

RESEARCH ARTICLE

The chemical composition of *Salvia verticillata* L. subsp. *verticillata* from Turkey

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Abstract

Water-distilled essential oil was obtained from dried aerial parts of *Salvia verticillata* L. subsp. *verticillata* (Lamiaceae) from south eastern region of Turkey. The essential oil was analyzed simultaneously by GC-FID and GC-MS systems where 39 components were identified. Spathulenol (31.0%), α -pinene (8.2%), limonene (4.1%) and hexahydrofarnesyl acetone (3.8%) were found as the main constituents.

Keywords: *Salvia verticillata* subsp. *verticillata*, sage, Essential oil, Spathulenol, Sesquiterpene

Introduction

The genus *Salvia* L. (Lamiaceae) is represented by 98 species, four subspecies and three varieties, of which 56 are endemic in Turkey (Celep and Kahraman 2012). *Salvia* species are commonly used in Anatolia for colds, stomach aches, sore throats (Tabanca et al., 2006; Ozek et al., 2010; Askun et al., 2010), to treat inflammatory skin diseases, to stop bleeding or as an antiseptic for wounds (Suntar et al., 2011). The most well-known species, *S. fruticosa* Mill. (Syn: *S. triloba* L.), locally known as “adaçayı”, “elmaçayı”, is consumed as a hot tea and wildcrafted for sale in local markets in Turkey (Demirci et al., 2002; Askun et al., 2010; Suntar et al., 2011; Gurdal and Kultur, 2013). The essential oil of *S. fruticosa* is known as “elma yağı” in Turkey and has been reported to have carminative, stomachic, diuretic effects, to reduce sweating, and to treat foot infections (Demirci et al., 2002; Orhan & Aslan, 2009; Suntar et al., 2011). *Salvia* species have been traditionally used for various purposes in different parts of Turkey (Table 1). The decoction prepared from leaves and stems of *S. fruticosa* (“adaçayı”, “kara ot”) has been reportedly used for cold, tonsillitis, bronchitis, carminative, digestive and stomachache by Turkish migrants in Cologne, Germany (Pieroni et al., 205). The use of *S. fruticosa* for respiratory and digestive diseases are also extended to Cyprus (Gurdal & Kultur, 2013).

Salvia verticillata is morphologically quite close to *S. russellii* Bentham and differs from it with oblong to ovate leaves (not linear-oblong) and mucronate calyx teeth (Hedge, 1982). In the Flora of Turkey, *S. verticillata* was described as *S. uberrima* by Rechinger in 1941, later it was reduced to a synonym of *S. verticillata* subsp. *verticillata* (Hedge, 1982). The plant of Euro-Siberian origin is distributed in north and east part of Anatolia (Hedge 1982). *Salvia verticillata* L. has two subspecies (subsp. *amasiaca* (Freyn. & Bornm.) Bornm. and subsp. *verticillata*) and they differ from each other with leaf shape and indumentum structure (Hedge, 1982). Subsp.

verticillata is known as “dadırak” and subsp. *amasiaca* is locally called as “hart şalbası” in Turkey (www.bizimbittkiler.org.tr). Based on traditional literature in Turkish folk medicine, subsp. *amasiaca* is locally known as “yağlıkara” in Kayseri (central part of Turkey) (Sezik et al., 2001), “adaçayı” in Kırklareli (Thrace) (Kultur, 2007). In the Eastern part of Turkey, both subspecies are locally known as “Karabaş otu” and are used for cold and gastrointestinal disorders (Table 1) (Altundag & Ozturk, 2011).

Particular attention has been paid by our group to *Salvia* essential oils due to a wide range of chemical biodiversity (Baser, 2002). Therefore, in the present study, we have investigated the chemical composition of *S. verticillata* subsp. *verticillata* essential oil from Turkey.

Table 1. Traditional uses of *Salvia* species from Turkey

Species	Local Name/ Locality	Used parts	Preparations/ utilization method	Use	References
<i>S. absconditiflora</i> (Montbret & Aucher) Greuter & Burdet	Adaçayı/ Sivas & Yozgat: central Anatolia	WP	Dec or Inf, Int	Cold and sore throat	Ozudogru et al., 2011
<i>S. absconditiflora</i> (Montbret & Aucher) Greuter & Burdet	Öksürük otu/ Kahramanmaraş: southern-eastern Anatolia	L	Inf, Int	Cough and bronchitis	Demirci & Ozhatay, 2012
<i>S. absconditiflora</i> (Montbret & Aucher) Greuter & Burdet	Boz şabla, kara şabla, sarı şabla/ Niğde: central Anatolia	AP	Inf, Int	Cold	Ozdemir & Alpınar, 2015
<i>S. absconditiflora</i> (Montbret & Aucher) Greuter & Burdet	Kara ot, garaot, garaod/ Manisa: western Anatolia	AP F L	Inf, Lcw, Mx, Ca	Stomachache, cold, flu, bronchitis, asthma, herbal tea	Sargin et al., 2015
<i>S. aramiensis</i> Rech. Fil.	Adaçayı/ Antakya: southern Anatolia	L	Dec, oral	Bronchitis, cold, flu, antidiabetic	Guzel et al., 2015
<i>S. cadmica</i> Boiss.	Meryemana adaçayı/ Niğde: central Anatolia	AP	Crushed, Ext	Bleeding	Ozdemir & Alpınar, 2015
<i>S. candidissima</i> Vahl. subsp. <i>candidissima</i>	Galabor/ eastern Anatolia	L	Inf, Int	Cold	Altundag & Ozturk, 2011
<i>S. dichroantha</i> Stapf.	Yağlıkara/ Kayseri: central Anatolia	L	Inf, Int	Abdominal pain, stomachache	Sezik et al., 2001
<i>S. fruticosa</i> Mill.	Adaçayı, boşalba/ Canakkale: western Anatolia	L	Dec	Antiseptic, dyspepsia, cold, tonsillitis	Uysal et al., 2012
<i>S. fruticosa</i> Mill.	Adaçayı, almakeyik, almageyik/ Muğla: western Anatolia	L	Inf, Int	Stomachache, flatulence, cold, tonsillitis, laxative, antipyretic	Gurdal & Kultur, 2013
<i>S. hydrangea</i> DC.	Koçotu/ eastern Anatolia	Herb	Inf	Cold, diabetes, stomach disorders, antipyretic, emmenagogue	Altundag & Ozturk, 2011
<i>S. hypargeia</i> Fisch. & C.A. Mey.	Kök çayı/ Niğde: central Anatolia	R	Inf, Int	Cold	Ozdemir & Alpınar, 2015
<i>S. multicaulis</i> Vahl	Adaçayı/ Elazığ: eastern Anatolia	AP	Dec	Diabetes disease	Cakilcioglu & Turkoglu, 2010
<i>S. multicaulis</i> Vahl	Adaçayı/ eastern Anatolia	Herb	Dec, Int	Cold, antiinflammatory	Altundag & Ozturk 2011

Species	Local Name/ Locality	Used parts	Preparations/ utilization method	Use	References
<i>S. multicaulis</i> Vahl	Adaçayı, dağ çayı/ Malatya: eastern Anatolia	F, L	Dec, Int	Cold, flu, digestive, tonsillitis	Tetik et al., 2013
<i>S. multicaulis</i> Vahl	Ada çayı/ Elazığ: eastern Anatolia	AP	Dec, Dpt	Cold, flu	Hayta et al., 2014
<i>S. multicaulis</i> Vahl	Boz kulak, mavi-mor şabla/ Niğde: central Anatolia	AP	Inf, Int	Sedative	Ozdemir & Alpınar, 2015
<i>S. nemorosa</i> L.	Gemtaş/ eastern Anatolia	Herb	Pounded, Ext	Hemostatic	Altundag & Ozturk, 2011
<i>S. russellii</i> Benth	Şaplamaotu/ Niğde: central Anatolia	Herb	Dec, Inf, Int	Cold, catarrh	Sezik et al., 2001
<i>S. sclarea</i> L.	Dağ çayı/ eastern Anatolia	L	Inf, Int	Cold	Altundag & Ozturk, 2011
<i>S. sclarea</i> L.	Misk adaçayı, yağlı kara/ Niğde: central Anatolia	F B, L	As a spice, Int, Inf, Int	Digestive Diarrhea, sedative	Ozdemir & Alpınar, 2015
<i>S. sclarea</i> L.	Polaç/ Malatya: eastern Anatolia	AP	Raw	Antacid	Tetik et al., 2013
<i>S. tomentosa</i> Mill.	Şalba, şabla, boz şalba, boz sabla, borçağla/Isparta: southern Anatolia	L EO L L	Inf, Int Ext Dec, Ext Inf, Int Inf, Int	Stomachache Inflamed wounds Inflamed wounds Asthma Analgesic	Tuzlaci & Erol, 1999
<i>S. tomentosa</i> Mill.	Adaçayı/ Denizli: western Anatoli	AP AP	Dec Dec	As food Stomach diseases	Kargioglu et al., 2010
<i>S. tomentosa</i> Mill.	Boş yaprağı, adaçayı/ Balıkesir: western Anatolia	L	Inf, Int	Cold, flu, tonsillitis	Polat & Satil, 2012
<i>S. tomentosa</i> Mill.	Kurtluca otu, yakı otu/ Manisa: western Anatolia	L	Ext, Poul	Abdominal pain	Bulut & Tuzlaci, 2013
<i>S. tomentosa</i> Mill.	Yakı otu, yakı şablasi, yaka çalpası, şalpa/ Manisa: western Anatolia	L AP	Int, Mx, Ca, Ms, Bs, Grg	Angina, bronchitis, pharyngitis and laryngitis, cold and flu, gall bladder stones, diarrhea, sedative, insomnia, carminative, costiveness and intestinal spasm, dyspepsia, herbal tea	Sargin et al., 2015
<i>S. tomentosa</i> Mill.	Adaçayı, yara otu, buhur ağacı/ Antakya: southern Anatolia	L	Olea, Ext Inf, Int	This oleate is applied to wounds in muslin pad Infusions used for the treatment of stomach pain	Guzel et al., 2015
<i>S. verbenaca</i> L.	Şalba/ Kahramanmaraş: southern-eastern Anatolia	L	Inf, Ext	Fungal infections	Demirci & Ozhatay, 2012
<i>S. verticillata</i> L. subsp. <i>amasiaca</i> Bornm.	Yağlıkara/Kayseri: central Anatolia	L	Inf, Int	Abdominal pain, stomachache	Sezik et al., 2001
<i>S. verticillata</i> L. subsp. <i>amasiaca</i> Bornm.	Adaçayı /Kırklareli: Thrace	L	Dec, Int	Cardiovascular diseases	Kultur, 2007

<i>S. verticillata</i> L. subsp. <i>amasiaca</i> Bornm.	Karabaş otu/ eastern Anatolia	Herb	Dec, Inf, Int	Laxative, cold, nausea	Altundag & Ozturk, 2011
Species	Local Name/ Locality	Used parts	Preparations/ utilization method	Use	References
<i>S. verticillata</i> L. subsp. <i>verticillata</i> .	Karabaş otu/ eastern Anatolia	Herb	Dec, Int	Catarrh, cold, laxative	Altundag & Ozturk, 2011
<i>S. virgata</i> Jacq.	Ballıbaba/ Denizli: western Anatoli	AP	Dec	As food	Kargioglu et al., 2010
<i>S. virgata</i> Jacq.	Kazan karası/Sivas & Yozgat: central Anatolia	Stem	Era	As food	Ozudogru et al., 2011
<i>S. virgata</i> Jacq.	Yaban çayı/ Kars: eastern Anatolia	L Stem	Inf	Cardiac diseases	Gunes & Ozhayat, 2011
<i>Salvia viridis</i> L.	Adaçayı/ Denizli: western Anatolia	AP	Dec	As food	Kargioglu et al., 2010

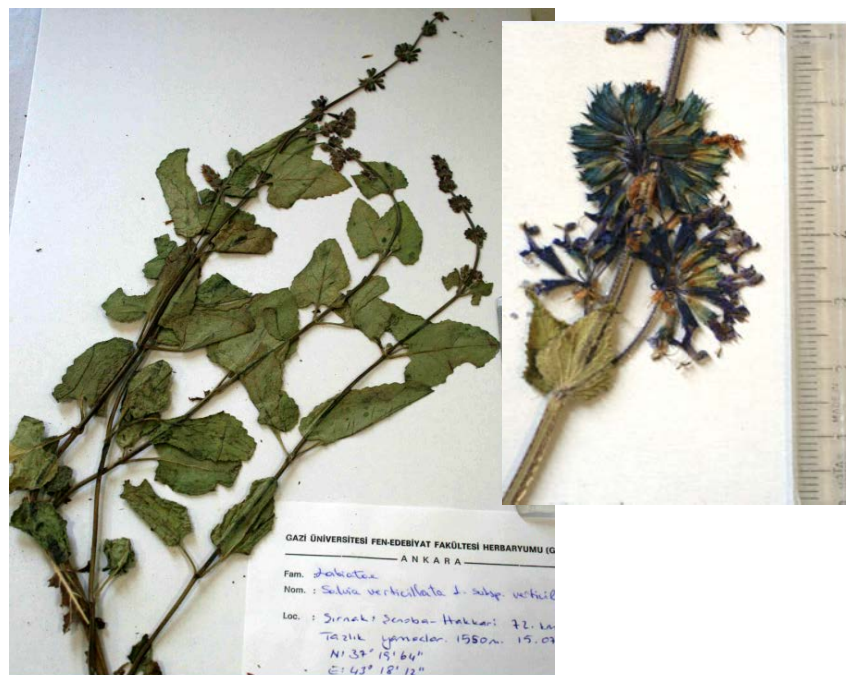
Plant parts used: AP: aerial parts; B: brances; EO: essential oils; F: flowers; L: leaves; R: roots; WP: whole parts; Preparations and utilization methods: Bs: skin bath; Ca: cataplasm; Dec: decoction; Dpt: drink one water glass of plant after meal; EO: essential oil; Era: fresh stems are eaten after peeling; Ext: externally; Grg: gargle; Inf: infusion; Int: Internally; Lcw: leaves are eaten by chewing;); Ms: mash; Mx: mixture (pickles, jam, salad, tzatziki, mixed paste, mixed ointments, mixed mash by adding other plants; Olea: oleate, *S. tomentosa* leaves are prepared by soaking into warmed olive oil and filtered; Poul: poultice

Materials and Methods

Plant material

The aerial parts of *S. verticillata* subsp. *verticillata* were collected while flowering from Şırnak: Şenobo to Hakkari, 72 km, 1550 m, rocky slopes, Aytaç 8194 et al. collected on July 15th, 2001. A voucher specimen has been deposited at the GAZI Herbarium in Ankara, Turkey.

Figure 1. Herbarium specimen of the plant material.



Isolation of the essential oil

The air-dried plant materials (flowers, leaves, and stems) were hydrodistilled for 3 hours using a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulfate and stored at 4 °C in the dark until analyzed. The oil yield was calculated as 0.05%, v/w on dry weight basis.

Gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) analysis conditions

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240 °C at a rate of 1 °C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450.

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analysis results are given in Table 2.

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention indices (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library) (McLafferty & Stauffer, 1989; König, Joulain, & Hochmuth, 2004)) and in-house “Başer Library of Essential Oil Constituents” built up by genuine compounds and components of known oils, as well as MS literature data (Joulain & König, 1998; ESO 2000, 1999) was used for the identification of oil components.

Results and Discussion

The chemical composition of the essential oil from aerial parts of *S. verticillata* subsp. *verticillata* was characterized by GC-FID and GC-MS analysis. The results are shown in Table 2. Thirty-nine components were identified representing 83.8% of the sample. The major components were spathulenol (31.0%), α -pinene (8.2%), limonene (4.1%) and hexahydrofarnesyl acetone (3.8%).

Literature survey indicated that the oil of *S. verticillata* consisted mainly of the sesquiterpenes β -caryophyllene, germacrene D and α -humulene (Table 3). The chemical composition of *S. verticillata* subsp. *verticillata* from Turkey has previously been investigated and germacrene D (10-16%) was reported as the main component (Baser, 2002). The other subspecies of *S. verticillata* essential oil, subsp. *amasiaca*, showed significant quantitative and qualitative differences. For example; β -pinene, α -pinene, β -phellandrene, limonene and 1,8-cineole were the main components in Bitlis samples (eastern part of Turkey) (Altun et al., 2007) while β -pinene, 1,8-cineole, α -copaene, α -gurjunene were the major components in another Bitlis sample (Askun et al., 2010). Other two samples of subsp. *amasiaca* from the central part of Turkey (Eskişehir and Sivas) displayed quite different chemical profiles (Table 3). In Eskişehir sample, hydrodistilled essential oil showed germacrene D, β -caryophyllene and hexadecenoic acid as major constituents (Kunduhoglu et al., 2011), whereas volatiles obtained by a thermal desorption-GC-MS technique gave a different profile for a Sivas sample with palmitic acid, 7-methyl-Z-tetradecen-1-ol-acetate, heptacosane as the main constituents (Hatipoglu et al., 2016).

Table 2. The chemical composition of essential oil of *S. verticillata* subsp. *verticillata*

RRI	Compound	%
1032	α-Pinene	8.2
1093	Hexanal	0.5
1118	β -Pinene	2.0
1174	Myrcene	1.4
1176	α -Phellandrene	0.6
1203	Limonene	4.1
1213	1,8-Cineole	2.5
1255	γ -Terpinene	1.1
1280	<i>p</i> -Cymene	1.0
1532	Camphor	0.6
1553	Linalool	2.2
1591	Bornyl acetate	0.3
1611	Terpinen-4-ol	0.6
1612	β -Caryophyllene	0.7
1670	<i>trans</i> -Pinocarveol	0.2
1706	α -Terpineol	1.0
1719	Borneol	0.4
1725	Verbenone	0.8
1751	Carvone	0.1
1758	<i>cis</i> -Piperitol	0.2
1772	Citronellol	0.6
2008	Caryophyllene oxide	1.9
2037	Salvial-4(14)-en-1-one	0.4
2071	Humulene epoxide-II	0.9
2098	Globulol	0.6
2104	Viridiflorol	0.6
2131	Hexahydrofarnesyl acetone	3.8
2144	Spathulenol	31.0
2187	T-Cadinol	0.7
2198	Thymol	0.4
2211	Clovenol	1.8
2239	Carvacrol	2.1
2255	α -Cadinol	0.6
2278	Torilenol	0.5
2324	Caryophylla-2(12),6(13)-dien-5 β -ol (=Caryophylladienol II)	1.8
2369	Eudesma-4(15),7-dien-4 β -ol	0.5
2389	Caryophylla-2(12),6-dien-5 β -ol (=Caryophyllenol I)	2.8
2392	Caryophylla-2(12),6-dien-5 β -ol (=Caryophyllenol II)	2.0
2931	Hexadecanoic acid	2.3
	Total	83.8

RRI Relative retention indices calculated against *n*-alkanes, % calculated from FID data, tr :Trace (< 0.1 %)

Table 3. Main components of *S. verticillata* essential oils based on the literature

Salvia Species	Main compounds	Plant Parts*	Extraction technique**	Country	References
<i>verticillata</i>	β -caryophyllene 24.7%, γ -muurolene 22.8%, limonene 8.9%, α -humulene 7.8%, germacrene B 6.6%	AP	SD	Iran	Sefidkon & Khajavi, 1999
<i>verticillata</i>	β -caryophyllene 13.3%, γ -muurolene 10.3%, <i>trans</i> -chrysanthenol 6.1%	AP	HD	Serbia	Chalchat et al., 2001
<i>verticillata</i> subsp. <i>amasiaca</i>	β -caryophyllene 17%	AP	HD	Turkey	Baser, 2002
<i>verticillata</i> subsp. <i>verticillata</i>	germacrene D 10-16%				
<i>verticillata</i>	β -pinene 30.7%, <i>p</i> -cymene 23.0%, lauric acid isopropyl ester 16.8%	AP	HD	Greece	Pitarokili et al., 2006
<i>verticillata</i>	germacrene D 48%, β -caryophyllene 13.4%, α -cadinol 10.4%, α -humulene 7.2% germacrene D 24.6%, β -caryophyllene 19%, bicyclogermacrene 16.7%, α -humulene 10.2% β -caryophyllene 10.2%, β -cubebene 8.6%, spathulenol 6.5%	AP	HD	Vrdnik, Serbia Rimski Sanac, Serbia Tara mount., Serbia	Krstic et al., 2006
<i>verticillata</i>	β -caryophyllene 31.5%, germacrene D 16.2%, limonene 15.5%, α -pinene 10.4%, α -humulene 9.4%	AP	HD	Iran	Yousefzadi et al., 2007
<i>verticillata</i> subsp. <i>amasiaca</i>	β -pinene 23.0%, α -pinene 21.6%, β -phellandrene 13%, limonene 11%, 1,8-cineole 10.9%	AP	HD	Bitlis, Turkey	Altun et al., 2007
<i>verticillata</i>	1,8-cineole 38.3%, camphor 23.0%	AP	HD	Iran	Forouzin et al., 2009
<i>verticillata</i>	α -pinene 10.7%, limonene 5.9%, camphor 5.2%	-	HS-GC-MS	Poland	Rzepa et al., 2009
<i>verticillata</i> subsp. <i>amasiaca</i>	β -pinene 21.4%, 1,8-cineole 16.1%, α -copaene 5.4%, α -gurjunene 4.6%	AP	HD	Bitlis, Turkey	Askun et al., 2010
<i>verticillata</i>	β -caryophyllene 65.3%, α -humulene 25.4%	AP	HD	Brumov-Bylnice-Czech Republic (CR)	Smekalova et al., 2010
	β -caryophyllene 64.5%, α -humulene 26.6%			Brezova, (CR)	
	β -caryophyllene 62.2%, α -humulene 25.2%			Suchovské Mlýny, (CR)	
	β -caryophyllene 64.0%, α -humulene 23.8%			Radobýl, (CR)	
	β -caryophyllene 58.3%, α -humulene 23.4%			Rydvaltice, (CR)	
	β -caryophyllene 68.5%, α -humulene 25.9%			Březina lom, (CR)	
	β -pinene 29%, β -caryophyllene 20.22%, α -humulene 15.6%, limonene 13.9%			Macošská stráň, (CR)	
<i>verticillata</i>	α -pinene, β -pinene, β -caryophyllene, caryophyllene oxide, germacrene D	-	-	Poland	Strzalka et al., 2011
<i>verticillata</i> subsp. <i>amasiaca</i>	germacrene D 36.6%, β -caryophyllene 7.6%, hexadecenoic acid 6.7%	AP	HD	Eskişehir, Turkey	Kunduhoglu et al., 2011

<i>Salvia</i> Species	Main compounds	Plant Parts*	Extraction technique**	Country	References
<i>verticillata</i>	β -caryophyllene 17.8%, β -phellandrene 14.2%, α -humulene 10.2%, α -pinene 5.7%, germacrene D 5.2%	AP cult	HD	Iran	Nasermoadeli et al., 2013
	β -caryophyllene 14.7%, α -gurjunene 12.8%, germacrene D 8.7%, α -humulene 7.7%, β -phellandrene 6.6%, β -pinene 6.5%, bicyclogermacrene 6.4%	AP wild			
	β -caryophyllene 24.4%, β -phellandrene 9.1%, α -humulene 8.6%, bicyclogermacrene 6.3%, spathulenol 6.0% and β -pinene 5.00%	AP	HD	Iran	Dehaghi et al., 2014
<i>verticillata</i>	β -caryophyllene 41.0%, α -humulene 14.0%, germacrene D 13.0%	AP	HD	Ardebil, Iran	Rajabi et al., 2014
	β -caryophyllene 24.0%, spathulenol 11.0%, caryophyllene oxide 10.0%, α -humulene 8.1%, germacrene D 6.4%, α -cedrene 5.7%			Khoi, Iran Azarbaijan-Gharbi, Iran	
	β -caryophyllene 17.0%, spathulenol 17.0%, caryophyllene oxide 7.0%, α -humulene 5.4%, γ -gurjunene 4.3%, germacrene D 3.5%			Tehran, Iran	
<i>verticillata</i> subsp. <i>amasiaca</i>	Palmitic acid 17.8%, 7-methyl-Z-tetradecen-1-ol-acetate 10.0%, heptacosane 8.4%	AP	TD-GC-MS	Sivas, Turkey	Hatipoglu et al., 2016

*AP: aerial parts; cult: cultivated; **HD: hydrodistillation; TD-GC-MS: thermal desorption-GC-MS

Salvia species (sage) are well-known as aromatic and medicinal plants world-wide. They have an important role not only in folk medicine, but also in cosmetics, phytotherapy, and the flavoring of food products (Bozin et al., 2007). In this study, we investigated the chemical composition of *S. verticillata* subsp. *verticillata* essential oil from Şirnak (southern eastern part of Turkey). The chemical composition of *Salvia* essential oils is highly variable, depending on geographic origin, plant part, harvesting, drying, storage, genetic factors and oil extraction process. Chemical variability of essential oils directly affects their biological activity. More research is necessary to better understand the variabilities in the chemical profile of *S. verticillata* and other *Salvia* species.

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