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Full Length Research Paper

Chemotaxonomy of six *Salvia* species using essential oil composition markers

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Chemotaxonomy of six *Salvia* L. species in Iran has been controversial. The group includes the taxa *Salvia atropatana* Bunge., *Salvia oligophylla* Auch. ex Benth., *Salvia aethiopis* L., *Salvia sclarea* L., *Salvia reuterana* Boiss., *Salvia macrosiphon* Boiss., of which *Salvia reuterana* and *Salvia oligophylla* are endemic to Iran. The essential oils obtained from aerial parts of species were analyzed by using gas chromatography (GC) and gas chromatography- mass spectrum (GC-MS). Two hundred and seventy components were analyzed and cluster analysis of oil components was done by average linkage. The results showed that *S. oligophlla* and *S. aethiopis* separated, due to its high amount of occidentotal, *iso*- Longifolene, *β*- Acoradiene, in *S. oligophylla*. Similarity in essential oil composition of the *S. aethiopis* and *S. sclarea* grouped them to one sub cluster. Also, a comparison of these results with previous works on *Salvia* species revealed that the major constituents of these taxa are different. Evidence from volatile oil compounds may have contributed to environmental factors such as available water and other climatic factors.

Key words: *Salvia*, volatile constituent, chemotaxonomy, *Lamiaceae*, Iran.

INTRODUCTION

The genus *Salvia* with over 900 species is probably the largest member of the family *Lamiaceae* and is found in both subtropical and temperate parts of the world (Walker and Sytsma, 2007). The two largest centers of the genus are in America and in South-West Asia (Walker and Sytsma, 2007). Fifty eight annual or perennial species of the genus are found in Iran of which 17 are endemic (Mozafarian, 1996; Rechinger, 1987). Some *Salvia* species are used in folk medicine for the treatment of asthma, eczema, psoriasis and tuberculosis. The genus is named "*Salvia*", derived from latin "Salveo", which means to "save, to recover" (Aktas et al., 2009). The antibacterial, antituber culous and antiphlogistic activities of the constituents of *Salvia* species are well established (Abravesh et al., 2005). Also, there are several reports of phytochemical analysis of species belonging to *Salvia* (Bigdeli et al., 2005; Gulluce et al., 2006; Habibi et al., 2004; Lari et al., 2005; Rustaiyan et al., 2000). These scientific studies showed the presence of many compounds belonging mainly to the groups of phenolic acids, phenolic glycosides, flavonoids, anthocyanins, terpenoids and essential oils (Walker and Sytsma, 2007). However, the composition of volatile compounds is known for a number of species (Chalchat et al., 2001; Mirza et al, 2006; Rustaiyan and Komeilizadeh, 1997; Sajjadi and Ghannadi, 2005; Skatsa, 2001), but few data are found in chemotaxonomy studies of this genus. On the other hand, chemistry of volatile compounds has been proved particularly helpful in assessing taxonomic relationships of several genera in *Lamiaceae* (Walker and Sytsma, 2007). The present study aims is to investigate the volatile compounds of six *Salvia* species (*Salvia atropatana, Salvia oligophylla, Salvia aethiopis, Salvia sclraea, Salvia reuterana, Salvia macrosiphon*), that have similarity in morphological characters especially in their leaves, trichomes and flowers. The results are compared

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Table 1. List of *Salvia* species and their locality.

Species	Locality
S. aethiopis L.	Ardebil: Khalkhal to Asalem, 2100 m, Mazooji 12190 IAUH
S. atropatana Bunge.	Tehran: Damavand to Firoozkooh, Gadok, 2050 m,Mazooji 12193 IAUH
S. sclarea L.	Mazandaran: Firoozkooh to Polesefid, 200m, Mazooji 12202 IAUH
S. macrosiphon Boiss.	Tehran: Damavand to Firoozkooh,2150 M, Mazooji 12197 IAUH
S. oligophylla Auch. ex Benth.	Zanjan: Qazvin to Zanjan, 1500 m, Mazooji 12194 IAUH
S. reuterana Boiss.	Tehran: Poonak, 1800m, Mazooji 12198 IAUH

with the taxonomy of the taxa to extract evidence of possible chemotaxonomic significance.

MATERIALS AND METHODS

Six taxa were collected during the flowering period from natural habitats of Iran between 2008-2009 (Table 1). The taxonomic description of these species follows Hedge (1982b). For isolation of the volatile oils, the aerial parts of the plants were dried at room temperature and hydrodistilled for 5 h using a clevenger-type apparatus. The oil was dried over anhydrous sodium thiosulfate and kept at 4℃ in sealed brown vials until required. Analytical gas chromatography was capillary column DB-5 (30 m. 0.25 mmid, 0.25 µm film thickness); carrier gas, He; split ratio, 1: 25, and using a flame ionization detector. The column temperature was programmed at 50℃ for 1 min, and then heated to 265℃ at a rate of 2.5℃ /min, then kept constant at 265℃ for 20 min. Gas chromatography- mass spectrum (GC-MS) was performed on a thermoquest 2000 with quadruple detector, on capillary column DB-5 (GC), carried gas, He; flow rate, 1.5 ml/min. The column was held at 50℃ for 1 min, and programmed up to 265℃ for 20 min. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the oils were identified by comparison of their mass spectra and retention indicates with Wiley library. Hierachial clustering analysis of main components between species was performed using SPSS software with average linkage method and the resulting dendrogram was illustrated.

RESULTS

The oils isolated by hydrodistillation from the aerial parts of *S. atropatana, S. oligophylla, S. aethiopis, S. sclraea, S. reuterana, S. macrosiphon* were obtained in yields of 0.2, 0.45, 23, 0.51, 0.49 and 0.5% (w/w), respectively. The composition of the oils of the *Salvia* species is listed in Table 2, in which the percentage and retention times of the total components are given. 28 constituents representing 97.21% of the total components in the oil of *S. aethiopis*, characterized by *α*–Copanaene (16.64%), Germacrene D (16.54%), Trans Caryophyllene (8.22%), Caryophyllene oxide (5.09%) and *α*- Humulene (4.66%). *S. atropatana* oil contained Caryophyllene oxide (19.26%), *α*- Cubebene (12.97%), *trans*- Caryophyllene (6.16%), Germacrene D (5.16%), Phytol (3.94%), Longifololacetate (3.94%) and *δ*-Cadinene (2.17%) among the 57 constituents characterized, comprising 97.37% of the total components detected. Germacrene D (12.67%), followed by *trans*-Caryophyllene (7.66%), Linalool L (7. 41%), (+) Spathulenol (6.94%), *α*- Copaenea (4.18%) and Sclareoloxide (4. 02%) were the main constituents, among the 56 characterized comprising 98.26% of the total components detected in the oil of *S. sclerea*. Also, *S. reuterana* oil contained Germacrene D (11.17%), (11*trans*,13*cis*) -11813 -Labdadien-8-01 (7.2%), Neoisolongifolene (6.56%), *β*-Elemene (6.29%), Sclareoloxide (4.18%), *β*- Eudesmol (3.28%) and Hexyl n-valerate (3.61%) among the 73 constituents characterized from 82.78% of the total components detected. In *S. oligophylla*, Occidentotal (24%), iso-longifolene (9.86%), *β*- Acoradiene (5.58%), Manool (3.99%), Hexadecanoate Methyl (3.88%), *β*-chamigrene (2.81%) and Lyral (2.4%) were the main constituents among the 72 characterized comprising 93.50% of the total components. And in *S. macrosiphon*, 67 constituents representing 97.28% of the total components in the oil was characterized by Sclareol (8.60%), (+) Spathulenol (5.86%), (-)-Aristolenel (5.71%), *β*-Elemene (5.44%), Hexyl n-valerate (4.84%), Germacrene D (4.31%) and *β*-Eudesmol (3.88%). To evaluate whether the identified constituents may be useful in reflecting taxonomic relationships in these *Salvia* species or not, cluster analysis was performed with Average Linkage. As demonstrated in Figure 1, three main clusters were detected. *S. reuterana* and *S. macrosiphon* are well segregated from others at the linkage distance 21. Other species divided into two sub clusters at the linkage 15. *S. atropatana* is the first sub cluster and second one containing three other species. *S. sclarea* and *S. aethiopis* near togather at the linkage 1 and *S. oligophylla* is separate from them at the linkage 14.

DISCUSSION

Like many other studies of volatile compounds of *Salvia* species (Torres et al., 1997; Viljoen et al., 2006; Sajjadi and Ghannadi, 2005; Bigdeli et al., 2005; Gulluce et al., 2006; Habibi et al., 2004; Lari Yazdi et al. 2005), the chemical composition in the taxa varied with regard to predominance of sesquiterpens and monoterpene such as Occidental and Germacerne D, respectively. Also, the

Table 2. Chemical composition (%) of the essential oils of six *Salvia* species.

Component	*S. aethiopis* (%)	*S. atropatana* (%)	*S. macrosiphon* (%)	*S. oligophylla* (%)	*S. reuterana* (%)	*S. sclarea* (%)
α-Cubebene	1.5	13	-	0.4	1.3	0.6
α-Copanaene	16.6	-	0.9	-	-	-
cis-isoeugenol	9.2	-	-	-	-	-
trans-Caryophyllene	12	6.1	-	1.3	-	7.6
β-Cubebene	1.1	-	0.3	-	0.6	-
α-Humulene	4.7	-	0.6	-	-	-
Germacrene D	16.5	5.1	4.3	-	11.2	12.7
Valencene	1.5	-	1.4	-	0.9	-
Germacrene A	1	-	-	-	-	-
α-Amorphene	1.5	-	0.7	-	-	-
δ-Cadinene	8.2	2.1	0.7	-	0.6	1.6
β-Calacorene	0.6	-	-	-	-	-
Caryophyllene oxide	5	19.3	-	-	1.2	0.4
β-Copaen-4-*α*-ol	0.6	-	-	1	-	-
Salvial-4(14)-en-1-one	1	1.8	0.7	-	1.1	0.6
Longipinocarvone	0.4	-	-	-	-	0.3
Cadalene	0.5	-	-	-	-	0.2
α-Cadinol	0.8	-	-	-	-	-
t-Muurolol	1.2	-	-	-	-	1.5
Valeranone	0.5	-	-	-	-	-
Caryophyllenol acetate	0.6	2.2	-	-	-	-
(+)-Oplopanone	1.4	-	-	-	-	-
12-Norcyercene-B	0.5	1.1	-	1.1	-	0.5
(*trans*)-Nerolidolol acetate	0.3	-	-	-	-	-
2-Pentadecanone	0.3	1.3	-	-	-	-
Sclareoloxide	0.5	-	-	-	4.1	4
Hexadecanoid acid	0.7	1.3	-	0.4	-	0.3
Linalool	-	0.3	-	-	-	-
α-Terpinol	-	0.4	-	-	-	-
β-Bourbonene	-	0.6	-	-	1.3	-
9,10-Dehydro-Isolongifolene	-	0.9	-	-	1	-
Germacrene D-ol	-	0.3	-	-	-	-
γ-Gurjunene	-	0.5	-	-	-	-
α-Cubebene	1.5	13	-	0.4	1.3	0.6
α-Copanaene	16.6	-	0.9	-	-	-
cis-isoeugenol	9.2	-	-	-	-	-
trans-Caryophyllene	12	6.1	-	1.3	-	7.6
β-Cubebene	1.1	-	0.3	-	0.6	-
α-Humulene	4.7	-	0.6	-	-	-
Germacrene D	16.5	5.1	4.3	-	11.2	12.7
Valencene	1.5	-	1.4	-	0.9	-
Germacrene A	1	-	-	-	-	-
α-Amorphene	1.5	-	0.7	-	-	-
δ-Cadinene	8.2	2.1	0.7	-	0.6	1.6
β-Calacorene	0.6	-	-	-	-	-
Caryophyllene oxide	5	19.3	-	-	1.2	0.4
β-Copaen-4-*α*-ol	0.6	-	-	1	-	-

Table 2. Contd.

Salvial-4(14)-en-1-one	1	1.8	0.7	-	1.1	0.6
Longipinocarvone	0.4	-	-	-	-	0.3
Cadalene	0.5	-	-	-	-	0.2
α-Cadinol	0.8	-	-	-	-	-
t-Muurolol	1.2	-	-	-	-	1.5
Valeranone	0.5	-	-	-	-	-
Caryophyllenol acetate	0.6	2.2	-	-	-	-
(+)-Oplopanone	1.4	-	-	-	-	-
12-Norcyercene-B	0.5	1.1	-	1.1	-	0.5
(*trans*)-Nerolidolol acetate	0.3	-	-	-	-	-
2-Pentadecanone	0.3	1.3	-	-	-	-
Sclareoloxide	0.5	-	-	-	4.1	4
Hexadecanoid acid	0.7	1.3	-	0.4	-	0.3
Linalool	-	0.3	-	-	-	-
α-Terpinol	-	0.4	-	-	-	-
β-Bourbonene	-	0.6	-	-	1.3	-
9,10-Dehydro-Isolongifolene	-	0.9	-	-	1	-
Germacrene D-ol	-	0.3	-	-	-	-
γ-Gurjunene	-	0.5	-	-	-	-
(-)-Isoledene	-	0.5	-	-	-	-
α-Humulene	-	0.5	-	-	-	-
(+)-*β*-Guaiene	-	0.5	-	-	-	-
β-Ionone	-	0.6	-	-	-	-
Bicyclogermacrene	-	1.3	1.8	-	-	-
trans, trans-*α*-Farnesene	-	1.5	-	-	-	-
α-Calacorene	-	0.9	0.4	-	-	-
Caryophyllene alcohol	-	1.1	-	-	-	-
Vulgarol B	-	0.5	-	-	-	0.3
1,5-epoxysalvial-4(14)-ene	-	1.2	-	-	-	0.6
Alloarmadendrene	-	0.4	0.4	-	-	-
Isoaroma dendrene epoxide	-	0.4	-	-	0.7	0.5
Hexadecane	-	1.1	-	-	-	-
cis-*cis*-*α*-Bisabolene epoxide	-	1.1	-	-	-	-
Alloarmadendrene oxide	-	0.8	0.4	-	0.9	-
Diepi-*α*-Cedrenepoxide	-	0.2	-	-	-	-
3-*iso*-Thujopsanone	-	0.4	-	-	-	-
Valerianol	-	0.9	0.8	-	-	-
(+)-*β*-Costol	-	0.5	-	-	-	0.2
Patchouli alcohol	-	1.1	-	-	-	-
Ledenoxide	-	0.9	-	-	-	-
Ethyl citrate	-	0.7	-	-	-	-
Diepicedrene	-	0.7	-	-	-	-
14-Hydroxy-*α*-Humulene	-	1.8	-	-	-	-
Benzyl benzoate	-	0.5	-	-	-	-
14-oxy-*α*-Muurolene	-	0.6	-	-	-	-
Cedryl acetate	-	0.2	-	-	-	-
1-Octadecene	-	0.3	1	-	-	-
8-Cedren-13-ol acetate	-	0.5	-	-	-	-
Ambrosin	-	0.6	0.8	-	-	-

Table 2. Contd.

Longifolol acetate	-	3.3	-	-	-	-
cis-Sclareoloxide	-	0.6	-	-	-	-
1-Heptadecene	-	0.2	-	-	-	-
Sandracopimararadiene	-	0.7	-	-	-	-
1-Nonadecene	-	0.3	-	-	-	-
Palmitaldehyde, diallyl acetal	-	0.4	-	-	-	-
8,3-epoxylabd-14-en-2a-ol	-	1.4	-	-	-	-
Sclareol	-	0.3	8.6	-	-	5.3
Pentacosane	-	0.3	-	-	-	-
Acetic acid hexyl ester	-	-	0.7	-	1.4	-
Butyl 2-methyl butanoate	-	-	0.2	-	0.3	-
3,7-Dimethyl 1,3,6-Octatriene	-	-	1.5	-	-	-
1-Octanol	-	-	1.2	-	0.8	-
Nonanal	-	-	1.2	-	0.4	-
2-Methyl propanoic acid hexyl ester	-	-	1.3	-	-	-
Acetic acid octyl ester	-	-	0.7	-	0.8	-
Hexyl n-Valerate	-	-	4.8	-	3.6	-
α-Terpinene	-	-	2.6	-	-	-
(+)-Calarene	-	-	0.6	-	-	1.3
β-Elemene	-	-	5.4	-	-	-
(-)-Aristolene	-	-	5.7	-	-	-
γ-Elemene	-	-	0.2	-	-	-
Gymnomitrene	-	-	0.7	-	-	-
trans-*β*-Farnesene	-	-	0.5	-	-	-
β-Selinene	-	-	2.2	-	-	-
Cadinene	-	-	1.8	-	1.8	-
Aromadendrene	-	-	1.1	-	0.8	-
α-Farnesene	-	-	0.6	-	0.4	-
Germacrene B	-	-	0.2	-	-	-
β-Damascone	-	-	0.4	-	0.3	-
Longifolenaldehyde	-	-	0.2	-	-	-
α-Selinene	-	-	0.7	-	0.4	-
(+) Spathulenol	-	-	5.8	-	-	6.9
Ledene	-	-	1	-	0.7	-
Ledene oxide	-	-	0.4	-	-	-
α-Gurjunene	-	-	0.6	-	0.7	-
cis-*cis*-*α*-Bisabolene	-	-	1.9	-	-	-
Valencene	-	-	-	-	-	-
β-Eudesmol	-	-	3.9	-	3.2	1.1
Isospathulenol	-	-	0.4	-	0.4	-
Aromadendrene oxide	-	-	0.4	-	-	-
Isobutamben	-	-	1.4	-	-	-
(+)-Oxo-*α*-Ylangene	-	-	0.2	-	-	-
Heptadecane	-	-	0.2	-	-	-
α-Cedrene oxide	-	-	0.3	-	-	-
9-Aristolen-1-*α*-ol	-	-	0.4	-	-	-
Oplopenone	-	-	0.8	-	-	-
4,5,*α*,*α*-Eudesmane	-	-	0.3	-	-	-
Tetradecanoic acid	-	-	0.3	-	-	-

Table 2. Contd.

Isoledene	-	-	0.3	-	-	-
9-Hydroxy-Isolongifolene	-	-	0.2	-	-	-
7,8-Epoxy-*α*-Ionone	-	-	0.7	-	-	-
trans-cis-α-Bergamotol	-	-	0.3	-	-	-
trans-β-Farnesene	-	-	0.6	-	-	-
β-Sesquiphellandrene	-	-	0.5	-	0.4	-
n-Hexadecanoic acid	-	-	0.3	-	-	-
Palmitic acid	-	-	1.6	-	-	1.4
Neryl Linalool Isomer	-	-	0.4	-	-	-
β-Patchoulene	-	-	0.8	-	-	-
Cedren-13-ol	-	-	1	-	-	-
Isolongifolol	-	-	0.9	-	-	-
β-*Iso*-Methyl Ionone	-	-	0.3	-	-	-
Diethylstilbestrol	-	-	0.8	-	-	-
Ethyl linoleate	-	-	0.3	-	-	-
2-*β*-Pinene	-	-	-	-	1.2	-
cis Ocimene	-	-	-	-	0.2	0.3
1,3,6-Octatriene, 3,7-Dimethyl	-	-	-	-	2.4	-
3-Methyl butanoic acid	-	-	-	-	0.3	-
2-Methyl propanoic acid	-	-	-	-	1.3	-
α-Terpineol	-	-	-	-	0.2	3.3
Hexyl 2-Methyl Butanoate	-	-	-	-	2.4	-
δ-Elemene	-	-	-	-	2.5	-
β-Elemene	-	-	-	-	6.3	-
Benzene butanal	-	-	-	-	0.3	-
Neoisolongifolene	-	-	-	-	6.5	-
Calarene	-	-	-	-	0.2	-
1-Butanol 3-Methyl Benzoate	-	-	-	-	0.3	-
α-Muurolene	-	-	-	-	0.4	-
Copaene	-	-	-	-	0.5	-
α-Amorphene	-	-	-	-	0.4	0.7
α-Saliene	-	-	-	-	0.7	-
Epi ligulyl oxide	-	-	-	-	0.7	-
(+)-*β*-Guaiene	-	-	-	-	0.3	-
Widdrene	-	-	-	-	0.2	-
2,6-Dimethoxytoluene	-	-	-	-	0.7	-
Cadina-1(10),6,8-triene	-	-	-	-	0.4	-
Benzoic acid hexyl ester	-	-	-	-	1.3	-
Tetradecanal	-	-	-	-	0.4	-
(-)-Spathulenol	-	-	-	-	1.6	-
α-Eudesmol	-	-	-	-	1	-
Epizonaren	-	-	-	-	0.7	-
9-Cedranone	-	-	-	-	0.3	-
Dihydro neoclovene	-	-	-	-	0.6	-
1,3,5,6-tetramethyladamantane	-	-	-	-	1.1	-
Isolongifolen-5-one	-	-	-	-	0.2	-
Caryophylla-3,8(13)dien-5-α-ol	-	-	-	-	0.3	-
Valerenol	-	-	-	-	0.4	-
Citronella	-	-	-	-	0.3	-

Table 2. Contd.

Benzenpropanoic acid methyl ester	-	-	-	-	0.3	-
6,10,14-trimethyl2-Pentadecanone	-	-	-	-	0.3	-
α-Bisabolol	-	-	-	-	0.5	-
4-Ethyl1,2-Dimethyl Benzene	-	-	-	-	1.1	-
Amorphane	-	-	-	-	0.5	-
(-)-α-Selinene	-	-	-	-	0.4	-
α-Longipinene	-	-	-	3.4	0.3	-
(11trans,13cis)-11813-Labdadien-8-ol	-	-	-	-	7.2	-
β-Myrcene	-	-	-	-	-	0.7
dl-Limonene	-	-	-	-	-	0.2
δ-3- Carene	-	-	-	-	-	0.3
α-Terpinolene	-	-	-	-	-	0.2
Linalool L	-	-	-	-	-	7.4
Neral	-	-	-	-	-	0.6
Linalyl acetate	-	-	-	-	-	6.8
Bicycloelemene	-	-	-	-	-	1.1
Neryl acetate	-	-	-	0.5	-	0.9
α-Copaene	-	-	-	-	-	4.2
Geranyl acetate	-	-	-	-	-	2.6
α- Caryophyllene	-	-	-	-	-	0.9
Bicyclogermacrene	-	-	-	-	-	3.4
12-nor-Caryophyll-5-en-2-on	-	-	-	-	-	0.2
Calacorene	-	-	-	-	-	0.3
1,4-Methanoazulen-7-one	-	-	-	-	-	0.3
o-Menth-8-ene	-	-	-	-	-	0.5
α-Copaene-8-ol	-	-	-	-	-	0.6
1-Bromoadamantane	-	-	-	-	-	0.7
3-1-Adamanthylsydnone	-	-	-	-	-	0.4
14-hydroxy-epi(trans)-Caryophyllene	-	-	-	-	-	0.3
β-Vetivenene	-	-	-	-	-	0.3
Cyercene I	-	-	-	-	-	0.7
trans-Longipinocarveol	-	-	-	-	-	0.4
Farnesol 2	-	-	-	-	-	0.6
Estrone	-	-	-	-	-	1.2
Neopentylidenecyclohexane	-	-	-	-	-	0.7
Neocembrene	-	-	-	-	-	0.3
γ-cis-Sesquicyclogeraniol	-	-	-	-	-	0.4
cis-Farnesol	-	-	-	-	-	0.4
Farnesol A	-	-	-	-	-	0.2
Tricyclene	-	-	-	0.3	-	-
Camphene	-	-	-	0.7	-	-
Terpinolene	-	-	-	0.6	-	-
Camphenol	-	-	-	0.3	-	-
1,3,8- *ρ*-Menthatriene	-	-	-	0.4	-	-
cis-Limonene oxide	-	-	-	0.4	-	-
Camphor	-	-	-	0.3	-	-
ρ-Menth-3-en-8-ol	-	-	-	1.4	-	-
Lavandulol	-	-	-	0.5	-	-
Terpin-4-ol	-	-	-	0.6	-	-

Table 2. Contd.

Thuj-3-en-10-al	-	-	-	0.3	-	-
cis-Pinocarveol	-	-	-	0.4	-	-
trans-Dihydro Cavone	-	-	-	0.3	-	-
neo-3-Thujyl acetate	-	-	-	1.2	-	-
neo-iso-3-Thujyl acetate	-	-	-	0.9	-	-
iso-Dihydro Carveol Acetate	-	-	-	0.6	-	-
Cyclosativene	-	-	-	-	-	-
β-Pathcholene	-	-	-	0.4	-	-
iso-Longifolene	-	-	-	9.9	-	-
α-Funebrene	-	-	-	0.2	-	-
Longifolene	-	-	-	0.2	-	-
α-Santalene	-	-	-	0.7	-	-
Bakerol	-	-	-	0.3	-	-
α-Pathcholene	-	-	-	1.3	-	-
β-Acoradiene	-	-	-	5.6	-	-
γ-Gurjunene	-	-	-	0.7	-	-
β-Chamigrene	-	-	-	2.8	-	-
β-Dihydro agarofuran	-	-	-	0.3	-	-
Butylated hydroxytoluene	-	-	-	0.6	-	-
6,4-*oxido*-Acor-4-ene	-	-	-	0.3	-	-
8,14-Cedranoxide	-	-	-	0.3	-	-
α-Agarofuran	-	-	-	1	-	-
Occidentalol	-	-	-	24	-	-
Longiborneol	-	-	-	0.4	-	-
cis- *β*-Elemenone	-	-	-	0.9	-	-
cis-Isolongifolanone	-	-	-	0.5	-	-
β-Biotol	-	-	-	0.2	-	-
5-Cedranone	-	-	-	0.2	-	-
1-epi-Cubenol	-	-	-	0.3	-	-
α-Acorenol	-	-	-	0.9	-	-
β- Acorenol	-	-	-	0.4	-	-
(cis)-Methyl Jasmonate	-	-	-	0.3	-	-
Desmethoxy Encecalin	-	-	-	0.2	-	-
Vulgarone B	-	-	-	1	-	-
Isoelemicin	-	-	-	0.3	-	-
3-Thujopsanone	-	-	-	0.2	-	-
Lyral	-	-	-	2.4	-	-
Khusinol	-	-	-	0.7	-	-
Occidentalol acetate	-	-	-	0.3	-	-
(cis)-α-Santalol	-	-	-	0.2	-	-
Guaiac acetate	-	-	-	0.2	-	-
- ol9)-en—15(8Cedr-	-	-	-	0.2	-	-
α- Bisabolo oxide	-	-	-	0.2	-	-
Cedryl menthyl ketone	-	-	-	0.2	-	-
Bicyclo vetivenol	-	-	-	0.2	-	-
Santalol acetate	-	-	-	0.4	-	-
O-Methyl α iptzolP	-	-	-	1.4	-	-
Methyl hexadecanoate	-	-	-	3.7	-	-
-Isopimaradiene15	-	-	-	0.2	-	-
Dolabradiene	-	-	-	0.4	-	-
Manoyl oxide	-	-	-	0.2	-	-
Manool	-	-	-	4	-	-

Table 2. Contd.

-one 2Laurenan-	-	-	-	0.5	-	-
Incensole acetate	-	-	-	0.2	-	-
Abietol	-	-	-	0.3	-	-
n-Tetracosane	-	-	-	0.3	-	-
Total	88.7	90.3	86.9	87.7	92.4	90.2
KI= Kovats Index						

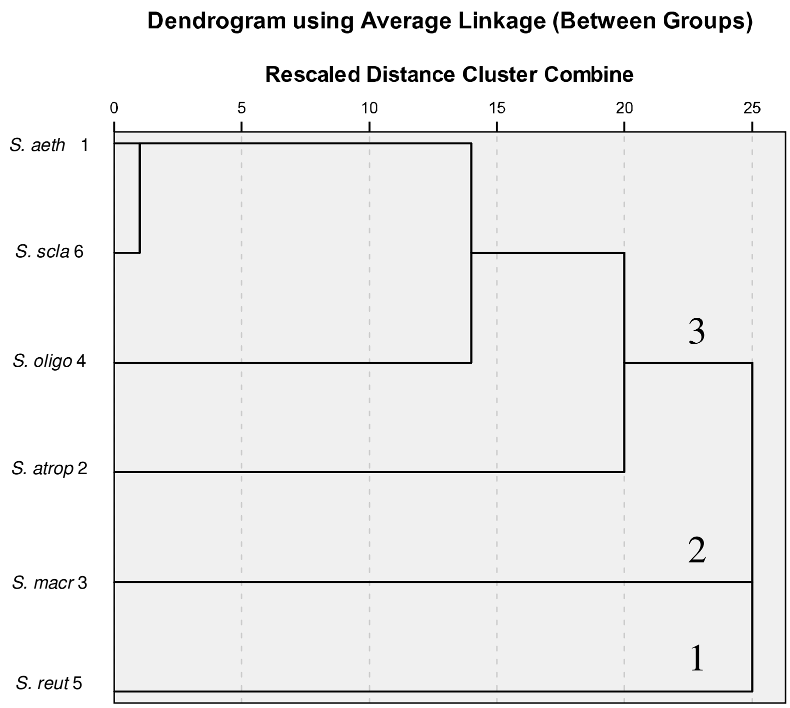

Figure 1. Cluster analysis of six *Salvia* species resulted from analysis of chemical components data using average linkage (Abbreviation: *S. aethiopis*= S. *aeth*, *S. atropatana* = *S. atrop*, *S. macrosiphon* = *S. macr*, *S. oligophylla* = *S. oligo*, *S. sclarea* = *S. scla*, *S. reuterana* = *S. reut*).

chemical data results presented here, was comparised with systematic classification of these six *Salvia* species. In the Flora Iranica, *Salvia* genus is divided into five Grex. *S. atropatana, S. oligophylla, S. aethiopis S. sclarea* are placed in Grex E and *S. reuterana, S. macrosiphon* are placed in Grex D. As demoestrated in Table 1, α-Cubebene, *trans*-Caryophyllene, 12- Norcyercene-B and Hexadecanoid acid have obvious taxonomic significance in Grex E because these compounds are not found in Grex D. In Grex E, Leaf characters, hair types and nutlet shape in *S. sclarea* appears more similar to *S. aethiopis*. Also these two species display the stamina character of total fusion of the posterior thecae into what Claβen-Bockhoff et al. (2004), described as "stamen type v". This morphology creates the classic *Salvia* lever mechanism, where the pollinator is forced to push against the fused posterior thecal tissue. Cluster analysis based on chemical composition shows that these two species are nearly together at the linkage 1 (Figure 1). They have similar composition especially in the main components, so our results supported this classification. The ordination of these two taxa based on first two principal components support this idea (Figure 2). Another purpose of the study was to examine the differences between S. *aethiopis* and *S. oligophylla*. As demonstrated in Figure 1, *S. aethiopis* and *S. oligophylla* are separated into two sub cluster. These two species have many similarities in leaf shape, trichomes of leaf and stem and color of corolla, but our data suggests that they have different chemical composition. The main compounds of *S. aethiopis* are α-Copanaene (16.6%), Germacrene D (16.5%) and Caryophyllene oxide (5%), but these compositions are not found in *S. oligophylla*. In this species, endemic species to Iran, Occidental is the major

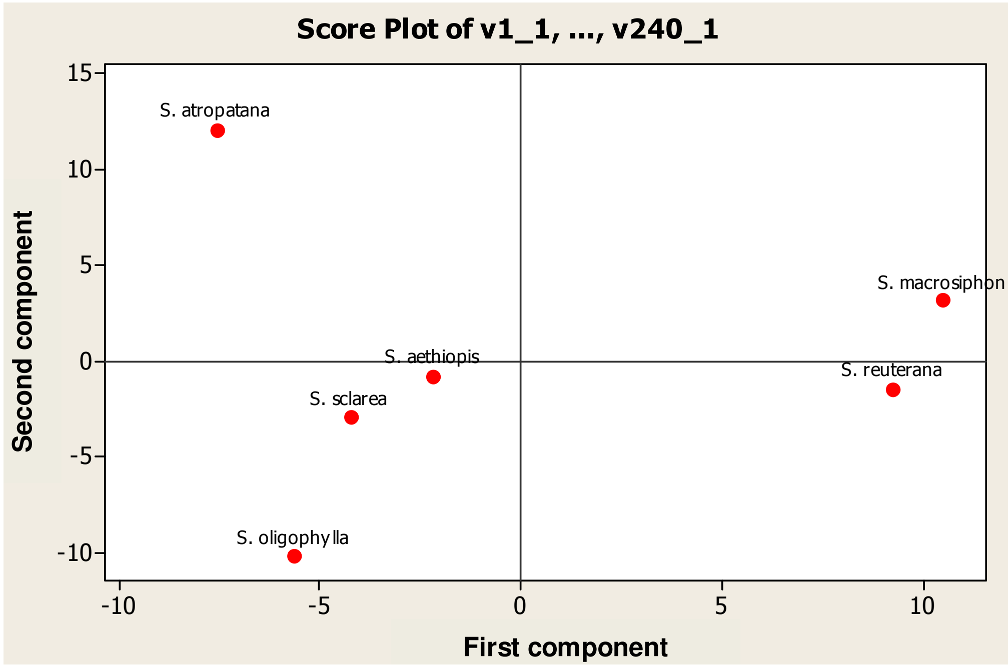

Figure 2. Ordination of six *Salvia* species based on first two principal components using essential oil data.

component of essential oil. To this end, the study of chemical composition confirms that they are two different species. On the other hand, the finding reported by Torres et al. (1997) and Gulluce et al.(2006) demonstrated that in *S. aethiopis*, the most abundant were *β*-Caryophyllene (24.6%) and bicyclogernacrene (41.5%) (Gulluce et al., 2006; Rustaiyan et al., 2000). Chalchat et al. (2001) reported *β*- Caryophyllene (27.5%) and Germacrene D (6.4%) as the main components of *S. aethipois* (Lari et al., 2005). Our study showed clear qualitative and quantitative differences. In our samples, *α*-Copanaene, Germacrene D and caryophyllene oxide were much greater than in other works. These results showed that variability in yield and chemical composition of the essential oils in *Salvia* species may be linked to the local environmental factors such as availability water and other unique climatic factors. Morphological comparison between *S. atropatana* and three other species in Grex E, shows that indumentum, petiole length, color of corolla, nutlet shape and densely white eglandular lanate in the stem of this species are an important diagnostic morphological characters that can separate it from closer species. Based on chemical composition (Figure 1), this species separates from other species in sub cluster 3 at the linkage 20. Also, Caryophyllene oxide (19.3%) and *α*-Cubebene (13%) are two new compounds isolated in this species. Mirza and Ahmadi, were found *β*-Caryophyllene, Sclareol and Hexyloctanoate to be the major constituents in this species (Mirza and Ahmadi, 2000). Anatomical observation on nutlets of some *Salvia* species showed that *S. aethiopis* and *S. sclarea* can be differentiated from *S. atropatana* based on thickness of pericarp layer and parenchymatous layers of colour (Nejad et al., 2007). To this end, our results highly support the classification of these species in Flore Iranica.. In Grex D, as it is evidence from cluster analysis, *S. macrosiphon* and *S. reuterana* species clearly warrants two separate groups. *S. macrosiphon* has been reported to be rich in *α*- Gurjuene, *β*- Cubebene and Germacrene B, but our results showed that the main components of this taxon is Schareol (8. 60%), (+) Spathulenol (5.86%), (-)- Aristolenel (5.71%), *β*-Elemene (5.44%), Hexyl n-valerate (4.84%), Germacrene D (4.31%) and *β*-Eudesmol (3.88%) and *α*- Gurjuene, *β*-Cubebene and Germacrene B are lower than previous works (Skatsa et al., 2001). Also the main compounds found in *S. reuterana*, were Germacren D and *β*-caryophyllene and of which, in general concurs with the findings of Esmaeili et al. (2008), in that, the oil Germacren D was the major constituent. However in this present study, because the most abundant components are Neoisolongifolene (6.56%), *β*-Elemene (6.29%), the above two *Salvia* species may exhibit outward similarities in morphology characters, but they have different key chemical composition and thus the type of oils can be utilized to separate the two species from each other. PCA results are highly congruent with chemical analysis (Figure 2). Our results suggest that chemical composition may be useful to identify a number of systematic quiries in regard to other *Salvia* species.

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