ESSENTIAL OIL VARIATION OF *Salvia officinalis* AERIAL PARTS DURING ITS PHENOLOGICAL CYCLE

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In this paper the variation in the quantity and quality of the essential oil of Salvia officinalis *during its life cycle stages is reported. The oils were obtained by hydrodistillation of air-dried samples. The yield of essential oil (w/w %) in different stages was in the order: floral budding (0.9%) > vegetative (0.7%) > flowering (0.5%) > immature fruit (0.4%) > ripen fruit (0.2%). The essential oils were analyzed by GC and GC-MS. In total, 36, 41, 40, 38, and 41 constituents were identified and quantified in the subsequent stages, respectively. Oxygenated monoterpenes were the main group of compounds in the fruiting set (56.9%), vegetative (48.5%), flowering (47.7%), and floral budding (45.3%) stage. 1,8-cineole as one of the major constituents of all samples was lower in the vegetative stage and gradually increased in subsequent harvesting times to reach a maximum in flowering and then decreased in the fruiting set. In contrast, the globulol content was higher in the first stage and decreased drastically during fruit maturation.*

Key words: *Salvia officinalis* L., Lamiaceae, phenological cycle, essential oil variation.

The genus *Salvia* L. (sage) of the family Lamiaceae comprises nearly 900 species spread widely throughout the world, which correspondingly display marked morphological and genetic variation according to their geographical origin [1–3]. *Salvia officinalis* L., with the common Persian name Maryamgoli, is a perennial woody sub-shrub native to the Mediterranean region that is now extensively cultivated all over the world [4–6]. Sage is well known as a common medicinal and aromatic plant widely used in food, perfumery, and herbal products [7, 8]. Various Iranian folk traditions have used the plant for treatment of several gastrointestinal disorders, infected wounds, and skin disorders [9]. *S. officinalis* essential oil is applied in the treatment of a large range of diseases such as those of the nervous system, heart and blood circulation, and respiratory [10, 11].

The chemical composition of *Salvia* spp. depends largely on the species from which it is obtained [1]. Within the *Salvia* species, the essential oil composition differs significantly depending on the individual genetic variability, different plant parts, and developmental stages [12]. The presence and concentration of certain chemical constituents also fluctuates according to the season, climatic condition, and the site of plant growth [13].

Most of the *Salvia* species have been studied for their fragrances, but *S. officinalis* is one of the most commercially important of these plants. Good quality sage oils contain a high percentage (> 50%) of the epimeric α- and β-thujones and a low proportion (<20%) of camphor [14]. Manool is usually found in small quantities (0.1–5.9%) in *Salvia officinalis* essential oils [15, 16]. However, it was found in relatively high amounts (14.7%) in the plant originating from Cuba [17]. A variety of sesquiterpenes and a diterpene are also found in Dalmatian sage oils [18, 19]. It seemed likely that different developmental stages of *S. officinalis* would have different oil compositions, because *S. sclarea* L. (clary sage) contained different mixtures of volatile components from leaves than the flowering parts [18]. To the best of our knowledge there is no report on the essential oil analysis of *S. officinalis* during its phenological cycle; therefore, in this study we report the variation of the essential oil composition from the aerial parts of the plant harvested at different developmental stages.

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TABLE 1. Essential Oil Composition of *Salvia officinalis* L. During Its Phonological Cycle

Compounds	RI	Vegetative	Floral budding	Flowering	Fruiting set	
					Immature	Ripen
Tricyclene	926	0.1	0.2	0.3	0.2	0.3
α-Pinene*	935	2.7	3.1	4.6	3.3	3.5
Camphene	949	3.4	2.5	4.7	4	1.6
Sabinene	970	Tr.	Tr.	Tr.	Tr.	0.3
β-Pinene*	977	8	12.3	16.4	10.5	7.1
Myrcene	982	0.5	0.6	0.7	0.5	Tr.
α-Terpinene	101	0.2	0.1	Tr.	-	0.2
p-Cymene	101	-	Tr.	Tr.	Tr.	0.2
(*E*)-Ocimene	103	1.3	1	0.9	0.6	-
γ-Terpinene*	105	0.3	0.2	0.2	0.1	0.5
Terpineolene	108	0.1	Tr.	Tr.	0.1	0.2
1,8-Cineole*	102	16.8	19.3	22.3	19.5	15.3
trans-Sabinene hydrate	105	0.3	0.3	0.4	Tr.	-
Linalool	108	Tr.	0.1	0.1	Tr.	0.3
α-Thujone	109	12.8	13.2	9.1	9.5	25.1
β-Thujone	110	1.1	2.7	2.1	1.1	5.0
α-Campholenal	111	1.1	-	-	-	-
Camphor	113	7.1	2.1	1.6	2.8	6.3
Borneol*	116	8.4	6.9	11	14.6	3.5
4-Terpineol	116	0.4	0.4	0.8	1.3	0.6
α-Terpineol	118	0.2	0.1	0.1	0.2	0.2
Myrtenol	118	0.1	0.1	Tr.	-	-
Isobornyl acetate	127	0.2	0.1	0.2	0.3	0.6
α-Cubebene	135	-	0.1	0.1	Tr.	0.1
α-Ylangene	137	-	Tr.	Tr.	-	Tr.
α-Copaene	138	-	0.1	0.1	Tr.	0.2
β-Bourbonene	139	-	Tr.	Tr.	Tr.	0.1
β-Caryophyllene*	142	10.5	10.6	4.9	3.6	7.3
β-Cedrene	143	-	0.1	0.1	Tr.	0.2
(*E*)-α-Bergamotene	144	0.1	0.1	Tr.	0.1	0.6
α-Humulene	146	8.2	9.2	8.7	12.1	11.6
γ-Gurjunene	147	0.1	0.1	Tr.	-	Tr.
γ-Muurolene	147	Tr.	0.3	0.4	0.2	0.4
Germacrene-D	148	Tr.	0.1	0.1	Tr.	Tr.
γ-Elemene	150	0.1	0.1	0.1	0.1	0.5
γ-Cadinene	151	Tr.	0.1	0.2	0.1	0.2
δ-Cadinene	152	Tr.	0.4	0.4	0.2	0.5
α-Calacorene	153	-	-	-	-	0.1
Spathulenol	157	-	-	-	-	0.1
Caryophyllene oxide*	158	0.5	0.3	Tr.	-	0.5
Globulol	159	10.4	8.1	6.4	10	2.6
Citronellyl propionate	160	0.6	0.4	0.3	0.1	0.6
β-Eudesmol	165	Tr.	-	-	Tr.	-
(Z)-α-Santalyl acetate	179	-	Tr.	Tr.	Tr.	0.1
Manool	203	2.6	2.2	1.4	2.1	1.8
Total		98.2	97.6	98.7	97.2	98.3

All compounds were identified by the method of retention indices relative to C_6-C_{24} *n*-alkanes on the DB-1 column and mass spectrum.

*Coinjection with an authentic sample.

Tr.: trace <0.1%.

TABLE 2. Compound-Class Composition of the Essential Oil of *Salvia officinalis* During Its Phenological Cycle

Compound class	Content, %				
	Vegetative	Floral budding	Flowering	Fruiting set	
				Immature fruit	Ripen fruit
Monoterpene hydrocarbons	16.6	20	27.8	19.3	13.9
Oxygenated monoterpenes	48.5	45.3	47.7	49.3	56.9
Sesquiterpene hydrocarbons	19	21.3	15.1	16.4	21.7
Oxygenated sesquiterpenes	14.1	11	8.1	12.2	5.8
Total	98.2	97.6	98.7	97.2	98.3

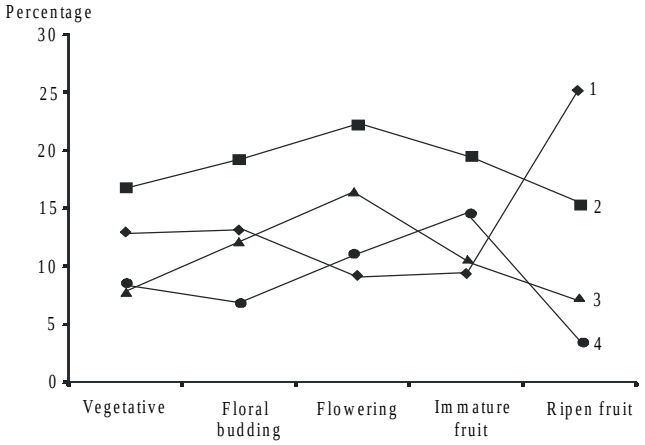

Fig. 1. Major compound variation of *Salvia officinalis* essential oil during phenological cycle; 1 - α-thujone, 2 - 1,8-cineole, 3 - β-pinene, 4 - borneol.

The yield of essential oil (w/w %) based on the dry weight of the plant in different stages was in the order: floral budding (0.9%) > vegetative (0.7%) > flowering (0.5%) > immature fruit (0.4%) > ripen fruit (0.2%). As can be seen, the amount of essential oil is at its highest level at the floral budding stage where the oil is intensively biosynthesized, and decreased gradually at the fruiting phase, as observed in other plant species [20–22]. The percentage compositions of the essential oils are listed in Table 1 along with the retention indices of the identified compounds, where all constituents are arranged in the order of their elution on the DB-1 column. A comparison of the composition of the essential oils during the mentioned stages revealed both quantitative and qualitative differences. In total, 36, 41, 40, 38, and 41 constituents were identified and quantified in the vegetative, floral budding, flowering, immature fruit, and ripen fruit stages, representing 98.2, 97.6, 98.7, 97.2, and 98.3% of the total oil, respectively. Twenty-eight compounds were common in all of the samples and represented from the lowest amount in the ripen fruit stage (89.6%) to the highest level in the flowering phase (97.2%).

The main constituents identified and their percentages range were as follow: α-thujone (9.1–25.1%), 1,8-cineole (15.3–22.3%), β-pinene (7.1–16.4%), borneol (3.5–11%), β-caryophyllene (0.1–10.6%), globulol (2.6–10.4%), and α-humulene (0.6–9.2%).

As can be seen in Fig. 1, the quantity of the major compound of *S. officinalis* oil was changed during the growth cycle, e.g., α-thujone increased drastically at the ripen fruit phase, while the 1,8-cineole and β-pinene percentages increased gradually from the vegetative to the flowering stage and then decreased at the fruiting set. The total pinene percentage was lower in the vegetative phase and increased gradually in subsequent harvests to reach a maximum in the flowering stage, whereas the total thujone was higher in the essential oil of the ripen fruit phase.

Maximum *trans*-ocimene (1.3%) and camphor (7.1%) percentages in essential oils were observed at the vegetative stage, whereas the highest amount of borneol (11%) was found in the oil of the flowering phase. In the present study 1,8-cineole was the main compound during the vegetative to the flowering stage (16.8 to 22.3%), whereas the major compound of ripen

fruit oil changed to α-thujone at the last phenological stage. α-Campholenal (1.1%) was only found in the oil of the vegetative stage. In contrast, *p*-cymene, α-cubebene, α-copaene, and β-bourbonene were not detected in the oil of the vegetative phase. Borneol and α-humulene increased during fruit formation, whereas camphene, β-caryophyllene, and globulol decreased to the lowest percentage in the oil of this stage. In an earlier report on the oil composition of *S. officinalis* cultivated in two different sites of Portugal [5], globulol was not found, while in our study this compound was detected in the amount of 10.4% at the vegetative stage. The classification of the identified compounds based on functional groups is summarized in Table 2. As can be seen, oxygenated monoterpenes were the main group at different phenological stages, reaching the maximum amount of 56.9% in ripen fruit oil. In conclusion, our results showed that the variation in the *S. officinalis* oil may be linked, in part, to different developmental stages and harvesting times along with other parameters such as edaphic and climatic factors, geographic origin, and cultivation site.

EXPERIMENTAL

Plant Material and Isolation Procedure. This experiment was conducted during 2001–2004 at the field of Medicinal Plants and Drugs Research Institute of Shahid Beheshti University located in Evin (35°48′ 285″N, 51° 23′ 494″E and altitude 1785 m) in the North of Tehran, Iran. *S. officinalis* L. seeds obtained from the seed bank of Medicinal Plants and Natural Products Research Institute, Iranian Academic Center for Education, Culture, and Research (ACECR) and were sown in the greenhouse (25–30°C) in the last week of February 2001. Nine-week-old seedlings were transplanted at 50 cm row-to-row and 30 cm plant-to-plant spacing in the experimental field in May 2001. The aerial parts were harvested early in the morning from a 3-year-old cultivated population by randomized collection of 10 individuals for each development stage. For collection at the vegetative stage, only shoots with leaves and without floral buds were harvested. For the floral budding stage, only shoots with floral buds and some young flowers were collected. At the flowering stage, the entire flower on the shoot was opened and, in some case, the ovary of the flower was already fecundated but was still green. The samples of the fruiting stage were collected at two different times of fruit maturation, i.e., immature (shoots with young fruits 15 days after flowering) and ripen (shoots with brownish achenes just in deciduous time). Plant material was taken immediately to the laboratory to be dried at ambient temperature. A voucher specimen (MP-816) was deposited at the Medicinal Plants and Drugs Research Institute Herbarium of Shahid Beheshti University.

The essential oil of air-dried samples (100 g) of each stage was isolated by hydrodistillation for 3 h using a Clevenger-type apparatus. The distillated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until the analysis. The oils had a light yellow color with a distinct sharp odor.

Oil Analysis Procedure. GC analysis was performed using a Thermoquest gas chromatograph with a flame ionization detector (FID). The analysis was carried out using a fused silica capillary DB-1 column (60 m $\times$ 0.25 mm i.d.; film thickness 0.25 μm). The injector and detector temperatures were kept at 250°C and 300°C, respectively. Nitrogen was used as carrier gas a flow rate of 1 mL/min; oven temperature programme was 60–250°C at the rate of 5°C/min, and finally held isothermally for 10 min.

GC-MS analysis was performed using a Thermoquest-Finnigan gas chromatograph equipped with the same column, coupled with a TRACE mass ion trap detector. Helium was used as carrier gas with an ionization voltage of 70 eV. Ion source and interface temperatures were 200°C and 250°C, respectively. Mass range was from 43 to 456 *m/z*. Gas chromatographic conditions were as given for GC.

Identification of Compounds. The constituents of the essential oils were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes (C_6–C_{24}) and the oil on a DB-1 column under the same chromatographic 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 [23]. For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

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