Ajuga chamaecistus Ging subsp. tomentella Rech.f. J. Essent. Oil Bearing Plants. (2010) 13: 45-51.

(53) Mohammadhosseini M, Pazoaki A, Zamani HA and Akhlaghi H. Chemical composition of the essential oil from aerial parts of Ajuga chamaecistus Ging subsp. Scopria in Brackish regions of Iran. J. Essent. Oil Bearing Plants. (2011)14: 101-5.

(54) Azizan J, Fallah- Bagher-Shaidaei H and Kefayati H. Chemical constituents of the essential oil of Ajuga chamaepitys growing in Iran. J. Essent. Oil Res. (2002)14: 344-5.
Baser KHC, Kurcuoglu M and Erdemgil FZ. The essential oil of *Ajuga bombycina* from Turkey. *Chem. Nat. Compounds.* (2001) 37: 242-4.

This article is available online at http://www.ijpr.ir