Medicinal and Aromatic Plant Science and Biotechnology ©2009 Global Science Books

Changes in Essential Oil Content and Composition of Clary Sage (*Salvia sclarea*) Aerial Parts during Different Phenological Stages

Mohammad J. Saharkhiz[1*] • Askar Ghani[2] • Mohammad Hassanzadeh-Khayyat[3]

[1] Department of Horticultural Sciences, Shiraz University, Shiraz, Iran
[2] Department of Horticultural Sciences, Ferdowsi University of Mashhad, Iran
[3] Department of Medicinal Chemistry, School of Pharmacy, Pharmaceutical Sciences Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
Corresponding author: * **jamalsaharkhiz@yahoo.com / saharkhiz@shirazu.ac.ir**

ABSTRACT

The variations in quantity and quality of essential oils (EOs) of *Salvia sclarea* L. (Lamiaceae) were examined at different phenological stages (i.e. rosette, stem initiation, full flowering, and fruit set) of the life cycle of species. The EOs of air-dried samples were obtained by hydrodistillation. The yields of EOs (w/w %) were 0.23, 0.15, 1.36, and 1.35% at rosette, stem initiation, full flowering, and fruit set stages, respectively. The EOs were analyzed by GC and GC-MS. A total of 17, 27, 19, and 51 components were identified and quantified at the above mentioned stages, respectively. The proportions of linalool and linalyl acetate, as major oil constituents were highest at the full flowering stage. However, the main group of compounds at the rosette stage was the monoterpene esters (53.36%) and at stem initiation the sesquiterpenes (57.7%). Moreover, monoterpene alcohols were the predominant compounds at full flowering (43.7%) and at fruit set (34.88%) stages.

Keywords: growth stages, hydrodistillation, linalool, linalyl acetate, γ-muurolene

INTRODUCTION

The genus *Salvia* is one of the most important genera of the family Lamiaceae and has an important centre of diversity in the Flora Iranica. In this flora, *Salvia* is represented by 60 species, of which 17 species are endemic to Iran (Hedge 1982). Clary sage (*Salvia sclarea* L.) with the common Persian name Maryam-goli kabir, is a xerophytic biennial or perennial plant (Mozaffarian 2004). It is native to southern Europe and Syria and is commonly cultivated in southern France, Russia, Hungary, Italy, and the UK (Lattoo *et al*. 2006). The whole plant, in particular the inflorescences, possess a very strong aromatic scent and the essential oil (EO) is characterized by a fresh floral and herbaceous odor that has economic value for cosmetics and as flavoring agent in food and liqueur preparations (Lawrence 1979; Jibao *et al*. 2006). Moreover, it is used in traditional herbal medicine as an antispasmodic, carminative, diuretic, and oestrogenic (Ulubelen *et al*. 1994). Extract of the whole plant contains seven diterpenes which have been shown to possess antimicrobial activity against *Staphylococcus aureus*, *Proteus mirabilis* and the yeast *Candida albicans* (Lattoo *et al*. 2006). It has also been reported to show a high radical scavenging activity and a potent inhibitor of growth of rust fungi (Banthorpe *et al*. 1990; Miliauskas *et al*. 2004). The EO is used in the preparation of alcoholic and non-alcoholic beverages, ice creams, candy-baked foods, and as a modifier in spice compounds (Burdock 1995). Furthermore, fresh leaves are used sparingly in soups, and in Middle Ages, the seeds were used to clear vision, from which it received its popular name, 'clary', or 'clear eye' (Chevallier 2001).

Chemical and biological diversity of aromatic and medicinal plants differs significantly depending on factors such as cultivation area, climatic conditions, genetic modifications, different plant parts, and developmental stages (Franz 1993; Miliauskas *et al*. 2004). In recent years, numerous publications have reported the chemical compositions of the EOs of medicinal and aromatic plants indicating that growth stage and harvesting time have major impact on the EO content and chemical composition (Saharkhiz *et al*. 2005; Lattoo *et al*. 2006; Msaada *et al*. 2007; Moghaddam *et al*. 2007). The analysis of the EO of *S. sclarea* from India at different stages of inflorescence maturity (i.e. bud, anthesis, fertilization, pre-full bloom, full bloom, and maturation stages) has shown that linalool and linalyl acetate are the two major components and that both EO yield and composition varied according to these stages (Lattoo *et al*. 2006). To the best of our knowledge literature pertaining to the EO content and composition of Iranian-grown clary sage is not available. It is therefore imperative to determine the proper time for plant harvest by analyzing the oil yield and composition during various growth stages of this plant.

The present study was initiated to evaluate the variation of EO composition of clary sage at four different phenological stages (such as at rosette, stem initiation, full flowering, and fruit set stages).

MATERIALS AND METHODS

Plant material

Clary sage seeds were collected in July 2006 from plants growing wild in Golmakan Mountains at an altitude of 1070 m above mean sea level, near Mashhad, Iran. The plant species from which the seeds were collected was identified and authenticated by M.R. Joharchi, a plant taxonomist, at Ferdowsi University of Mashhad, Herbarium, Mashhad, Iran. A voucher specimen (FUMH, no. 18037) has been deposited in the herbarium.

Seeds were sown in pots in a greenhouse on December, 2006, which germinated after 15-20 days. The seedlings were kept in a cool greenhouse (5-10°C) over the winter to satisfy their chilling requirement. Equal height and vigour seedlings were transplanted in April, 2007 in plots of 1.5 m^2. The rows were 40 cm apart and there was a 30 cm distance between plants. All plots were furrow irrigated soon after planting. Plant samples were harvested at

Received: 4 May, 2009. Accepted: 8 July, 2009.

Original Research Paper

rosette (May), stem initiation (flower stem formation) (June), full flowering (July), and fruit set (end of July) stages.

Essential oil isolation

The aerial parts of plants were harvested at early morning, then air dried. Samples (50 g, four replicates for each stage) were hydrodistillated for 3 h using an all glass Clevenger-type apparatus, to extract EOs, according to the method outlined by the European Pharmacopoeia (Anonymous 1997). The extracted EO samples were dried over anhydrous sodium sulphate and stored in sealed vials at low temperature (4°C) before gas chromatography (GC) and gas chromatography-mass spectrometric (GC-MS) analysis.

GC and GC-MS analysis

The components of volatile oils from the aerial parts of the plants were identified using GC-MS analysis.

The gas chromatograph (GC) was a Shimadzu GC-17 equipped with a FID detector, fused-silica column (BP-5, 25m × 0.22 mm i.d, film thickness 0.25 mm). The operating conditions were oven temperature, 60-280°C with the rate of 8°C/min; injector temperature, 280°C, split ratio 1:10, with the carrier gas, He; detector temperature, 300°C.

The GC-MS apparatus was a Varian GC-MS consisting of a Varian star 3400 gas chromatograph equipped with a fused-silica column (DB-5, 30 m ×0.32 mm i.d., film thickness 0.25 mm; J &W Scientific Inc), interfaced with a mass spectrometric detector (Varian Saturn 3). The operating conditions were oven temperature, 60-280 °C with the rate of 3°C/min; injector temperature, 280°C; injector mode, split injection; with the carrier gas, He; flow rate, 2 mL/min; ionization mode, electron impact (EI) at 70 eV; interface temperature, 300°C; scan range, 40-300 u.

The oil components were identified from their retention indices (RI) obtained with reference to *n*-alkane series (Sigma, UK) on a DB-5 column, mass spectra with those of authentic samples, composition of their mass spectra and fragmentation patters reported in literature, computer matching with MS-data bank (Saturn version 4). Quantification of the relative amount of the individual components was performed according to the area percentage method without consideration of calibration factor (Adams 2001).

RESULTS AND DISCUSSION

The hydrodistillation of 50 g of aerial parts of clary sage showed that EOs at the rosette, stem initiation, full flowering, and fruit set stages were 0.23, 0.15, 1.36, and 1.35% based on dry weight, respectively (**Fig. 1**). The oil content decreased from rosette to stem initiation and then increased dramatically (more than five times) through the full flowering and fruit set stages. This increase was concomitant with an increase in monoterpene constituents, particularly linalool and linalyl acetate (from 0 to 53.1%). However, contents of EOs in this investigation were significantly more than those reported by Lattoo *et al*. (2006) and Chevallier (2001).

The compositions of EOs at different growth stages are shown in **Table 1** along with retention indices of the identified components that are arranged in the order of their elution from a DB-5 column. A comparison of the composition of the EOs during the growth stages revealed both quantitative and qualitative differences. A total of 17, 27, 19, and 51 compounds representing 99.34, 98.48, 98.26, and 97.19% of the total were detected at rosette, stem initiation, full flowering, and fruit set stages, respectively.

At the rosette stage, the oil consisted mainly of monoterpene esters (**Table 2**) which was represented exclusively by santalol acetate β-Z (51%) followed by sesquiterpenes (44.5%) that contained γ–muurolene (20.5%) as its main constituent. Moreover, monoterpene ethers were 1.53%, which represented a very small quantity. Also, the main components of the EO were santalol acetate β-*Z* (51%), γ-muurolene (20.5%), germacrene D (5.9%), and lanceol Z (5.6%).

At the stem initiation stage, 27 components were detected, accounting for 98.5% of EO content. The number of components at this stage was markedly higher than that of the first stage. The EO was comprised mainly of sesquiterpenes (57.7%) (**Table 2**), which were represented mostly by γ-muurolene (31.3%) and germacrene-D (8.5%). Other volatile compounds observed were monoterpene alcohols (18.7%), monoterpene esters (17%), monoterpene hydrocarbons (4.3%), and phenols (1%). Also, the major constituents of the EO were: γ-muurolene (31.3%), linalool (11.2), linalyl acetate (8%), and geranyl acetate (5.5%).

EOs at the full flowering and fruit set stages were predominated by monoterpene alcohols (43.7 and 34.9%, respectively) which consisted mainly of linalool at 30 and 21%, respectively. The remaining important fractions of these two stages were monoterpene esters (36.1-25.2%), monoterpene hydrocarbons (8.94-9.1%), and sesquiterpenes (6.1-18.3%). Interestingly, phenols known as potent antioxidant were detected as 2% at the full flowering stage (**Table 2**). Nineteen and 51 components representing 98.3 and 97.1% of the total were identified and quantified at these two stages, respectively. The major components of these stages were linalool (30-21%), α-terpineol (11-11.1%), linalyl acetate (23-12.7%), and geranyl acetate (8.4-6.5%) (**Table 1**).

Fig. 2 shows the quantity of the major compounds of clary sage oil during various growth and developmental stages γ-muurolene and germacrene-D, the two major EO components at the rosette stage, gradually increased towards stem initiation and then drastically decreased at full flowering and fruit set stages. Linalool, α-terpineol, and linalyl acetate, which were the main components at stem

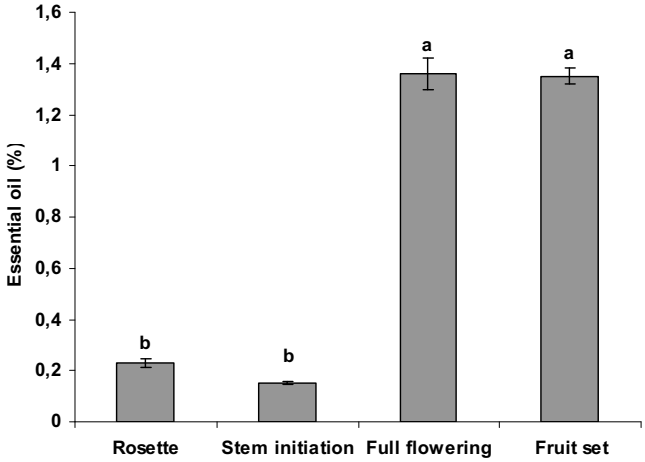

Fig. 1 The effect of different phenological stages on the essential oil content (%) of *S. sclareae*. Means followed by the same letter are not significantly different by Duncan's new multiple range test ($P \leq 0.05$).

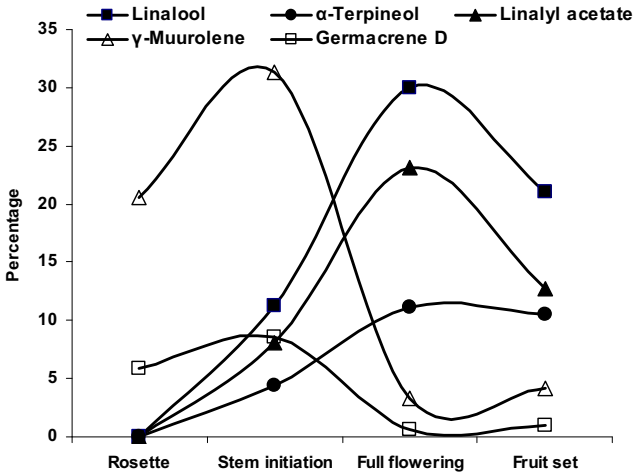

Fig. 2 Major compounds variation of *S. sclareae* essential oil during different phenological stages.

Table 1 Chemical composition (%, w/w) of *S. sclareae* essential oil at four phenological stages.

Components	Retention index[a]	Rosette	Stem initiation	Full flowering	Fruit set
α-Pinene	939	t	-	-	0.31
Sabinene	976	-	-	-	0.46
β-Pinene	980	-	-	-	1.16
Myrcene	991	-	1.11	2.96	2.23
α-Terpinene	1019	-	-	-	0.17
ρ-Cymene	1026		-	0.60	0.46
1,8-Cineole	1031	1.53	t	0.81	3.86
β-*Z*-Ocimene	1040	-	1.22	1.82	1.26
β-*E*-Ocimene	1051	-	1.99	3.18	2.12
γ-Terpinene	1061	-	-	0.30	0.42
cis-Sabinene hydrate	1068	-	-	-	0.27
Terpinolene	1089	-	t	0.68	0.70
Linalool	**1099**	**-**	**11.23**	**30.03**	**20.99**
trans β-Thujone	1110	-	-	-	4.84
cis-Limonene oxide	1132	-	-	-	0.11
trans Pinocarveol	1140	-	-	-	0.12
Camphor	1144	-	-	-	0.32
Pinocarvone	1162	-	-	-	0.10
Borneole	1166	-	-	-	0.54
α-Terpineol	**1190**	**-**	**4.38**	**11.13**	**10.58**
Myrtenol	1196	-	-	-	0.22
Nerol	1228	-	0.91	2.54	2.43
Geraniol	1250	-	2.16	-	-
Linalyl acetate	**1259**	**-**	**8.11**	**23.08**	**12.71**
Isobornyl acetate	1284	-	-	-	0.11
Thymol	1291	-	t	0.74	t
Carvacrol	1299	-	0.69	1.21	-
Δ-Elemene	1335	1.25	1.62	-	0.24
α-Terpinyl acetate	1349	-	-	-	0.19
Neryl acetate	1364	-	2.68	4.69	4.12
α-Ylangene	1373	-	2.25	-	-
α-Copaene	1374	1.97	-	-	4.12
Geranyl acetate	1383	-	5.49	8.37	6.48
β-Cubebene	1388	1.17	1.37	-	-
β-Elemene	1389	1.30	1.52	-	0.21
Longifolene	1413	4.89	7.35	-	-
β-Ylangene	1415	-	-	1.78	2.84
Caryoplyllene *E*	1423	0.63	0.75	-	0.13
α-Himachalene	1447	t	0.62	-	0.20
α-Patchulene	1457	-	0.71	-	0.37
γ-Muurolene	**1476**	**20.54**	**31.30**	**3.32**	**4.10**
Germacrene D	**1488**	**5.93**	**8.53**	**0.58**	**0.96**
β-Himachalene	1503	-	-	-	0.30
α-Chamigrene	1505	-	-	-	0.35
γ-Cadinene	1516	1.13	1.06	-	0.29
Nerolidol *E*	1560	-	-	-	0.31
Isoeugenol acetate *Z*	1569	2.28	0.81	-	0.27
Spathulenol	1574	-	-	-	0.52
Viridiflorol	1593	-	-	-	0.18
Nerolidol acetate *E*	1714	-	-	-	0.94
E,E farnesol	1720	-	-	-	0.38
Nuciferol *E*	1725	-	-	-	0.27
Santalol *E*-β	1740	-	0.62	0.44	0.58
E,Z farnesol	1746	t	-	-	1.96
Lanceol *Z*	1762	5.64	-	-	-
Santalol acetate α-*Z*	1777	t	-	-	0.27
β-Eudesmol acetate	1787	-	-	-	0.12
Santalol acetate β-*Z*	1819	51.08	-	-	-

t = trace (< 0.1%)

[a]The retention Kovats indices were determined on DB-5 capillary column.

initiation, full flowering, and fruit set stages, were not detected at the rosette stage. The biosynthesis of these components started after the rosette phase and reached to its highest level at full flowering followed by a decrease at fruit set stage (**Fig. 2**). In the present study, the maximum amount of the major EO components were detected in the EO of the full flowering phase and they included linalool (30%), linalyl acetate (23%), α-terpineol (11.1%), and geranyl acetate (8.3%). On the other hand, at the fruit set phase, the amount of major EO components significantly decreased and many new minor components were produced (**Table 1**).

As shown in **Table 1**, some components such as geranyl acetate, β-*E*-ocimene and longifolene were not detected either at a specific growth stage (especially at rosette stage) or showed a decrease or an increase trend in rosette, stem initiation, full flowering, and fruit set stages. This indicates that the significant changes in EO content and composition during different growth and developmental stages can be used as a marker of maturation processes.

There are few published reports regarding the evolution of the chemical composition of oil of *S. sclarea* during different growth stages. The analysis of the EO composition

Table 2 Volatile compounds classes and percentages at four phenological stages.

Classes	Rosette	Stem initiation	Full flowering	Fruit set
Monoterpene hydrocarbons	-	4.32	8.94	9.1
Aromatic hydrocarbons	-	0	0.6	0.57
Monoterpene alcohols	-	18.68	43.7	34.88
Monoterpene esters	53.36	17.09	36.14	25.21
Phenols	-	0.69	1.95	0
Monoterpene ketons	-	-	-	5.26
Monoterpene ethers	1.53	-	0.81	3.86
Sesquiterpenes	44.45	57.7	6.12	18.31
Total	99.34	98.48	98.26	97.19

of plants from subtropical regions of India revealed that linalool and linalyl acetate were the two major components of the EO during different inflorescence maturity (Lattoo *et al*. 2006), which is similar to the study reported here. However, santalol acetate β-*Z* was not found in previous work, while it was detected at 51% in the rosette stage in this study. Moreover, γ-muurolene was not observed by Lattoo *et al*. (2006), whereas its amount varied from 3.3 to 31.3% in the present work. However, sclareol was only detected in the EO of Indian clary sage and not found in this study. It seems that such variations in quantity and quality of components in the same species might be due to extrinsic factors such as different developmental stages and harvesting times along with parameters such as edaphic and climatic factors, geographic origin, cultivation site (Graven *et al*. 1991; Chalchat *et al*. 1995) and/or the occurrence of a chemotype (Gil *et al*. 2000).

CONCLUSION

In conclusion, the results reported herein revealed that there are considerable differences in the content and chemical composition of the oil of *S. sclarea* during its phenological cycle. It is strongly believed that these differences are due to variations in the metabolic pathways and consequently modifications in secondary metabolism which coupled with the plant growth and development. Further studies are still required to explore the molecular aspects of these variations during different maturation stages of the plant.

ACKNOWLEDGEMENTS

The authors wish to extend their thanks and appreciation to Department of Pharmacognosy, Mashhad University of Medical Sciences for GC and GC/MS analysis.

REFERENCES

Adams RP (2001) *Identification of EOs Components by Gas Chromatography/ Quadra pole Mass Spectroscopy.* Carol Stream, IL, Allured, pp 61, 63, 68, 70, 72, 77, 80, 81, 83, 89, 99, 138, 159, 185, 212, 231, 242, 259, 275, 287, 297, 12, 321, 367

Anonymus (1997) *European Pharmacopoeia* (3r Edn), Council of Europe, Strasbourg, France, pp 121-122

Banthorpe DV, Brown JT, Morris GS (1990) Accumulation of the antifungal diterpene sclareol by cell cultures of *Salvia sclarea* and *Nicotiana glutinosa*. *Phytochemistry* **29**, 2145-2148

Burdock GA (1995) CRC *Fenarolis Handbook of Flower Ingredients*, 3rd edn, vol 1. CRC Press: Tokyo, pp 93-95

Cai J, Lin P, Zhu X, Su Q (2006) Comparative analysis of clary sage (*S. sclarea* L.) oil volatiles by GC-FTIR and GC-MS. *Food Chemistry* **99**, 401-407

Chalchat JC, Garry RP, Muhayimana A (1995) Essential oil of *Tagetes minuta* from Rwanda and France: Chemical composition according to harvesting location, growth stage and part of plant extracted. *Journal of Essential Oil Research* **7**, 375-386

Chevallier A (2001) *The Encyclopedia of Medicinal Plants*, Dorling Kindersley, London, 265 pp

Franz C (1993) *Volatile Oil Crops: Their Biology, Biochemistry and Production*. Hay RKM, Waterman PG (Eds), Longman, Harlow, UK, 63 pp

Gil A, Ghersa CM, Leicach S (2000) Essential oil yield and composition of *Tagetes minuta* accessions from Argentina. *Biochemical Systematics and Ecology* **28**, 261-274

Graven EH, Webber L, Benians G, Venter M, Gardner JB (1991) Effect of soil type and nutrient status on the yield and composition of tagetes oil (*Tagetes minuta* L.). *Journal of Essential Oil Research* **3**, 303-307

Hedge IC (1982) *Labiatea.* In: Rechinger CH (Ed) *Flora Iranica* (Vol 150), Akademische Druck-u Verlagsanstalt, Graz, Austria, pp 403-476

Lattoo SK, Dhar RS, Dhar AK, Sharma PR, Agarwal SG (2006) Dynamics of essential oil biosynthesis in relation to inflorescence and glandular ontogeny in *Salvia sclarea*. *Flavour and Fragrance Journal* **21**, 817-821

Lawrence BM (1979) Commercial production of non-citrus EOs in North America. *Perfumer and Flavorist* **3**, 21-33

Msaada K, Hosni K, Ben Taarit M, Chahed T, Kchouk ME, Marzouk B (2007) Changes on the essential oil composition of coriander (*Coriandrum sativum* L.) fruits during three stages of maturity. *Food Chemistry* **102**, 1131-1134

Miliauskas G, Venskutonis PR, van Beek TA (2004) Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry* **85**, 231-237

Moghaddam M, Omidbaigi R, Sefidkon F (2007) Changes in content and chemical composition of *Tagetes minuta* oil at various harvest times. *Journal of Essential Oil Research* **19**, 18-20

Mozaffarian V (2004) *A Dictionary of Iranian Plant Names*, Tehran, Farhange Moaser Press, pp 479-480

Saharkhiz MJ, Omidbaigi R, Sefidkon F (2005) The effect of different harvest stages on the essential oil of Ajowan (*Trachysperum ammi* Sprague) cultivated in Iran. *Journal of Essential Oil-Bearing Plants* **8**, 300-303

Ulubelen A, Topcu G, Eris C (1994) Terpenoids from *Salvia sclarea*. *Phytochemistry* **36**, 971-974