Nat. Volatiles & Essent. Oils, 2019; 6(1): 40-46

NVEO
Natural Volatiles & Essential Oils

RESEARCH ARTICLE

Characterization of *Salvia verticillata* L. subsp. *amasiaca* (Freyn & Bornm.) Bornm. essential oil from Turkey

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Abstract

The essential oil obtained by hydrodistillation from the aerial parts of *Salvia verticillata* L. subsp. *amasiaca* (Freyn&Bornm.) Bornm. from Turkey was analyzed by GC-MS. Overall, 21 compounds were identified representing 97.27% of the total oil. 1,8-Cineole 15.9%, *trans*-caryophyllene 13.3%, spathulenol 8.3%, germacrene-D 7.5%, carvacrol 6.3% and β-pinene 4.9 % as main constituents in the oil.

Keywords: *Salvia verticillata,* Lamiaceae, essential oil, GC-MS

Introduction

Lamiaceae is the third largest family based on the taxon number and fourth largest family based on the species number in Turkey (Celep and Dirmenci, 2017). *Salvia*, the largest genus of Lamiaceae, includes about 945 species, widespread throughout the world. This genus is represented, in the flora of Turkey by 100 species and 107 taxa, 54 % of which are endemic (Davis,1982; Güner et al., 2000; Chalchat et al., 2001; Celep and Dirmenci, 2017; Başer and Kırımer, 2018). The second largest geographical distribution of Lamiaceae taxa occurs in Central Anatolia (rate of endemism 36%), after the Mediterranean region (Celep and Dirmenci, 2017).

The interest in *Salvia* has increased remarkably over the last 15 years, due to the diversity of species, world distribution and high ecological, structural and functional diversity (Claßen-Bockhoff, 2017). The genus *Salvia* has recently attracted great attention due to its notable biological activities. In Turkish folk medicine, Salvia species, also known as "adaçayı, elmaçayı, karabaşotu, dadırak, hart şalbası and yağlıkara" are used as diuretic, carminative, antiseptic, against colds, stomachache, sore throat, inflammations in the mouth and infections. They are also consumed as an herbal tea and used as food flavor (Adams, 2001; Aşkun et al., 2010; Tabanca et al.,2017). Two subspecies are currently recognized, based on the colour of the inflorescence axis: *S. verticillata* subsp. *verticillata*, with a wide distribution range in Europe and *S. verticillata* subsp. *amasiaca* (Freyn & Bornm.) Bornm, limited to Turkey and Western Asia (Giuliani et al., 2018). Especially in the form of infusion and decoction of *Salvia* subsp. *amasiaca* is used in the treatment of diseases such as abdominal pain, stomachache (Sezik et al. 2001) cardiovascular diseases (Kultur, 2007), laxative, colds, nausea (Altundağ and Ozturk, 2011). Previous studies showed that the essential oils and extracts *Salvia verticillata* subsp. *amasiaca* have biological activity such as antioxidant, antimicrobial, anticholinesterase, antidiabetic (Tepe at al.,2007; Eidi et al., 2011; Kunduhoglu et al.,2011; Erbil and Dığrak, 2015) and antimycobacterial activities (Aşkun et al., 2010).

Although Turkey is a country rich in endemic *Salvia* species, only a few studies have been conducted on the essential oil components of *Salvia verticillata* subsp. *amasiaca*. In the present study, *S. verticillata* subsp. *amasiaca* essential oil, one of the two subspecies of *Salvia verticillata*, which is thought to be especially important due to chemical variation, was collected and investigated from the Sivas region.

Plant material

Aerial parts of *Salvia verticillata* subsp. *amasiaca* were collected from Gürün, Yazyurdu village (August/1550 m), Sivas, Turkey. The voucher specimen was identified by Dr. Erol Dönmez at the Department of Biology, Cumhuriyet University, Sivas-Turkey and deposited at the Herbarium (CUFH-Voucher No: ED 11019).

Isolation of the essential oil

The air-dried and finely ground aerial parts were distilled for 3h using a Clevenger-type apparatus. The oil (yielded 0.02% v/w) was dried over anhydrous sodium sulphate and stored at +4 °C.

Gas chromatography- mass spectrometry (GS/MS) analysis

The chemical composition of the *Salvia verticillata* subsp. *amasiaca* essential oil was analysed using a Shimadzu QP-5000 gas chromatograph-mass spectrometer equipped with a GL Science capillary column TC-5 (30 m × 0.25 mm i.d.,0.25 mm) and a 70 eV EI Quadrupole detector.

Helium was the carrier gas, at a flow rate of 1.9 mL/min. Injector and MS transfer line temperatures were set at 250 and 280°C, respectively. The column temperature was initially at 40°C held for 2 min, then gradually increased to 125°C at a 2°C/min rate, held for 2 min, and finally increased to 250°C at 5°C/ min held for 2 min. Diluted samples (1:100 v/v, in acetone) of 1.0 µL were injected manually and splitless.

Identification

The components were identified by comparison with their relative retention indices and MS (NBS75K library data of the GC–MS system) as well as the literature (Adams, 2001).

Results and Discussion

In this present study, the composition of *Salvia verticillata* subsp. *amasiaca* oil was analysed by GC-MS, compounds representing 97.27% of the oil were identified, with 1.8-cineole 15.9%, trans- caryophyllene 13.3%, spathulenol 8.3%, germacrene D 7.5 %, carvacrol 6.3 % and β-pinene 4.9 % as main constituents as seen at Table 1.

Previous reports indicate that β-Pinene, α-pinene ,1,8-cineole caryophyllene, carvacrol, spathulenol and germacrene-D are the main and/or characteristic constituents of *Salvia verticillata* subsp. *amasiaca* essential oil (Başer,1993; Başer,2002; Başer and Kırımer, 2006; Altun et al., 2007; Aşkun et al.,2010; Kunduhoglu et al.,2011; Başer and Kırımer, 2018).

Table 1. Components of *S. verticillata* subsp. *amasiaca* essential oil

RRI	Retention time (R_t)[a]	Compounds	%
954	13.555	camphene[b]	1.85
975	15.067	sabinene[b]	2.45
977	15.183	β-pinene[c]	4.89
990	16.418	β-myrcene[b]	2.05
1024	18.268	*p*-cymene	3.45
1030	18.531	1,8-cineole[b]	15.89
1044	19.820	*cis*-β-ocimene	3.15
1096	23.808	linalool	3.97
1102	23.900	thujyl alcohol[c]	3.97
1126	24.173	pulegone	2.76
1138	25.800	*cis*-sabinol	3.81
1165	27.712	borneol	3.54
1194	36.580	myrtenol	1.70
1295	38.625	carvacrol[b]	6.28
1375	41.486	α-copaene[c]	2.98
1406	45.983	*trans*-caryophyllene	13.25
1435	47.202	aromadendrene[b]	2.98
1480	49.255	germacrene D[b]	7.47
1486	52.125	β-selinene[c]	0.57
1620	53.543	spathulenol	8.28
1719	56.125	farnesol	1.95
		Σ Monoterpene compounds	59.79
		Σ Sesquiterpene compounds	37.48
		Total identified	97.27%

RRI Relative retention indices calculated against *n*-alkanes. % calculated from FID data. [a]Retention time (as min). [b]Compounds listed in order of elution from a OV-5 column. [c]Identification of components based on standard compounds.

In order to assess the variability in the essential oil composition of our sample, a qualitative comparison was performed with respect to the profiles known in the literature, referring to 23 populations from a broad geographical range, from the Yugoslavia, Serbia, Romania, Greece, Italy, Turkey region to the Iran (Table 2). Essential oil data of *S. verticillata* subsp. *amasiaca* in the literature is limited to a few studies in Turkey (Başer et al., 1993; Başer, 2002; Başer and Kırımer,2006; Altun et al.,2007; Aşkun et al., 2010; Kunduhoglu et al., 2011; Hatipoglu et al., 2016; Başer and Kırımer,2018).

The essential oil yields obtained in the present and the other previous studies in literature were extremely variable from levels below the limit of quantification up to 1.3% (Altun et al., 2007) in Turkish populations. At the same time, the total number of the identified compounds was very different.

According to the present literature, *S. verticillata* volatile chemicals appeared to be characterized by a high level of complexity. A general chemical polymorphism exists due to the geographical provenance, the processed plant parts, the employed techniques and the investigated subspecies.

However, monoterpene hydrocarbons constituted the most represented class in a few studies taken into consideration in accordance to our data (Altun et al. ,2007; Aşkun et al., 2010) in *S. verticillata* subsp. *amasiaca*.

In Turkey, Başer et al., (1993) reported that carvacrol 27.5% and spathulenol 17% were the main components in *S. verticillata* subsp. *amasiaca*. At the same time, β -caryophyllene 17% (Başer, 2002; Başer and Kırımer, 2006) and germacrene-D 37% (Başer and Kırımer, 2018) were also determined. Altun et al., 2007 reported, β-pinene 23.0%, α-pinene 21.6%, ß-phellandrene 13%, limonene 11%, 1,8-cineole 10.9% in a Bitlis sample.

Askun et al., (2010) determined different main constituents β-pinene 21.4%, 1,8-cineole 16.1%, α-copaene 5.4%, alloaromadendrene 5.1%, α-gurjunene 4.6% in *S. verticillata* subsp. *amasiaca* collected from the Bitlis-Tatvan geographical region. Kunduhoğlu et al., (2001) reported that *S. verticillata* subsp. *amasiaca* collected from Bilecik, Bozüyük, Kozpınar had high levels of germacrene D 36.6%, β-caryophyllene 7.6%, hexadecanoic acid 6.7% and β-copene 5.7%. The contents of *S. verticillata* subsp. *amasiaca* essential oil have been reported mainly hydrocarbons and their derivatives by Hatipoglu et al., 2016. According to our survey of the available literature on the composition of *S. verticillata* subsp. *amasiaca* our data partially agrees with the previous studies (Table 2).

Table 2. Previous reports on *S. verticillata* and *S. verticillata* subs*p. amasiaca** essential oils

Main compounds (%)	**Origin**	**References**
β-caryophyllene 24.7%, γ-muurolene 28.8%, limonene 8.9%, α-humulene 7.8%	Iran	Şefidkon et al., 1999
β-caryophyllene 13.3%, γ-muurolene 10.3%, *trans*-chrysanthenol 6.1%	Yugoslavia	Chalcat et al., 2001
Germacrene D 48%, (*E*)-caryophyllene 13.4%, α-humulene 7.2%, α -cadinol 10.4%	Serbia North Serbia	Krstic et al., 2006
α-Pinene 30.7%, p-cymene 23%, β-pinene 7.6%, lauric acid isopropyl ester 16.8%	Greece	Pıtarokili et al., 2006
β-Caryophyllene 31.5%, germacrene D 16.2%, limonene 15.5%, α-pinene 10.4%, α-humulene 9.4%	Iran	Yousefzadi et al., 2007
β-Caryophyllene 13.5%, germacrene-D 22.8%,valeronene 8.9%, γ-elemene 4.6%	Romania	Pădure et al., 2008
β-Caryophyllene 16%, α-caryophyllene 14.5%, spathulenol 8.6%	Eastern Romania	Coisin et.al, 2012
E-Caryophyllene 14.7-17.8%, a-gurjunene 12.8- 3.4%, β- phellandrene 6.6-14.2%, α-humulene 7.6- 10.11 %, germacrene D 5.1 %, sabinene 4.5 %	Iran	Nasermoadeli et al., 2013
E-Caryophyllene 16.9–40.9%, spathulenol 0–17.5%, α-humulene 5.4–14.3%, bicyclo-germacrene 3.4–6.4%	Iran	Rajabi et al., 2014
trans-Caryophyllene 24.4%, β-phellandrene 9.0%, α-humulene 8.6%, bicyclogermacrene 6.3%, spathulenol 5.9%, β-pinene 5%	Iran	Khosravi Dehaghi et al., 2014
1,8-Cineole 38.3 %, camphor 22.9%	Iran and West Azerbaijan)	Forouzin et al., 2015
Germacrene D 13.8%, spathulenol 10%, limonene 4.5%, 1,8- cineole 4.5%	Turkey (Elazıg, Baskil)	Doğan et al., 2015
trans-Caryophyllene 18.8%, Germacrene-D 9.49%, spathulenol 7.5%, sabinene 6.5%, α-caryophyllene 5.8%	Iran (the central Alborz)	Mahdavi et al., 2015
Spathulenol 31.0%, α-pinene 8.2%, limonene 4.1%) and hexahydrofarnesyl acetone 3.8%	Turkey (Şırnak, Hakkari)	Tabanca et al., 2017
Germacrene-D 39.5-40.7 %, bicyclogermacrene 11.5-14.8%, β-caryophyllene 7.5-11.9%, spathulenol 3.1-6.6%, α-humulene 2.7-5.6%,	Milan, Italy	Giuliani et al.,2018
Carvacrol 27. 5 %, spathulenol 17%	Turkey	Başer et al., 1993
β -Caryophyllene 17%	Turkey	Başer, 2002
β -Caryophyllene 17%, Carvacrol 27 %,spathulenol 17%	Turkey	Başer and Kırımer, 2006
β -Pinene 23.0%, α-pinene 21.6%, ß-phellandrene 13%, limonene 11%, 1,8-cineole 10.9%	Turkey (Bitlis Tatvan)	Altun et al., 2007

β-Pinene 21.4%, 1,8-cineole 16.1%, α-copaene 5.4%, alloaromadendrene 5.1%, α-gurjunene 4.6%	Turkey (Bitlis Tatvan)	Aşkun et al., 2010
Germacrene-D 36.6%, β-caryophyllene 7.6%, hexadecenoic acid 6.7%, β-copaene 5.7%, spathulenol 4.5%	Turkey (Bilecik, Bozüyük Kozpınar)	Kunduhoglu et al., 2011
β-Caryophyllene 17%, germacrene-D 37%	Turkey	Başer and Kırımer,2018

Contrary to our study, the investigations on the spontaneous populations of *S. verticillata* subsp. *amasiaca* from Turkey (Başer et al., 1993; Başer and Kırımer, 2006; Kunduhoğlu et al., 2011; Hatipoglu et al., 2016; Başer and Kırımer, 2018) documented a dominance of sesquiterpenes in the essential oils. At the same time, the investigations on the populations of *S. verticillata* subsp. *verticillata* in the literature give similar results (Table 2).

The only ubiquitous compound is beta-caryophyllene, even if detected in variable relative amounts. Among the most frequent compounds, that are also the most abundant ones, germacrene-D, spathulenol and carvacrol must be cited.

Conclusion

In conclusion, dominance of sesquiterpenes may be specific to both *S. verticillata* subsp. *verticillata* and *S. verticillata* subsp. *amasiaca.* In order to find an answer to this problem, it is necessary to characterize additional samples from two sub-species.

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Received: 09.08.2017
Accepted: 01.10.2018