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Essential oil variation within and between *Stachys inflata* Benth. and *Stachys lavandulifolia* Vahl. populations from Iran

Seyed Mehdi Shahgolzari*, Afagh Yavari

Department of Biology, Payame Noor University, Tehran, Iran

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Key words: Essential oils, GC–MS, *Stachys inflata*, *Stachys lavandulifolia*.

Abstract

Although several species of *Stachys* possess characteristic essential oils, only a few published studies have described within and between populations of their volatile constituents. The aim of the present study was to evaluate the chemical composition of essential oils from two chemically unexplored *Stachys* species, *Stachys lavandulifolia* and *Stachys inflata*. Populations of these species were collected from Touyserkan, Hamedan province west of Iran. The essential oils of the dried flowering aerial parts of two species were isolated by hydrodistillation and analysed by means of GC and GC–MS. In this survey, 12 populations of both species were studied. Then data obtained were analyzed by Pc-ord software. 64 oil components were identified. 6, 10, 14-trimethyl-2-pentadecanone, Dibutylphthalate, isobutyl phthalate, spathulenol, trans-caryophyllene and α-copaene was observed in all populations of two species. δ-3-carene, Mytenal and α-thujene only in *S. lavandulifolia* and E-Citral (Geranial), n-docosane, neryl acetate, Nerylphenylacetate, Z-citral (Neral) only in *S. inflata* was observed. No similarity was observed between the chemical profile of essential oils obtained from *S. lavandulifolia* and *S. inflata*. The results of the present study indicated that essential oil components of *S. lavandulifolia* and *S. inflata* could be varied within and between populations.

***Corresponding Author:** Seyed Mehdi Shahgolzari ✉ Mehdi.shahgolzari@gmail.com

Introduction

The genus *Stachys*, one of the largest genera of the Labiatae (Lamiaceae) family with around 300 taxa, is widely distributed across the world from tropical to subtropical regions (Piozzi and Bruno, 2009 &, 2011). The genus is geographically wide spread and found mostly in the Mediterranean, South Western Asia, North and South America and South Africa. The native species are, however, absent in New Zealand and Australia (Bhattacharjee, 1980). *Stachys lavandulifolia* Vahl. and *Stachys inflata* Benth. type of Stachys genus (Lamiaceae family) has been widely studied based on its essential oil and therapeutic characteristics over the recent decades. In Previous studies on *S. lavandulifolia* (Ghannadi and Alemdoost, 1996; Alamdoost, 1996; Feizbaksh *et al*., 2003; Javidnia *et al*., 2004; Hajhashemi *et al*., 2006; Meshkatalsadat *et al*., 2007; Amiri *et al*., 2009; Asadi *et al*., 2010; Nadaf *et al*., 2011; Sadrmomtaz *et al*., 2011; Pirbalouti *et al*., 2011; Jafarzadeh *et al*., 2012; Soleimani- Meimand *et al*., 2013; Aghaei *et al*., 2013; Pirbalouti and Mohammadi, 2013; Meimand *et al*., 2013; Tundis *et al*., 2015) and *S. inflata* (Garjani *et al*., 2004; Sajjadi and Somae, 2004; Norouzi-Arasi *et al*., 2005; Ebrahimabadi *et al*., 2009; Nabizahedasl *et al*., 2010; Rustaiyan *et al*., 2011; Omidbaigi *et al*., 2013; Yavari and Shahgolzari, 2013; Shahbazi *et al*., 2014; Talebi *et al*., 2014) different compounds of oil were reported. The differences in qualitative and quantitative compositions of the essential oils were found on both specific and population levels. According to existing references and information, individuals of this species are present in many stations with different ecological conditions. We are becoming increasingly aware that an individual cannot be considered out of the context of its environment. With regard to the wide distribution of *S. lavandulifolia* and *S. inflata* in Iran we wanted to know if there is essential oil diversity within and between populations of these species in studied area.

Material and methods

Location

12 populations of wild *S. lavandulifolia* and *S. inflata* were collected in different habitats from Touyserkan, Hamedan province west of Iran (May 2013).

Plant material and essential oil preparation

The aerial parts of different populations *S. lavandulifolia* and *S. inflata* were collected at full flowering stage. A voucher specimen (code numbers of populations 1001-1012) of population was deposited at the Herbarium of Biology Department, Payame Noor University, Touyserkan, Hamedan, Iran. The air-dried materials were finely ground, then subjected to hydro-distillation for 3 hours with water as solvent, using a Clevenger apparatus according to the standard procedures. The oils were stored at 4 ^{o}C for further analyses.

Gas chromatography–mass spectrometry (GC–MS)

Volatile components were identified by GC-MS using a Finnigan TRACE GC-MS (Thermo Quest Finnigan Co., USA) equipped with a DB-1 capillary column (60 m × 0.25 mm × 25µm). Helium (flow rate, 1.1 ml/min) was used as the carrier gas, and injection volumes were 0.2 µl. The oven temperature was raised from 60 ^{o}C to 250 ^{o}C at a rate of 5 ^{o}C /min, held at 250 ^{o}C for 5 min; transfer line temperature was 250 ^{o}C. Split ratio was 100. The quadruple mass spectrometer was scanned over the 40-460 amu with an ionizing voltage of 70 eV and the injector temperatures were kept at 250 ^{o}C. The constituents of the oil were identified by calculation of their retention indices under programmed temperature conditions for n-alkanes (C8-C24) and the oil on a DB-1 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature (Adams, 2001).

Chemical variability

Analytical data for cluster analysis were treated by means of the Pcord-4 with group linkage method (nearest neighbor).

Results and discussion

The essential oil compositions of the *Stachys* genus have been well documented in the literature, but not for all the species have been described in detail. The main components of the essential oil of the species were observed to be germacrene D, caryophyllenes, cadinene, spathuleneol and caryophyllene. The moderate antibacterial activity of ß-caryophyllene and germacrene D were reported. Germacrenes were produced as antimicrobial and insecticidal agents from *Stachys* species (Omura *et al*., 2006; Goren *et al*., 2011). α- pinene, ß-caryophyllene, linalool oxide and caryophyllene oxide were tested against several bacterial strains.

Table 1. Essential oil composition of *S. lavandulifolia* and *S. inflata* populations from studied area (%).

Compounds		Populations of *S.lavandulifolia*							Population of *S.inflata*						
		Khangormaz		Serkan		Sarabi			Khangormaz		Serkan		Sarabi		
	IR	P1	P2	P3	P4	P5	P6	Average	P7	P8	P9	P10	P11	P12	Average
1,8-Cineol	1038	1.47	1.57	0.23	0.62	0.45	0.4	0.79	0	0	0	0.2	0.2	5	0.9
4a-α,7-α,7a-α-Nepetalactone	1365	0	0.06	0.07	0.09	0.03	0	0.04	0	0.06	0	0.2	0.1	0	0.07
4-Terpineol	1186	0.12	0.03	0	0.05	0	0	0.04	0	0	0	0	0	0.6	0.11
6,10,14-trimethyl-2-pentadecanone	1848	1.05	0.26	2	1.87	0.47	1.4	1.17	0.54	1.34	5	1.3	0.9	0.3	1.6
6,9-Guaiadiene	1454	0	0.06	0.04	0.13	0	0.1	0.06	0	0	0	0.2	0.1	0	0.09
ar-Curcumine	1492	0	0.03	0.13	0.02	0.02	0.2	0.07	1.3	0.08	0	0.5	0.1	6.1	1.34
bicyclo Elemene	1350	0	0.43	0.02	0.38	0.26	0.1	0.2	0	0	0	0.2	0.6	0	0.19
bicyclogermacrene	1517	0	13.17	0.4	1.96	6.84	0.8	3.87	0	0.05	5	6.9	18	0	4.94
Caryophyllene oxide	1608	16.4	3.3	16.3	4.26	5.19	0	7.57	0	5.84	18	4.3	2.6	6.1	6.18
Cembrene	1964	0	0.25	0.18	0.37	0.74	0.8	0.38	0	0.7	0	0.4	0.1	0	0.27
cis-sabinene hydrate	1072	0.09	0.51	0.01	0.05	0.02	0	0.12	0	0	0	0	0.1	0.1	0.03
cis-sesquisabinene hydrate	1565	0	0.04	0.14	0.21	0.11	0.2	0.12	0	0	0	0	0.1	0	0.02
cis-ß-bisabolene	1551	0	0.27	0	0.09	0.17	0	0.1	0	0.05	0	0.2	0.2	0	0.1
cryptone	1194	0.04	0	0	0	0	0.2	0.03	0	0	0	0.2	0.1	0	0.05
cumin aldehyde	1248	0.32	0.27	0.11	0.39	0.06	0.2	0.23	0	0	0	0.5	0.2	0	0.16
δ-3-carene	1017	0	0.63	0	0.07	0	0	0.12	0	0	0	0	0.1	0	0
Dibutylphthalate	1969	4.72	1.45	1.02	0.8	4.1	2.1	2.36	15.1	34.66	5	2.6	5.9	2.5	10.9
E-Citral(Geranial)	1272	0	0	0	0	0	0	0	3.95	0	0	0	0	26	5.07
E-Nerolidol	1569	0	0.42	1.44	1.18	0.72	0.8	0.75	0.18	0.08	0	0	0.2	0.7	0.2
E-ß-Farnesene	1460	0	2.3	1.56	4.76	3.67	2.2	2.42	0	0.22	1	0.3	0.9	0	0.33
E-ß-Ionone	1496	0	0.16	0.53	0.2	0.29	0.3	0.24	0	0.08	2	2.1	1	0	0.9
Germacrene D	1501	0.22	8.21	0.13	2.74	4.94	0	2.71	0.56	0.27	13	6.5	23	0.1	7.1
humulene epoxide	1633	0.13	0.22	0.7	0.17	0.15	0	0.24	0	0.25	0	0.7	0.3	0	0.22
Intermedeol	1684	1.63	0.08	1.17	0	0	0.7	0.6	0	0.91	4	0	0	0	0.88
isobutyl phthalate	1874	18.4	3.24	4.12	0.75	0.72	16	7.22	8.45	24.68	1	1.1	0.8	0.1	5.97
isospathulenol	1658	0	0.87	2.8	2.01	1.84	2.4	1.65	0	0.82	2	6	1.7	0	1.72
limonene	1035	0	14.31	0.13	0.31	2.36	0.6	2.95	1.15	0.24	0	0	2.4	5.2	1.5
linalool	1100	0.77	0.09	0.12	0.22	0.06	0	0.22	0	0	0	0	0.1	0	0.02
Mytenal	1205	0.16	0.18	0.04	0.21	0.11	0	0.12	0	0	0	0	0	0	0
n-docosane	2204	0	0	0	0	0	0	0	0.25	0	0	0	0	0	0.05
neryl acetate	1381	0	0	0	0	0	0	0	0.48	0	0	0	0	0.9	0.23
Nerylphenylacetate	2013	0	0	0	0	0	0	0	6.64	0	0	0	0	0.2	1.13
n-tetradecane	1401	0	0.28	0.17	0.77	0.46	0.8	0.41	0	0.03	1	0.3	0.9	0	0.41
P-Cymene	1029	0	0.1	0	0.02	0	0.1	0.03	0	0	0	0	0.1	0.6	0.13
pirollene	1103	0	0.1	0.04	0.22	0.1	0.1	0.09	0	0	0	0	0	0.1	0.02
pristane	1709	0.63	0.22	1.16	0.72	0.99	0.6	0.72	0	1.91	1	1.3	0.7	0	0.85
sabinene	978	0	0.86	0	0.07	0.07	0	0.17	0	0	0	0	0.2	0.3	0.08
Sesquisabinene	1466	1.78	0	0	0	0	0	0.3	0.19	0	0	0	0	0.4	0.1
spathulenol	1600	0.94	10.28	50.7	40	26.2	52	30	41.6	20.15	21	47	19	9.5	26.3
terpinolene	1049	0	0.93	0.09	1.03	0.55	0.1	0.45	0	0.09	1	0	0.1	0	0.17
trans- verbenol	1154	0.62	0.64	0.12	2.19	0.55	0.2	0.72	0	0.06	0	0.8	0.3	0	0.21
trans-caryophyllene	1441	14.4	14.58	7.98	17.7	25	7.8	14.6	4.15	2.15	7	0.9	3.6	4	3.59
trans-pinocarveol	1150	0	0.16	0.31	0.14	0.25	0.1	0.16	0	0	0	0	0.2	0	0.05
trans-α-bergamotene	1447	0.3	0.47	0.42	1.41	0.93	0.4	0.65	0	0.07	0	1.1	0.6	0.1	0.39
Viridiflorol	1617	0	0.3	0.68	0.41	0.48	0.9	0.47	0	1.03	3	4.4	1.4	0	1.64
Z-citral (Neral)	1244	0	0	0	0	0	0	0	2.86	0	0	0	0	20	3.78
α-bisabolol	1696	0	0.03	0.07	0.02	0.1	0.1	0.05	0	0.21	0	0	0	0	0.04
α-Cadinol	1674	0	0.17	1	0.67	0.24	0	0.35	1.6	0.69	0	4.5	1	1.7	1.56
α-campholenal	1132	0.39	0.43	0.1	0.91	0.46	0.2	0.42	0	0	0	0	0.2	0	0.05

α-copaene	1392	0.38	1.23	1.14	2.1	0.76	2.4	1.34	0.23	0.07	2	1	1.4	1.1	0.88
α-humulene	1474	0.38	0.14	0.09	0.37	0.39	0.2	0.26	0	0	0	0.8	0.8	0	0.34
α-phellandrene	1010	0	0.28	0	0	0.03	0	0.06	0	0	0	0	0.1	0	0.01
α-pinene	940	0.29	1.5	0	0.1	0.07	0.1	0.35	0	0.01	0	0	3	0.2	0.53
α-Terpineol	1197	0.33	0.27	0.16	0.68	0.32	0.1	0.31	0	0.11	0	0	0	1.2	0.22
α-thujene	931	0	0.17	0	0.02	0	0	0.03	0	0	0	0	0	0	0
ß-bourbonene	1403	0	0.45	0.16	0.83	0.29	0.6	0.38	0.07	0.02	2	0.9	0.1	0	0.51
ß-elemene	1405	0.05	0.34	0.16	0.44	0.47	0.4	0.31	0	0.12	0	0	0.1	0	0.04
ß-Myrcene	991	0	9.39	0.06	0.91	2.49	0.1	2.15	0	0.04	0	0	0.3	0	0.06
ß-phellandrene	1036	0	0	0	2.21	1.93	0.4	0.75	0	0	0	0	1.4	0	0.25
ß-Pinene	985	0	1.06	0	0.14	0.31	0.2	0.28	0	0.01	0	0	2.5	0	0.43
γ-Terpinene	1063	0.11	0.24	0	0.1	0.07	0.1	0.11	0	0.04	0	0	0.2	0	0.03
δ-Cadinene	1538	0	2.2	0.21	1.49	1.4	1.1	1.07	0.74	0.4	2	2	2	0.5	1.27
Number of compounds	------	27	50	44	53	49	42	--------	19	29	19	31	49	26	------
Total oil	-----	66.1	99.23	98.2	99.5	98.2	98	93.3	90.1	97.54	98	99	99	94	96.2

The essential oil composition of *Stachys* species mainly consists of sesquiterpenes and oxygenated sesquiterpenes. Moreover, the monoterpenes such as α-pinene, ß-pinene, phellandrene and carvacrol were also extracted from *Stachys* species. *S. lavandulifolia* and *S. inflata* are medical plants that can be a potential source of monoterpenes and sesquiterpenes. According to previous studies, various components of essential oil on this both species have been reported. This shows that the volatile oil composition of those, in Iran is extremely variable (Naghibi *et al.*, 2005; Semnani *et al.*, 2006; Arasi *et al.*, 2006; Goudarzi *et al.*, 2011).

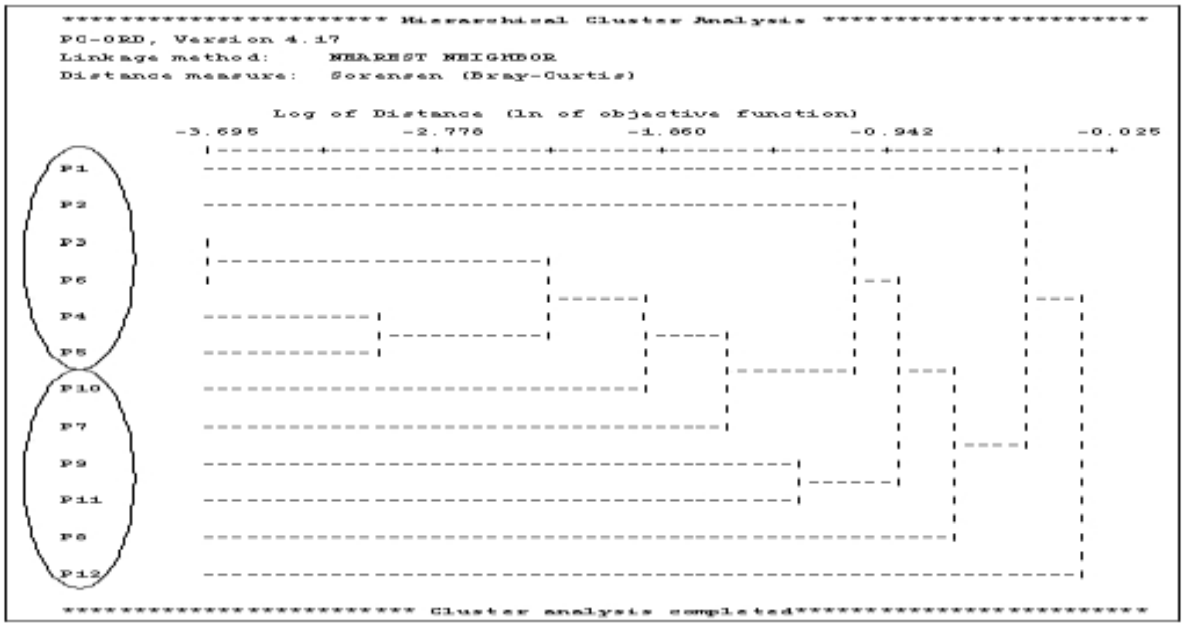

Fig. 1. Dendrogram representing similarity relationship between S. *lavandulifolia* and *S. inflata* species based on essential oil constituents.

The essential oil compositions of our samples for *S. lavandulifolia* and *S. inflata* are shown in Table 1. According to the GC mass analysis a total of 62 compounds were identified in *S. lavandulifolia* and *S. inflata* together. The dendrogram (Fig. 1) represents graphically the relationships between the populations of *S. lavandulifolia* and *S. inflata* and the groups, based on their essential oil composition (namely analysis of the two species together). 6, 10, 14-trimethyl-2-pentadecanone, Dibutylphthalate, isobutyl phthalate, spathulenol, trans-caryophyllene and α-copaene was observed in all populations of both species. Based on these results, the essential oil variation patterns in *Stachys* species exhibited more

diversity. The highest oil compounds in two *Stachys* species were Spathulenol. The highest percentage was recorded in population 3 (50.7%) and the lowest in population 1 (0.94%). To assess the use of essential oil constituents in identifying taxonomic relationships between species, multivariate analysis by nearest neighbor complete linkage cluster analysis were initially performed with oil constituent. Therefore, two groups of populations were identified. δ-3-carene, Mytenal and α-thujene only in *S. lavandulifolia* and E-Citral(Geranial), n-docosane, neryl acetate, Nerylphenylacetate, Z-citral (Neral) only in *S. inflata* was observed. No similarity was observed between the chemical profile of essential oils obtained from *S. lavandulifolia* and *S. inflata*. Figure 1 shows the separation between populations. On the other hand, the two species from *Stachys* were clustered in 2 groups, and were clearly different from the others.

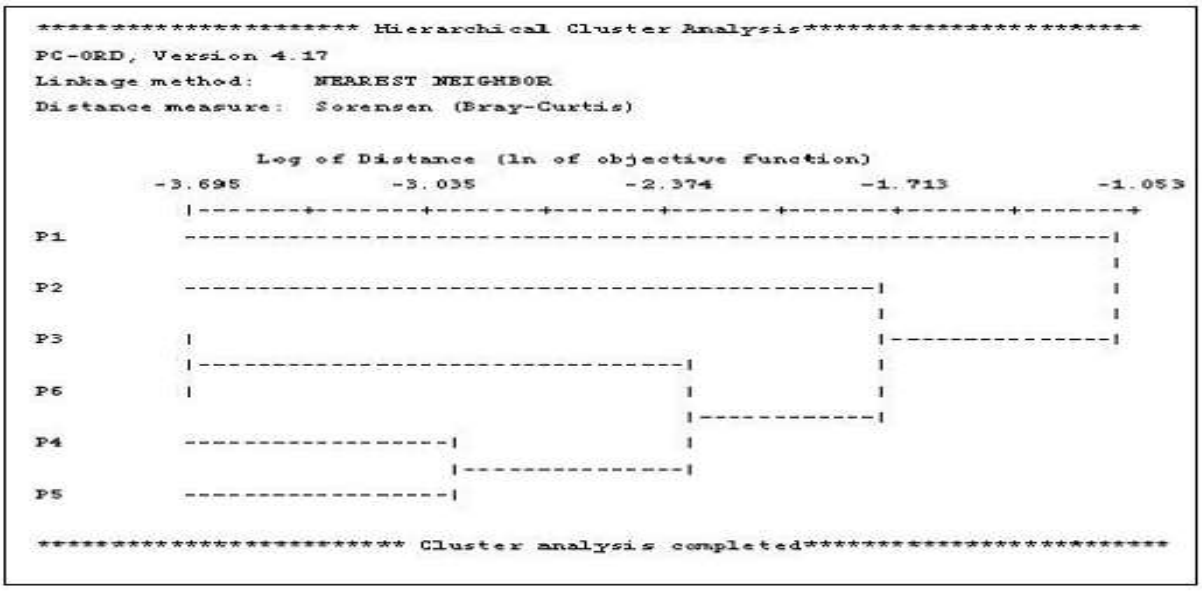

Fig. 2. Dendrogram representing similarity relationship between S. *lavandulifolia* populations based on essential oil constituents.

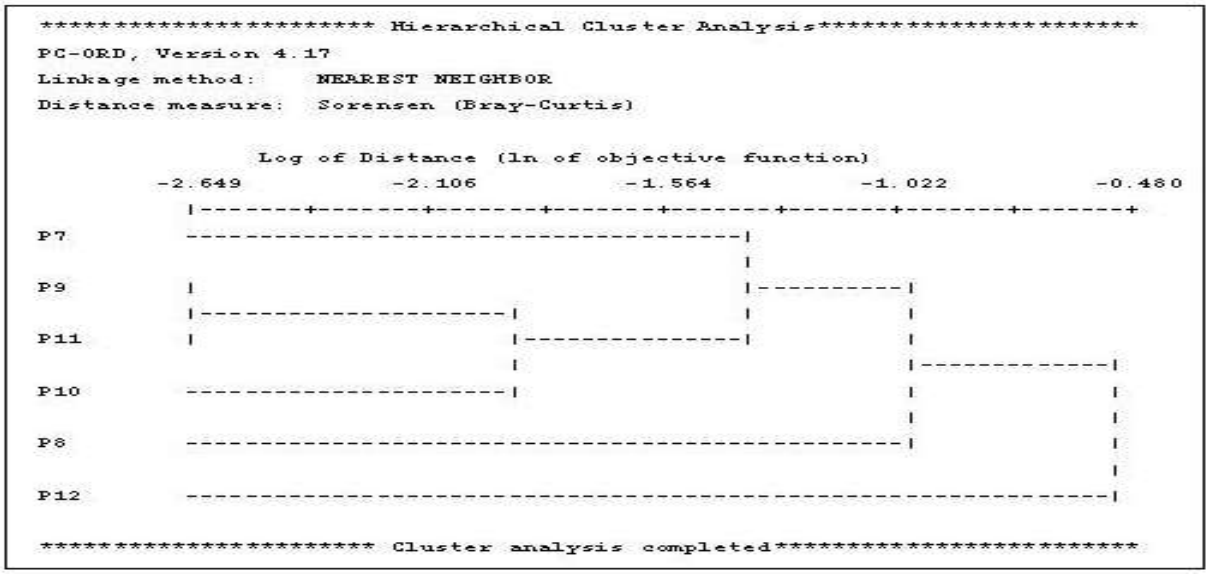

Fig. 3. Dendrogram representing similarity relationship between *S. inflata* populations based on essential oil constituents.

The dendrogram (Fig. 2, 3) represents graphically the relationships within the populations and the groups of both species, separately. The major components of *S. lavandulifolia* according to average essential oil in populations were bicyclogermacrene (3.87%), Caryophyllene oxide (7.57%) isobutyl phthalate (7.22%), spathulenol (30%) and trans-caryophyllene (14.6%). The highest total essential oil was recorded in populations 4 (99.5%) and the lowest ratio was obtained in populations 1 (66.1%).

The numbers of identified compounds in the essential oil of the 6 populations ranged from 27 to 53. Total percentages of identified compounds however, varied from 66.1% to 99.5%. The average value of the totally identified compounds was 93.3% (Table 1). The major components of *S. inflata* according to average essential oil in populations were Z-citral (Neral) (3.78%), bicyclogermacrene (4.94%), Caryophyllene oxide (6.18%) isobutyl phthalate (5.97%), spathulenol (26.3%), Dibutylphthalate (10.9) and trans-caryophyllene (3.59%).

The highest total essential oil was recorded in populations 10 and 11 (99%) and the lowest ratio was obtained in populations 7 (90.1%). The numbers of identified compounds in the essential oil of the 6 populations ranged from 19 to 49. Total percentages of identified compounds however, varied from 90.1% to 99%. The average value of the totally identified compounds was 96.2% (Table 1). Gören (2014) reports, α-pinene, b-myrcene, 4-hydroxy-4- methyl-2-pentanone, hexadecanoic acid for *S. lavandulifolia* and Hexadecanoic acid, germacrene D, α- pinene, bicyclogermacrene, Δ-3- carene, limonene, linalool, spathulenol for *S. inflata* as main compounds that is different from our surveys.

Conclusion

The secondary metabolites are of great value in identifying the relationships between plants and classification. Moreover, oil compounds have been used effectively for interpreting the taxonomic status within and between medicine plants. The diversity within and between the species was determined by using essential oil analysis could prove useful for the classification of two species in relation with locality of species. Oil constituents in the *Stachys* species show excessive diversity in Iran and these compounds strongly differentiated them. The application of single linkage clustering produced two large clusters within the population, it is therefore concluded that essential oil could be useful markers in population identification and classification. These results suggested that essential oils data could clearly separate different populations of two species from each other. Essential oil analyses of *S. lavandulifolia* and *S. inflata* species found in Touyserkan, Hamedan province west of Iran have revealed high chemical polymorphism in within and between populations, possibly related to edaphic, genetic and ecological influences.

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