

The Effect of Cuttings Stages on Components and Content of Essential Oils from *Salvia viridis* L.

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ABSTRACT

Salvia viridis L. is annual herb which belongs to Lamiaceae family and is distributed particularly in Mediterranean region. This study was conducted to evaluate the effects of three different cutting stages on its components and essential oil ratio from aerial parts of *S. viridis*. The seedlings obtained from the seeds of wild-growing *S. viridis* transplanted to the experimental area. Plants were harvested in three different stages (the beginning of flowering, 50% of flowering and full flowering). The essential oils were obtained by hydrodistillation and analyzed by GC and GC/MS. The essential oil ratio ranged from 0.023% (the full flowering stage) to 0.130% (the beginning of flowering stage). According to the results of analysis, β -caryophyllene, germacrene D and caryophyllene oxide were recorded as major components in the essential oils from the different cutting stages. The results of our study showed differences in the content and chemical composition of the essential oil from *S. viridis* depending on the developing stages of the plant harvested.

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Salvia viridis L.'den Elde Edilen Uçucu Yağın Miktarı ve Bileşenleri Üzerine Biçim Zamanlarının Etkisi

ÖZET

Salvia viridis L. Lamiaceae familyasından tek yıllık bir bitki olup, özellikle Akdeniz Bölgesinde yayılış göstermektedir. Bu çalışma, *S. viridis*'in toprak üstü aksamından elde edilen uçucu yağın oranı ve bileşenleri üzerine üç farklı biçim döneminin etkilerini değerlendirmek için yürütülmüştür. Doğal ortamda yetişen *S. viridis* tohumlarından elde edilen fideler deneme alanına dikilmiştir. Bitkiler üç farklı dönemde (çiçeklenme başlangıcı, %50 çiçeklenme ve tam çiçeklenme) hasat edilmiştir. Su distilasyonu ile elde edilen uçucu yağlar GC ve GC/MS ile analiz edilmiştir. Uçucu yağ oranı %0.023 (tam çiçeklenme dönemi) ile %0.130 (çiçeklenme başlangıcı dönemi) arasında değişmiştir. Analiz sonuçlarına göre, β -caryophyllene, germacrene D ve caryophyllene oxide farklı biçim dönemlerinden elde edilen uçucu yağlarda ana bileşenler olarak kaydedilmiştir. Bu çalışmanın sonuçları *S. viridis*'den elde edilen uçucu yağın miktarı ve kimyasal kompozisyonundaki farklılıkların hasat edilen bitkinin gelişim dönemlerine bağlı olduğunu göstermiştir.

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INTRODUCTION

Salvia L. (or Sage) belonging to the Lamiaceae family has almost 1000 species which spread all around the world. Different *Salvia* species have been known as an important medicinal and culinary herb since ancient times. There are 97 species of *Salvia* in Turkey, and 47 of these species are endemic (Davis,

1982; Dweck, 2000; Ozdemir et. al., 2009). One of them is *Salvia viridis* L. syn. *S. horminum* L. (annual clary, bluebeard, Joseph sage, painted sage). This species is distributed particularly in the Mediterranean region. It is mainly distributed rocky slopes, sand dunes, fields and arid lands.

It is an annual herb with simple or branched stems, simple leaves, and lilac-purple to white corolla. It is in flower from March to July. The parts used of *S. viridis* are leaves, flowering spikes, seeds, and oil (Bown, 2002). This species having a long flowering period has been cultivated as an ornamental plant in Britain. Also, it is used as cut flowers and dried flowers (Pogroszewska and Laskowska, 2008). Its flowering spikes are used as folk medicine in Anatolian (Baytop, 1984). The leaves and seeds of and the essential oil from this species have been used to increase the quality of liquor, and flavor certain wines and beers. In addition, it is known that it has an important potential honey production.

The essential oils from many *Salvia* species contain the compounds of the terpene class such as α - and β -pinene, camphor, phelladrene, cineol and bornyl acetate as main components. *S. viridis* is very rich in pinenes, and β -pinene (32.5%) and α -humulene (15.3%) were main compounds (Kokkalou et al., 1982). Similarly, a tri-terpenic alcohol 2 β , 3 β -dihydroxyolean-13(18)-en were isolated in the essential oil from the aerial parts of *S. viridis* (Dweck, 2000; Abdallah et al., 2013). The essential oils yield from fresh flower, leaf and stem parts of *S. viridis* were recorded as 0.28%, 0.17% and 0.12% (v/w), respectively. The major components of the essential oils were identified trans-muurola-4(14),5-diene (18.5%), myrcene (17.2%), β -copaene (12.6%), δ -3-carene (5.1%) and β -bourbonene (5.0%) in the flower, β -pinene (26.4%), β -copaene (13.3%), trans-muurola-4(14),5-diene (9.0%), zonarene (3.8%) and α -humulene (3.6%) in the leaf, and germacrene D (16.0%), palmitic acid (11.4%), (E)-caryophyllene (9.8%), caryophyllene oxide (7.3%) and δ -cadinene (5.7%) in the stem. The eudesmenol sesquiterpenoid intermedeol has been recorded in small concentrations in the floral essential oil of this species (Yayli et al., 2010). In addition, α -amirin, β -amirin

and olean-(13)18-en-2 β , 3 β -diol involved in essential oil components (Ucar, 2014).

Essential oils are used in many fields, such as food, medicine, drug, cosmetics, and perfumes. The qualities in essential oil-bearing plants are determined by their essential oil content and composition (Zawiślak, 2013). The content and composition of essential oil obtained from these plants vary depending on several factors, such as cultivation area, climatic conditions, genetic modification, different plant parts, developmental stages, and harvesting time. Because these factors affect the biosynthetic pathways of plant, the proportion of essential oil components varies (Lakušić et al., 2013).

The aim of this study was to determine the effect of different developing stages on essential oil content and composition of the oil from *S. viridis* L.

MATERIAL and METHODS

This research was carried out at the experimental area of Mudurnu S.A. Vocational Higher School of Abant İzzet Baysal University (Mudurnu-Bolu/Turkey) in 2010-2011. The soil characteristics of experimental area were determined as clay and loam, water saturation of 51.7%, total salt 0.09%, pH 7.25, lime 49.5%, phosphorus 148.6 kg ha⁻¹, potassium 537.3 kg ha⁻¹ and, organic matter 1.36% by Bolu Directorate of Provincial Food Agriculture and Livestock. According to data from Turkish State Meteorological Service, total rainfall, mean relative humidity, and temperature were recorded as 754.5 and 487.0 mm, 12.8 and 10.2 °C, and 75.1 and 77.0% in 2010 and 2011, respectively (Table 1).

Compared to the previous year, the amount of rainfall recorded in 2011 was very low. This situation affected adversely the plant growth. The seeds of wild-growing *S. viridis* L. were collected from Seben district of Bolu province (40° 22.580' N. 31° 36.723' E, 690 m, 09.07.2009).

Table 1. Monthly rainfall, average temperature and relative humidity values recorded in the experimental area during 2010-2011 years and in long term.

Months	Rainfall (mm)			Average temperature (°C)			Relative humidity (%)		
	Long term	2010	2011	Long term	2010	2011	Long term	2010	2011
January	55.7	52.7	31.6	1.0	3.1	1.9	77.2	83.3	88.7
February	44.2	108.7	14.2	1.9	5.9	2.6	74.1	79.4	80.4
March	45.6	66.0	60.5	4.9	6.6	5.0	70.9	76.2	78.7
April	50.5	64.3	84.5	9.8	10.3	7.7	68.7	75.1	83.5
May	59.5	43.7	67.6	13.9	15.5	13.5	70.7	67.6	80.4
June	47.2	118.5	73.0	17.4	18.5	17.3	70.4	77.9	76.8
July	33.1	44.7	14.2	19.7	21.8	21.8	69.8	73.3	68.3
August	27.6	4.5	7.2	19.7	24.0	19.4	69.8	64.8	71.0
September	24.5	27.2	14.1	16.0	18.1	17.2	70.7	74.9	66.7
October	45.5	136.0	62.8	11.7	11.0	10.0	74.4	84.2	74.9
November	48.5	15.7	5.2	6.5	11.8	2.5	75.3	65.3	76.9
December	60.5	72.5	52.1	2.8	6.6	3.1	77.8	79.4	77.2

The seeds were sown at a depth of 1-2 cm in plastic cases containing peat. On reaching an adequate height of average 10-15 cm average 2 months after sowing in the greenhouse, the seedlings were transplanted to the experimental area. The trial was a randomized complete block design with three replications. In sowing, row width and interrow spacing were 60 cm and 40 cm, respectively, and plot size was 14.4 m². Firstly, plants were irrigated with a hose daily for the first two weeks. Later, after each cuttings, when needed the irrigation were applied. Weeding was done manually and no fertilizer application was made on the experimental area. Plants were harvested in three different stages; the beginning of flowering (BF-at the beginning of June), in 50% of flowering (50% F-in the middle of July), and the full flowering (FF-at the beginning of August). The plants were cut at a height of about 10 cm above ground. Two cuttings and one cutting were taken from *S. viridis* in 2010 and 2011, respectively. In 2011, the second cutting could not be made because the plants did not grow enough after the first cutting.

Essential Oil Analysis

After each harvest, the aerial parts or herbage of the plants were dried in the shade at room temperature. Average 50 g of grounded dried plant materials was extracted using a Clevenger-type apparatus for 3 h in 700 ml water. The result data (% , v/w) were calculated as volume of essential oils per 50 g of plant dry matter.

Gas Chromatographic-Mass Spectrometric Analysis of Essential Oil

The chemical composition of the essential oils investigated was determined using a Hewlett Packard 6890 N GC, equipped with a capillary column HP 5MS (30 m x 0.25 mm x 0.25 µm film thickness), a Hewlett Packard 5973 mass selective

and FID detectors. The electron ionization energy of 70eV for GC/MS detection and He (1mL min⁻¹) as the carrier gas was used. The temperatures of the injector and detector were set at 220 °C and 290 °C, respectively. The temperature of the column was initially set at 50 °C for 30 min, and then increased gradually to 150 °C at a 3 °C min⁻¹rate, held for 10 min, and finally reached to 250 °C. Diluted samples (1/100 in acetone, v v⁻¹) of 1.0 µL were injected automatically at 250 °C, and in spitless mode. The chemical composition of the essential oils was identified by matching their retention times and mass spectra with those obtained from the libraries of Wiley, NIST and Flavor's spectral and literature data. Relative percentages of the separated chemical components were calculated using FID chromatograms.

Statistical Analysis

The results obtained from essential oil analysis were expressed as the means of three replications. All data were processed by analysis of variance (ANOVA), and the means were compared with LSD (Least Significant Difference). The statistical analysis was performed using TARIST software program (Acıkgöz et. al., 2010).

RESULT and DISCUSSION

The essential oil contents and components identified in herbage of the plants are listed in Table 2 and 3 together with their relative percentages, in order of their retention indices. The essential oil ratio ranged from 0.023 to 0.130% on the dry weight basis depend different cutting stages.

The differences among essential oils from the first cutting of 2010 year and 2011 year were significant (<0.05, <0.01) (Table 2). Essential oil content from aerial parts of *S. viridis* of 0.1% and 0.27% was recorded by Demirci, et. al., (2002) and Ozek, et. al., (2010), respectively.

Table 2. Mean content of essential oil extracted using a Clevenger-type apparatus in the different developing stages of *S. viridis* L. (2010 and 2011 years).

Essential Oil Content (% of dry weight)					
Developing Stages (DS)	2010- Cutting (C)	First 2010- Cutting (C)	Second Cutting	Average	2011-First Cutting
BF	0.130 ^{a*}	0.043 ^a		0.087 ^a	0.050 ^a
50%F	0.100 ^b	0.030 ^a		0.065 ^b	0.027 ^b
FF	0.050 ^c	0.027 ^a		0.038 ^c	0.023 ^b
Average	0.093 ^a	0.033 ^b			
LSD (0.05)	DS X C: 0.010				DS:0.009

The essential oil contents followed by the same letter within each column are not significantly different
*Significant at $p \leq 0.05$

Table 3. Chemical components of the hydro-distilled essential oils from the dried aerial parts of *S. viridis* L. harvested in the three different stages (%).

Components	RT	Percent of the essential oil components			
		2010		2011	
		First Cutting	Second Cutting	First Cutting	
α -pinene	9.83	BF	-	1.89	-
		50%F	-	1.37	-
		FF	-	-	-
sabinene	11.55	BF	-	-	-
		50%F	-	5.28	-
		FF	-	-	-
β -pinene	11.67	BF	2.62	-	-
		50%F	0.89	8.77	1.06
		FF	2.69	6.10	2.97
limonene	14.03	BF	-	-	-
		50%F	-	-	-
		FF	2.65	-	-
α -cubebene	28.87	BF	0.66	-	-
		50%F	-	-	-
		FF	-	-	-
α -copaene	29.96	BF	1.44	4.34	3.07
		50%F	-	-	1.98
		FF	-	1.13	1.08
β -bourbonene	30.35	BF	2.39	2.93	4.20
		50%F	2.40	6.25	5.78
		FF	8.05	9.47	3.97
β -caryophyllene	31.78	BF	19.04	11.39	27.08
		50%F	5.55	7.44	17.64
		FF	12.44	23.26	22.51
β -cubebene	32.25	BF	1.17	1.85	-
		50%F	-	-	1.37
		FF	-	0.63	1.16
α -humulene	33.20	BF	6.28	4.60	8.34
		50%F	1.29	2.05	6.03
		FF	3.95	7.50	6.18
α -amorphene	34.22	BF	13.83	1.13	2.12
		50%F	2.38	5.78	3.30
		FF	9.37	5.48	1.88
germacrene D	34.35	BF	11.01	6.65	17.01
		50%F	4.06	8.72	11.68
		FF	6.96	14.13	29.04
δ -muurolene	34.89	BF	4.02	-	2.15
		50%F	3.27	-	2.29
		FF	-	-	-
bicyclogermacrene	34.99	BF	-	1.56	1.92
		50%F	-	4.64	-
		FF	-	0.69	3.67
α -muurolene	35.15	BF	1.66	1.36	-
		50%F	-	-	-
		FF	-	-	-
δ -cadinene	36.06	BF	8.86	1.36	2.15
		50%F	3.10	3.11	3.25
		FF	4.63	6.48	2.54
germacrene B	37.37	BF	2.67	-	-
		50%F	2.70	4.65	-
		FF	3.33	-	1.88
spathulenol	38.17	BF	-	8.59	1.67

		50%F	-	7.47	1.48
		FF	-	1.13	1.57
caryophyllene oxide	38.36	BF	10.50	31.30	21.53
		50%F	20.47	15.35	26.11
		FF	19.14	20.79	8.41
β -selinene	40.22	BF	-	-	-
		50%F	2.14	-	-
		FF	-	-	-
α -cadinol	40.35	BF	-	-	-
		50%F	11.00	2.93	-
		FF	5.85	-	-
caryophyllenol	40.74	BF	-	-	-
		50%F	5.91	2.20	-
		FF	2.87	-	-
2-pentadecanone	43.62	BF	0.64	2.33	-
		50%F	1.64	1.19	1.02
		FF	2.30	1.03	-
Total		BF	85.62	81.28	91.24
		50%F	66.80	87.20	82.99
		FF	84.23	97.82	86.86

RT= Retention Time; BF= the Beginning of Flowering; 50%F= the 50% of Flowering; FF= the Full Flowering; - = not detected.

In a study carried out by Abdallah, et. al., (2013), the essential oil content obtained from the fresh and dried aerial parts of *S. viridis* was 1.2% and 0.8%, respectively (Abdallah et. al., 2013). Also, the essential oils ratios from fresh flower, leaf and stem parts of *S. viridis* were recorded as 0.28%, 0.17% and 0.12% (v/w), respectively (Yayli et al., 2010). These results are consistent with our findings. According to the average of three cuttings, the essential oil ratio was in the order: BF (0.069%) > 50% F (0.046%) > FF (0.031%). Essential oil ratio was affected by cutting stages. Amount of essential oils from the first cutting in 2010 year were higher than the others cuttings (Table 2). The yield of essential oil of *S. officinalis* harvested in the different stages was recorded as 0.9% in the floral budding, 0.7% in the vegetative, 0.5% in the flowering, 0.4% in the immature fruit and 0.2% in the ripen fruit (Mirjalili et. al., 2006). Amiri, (2007) stated that the yield of essential oil obtained by hydrodistillation from *S. bracteata* were 0.57%, 0.3% and 0.2% in pre-flowering, flowering, and post flowering stages, respectively. Also, Rayouf, et. al., (2013) recorded the essential oil from the aerial parts of *S. argentea* depending on at vegetative, full flowering, and fruiting stages, and the highest content of essential oil (0.15%) was obtained at full flowering (Rayouf et. al., 2013). Twenty-three components were identified in *S. viridis* essential oil. The thirteen having 5% or higher proportion in the total essential oil were recorded as main components. β -caryophyllene, germacrene D, and caryophyllene oxide were the first three components with the highest value. The percentage of β -caryophyllene and germacrene D in 2010 were lower than in 2011. The content of caryophyllene oxide in the essential oil

obtained in 2010 was higher than 2011. Also, the highest value of β -caryophyllene, germacrene D and caryophyllene oxide were obtained from FF stage, FF stage and BF stage of second cutting, respectively in 2010, and BF stage, FF stage and 50% F stage, respectively in 2011 (Table 3). β -caryophyllene has several biological activities such as anti-microbial, anti-oxidant and anti-carcinogenic (Kuwahata et. al., 2012), caryophyllene oxide exhibits anti-inflammatory and anti-carcinogenic activities (Yang et. al., 1999), and germacrene D has insecticidal properties (Nandi, 2012). It was reported that the qualitative and quantitative changes in the essential oil composition of *S. officinalis*, *S. fruticosa* and *S. sclarea* during stages of inflorescence maturity (Pitarevic et al., 1984; Müller-Riebau et al., 1997; Lattoo et al., 2006).

Although there are numerous investigations on essential oil content and composition of sage of commonly used and economically important species such as *S. officinalis*, *S. tomentosa* and *S. fruticosa*, there is limited research on the other species (for example, *S. viridis*). In a study by Yayli, et. al., (2010), the major components of the oils from *S. viridis* were β -pinene (26.4%) in leaf, trans-muurolo-4(14),5-diene (18.5%) in flower, and germacrene D (16.0%) in stem. α -cadinene (11.4%), β -pinene (9.7%), trans-isolimonene (6.0%), α -phellandrene (2.9%), 4-terpineol (3.6%) and thymol (2.7%) were detected as the main components in the oil from the dried aerial parts of *S. horminum* was reported by Abdallah, et. al., (2013). The above-mentioned authors recorded that β -pinene was the major component of *S. viridis* essential oil (9.7-26.4% of the total oil). In our study, the content of β -pinene in the essential oils varied depending on the cutting stages. The highest rate of β -pinene was

obtained from *S. viridis* plants in the 50% F stage (the second cutting) in 2010 and FF stage in 2011 (Table 3). Compared to other sage species, there is less research on *S. viridis*. When the results of previous researches together with our findings are generally, it is observed that the essential oil from *S. viridis* is rich in pinens, and β -pinene, α -humulene, trans-muuro-4(14),5-diene, myrecene, β -copaene, germacrene D, (E)-caryophyllene and caryophyllene oxide were recorded main compounds (Kokkalou et al., 1982; Yayli et al., 2010; Abdallah et al., 2013).

It is known that several factors (genotype, different plant parts, plant growth stage, environmental factors, area of plant growth, harvest time etc.) are affecting composition and content of essential oil from herbal plants (Mirjalili et al., 2006). The results of our study showed differences in the content and chemical composition of the essential oil from *S. viridis* depending on the developing stages of the plant harvested.

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