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Identification of the volatile composition of *Stachys lavandulifolia* Vahl. and *Salvia spinosa* L. in Isfahan climatic conditions

Amin Hadipanah[1], Ahmad Reza Golparvar[2]*, Ali Mehras Mehrabi[3], Mehrdad Jafarpour[4]

[1]*Department of Horticultural, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2]*Department of Agronomy and plant Breeding, College of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran*

[3]*Department of Plant Breeding, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran*

[4]*Department of Horticulture, College of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran*

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Abstract

Stachys lavandulifolia Vahl. and *Salvia spinosa* L. are perennial shrub and aromatic plant belongs to Lamiaceae family. *S. lavandulifolia* and *S.spinosa* is an endemic species grown in Iran.The aim of this study was to identify of the chemical components of *S. lavandulifolia* and *S.spinosa* in Isfahan climatic conditions.The aerial parts of the plants were collected (Kamu mountain) Isfahan province, during 2014. The essential oil was extracted by a Clevenger approach and analyzed using GC/MS. In total, 17 and 21 compounds were identified in the essential oil from the aerial parts *S. lavandulifolia* and *S.spinosa*, respectively.The results obtained in our study indicated that the major components in the oil *S. lavandulifolia* were α-pinene (49.24%), β-pinene (22.52%), β-phellandrene (11.71%), α-copaene (6.70%) and β-myrcene (2.02%).The major components in the oil*S.spinosa* were α-terpinolene (32.73%), β-ocimene (30.91%), β-patchoulene (12.77%), β-bourbonene (4.26%) and 1,8-cineol(2.88%). The results of the present study indicated that essential oil components of *Stachys lavandulifolia* Vahl. and *Salvia spinosa* L. can be varied with genetic as well as environmental conditions.

***Corresponding Author:** Ahmad Reza Golparvar ✉ dragolparvar@gmail.com

Introduction

Stachys lavandulifolia Vahl. and *Salvia spinosa* L. are perennial shrub and aromatic plant belongs to Lamiaceae family. The genus Stachys consists of about 300 species, and is justifiably considered as one of the largest genera of Lamiaceae that widespread throughout the world (Mabberley, 2008). In Iran, 34 species of this genus are present, among which, 13 are endemic. *S. lavandulifolia* is a native plant, which is known as Chay-e-kohi in Persian and Betony in English (Rechiger, 1982; Mozaffarian, 2008). The genus Salviathe largest genus represents a cosmopolitan assemblage of nearly 1000 species worldwide, there are 58 Salvia species growing naturally in Iran, 17 species endemic (Walker *et al*., 2004; Mozaffarian, 2008).

The genus Stachys had 2n=68 chromosomes (Asghari Zakaria and Zare, 2013). The genus is distributed mainly in warm temperate regions of the Mediterranean and Southwest Asia, Southern Africa, North and South America (Rechiger, 1982; Raymond*et al.,* 2004). In pharmacological studies *Stachys*species showed variety of effects; inflammatory diseases, sedative, antispasmodic, diuretic, gastrointestinal disorders, anxiolytic, cough, ulcers, fevers, diarrhea and antibacterial (Zargari, 1992; Gharib Naseri, 2011).

Salvia species are used in traditional medicines all around the world, possessing antioxidant, antibacterial, antidiabetic, anti-tumer, antiplasmodial and anti-inflammatory activities (Kamatou *et al.,* 2008). Study Amin *et al*., (2006) showed the major components (*Salvia spinosa* L.) a potent antimicrobial activity against *Pseudomonas aeroginsa* (2.06 µL/disc).

The chemical composition of plants is known to be influenced by several external factors including climate, as some compounds may be accumulated at a particular period to respond to environmental changes. Plant material collected at different times of the year may contain different novel compounds with other bioactivities. The effects of seasonal variations on the chemical and biological characteristics of some essential oils of the family Lamiaceae have been reported in the literature (Kofidis *et al.,* 2004; Salehi*et al.,* 2014; Golparvar*et al.,* 2015).

Ghasemi Pirbaloti and Mohammadi (2013) reported the main components found in the oil of *Stachys lavandulifolia* Vahl. collected throughout two provinces (Isfahan and Chaharmahal va Bakhtiary) were α-thujone (0.3%-32.3%), α-pinene (trace to 37.3%), myrcene (0.5%-15.9%), β-phellandrene (1.1%-37.9%), germacrene D (0.4%-11.3%), Δ-cadinene (trace to 11.6%) and 1, 4-methano-1 H-indene (trace to 10.1%). In a different report, the major components in *Stachys lavandulifolia* Vahl. Essential oil collected from Azerbaijan province were α-Pinene (22.03%), germacrene-D (10.32%), β-phellandrene (8.91%), β-pinene (8.21%) and spathulenol (6.78%) (Mazinani *et al.,* 2013).

According to Amin *et al*., (2006) the major components (*Salvia spinosa* L.) collected from Baraghan (Tehran) province were 1,8-cineole (32.87 %), β-ocimene (20.03%), germacrene-D (10.66%), 2-butyl thiophene (9.83%), trans caryophyllene (5.01%) and 3-butyl thiphene (3.49%).

Therefore, the aims of this study were to identify the chemical components of *Stachys lavandulifolia* Vahl. and *Salvia spinosa* L. in Isfahan climatic conditions.

Materials and methods

Plant material

The aerial parts of the plant samples of *Stachys lavandulifolia* Vahl. and *Salvia spinosa* L. were collected from (KamuMountain) Isfahan province during 2014. Kamu is a city in Qamsar district, Kashan County, Isfahan province, in center Iran (33°, 36_N and 51°, 14_E). The samples of the plants were identified by regional floras and authors with floristic and taxonomic references, and voucher specimens were deposited at the Herbarium of Agriculture Researches Islamic Azad University, Isfahan (Khorasgan), Iran.

Essential oil extraction

The fresh aerial of *S. lavandulifolia* Vahl. and *S.spinosa* L. were dried inside for six days at room temperature (25 ± 5 ˚C), and the ground to fine a powder using Moulinex food processor. The essential oil was extracted from 50 g of ground tissue in 1 L of water contained in a 2 L flask and heated by heating jacket at 100 ˚C for 3 h in a Clevenger–type apparatus according to the method recommended in BP (British Pharmacopoeia, 1988). Samples were dried with anhydrous sodium sulfate and kept in amber glass vials at 4˚C ± 1˚C until use.

GC/MS analysis

Compositions of the essential oils were determined by GC–MS. The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas with flow rate of 1.0 mL/min. The oven temperature was kept 20 °C at 50 °C for 4 min and programmed to 280 °C at a rate of 5 °C /min, and kept 20 °C constant at 280 °C for 5 min, at split mode. The injector temperature was at 20°C at 280 °C. Transfer 20 line temperatures 280 °C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 450. Identification of the essential oil components was accomplished based on comparison of retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (Adams 2007).

Results and discussion

Qualitative and quantitative analysis of the essential oils volatile profile are listed in Table 1, 2. In total, 17 and 21 compounds were identified in the essential oil from the aerial parts *S. lavandulifolia* and *S. spinosa*, respectively. The results obtained in our study indicated that the major components in the oil *S. lavandulifolia* were α-pinene (49.24%), β-pinene (22.52%), β-phellandrene (11.71%), α-copaene (6.70%) and β-myrcene (2.02%) (Table 1 and Fig 1). The major components in the oil*S.spinosa* were α-terpinolene (32.73%), β-ocimene (30.91%), β-patchoulene (12.77%), β-bourbonene (4.26%) and 1,8-cineol (2.88%) (Table 2 and Fig 2).

Table 1. Chemical compositions of essential oils of *Stachys lavandulifolia* Vahl.

No	Compound	RI	%
1	α-Pinene	935	49.24
2	β-Pinene	978	22.52
3	β-Myrcene	986	2.02
4	α-phellandrene	1005	0.95
5	β-Phellandrene	1030	11.71
6	β-Ocimene	1040	0.26
7	γ-terpinene	1059	0.38
8	Linalool	1095	0.27
9	α-Copaene	1369	6.70
10	β-bourbonene	1380	1.05
11	β-Cubebene	1391	1.46
12	β-elemene	1395	0.88
13	α-amorphene	1472	0.32
14	Germacrene D	1475	0.41
15	bicyclogermacrene	1490	0.26
16	Δ-cadinene	1520	0.65
17	Spathulenol	1570	0.85
	Total		99.93

RI: Retention indices determined on HP-5MS capillary column.

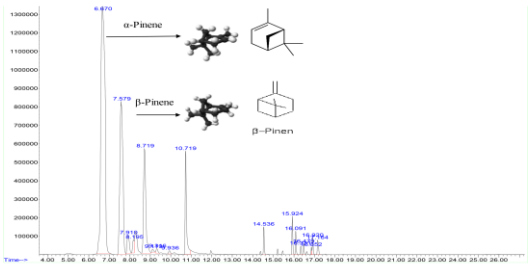

Fig. 1. The chromatograms found in essential oils of *Stachys lavandulifolia* Vahl (for peak identification see Table 1).

Table 2. Chemical composition of the essential oil from *Salvia spinosa* L.

No.	Compounds	RI	%
1	Sabinene	970	0.51
2	p-Cymene	1019	0.18
3	Acetaldehyde	1024	0.27
4	4-Amino butanoic acid	1025	0.25
5	1,8-cineol	1034	2.88
6	β-Cymene	1043	1.13
7	Butanoic acid, 2-methyl-, pentyl ester	1054	0.82

No.	Compounds	RI	%
8	β-Ocimene	1062	30.91
9	α-Terpinolene	1071	32.73
10	1,3-Cyclohexadiene, 1,5,5,6-tetram ethyl	1124	2.84
11	Butanoic acid, octyl ester	1175	0.88
12	Camphene	1235	1.08
13	Heptane, 3-methyl-	1285	2.23
14	Butanoic acid, 3-methyl-, hexyl ester	1294	0.29
15	Octane, 3,4-dimethyl	1298	0.72
16	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, (1α,2β,4β)	1345	2.29
17	β-Patchoulene	1368	12.77
18	β-Bourbonene	1385	4.26
19	N-Benzoyl-3-methylleucine	1394	1.26
20	4-Amino-furazan-3-yl-phenyl-methanone	1410	0.16
21	β-Gurjunene	1418	1.45
	Total		99.91

RI: Retention indices determined on HP-5MS capillary column.

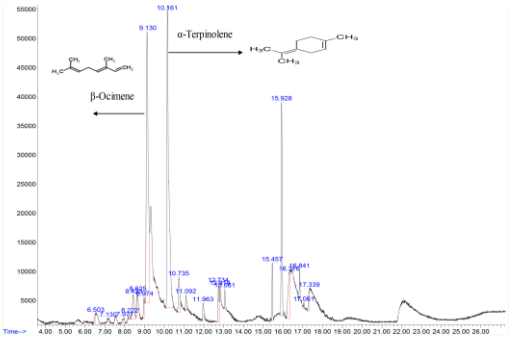

Fig. 2. The chromatograms found in essential oils of *Salvia spinosa* L. (for peak identification see Table 2).

The results of this study showed that monoterpenes and sesquiterpenes were the main constituent groups oilof S. lavandulifolia. In studies (Ghasemi Pirbaloti and Mohammadi, 2013) the main components found in the oil of *Stachys lavandulifolia* Vahl. collected throughout two provinces (Isfahan and Chaharmahal va Bakhtiary), Iran. Four groups were formed by the average linkage cluster analysis. The first cluster encompassed S. lavandulifolia Vahl accessions of Feryadan (Isfahan province), Ghalehsang, Sheyda and Broujen (Chaharmahal va Bakhtiari province), and exhibited high β-phellandrene (28.3%, 37.9%, 17.9% and 14.4%, respectively) and α-thujene (23.5%, 23.8%, 26.2% and 16.3%, respectively) contents. The second cluster enclosed accession of Kelishadrokh (Isfahan province), and displayed high α-thujene and α-phellandrene contents (32.3% and 14.0%, respectively). The third cluster enclosed accession of Naghan (Chaharmahal va Bakhtiari province), and displayed high myrcene and α-thujene contents (15.9% and 13.4%, respectively). The fourth cluster enclosed accession of Farsan (Chaharmahal va Bakhtiari province), and displayed high α-pinene and β-phellandrene contents (37.3% and 12.4%, respectively). Tajali (2012) reported the chemical compounds of the essential oil of *S. lavandulifolia* in two different habitats (Markazi province and Mazandaran province in Iran) during full flowering stage same components at two sites such as α-pinene (15.78-22%), β-pinene (3.41-5.07%), 1,8 cineole (14.03-22.94%), camphor (6.64-42.73%). In studies (Masoudi *et al.*, 2003) reported the main components found in the oil of *Stachys lavandulifolia* Vahl. collected from Iran was fifty-five compounds were observed, the major components found in the oil were α-pinene (20.1%), β-pinene (12.1%) and spathulenol (7.2%). In studies (Feizbaksh *et al.,* 2003) found α-pinene (20.1%), β-pinene (12.1%), spathulenol (7.2%) and germacrene D (5.3%) as the major components of oil of *S. lavandulifolia* Vahl collected from Ab-ali (Tehran province, Iran), this oil was rich in monoterpenoids (51.8%). The composition of the essential oil of *S. lavandulifolia* Vahl of the different stages of growth as pre-flowering, flowering and post flowering, the major components of essential oil were α-Pinene (27.25, 25.66, 8.52%), myrcene (17.33, 9.33, 23.85%), β-phellandrene (21.96, 37.49, 12.58%) and β-caryophylene (14.3, 8.38, 16.86%) respectively (Meshkatalsadat *et al.*, 2007).

In previous studies, the main components of *S. lavandulifolia* Vahl oil were reported to be germacrene-D (13.2%), β-phellandrene (12.7%), β-pinene (10.2%), myrcene (9.4%), α-pinene (8.4%) and Z-β-ocimene (5.8%) for Tehran population in Central Iran (Javidnia *et al.*,

2004), myrcene (20.9%), α-pinene (16.3%), α-terpinene (20%) and bicyclogermacrene (8.7%) for Lorestan population in West Iran (Amiri *et al.*, 2008), α-pinene (7.9%), 4-hydroxy-4-methyl-2-pentanone (9.3%) and hexadecanoic acid (5.9%) for Mazandaran population in North Iran (Semnani *et al.*, 2006) and β-caryophyllene and 1,8-cineole for *S. lavandulisolia* in Turkey (Sezik *et al.*, 1985). Baher Nik and Mirza (2005) reported the major components oil (*Salvia spinosa* L.) collected from Dizin (Tehran) province were β-ocimene (12.3%), β-caryophyllene (10.2%), isopentyl isovalerate (9.5%), α-gurjunene (7.2%) and isoarnyl, 2-methyl butyrate (7%). The oil obtained from the aerial parts of *S. lanigera* and *S. spinosa* wererich in thymol (54.9% and68.9%, respectively) (Flamini *et al.,* 2007). In this study, while thymol was not found in *S. spinosa* studied but β-ocimene was detected at the similar percentages with the results Baher Nik and Mirza (2005) and Amin *et al.*, (2006).Golparvar and Hadipanah(2013) reported the major components (*Salvia officinalis* L.) cultivated in Isfahan climatic conditions were; camphor (17.75%), thujone (13.25%), 1,8-cineole (13.03%), α-pinene (6%), β-thujone (5.85%), α-humulene (5.48%), β-caryophyllene (5.07%) and borneol (3.72%). Camparison between these results and the results of the other reports showed differences, probably due to that plant varieties or sites, as well as the time of harvesting. The variations in chemical composition of the essential oils with respect to season might have been due to the influence of phenological status, and environmental conditions can influence the regulation of the biosynthesis of essential oil (Abedi*et al.,* 2015).

Conclusion

In conclusion, the results obtained in our study indicated that the major components of oil of *S. lavandulifolia* Vahl collected from (Kamu mountain) Isfahan province such as α-pinene, β-pinene, β-phellandrene, α-copaene,β-myrceneand the chemical components of *Salvia spinosa* L. such as α-terpinolene, β-ocimene, β-patchoulene, β-bourbonene and 1,8-cineol. A comparison of our results with different reports, differences in the volatile composition of the plants could be attributed to genetic (genus, species, and ecotype), chemotype, distinct environmental and climatic conditions, seasonal sampling periods, geographic origins, plant populations, vegetative plant phases, and extraction and quantification methods.

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