ORIGINAL RESEARCH

Research on Crop Ecophysiology
Vol.14/1, Issue 1 (2019), Pages: 1 - 8

Assessment of Qualitative and Quantitative Composition of Essential Oil of Three *Salvia* Species

FARAHNAZ FARAJZADEH[1], HOSSEIN ALI ASADI-GHARNEH*[2], NASER SABAGHNIA[3]

1-Department of Horticulture, Faculty of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
2- Department of Horticulture, Faculty of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
3- Department of Plant Production and Genetics, Faculty of Agriculture, University of Maragheh, Iran

*Corresponding address Email: h.asadi@khuisf.ac.ir

Received: 25 July 2018 Accepted: 10 JANUARY 2019

ABSTRACT
Different species of *Salvia* are the most important medicinal plants. In this research essential oil composition of three *Salvia* species cultivated in climate condition in Marand region were investigated. The plants were harvested in flowering stage and after drying in room temperature, the essential oils of them were extracted with hydro-distillation technique and chemical compositions were analysed by a gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS). The highest and lowest amounts of essential oil yield were measured in *Salvia officinalis* and *Salvia virgata*, respectively. A total of 31 different components were identified accounting by 89.51%, 92.62% and 87.40% of the oils of *Salvia officinalis*, *Salvia nemorosa* and *Salvia virgata*, respectively. The main components were found in *Salvia officinalis* were α-thujone (37.35%), followed by camphor (18.59%), 1, 8-cineol (8.26%), β-thujone (5.53%), e-caryophyllene (4.15%), and α-humulene (3.50%). The oil of *Salvia virgata* contained, as main components, E-caryophyllene (37.51%), E- β-farnesene (15.26%), Caryophyllene oxide (8.57%) and camphor (5.16%). Major compounds in the volatile of *Salvia nemorosa* were e-caryophyllene, caryophyllene oxide, spathulenol and caryophylla-β-ol with 42.54, 23.94, 7.44 and 6.61%, respectively. Results indicated variation in the essential oil percent and composition among studied *salvia* species.

Keywords: Essential oil, *Salvia officinalis, Salvia nemorosa*, *Salvia virgata***.**

INTRODUCTION

The use of medicinal plants for diseases treatment purposes has been prevalent for centuries and is the basis of traditional Iranian medicine. Nowadays, due to the side effects of chemical drugs, use of medicinal plants is increasing (Mohammadi and Asadi-Gharneh, 2018). Iran, with 11 different climates and about 8000 valuable plant species, is a unique country in terms of plant materials and biodiversity (Omidbaigi, 2005).

The Lamiaceae family is one of the largest plant families widespread around the world. The *Salvia* genus, with about 1,000 species, constitutes the largest genus of this family, often highly aromatic and mainly distributed in Central and South America, West and East Asia. In different regions of Iran, 58 species of this genus have been found, which 17 species are endemic (Rechinger, 1992). The name of *Salvia* originates from the Latin word "Salvare", meaning healer, and in many pharmacopoeias, it is referred to as a medicinal plant (Omidbaigi, 2005; Blumenthal *et al*., 2000).

The essential oils of different species of *Salvia* are used in various pharmaceutical, food industries, cosmetics and medicinal plants (Ebadi, 2002; Kamatou *et al*., 2008). Different species of *Salvia* have antibacterial, anti-tumor, antioxidant and anti-inflammatory properties (Carta *et al*., 1996) and used in traditional medicine for the treatment of colds, bronchitis, gastrointestinal disorders and tuberculosis (Russo *et al*., 2013).

Secondary metabolites are the most important structure in medicinal plants that were produced by the plants under influence of various environmental condition and genetics. In terms of environmental factors, geographical distribution; climate and soil composition play an important role in the biosynthesis of secondary metabolites in medicinal plants (Omidbaigi, 2005; Aboukhalid *et al*., 2017).

The available literature reports numerous studies on the essential oils from *Salvia* species. Study of the essential oil composition of *Salvia virgate* in Tabriz weather condition showed that this specie contains fifteen different compounds and β-caryophyllene, germacrene-B, β-caryophyllene epoxide and spathulenol were the major constituents (Sefidkon and Mirza, 1999).

In a study, Kazemzadeh *et al* (2010) identified thirty four different compounds in the essential oil of *Salvia officinalis* and reported β-caryophyllene and 1, 8-cineol as the major constituents of this specie. Analysis of essential oil composition of *Salvia virgata* growing in central parts of Iran reported by Alizadeh (2013) revealed that this specie is rich in different composition of β-caryophyllene, caryophyllene oxide, sabinene, 1-octen -3-ol, terpinene-4-ol and alpha-thujene.

Golparvar and Hadipanah (2013) reported the major components of *Salvia officinalis* specie cultivated in Isfahan (Iran) were camphor, thujone and 1, 8-cineole. In another study, chemical composition of two species of *Salvia*, namely *Salvia officinalis* and *Salvia virgata* collected from Estahban region (in Fars province) were evaluated. Based on the results of their study, 42 and 29 compounds were identified in *Salvia officinalis* and *Salvia virgata*, respectively (Golparvar *et al*., 2017).

Saadatjoo *et al* (2018) evaluated diversity and composition of essential oils in some species of *Salvia*, collected from their natural habitats in Southwestern Iran, and reported considerable variation in essential oil content and composition within among studied *Salvia* species.

Since some of the chemical compositions that are produced by various environmental factors in plants are economically important and valuable as a main product of metabolism activities, the impact of environmental factors on the quantity and quality of these compounds should always be investigated.

The main purpose of this study is to evaluate cultivation site effects on yield and essential oil compositions of three *Salvia* species cultivated in climatic conditions of Marand, Iran.

MATERIALS AND METHODS

This research was carried out in 2018 in a privative field in Marand (38° 26' N latitude, 45° 46' E longitude, and 1334 m above sea level), Iran. Also, average of annual temperature, annual humidity, annual rainfall and wind speed of studied region are 14.3 C°, 37 %, 293 mm and 34 km/h, respectively. Seeds of *salvia* species including *S. officinalis, S. nemorosa* and *S. virgata* were purchased from Pakan-bazr Company, Iran.

For sowing the seeds, the soil was plowed and leveled. The area for each plot was 12 m^2. Seeds were sown at depth of 3-4 cm and irrigated immediately after seed sowing. After plants established, at 2-4 true leaves stage the plants were thinning to achieve ideal plant density. The plants were spaced 50 cm and 40 cm between and along the rows, respectively (Omidbaigi, 2005).

During the growth period all common agricultural practices were done for plants of three *Salvia* species. For measurement of physical and chemical properties of soil, the sample was taken in 0-30 cm depth of soil in the field and soil analysis was done according to standard methods and results are reported in Table 1.

Table 1. Physical and chemical properties of soil in studied region

Soil texture	EC (dS/m)	pH	O.C (%)	N (%)	P (mg/kg)	K (mg/kg)
Clay loam	0.75	7.83	1.80	0.08	103	880

The experiment was conducted in randomized block design (RBD) with 3 replications. The essential oil replications of each species were mixed together and a single sample for each species was submitted to identify chemical compounds (Moshrefi-Araghi *et al*., 2019).

Extraction and Identification of the Essential oil

For analysis of essential oils, the aerial parts of plants were harvested at flowering stage and were dried at room temperatures (25 ± 5 °C) and shade condition. Dried plant materials (100 g) were powder and the essential oil extraction was done by hydro-distillation technique for 3 hours using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia. The extracted essential oil was dehydrated with Na_2SO_4 and stored in sealed glass vials in the dark and 4 °C until analysis.

Analysis of essential oils was carried out in Research Institute of Forests and Rangelands, Tehran, Iran. The oils extracted from salvia species were analyzed using a Shimadzu- 9A Gas Chromatograph (GC). The detector was Flame Ionization Detector (FID) with 280° C temperature. The separation was achieved by capillary column, DB-5 (30 m × 0.25 mm, film thickness 0.25 μm). The column temperature was kept at 40 °C for 5 min and programmed to 250 °C at rate of 3°C/min. Helium was used as carrier gas at a flow rate of 1 mL/min. The temperature of the injection port was 260 °C.

The Gas Chromatography/Mass Spectrometry (GC/MS) was carried out on Varin-3400 GC coupled with Saturn II fused capillary column (30 m × 0.25 mm with film thickness 0.25 μm). The operating conditions programmed to 240 °C at rate of 3°C/min. The injector temperature was kept 250 °C.

Identification of the essential oils components was accomplished based on comparison of retention times with those of authentic standard and by comparison of their mass spectral fragmentation pattern (Willy and NIST Chen/Station data system and Adams (Adams, 2001).

RESULTS AND DISCUSSION

The analyses of essential oil revealed that different species of *Salvia* that cultivation in same region, display differences for their essential oil percentage and composition (Figure 1 and Table 2).

The essential oil yield in studied *salvia* species are shown in Figure 1. The percentage of essential oil was differing from 0.037 to 1.1 percent. The highest and the lowest values were measured in *S. officinalis* and *S. virgata*, respectively. In a previous report on *Salvia virgata* L. and *Salvia officinalis* L. from Estahban region (Fars province) in South of Iran, the essential oil yield was 1.1 and 2.4%, respectively. In another study, the essential oil yield of *Salvia virgata* L. genotypes were collected from natural habitats in Southwest of Iran reported between 0.03% and 0.04% (Saadatjoo *et al*., 2018). The sage (*Salvia officinalis* L.) oil yield of the fresh herb was 0.55 % and the oil yield of hydrosol (aromatic water) was 0.17 %, according to Baydar *et al* (2013) from the Isparta, Turkey.

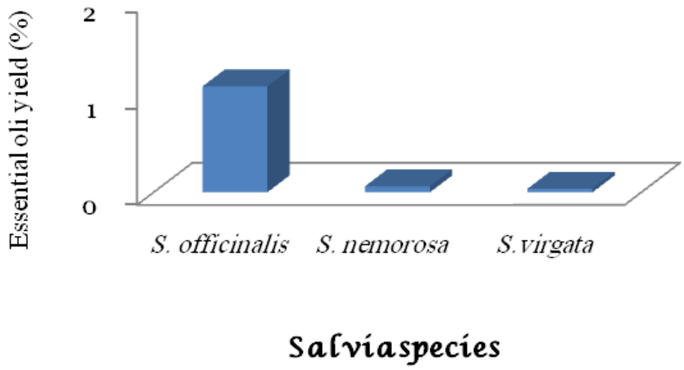

Figure 1. The essential oil yield of the studied *Salvia* species

The results of chemical analysis in essential oil of different *salvia* species by GC/MS including of components of essence, percentage and retention index are shown in Table 2. In *Salvia officinalis* seventeen different compound identified which represented 89.51% of total essential oil. The percentage of compound was varied from 0.18 to 37.35 percent. In this specie the highest compound were α-thujone (37.35%), followed by camphor (18.59%), 1, 8-cineol (8.26%), β-thujone (5.53%), e-caryophyllene (4.15%), and α-humulene (3.50%). Also the lowest compound belongs to cis-sabinene hydrate (0.18%) and linalool (0.30%).

Essential oil analysis in *S. nemorosa* showed different composition of secondary metabolites which represented 92.62% of the total essential oil. The chemical values differ from 0.23 to 42.54%. According to obtain results, the main constituents detected in *Salvia nemorosa* were e-caryophyllene, caryophyllene oxide, spathulenol and caryophylla-β-ol with 42.54, 23.94, 7.44 and 6.61%, respectively. On the other hand, the minimum values of components were observed in terpinene-4-ol (0.23%) and linalool (0.24%) (Table 2).

The analysis of essential oil in *Salvia virgta* identified compounds represented 87.40% of total oil and varied from 0.38 to 37.51 percent of essential oil and e-caryophyllene (37.51%), e- β-farnesene (15.26%), caryophyllene oxide (8.57%) and camphor (5.16%) were found in abundance. Also the lowest values of components are delta cadinene (0.38%), α-copaene (0.45%) and sabinene (0.48%).

Table 2. Chemical composition of the essential oils of three species of *Salvia* (*S. officinalis, S. nemorosa* and *S. virgata*) cultivated in Marand region

No	Compounds	RI	*S. officinalis* (%)	*S. nemorosa* (%)	*S. virgata* (%)
1	α-pinene	947	2.40	---	1.18
2	Camphene	964	2.58	---	---
3	β-pinene	980	1.12	---	---
4	Myrcene	1010	1.63	---	---
5	Sabinene	1012	---	---	0.48
6	Limonene	1058	1.50	---	---
7	1,8- Cineol	1067	8.26	---	---
8	γ-terpinene	1084	0.41	---	---
9	Cis sabinene hydrate	1102	0.18	0.33	---
10	P menthe 2,4(8) diene	1111	0.35	---	---
11	Linalool	1117	0.30	0.24	---
12	α-thujone	1143	37.35	0.91	2.15
13	β-thujone	1149	5.53	0.47	0.85
14	Camphor	1205	18.59	1.53	5.16
15	Brneol	1225	0.82	---	---
16	α-campholenal	1227	---	1.24	---
17	Terpinene-4-ol	1322	0.84	0.23	1.89
18	Thymol	1471	---	1.73	3.20
19	α-copaene	1474	---	---	0.45
20	e-caryophyllene	1479	4.15	42.54	37.51
21	α-humulene	1517	3.50	1.88	2.06
22	e- β-farnesene	1538	---	0.93	15.26
23	γ-cadinene	1541	---	---	1.13
24	Germacrene-D	1557	---	0.53	2.04
25	Delta cadinene	1637	---	---	0.38
26	Spathulenol	1657	---	7.44	---
27	Caryophyllene oxide	1667	---	23.94	8.57
28	(epi-α) bisabolol	1696	---	---	2.58
29	Humulene epoxide	1910	---	1.47	2.51
30	Caryophylla-4(14),b(15)-dien-5-α-ol	1942	---	0.60	---
31	Caryophylla-4(14),b(15)-dien-5-β-ol	1981	---	6.61	---
	Total	**---**	**89.51**	**92.62**	**87.40**

In overall, there were thirty one different components were identified in essential oils of studied *Salvia* species. In our study β-thujone (5.53%), caryophylla_4(14),b(15)_dien_5_β_ol (6.61%), spathulenol (7.44%), 1,8- cineol (8.26%), e-β-farnesene (15.2%), camphor (18.59%), caryophyllene oxide (23.94%), α-thujone (37.35%) and e-caryophyllene (42.54%) were found in the highest values. Also, our results indicated that, six compounds namely; α-thujone, β-thujone, camphor, terpinene-4-ol, e-caryophyllene and α-humulene are common compositions in three studied *Salvia* species. On the other hands, in *Salvia officinalis*, *Salvia nemorosa* and *Salvia virgata*, 8, 4 and 5 particular compound was observed (Table 2).

Baydar *et al* (2013) indicated that 1, 8-cineole, cis thujone, trans-thujone and camphor were the main components in *Salvia officinalis* essential oil, while in our study 1, 8-cineole, α-thujone and camphor were the abundant compounds. The amounts of 1, 8-cineole in our study was lower than measured by Bernotiene *et al* (2007) from plants collected from 8 gardens in Eastern Lithuanian. The amounts of alpha pinene, camphene, limonene, 1, 8-cineole, linalool, camphor and alpha humulene in our study, were highly supported with the ISO 9909, as an standard for medicinal uses of essential oil of *Salvia officinalis*. According to our results, while α-thujone was very similar to amount of reported by Golparvar *et al* (2017), the values of 1, 8-cineole, β-thujone and camphene had lower similarity. The essential oil of *Salvia officinalis* due to existence of 1,8- Cineol (8.26%) component, has antibacterial properties (Sefidkon and Mirza, 1999).

The major essential oil component of *Salvia nemorosa* from 21 different components were β-ocimene, β-caryophyllene and α- thujone (Mirza and Sefidkon, 1999).

In this study, e-caryophyllene, e-β–farnesene and caryophyllene oxide were identified as the major essential oil components in *virgata* specie, which amount of e-caryophyllene and e-β–farnesene were higher than what reported by Saadatjoo *et al* (2018), except for caryophyllene oxide which is lower. Compared with the results of our investigation, Golparvar *et al* (2017) demonstrated the higher levels of caryophyllene oxide and sabinene compounds. The main identified component from *Salvia virgata* in another study was β-caryophyllene and β-caryophyllene oxide (Sefidkon and Mirza, 1999). As a result of possessing valuable components such as 1, 8-cineole, thujone and camphor, the essential oil of *Salvia* species revealed antimicrobial, antioxidant and anti-cancer properties (Carta *et al*., 1996).

Although the amounts of secondary metabolites in medicinal plants are controlled by genes, accumulation and concentration of them are drastically influenced by environmental conditions. (Gurr, 1980). In fact, quantity and quality of essential oil composition in aromatic plants can be influenced by variety and ontogenic stage (Marotti and Piccaglica, 1994), as well as environmental factors including; temperature, light intensity, altitude, water availability, plant nutrition, soil type and preparation process (Rostaefar *et al*., 2018; Sajed *et al*., 2013; Gezeka *et al*., 2019). On the other hand; accessibility of different biochemical compounds are correlated with temperature and altitude of production site (Omidbaigi, 2005).

CONCLUSION

In this study, the quantity and quality of essential oil components of three *Salvia* species were evaluated. Results indicated variation in the essential oil yield and composition in studied *Salvia* species. To sum up, each *Salvia* species possesses different composition of essential oil that can be used for different purposes such as drug and food industries. Also according to our study, this region has appropriate condition for production of *Salvia* species, due to production of different valuable component in suitable amounts with compare to production of them in other regions.

REFERENCES

Aboukhalid K, Al Faiz C, Douaik A, Bakha M, Kursa K, Agacka Mołdoch M, Machon N, Tomi F, Lamiri A. 2017. Influence of environmental factors on essential oil variability in *Origanum compactum* Benth. growing wild in Morocco. Chemistry and Biodiversity, 14 (9). e1700158.

Adams RP. 2001. Identification of essential oil components by gas chromatography/mass spectroscopy: Carol Stream, IL Allured Publishing Co, 8(16): 76-81.

Alizadeh A. 2013. Essential oil constituents, antioxidant and antimicrobial activities of *Salvia virgata* Jacq. from Iran. Journal of Essential Oil Bearing Plants, 16: 172-182.

Baydar H, Sangun MK, Erbas S, Kara N. 2013. Comparison of aroma compounds in distilled and extracted products of sage (*Salvia officinalis* L.). Journal of Essential Oil Bearing Plants, 16(1): 39-44.

Bernotiene G, Nivinskiene O, Butkiene R, Mockute D. 2007. Essential oil composition variability in sage (*Salvia officinalis* L.). Chemija, 18: 38-43.

Blumenthal M, Goldberg A, Brinckmann J. 2000. Herbal medicine: expanded commission emonographs. Integrative medicine communications, USA.

Carta C, Moretti MD, Peana AT. 1996. Activity of oil *Salvia officinalis* against *Botrytis cinerea*. Journal of Essential Oil Research, 8: 399- 404.

Ebadi M. 2002. Pharmacodinamic basis of herbal medicine. Boca Raton: CRC Press.

European Pharmacopoeia.1983. Vol. 1, Maissoneuve, SA, Sainte Ruffine.

Gezeka G, Hashemia P, Kalaycıolua Z, Kaygusuza H, Sarıolua G, Dökerc S, Dirmencid T, Bedia Erima F. 2019. Evaluation of some Turkish *Salvia* species by principal component analysis based on their vitamin B2, mineral composition, and antioxidant properties. Food Science and Technology, 100: 287–293.

Golparvar AR, Hadipanah A. 2013. Identification of the components of sage (Sa*lvia officinalis* L.) and thyme (*Thymus vulgaris* L.) cultivated in Isfahan climatic conditions. Electronic Journal of Biology, 9(2): 42-45.

Golparvar AR, Hadipanah A, Gheisari MM, Naderi D, Rahmaniyan S, Khorrami M. 2017. Chemical composition and antimicrobial activity of essential oil of *Salvia officinalis* L. and *Salvia virgata* Jacq. Journal of Herbal Drugs, 8(2): 71-78.

Gurr MI. 1980. The biosynthesis of triacylglycerol. In: Stump PK (Eds). The Biochemistry of Plant. Academic, New York.

Kamatou GPP, Makunga NP, Ramogola WPN, Viljoen AM. 2008. South African *Salvia* species: a review of biological activities and phytochemistry. Journal of Ethnopharmacology, 119: 664-667.

Kazemzadeh Z, Habibi Z, Yousefzadeh M, Ashabi MA, Heydari M. 2010. Chemical composition and antibacterial activity of the essential oil of *Salvia* macrochlamys Boiss. Journal of Medicinal Plants, 9: 75-82.

Marotti M, Piccaglia R. 1994. Effects of variety and ontogenic stage on the essential oil composition and biological activity of Fennel. Journal of Essential Oil Research, 6: 57-62.

Mirza M, Sefidkon F. 1999. Essential oils composition of two *Salvia* species from Iran. *Salvia nemorosa* L. and *Salvia reuterana* Boiss. Flavour and Fragrance Journal, 14: 230-232.

Mohammadi M, Asadi-Gharneh HA. 2018. How the morphological properties of *Mentha longifolia* L. Huds. maybe affected by geographical differences. Journal of Photochemistry and Photobiology, B: Biology, 178: 237–242.

Moshrefi-Araghi A, Nemati H, Azizi M, Moshtaghi N, Shoor M, Hadian J. 2019. Assessment of phytochemical and agro-morphological variability among different wild accessions of *Mentha longifolia* L. cultivated in field condition. Industrial Crops and Products, 140: 698-715.

Omidbaigi R. 2005. Production and Processing of Medicinal Plants. Behnashr Publication. Mashhad, Iran. 347pp.

Rechinger KH. 1992. Flora Iranica. Akademische Druck-u Verlagsanstalt, Graz, Austria, pp. 495–504.

Rostaefar A, Hassani A, Sefidkon F. 2018. Effect of altitude on essential oil composition in different gender of *Juniperus communis* ssp. hemisphaerica growing wild in Amol. Eco-Phytochemical Journal of Medicinal plants, 21(6): 1-10.

Russo A, Formisano C, Rigano D, Senatore F, Delfine S, Cardile V, Rosselli S, Bruno M. 2013. Chemical composition and anticancer activity of essential oils of Mediterranean sage (*Salvia officinalis* L.) grown in different environmental conditions. Food and Chemical Toxicology, 55: 42–47.

Saadatjoo B, Saeidi K, Mohammadkhani A, Shirmardi HA. 2018. Assessment of variation in essential oil content and composition within and among *Salvia* sp. from Southwest Iran. Journal of Essential Oil Bearing Plants, 21(1): 237-245.

Sajed H, Sahebkar AH, Iranshahi M. 2013. *Zataria multiflora* Boiss. (Shirazi thyme) - An ancient condiment with modern pharmaceutical uses. Journal of Ethnopharmacol, 145: 686-698.

Sefidkon F, Mirza M. 1999. Chemical composition of the essential oils of two *Salvia* species from Iran, *Salvia virgata* Jacq. and *Salvia syriaca* L. Flavour and Fragrance Journal, 14(1): 45-46.