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Hydrodistilled Volatile Oils of the Flowers of *Salvia leriifolia* Bench. and *Salvia multicaulis* Vahl. as Two Growing Wild Plants in Iran

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The present report is a part of research to analyze and compare the constituent components of essential oil bearing plants in salt deserts of Iran comprising some species of *Salvia* genus. The volatile oils were extracted by hydrodistillation and were analyzed by gas chromatograph and gas chromatograph-mass spectrometer instruments. Recognition of the oil structure was afforded by comparison of the retention times and was confirmed by matching retention index, mass fragment and eight peak templates for each ingredient with those tabulated in authentic references. The flower oil of *Salvia leriifolia* Bench. was characterized by compounds constituting 97.6 % of the total oil, which were predominately γ-terpenene (62.2 %), *p*-cymene (11.1 %), α-terpinene (7.3 %) and myrcene (5 %). Twenty one compounds representing 94.3 % of flower oil of *Salvia multicaulis* Vahl. were identified among them 1,8-cineol (25.3 %), α-pinene (18.3 %), camphor (12.4 %), camphene (8.4 %) and bornyl acetate (7.9 %) were the major ones. Accordingly, in the both volatile oils, monoterpenes predominate over sesquiterpenes.

Key Words: *Salvia leriifolia* Bench., *Salvia multicaulis* Vahl., Essential oil, GC/MS, Monoterpenes, Sesquiterpenes.

INTRODUCTION

The genus *Salvia* comprises 700 herbs and shrubs, growing in the temperate and warmer zone of the world. Fifty eight species are found in Iran, among which 17 are endemic[1,2]. Some species of genus *Salvia* are used as medicinal aromatic and ornamental plants. *Salvia multicaulis* is an evergreen shrub growing to 0.3 m × 0.25 m, native to south-west Asia, particular eastern, central and southern Turkey. *Salvia officinalis* is one of the most widespread species and since ancient times has been used in the treatment of various disorders, such as berculosis, psoriasis and seborrhiec eczema[3,4]. *Salvia multicaulis* Vahl. (Fig. 1) is frost hardy to a least -10 ºC, but it does not like it too wet. The leaves are similar to those of the common sage, *S. officinalis*, but more rounded, rugose and covered in grey tomentum above and below. The flowering stems are erect to 30 cm with whorls of 4-10 violet flowers enclosed within reddish brown calyces, which are persistent, expanding and deepening in colour with formation of seed and subtended by reddish brown bracts. The main flowering period is May to June and often again in the autumn. Propagation is by seed, cuttings or layering[5]. The odor of *Salvia leriifolia* is very complex, strong and warm-balsamic-woody. Therefore, a lot of constituents will contribute to the total sensory impression. Previous chemical investigation of different species of *Salvia* has shown the presence of flavonoides[6], diterpenoides[7,8], sesterpenes[9,10] and essential oils[11-14].

Fig. 1. Presentation of *Salvia multicaulis*

However, in the recent decades improvement the combination systems like GC-MS and optimization of the instrumentation has encouraged chemists, phytochemists and biologists to initiate vital projects for distinguishing the essential oil fractions[15-18] and performance of the complementary investigations including antioxidant[19,20] and antibacterial activities[21-23].

The main goal of present study is the identification the constituents of volatile oils of flowers of *Salvia leriifolia* Bench. and *Salvia multicaulis* Vahl. which have been gathered from brackish regions of north-east and Semnan provinces of Iran, respectively.

EXPERIMENTAL

The plant material was collected during the flowering stage in May 2008 from Sabzavar, Province of Khorassan, north east of Iran and Shahmirzad in Semnan province of Iran, at an altitude of 1560 and 1500 m from sea levels (msL), respectively. A voucher specimen has been deposited in Herbarium of Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Essential oil isolation: Air-dried of flowers of *Salvia leriifolia* Bench. and *Salvia multicaulis* Vahl. (100 g) were separately subjected to hydrodistillation for 3 h using Clevenger-type apparatus to produce oils. The oils were dried over anhydrous sodium sulfate and stored in seald brown vials under darkness at low temperature (-10 ºC) before analysis.

GC analysis: Analytical gas chromatography was carried out on a Shimadzu 15 A gas chromatograph equipped with split/splitless injector (250 ºC) and a flame ionization detector (250 ºC). N_2 was as carrier gas (1 mL/min). The capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60 ºC for 3 min and then heated to 220 ºC with a 5 ºC/min rate and kept constant at 220 ºC for 5 min.

GC/MS analysis: A Hewlett-Packard 6890/5973 apparatus fitted with a fused silica HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm) was used. The column temperature was kept at 60 ºC for 3 min, programmed to 220 ºC at a rate of 5 ºC/min and kept constant at 220 ºC for 5 min. Helium was the carrier gas (1 mL/min). Mass spectra were taken at 70 eV.

RESULTS AND DISCUSSION

Qualitative and quantitative analyses: In this work, hydrodistilled volatile oils from crushed dry flowers of *Salvia leriifolia* Bench. and *Salvia multicaulis* Vahl. (Lamiaceae), were analyzed by GC and GC/MS. Physicochemical properties of the air-dried flowers of the plants have been demonstrated in Table-1. The chromatograms of homologous alkanes (C_9-C_{20}) and flower oil of *Salvia multicaulis* Vahl. have been shown in Figs. 2 and 3, respectively. Furthermore, the percentage compositions of the flowers oils are given in Tables 2 and 3, respectively.

Identification of the constituents of the oils was made by comparison of their retention times, mass spectral fragmentation pattern and retention indices (RI) relative to C_9-C_{21} *n*-alkanes with those given in the literature[24] and stored in a MS library (Wiley 275). Relative percentage amounts of the components were calculated from peak area using a Shimadzu C-R4A chromatopac on the DB-5 column without the use of correction factor.

TABLE-1
PHYSIOCHEMICAL SPECIFICATIONS OF THE OILS

Property	*Salvia leriifolia* Bench.	*Salvia multicaulis* Vahl.
Weight percent (w/w %)	0.75	0.66
Colour	Pale yellow	Yellow
Density[20] (g/mL)	0.42	0.53
$[\alpha]^{20}$	-6.40	-5.30
$[n]^{20}$	1.1	1.6

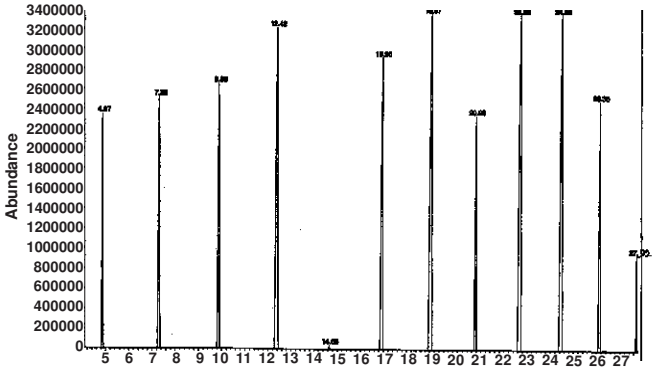

Fig. 2. Chromatogram of alkanes C_9-C_{20} in HP-5MS capillary column

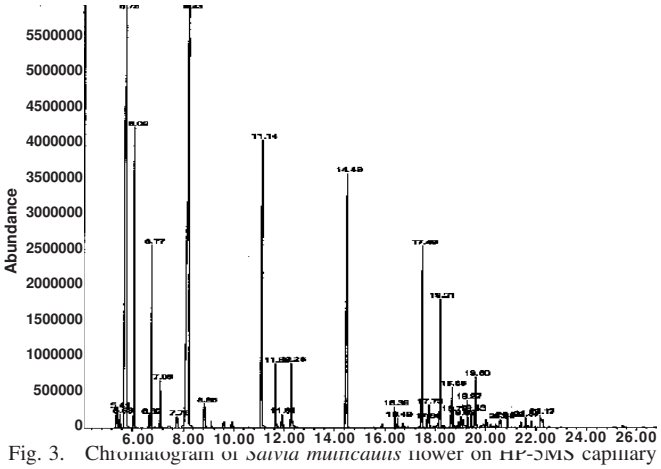

Fig. 3. Chromatogram of *Salvia multicaulis* flower on HP-5MS capillary column

TABLE-2
ESSENTIAL OIL COMPOSITION OF THE FLOWERS OF *Salvia leriifolia* BENCH[a]

No.	Compound	Retention index	Percentage
1	α-Pinene	936	3.3
2	Sabinene	975	2.0
3	Myrcene	993	5.0
4	α-Phellandrene	1006	0.7
5	α-Terpenene	1018	7.3
8	*p*-cymene	1027	11.1
9	Limonene	1031	2.1
10	Z-β-ocimene	1040	0.8
11	γ-Terpenene	1089	62.2
12	β-Bourbonene	1389	1.9
13	(E)-β-Farnesene	1458	1.2
		Total	97.6

[a]Compounds have been sorted according to retention indices on HP-5 MS and DB-5 capillary columns

TABLE-3
ESSENTIAL OIL COMPOSITION OF THE FLOWERS OF *Salvia multicaulis* VAHL[a]

No.	Compound	Retention indices	Flowers oil (%)
1	α-Thujene	931	0.37
2	α-Pinene	936	18.32
3	Camphene	953	8.45
4	Sabinene	976	0.3
5	β-Pinene	980	4.6
6	Myrcene	991	1.12
7	1,8-Cineol	1033	25.27
8	γ-Terpenene	1059	0.57
9	Camphor	1145	12.4
10	Borneol	1167	1.91
11	Terpenene-4-ol	1178	0.32
13	Bornyl acetate	1286	7.89
14	α-Yalengene	1374	0.48
15	α-Copaene	1376	0.21
16	β-Caryophyllene	1418	5.80
17	Calarene	1428	0.56
18	α-Humulene	1452	3.47
19	α-Amorphene	1470	1.02
20	α-Muurolene	1499	0.37
21	δ-Cadinene	1524	0.63
22	Caryophyllene oxide	1581	0.22
		Total Percentage	94.3

[a]Compounds have been sorted according to retention indices on HP-5 MS and DB-5 capillary columns

Mass fragments: Mass spectra of the major constituents in both oils under the optimized experimental conditions are given in Table-4.

TABLE-4
MASS FRAGMENTS OF THE MAIN COMPONENTS OF THE OILS

Compound	Fragments
γ-Terpenene	93 (100 %), 136.1 (38 %), 121.10 (32 %), 79 (22 %), 43 (32 %), 119 (30 %), 41 (24 %)
p-Cymene	119 (100 %), 134 (25 %), 91 (17 %), 120 (10 %), 39 (9 %), 41 (8 %), 117 (7 %), 77 (7 %)
α-Terpinene	93 (99.9 %), 91 (37.2 %), 136 (35.8 %), 121 (30.3 %), 77 (28.1 %), 92 (21.4 %), 79 (18.5 %), 43 (17.5 %), 41 (9.9 %), 105 (9.7 %)
Myrcene	93 (100 %), 41 (98 %), 69 (82 %), 79 (14 %), 77 (12 %), 91 (11 %), 53 (11 %), 67 (10 %)
1,8-Cineol	43 (100 %), 81 (64 %), 71 (58 %), 108 (52 %), 84 (49 %), 154 (44 %), 69 (43 %), 111 (42 %)
α-Pinene	93.10 (100 %), 92.10 (37.93 %), 91.10 (26.73 %), 79.10 (26.7 %), 77.05 (14.83 %)
Camphene	93 (100 %), 121 (71 %), 79 (39 %), 67 (32 %), 107 (31 %), 95 (22 %), 94 (21 %), 68 (21 %)
Bornyl acetate	95 (100 %), 43 (60 %), 136 (54 %), 93 (47 %), 121 (39 %), 41 (24 %), 108 (18 %), 27 (18 %)

Conclusion

Twenty seven compounds (97.6 %) were identified in the flower oil: nine monoterpene hydrocarbons (92.8 %), six oxygenated monoterpenes (1.9 %) and five sesquiterpene hydrocarbons (1.2 %) and trace of extra compounds (1.7 %). They were characrterized by the presence of γ-terpenene (62.2 %), *p*-cymene (11.1 %), α-terpinene (7.3 %) and myrcene (5 %).

Twnety one constituents (94.28 %) were identified in flower oil of *Salvia multicaulis* Vahl. (Lamiaceae): seven monoterpene hydrocarbons (33.73 %), 5 oxygenated monoterpenes (47.79 %), 8 sesquiterpene hydrocarbons (12.54 %) and 1 oxygenated sesquiterpene (0.22 %). 1,8-Cineol was the most abundant constituent (25.27 %), followed by α-pinene (18.32 %), camphor (12.40 %), camphene (8.45 %) and bornyl acetate (7.89%).

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