

Volatile Constituents from Different Parts of Three Lamiacea Herbs from Iran

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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 -caryophyllene (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), antifibrotic (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: *T. melissoides* 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 ailments 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

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(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 *T. polium* are found in literature. In the oil from *T. polium* collected in Athens (Greece), β -caryophyllene (17.7%) was shown to be the major constituent accompanied by δ -cadinene (9.3%), caryophyllene oxide (5.9%) and α -cadinol (5.4%) (25). The oil of *T. polium* subsp. *valentinum* from Spain, was found to contain α -pinene (15.8%) and β -pinene (11.7%) as major constituents (26). α -Muurolene (8.7%), α -cadinol (5.9%) and δ -cadinene (5.1%) were found to be the major constituents of the essential oil of *T. polium* from northern region of Saudi Arabia (27). The dominant compounds in *T. polium* from Corsica were α -pinene (28.8%), β -pinene (7.2%), and *p*-cymene (7.0%) (28).

The genus *Ajuga* is represented in the flora of Iran by five species in which *Ajuga chamaecistus* has contained several endemic subspecies including *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, antiarthritis, antipyretic, hepatoprotective, antibacterial, antifungal, antioxidant, cardiogenic (31), and antimalarial (32) properties and also their application in the treatment of joint diseases (33).

Many phytochemical studies on *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 *A. chamaecistus* Ging. ssp. *chamaecistus*, collected from Fasham, 35 km east of Tehran, which contained β -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 *Phlomis aucheri*, from stems, leaves, and roots from *Teucrium polium* and also from leaves of *Ajuga*

chamaecistus growing wild in Iran.

Experimental

Plant materials

The stems, leaves, and flowers of *Phlomis aucheri*, which is endemic to Iran, and stems, leaves and roots of *Teucrium polium*, were collected from Salehabad area, Province of Ilam, west of Iran, both in July 2013, during the flowering stage. The leaves of *Ajuga chamaecistus* were collected from Mehran, Province of Ilam, in July 2013. Voucher specimens have been deposited at the Herbarium of the Research Institutions 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 *P. aucheri* and also stems (110 g), leaves (80 g), and roots (65 g) of *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 *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 35×35×35 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 *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 ^a	RI ^b	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	<i>p</i> -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

Table 1. Continue.

No.	Compounds ^a	RI ^b	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 spathulenol	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		4.32	5.57	0.85
	Oxygenated monoterpenes		8.00	3.31	0.85
	Sesquiterpene hydrocarbons		38.04	71.54	78.95
	Oxygenated sesquiterpenes		13.48	6.98	6.21
	Other compounds		27.74	3.64	2.67
	Total		91.58	91.04	89.53

Note: ^aCompounds listed in order of elution from HP- 5 MS column;

^bRetention indices to C₈- C₂₄ n-alkanes on HP- 5 MS column.

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.

Gas chromatography analysis

Gas chromatography analysis was performed on Shimadzu 15A gas chromatograph equipped with a split /splitless injector (25 °C) and a flame

ionization detector (250 °C). Nitrogen was used as carrier gas () and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 μm). The column temperature was kept at 60 °C for 3 min and then heated to 220 °C with a 5 °C rate and kept constant at 220 °C for 5 min. Relative percentage amounts was calculated from peak area using a Shimadzu C- R4 A chromatopac without the use of correction factors.

Gas chromatography – mass spectrometry analysis

Analysis was done using a Hewlett-Packard 5973 with a HP- 5 MS column (30 m × 0.25mm, film thickness 0.25 μm). The column temperature was kept at 60 °C for 3 min and 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 e V.

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

monoterpene 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 β-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;

Table 2. Comparative percentage composition of the stem, leaf and root oils of *Teucrium polium*.

No.	Compounds ^a	RI ^b	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	<i>p</i> - 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	<i>trans</i> - Pinocarveol	1136	-	0.91	-
14	<i>cis</i> - 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	<i>cis</i> - Chrysanthenyl 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	<i>trans</i> - α - Bergamotene	1434	0.42	1.07	-
27	Geranyl acetone	1453	0.27	-	-
28	α - Humulene	1454	2.25	3.08	0.75
29	allo- Aromadendrene	1459	0.35	-	-
30	Germacrene D	1480	0.84	2.77	1.23
31	Bicyclogermacrene	1494	0.98	2.02	-
32	α - Muurolene	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	<i>trans</i> -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

Table 2. Continue.

No.	Compounds ^a	RI ^b	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	Khusinol	1675	-	0.54	-
51	(z, z)- Farnesol	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: ^aCompounds listed in order of elution from HP- 5 MS column; ^bRetention indices to C₈-C₂₄ n-alkanes on HP- 5 MS column; t= trace (< 0.1%)

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 monoterpenes. 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 of 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 monoterpenes (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 3. Percentage composition of the leaf oil of *Ajuga chamaecistus*.

No.	Compounds ^a	RI ^b	(%)
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- Hepten-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	(z)- 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 - Terpeneol	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)- Jasmene	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 - Muurolene	1476	0.58
46	Germacrene D	1480	10.11

Table 3. Percentage composition of the leaf oil of *Ajuga chamaecistus*.

No.	Compounds ^a	RI ^b	(%)
47	Bicyclogermacrene	1492	2.06
48	Cuparene	1502	0.19
49	β - Bisabolene	1507	0.71
50	γ - Cadinene	1513	0.50
51	δ - Cadinene	1524	1.15
52	Germacrene B	1556	0.21
53	(E)- Nerolidol	1564	0.56
54	Spathulenol	1574	6.10
55	Caryophyllene oxide	1581	1.74
56	β - Oplophenone	1606	0.85
57	Cedr- 8(15)- en- 9- α - ol	1644	0.28
58	(z)- methyl Jasmonate	1647	0.75
59	(z)- Nerolidol acetate	1675	0.42
60	α - Bisabolol	1683	1.17
61	Tetradecanoic acid	1760	0.65
62	Hexadecanol	1819	0.40
63	(E, E)- Farnesyl acetate	1841	0.24
64	6,10,14- trimethyl -2- Pentadecanone	1843	0.63
65	6,10,14- trimethyl-5,9,13- Pentadecatrien-2- one	1915	0.17
66	Hexadecanoic acid	1970	3.06
67	(z, z, z) 9,12,15- Octadecatrienonic acid-methyl ester	2010	0.22
68	(E)-5- Octadecene	2083	0.81
	Monoterpene hydrocarbons		26.38
	Oxygenated monoterpenes		16.25
	Sesquiterpene hydrocarbons		24.21
	Oxygenated sesquiterpenes		11.36
	Other compounds		14.40
	Total		92.6

Note: ^aCompounds listed in order of elution from HP- 5 MS column;

^bRetention indices to C₈- C₂₄ n-alkanes on HP- 5 MS column.

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 *p*-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).

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