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RESEARCH ARTICLE

Characterization of Essential Oil and Antioxidant Activities of Some Species of *Salvia* in Turkey

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Abstract

In this present study, the chemical composition and antioxidant activity of the essential oils of *Salvia aethiopis, Salvia blepharochlaena* and *Salvia euphratica* collected from Turkey were evaluated According to the GC-MS analyses, 20 components (representing 98.2%) were characterized in the essential oil of *S. aethiopis*. The major compounds of this essential oil were determined as α-copaene (18,2%), α-cubebene (12,4 %), spathulenol (12.3%), respectively. The main components of the *S. blepharochlaena* essential oil were determined as 1,8-cineole (26.8 %), *cis*-ocimene (15.3 %) and β-pinene (7.9%), respectively. Main components of *S. euphratica* oil were characterized as c*is*-sabinol (21.9 %), myrcenyl acetate (17.5 %), and 1,8 cineole (9.5 %). Antioxidant activity evaluations by β-carotene and radical scavenging assays showed that *Salvia* sp. essential oils prevent linoleic acid oxidation up to 19, 22, and 21%, respectively.

Keywords: *Salvia aethiopis, Salvia blepharochlaena*, *Salvia euphratica*, essential oil, antioxidant activity

Introduction

Medicinal and aromatic plants and their products have been used in medicines and cosmetics since time immemorial (Davis et al 1988; Demirci et al., 2005). With approximately 900 taxa, the genus *Salvia* L. is one of the important genera of Lamiaceae. The genus is represented by 94 taxa belonging to 89 species with about an 50% endemism ratio in Turkey (Baytop, 1999; Dönmez et al., 2001).

Some members of this species are also economically important since they are utilized largely in culinary foods, cosmetics and as traditional medicines (Baytop, 1999). The *Salvia* species are known as popular folk medicines, and are widely utilized in Anatolia where they are used against cold, stomach aches or sore throat among other uses (Baytop, 1999; Kintzios 2000; Ulubelen, 2003). Most commonly, *S. officinalis* is traditionally used to treat the symptoms of various digestive problems (Kintzios, 2000; Mirza and Sefidkon, 1999; Sefidkon and Mirza, 1999; Sefidkon and Khavi, 1999). The phytochemical compositions of *Salvia* sp. are studied and reported extensively, *S. aethiopis* volatiles were previously reported (Mirza and Sefidkon, 1999; Sefidkon and Mirza, 1999; Sefidkon and Khavi, 1999; Ulubelen, 2003; Tepe et al, 2006).

The aim of this present study was to investigate the chemical compositions of Turkish *S. aethiopis* L.*, S. blepharochlaena* Hedge et Hub.-Mor., *S*. *euphratica* Monbret et Aucher ex Bentham var. *euphratica* essential oils and their i*n vitro* antioxidant activities.

Materials and Methods

S. aethiopis was collected from Imranlı, Boğanak village (1400m), *S. blepharochlaena* was collected from Imranlı, Söğütlü village (1550m). *S*. *euphratica* var.

euphratica was collected from Divriği, Çayözü village (1500 m). The plants were collected during flowering . Voucher specimens were identified by Dr. Erol Dönmez of the Department of Biology, Cumhuriyet University, Sivas, and are deposited in the Herbarium of the Department of Biology (CUFH-Voucher No: ED 11006-11008-11009).

Essential oil isolation

The air-dried and finely ground aerial parts of *S. aethiopis, S. blepharochlaena* and *S. euphratica* were subjected to water distillation for 3 hours by the Clevenger distillation system with yields of 0.1 %, 0.2 %, 0.2 %, resp.). The essential oils obtained were dried over anhydrous sodium sulphate and kept at +4° C until further analyses and experiments.

Gas chromatography/ mass spectrometry (GC/MS) analysis

GC-MS-QP5000 (Shimadzu, Kyoto, Japan), equipped with a 70 eV EI quadrupole detector and a GL Sciences (Tokyo, Japan) capillary column TC-5 (30 m x 0.25 mm i.d., 0.25 µm film thickness) was used.

Helium was used as carrier gas and flow rate was set at 1.2 mL/min. The column temperature was initially set at 50 °C for 3 min., then raised to 280 °C at a ratio of 3 °C/min. for 5 min. Diluted sample in an amount of 1.0 µL [1:15 (v/v), in acetone] were injected. The chromatographically separated compounds were characterized both by using commercial standard compounds and common GC/MS libraries (NBS5K, Adams, 1995).

Radical scavenging assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical assay was used for the antioxidant activity by radical scavenging potential of the test samples. The tests were performed in triplicates as previously described (Burits and Bucar, 2000; Cuendet et al, 1997).

β-Carotene-linoleic acid assay

Using the β-carotene method antioxidant capacity was determined by measuring the inhibition of the essential oil and extract samples where the linoleic acid oxidation was measured as previously reported (Dapkevicius et al., 1998, Tepe et al. 2006, Yumrutas et al. 2012).

Results and Discussion

In the oil from aerial parts of *S. aethiopis,* 20 components were characterized representing 98.2% of the total constituents. The constituents of the volatile fraction of *S. aethiopis* and their percentages are given in Table 1. α-Copaene (18.2%), α-cubebene (12.4%), spathulenol (12.3%), germacrene-D (8.2%) were the main constituents detected. In the oils of *S. blepharochlaena* and *S. euphratica,* as shown in Table 1, the main components were 1,8-cineole (26.8%), *cis*-ocimene (15.3%), β-pinene (7.9%), respectively. Whereas in the oil of *S .blepharochlaena* and *S. euphraticta* the main components of were *cis*-sabinol (21.9%), myrcenyl acetate (17.5%), 1,8-cineole (9.5%), respectively.

There are a number of studies on the composition of the essential oil of *S. aethiopis*; Chalchat et al. (2001) reported that germacrene D (10.9%) and caryophyllene oxide (6.4%) were the main constituents of *S. aethiopis* essential oil from Serbia. Rustajian et al. (1999) reported spathulenol (8.3%), α-copaene (16.3%), β-cubebene (9.7%), β-caryophyllene (17.0%), and germacrene D (13.8%); Morteza-Semnani et al. (2005), reported similar composition, however, α-copaene (15.5%), β-caryophyllene (24.6%), germacrene D (13.5%) as main components from Iranian samples.

Velicovich and co-workers reported α-copaene (22.4% in leaf), germacrene D (13.5%), spathulenol (20.1% in stem), and bicyclogermacrene (29.0% in flower) as main components (Velikovic et al., 2003). In the other study Velickovic et al., (2002) reported that the ethanol extracts of the flower, stem and leaf of *S. pratensis*, *S. glutinosa* and *S. aethiopis* have some common contents, such as: β-caryophyllene, 1,8-cineole and camphene, β-pinene, β-thujone and α-thujone, respectively.

Gianouli and Kintzios (2000) reported that the volatile oils of flowering parts of *S. aethiopis* were examined by GC-MS, where the main components were characterized as germacrene D (4.95%), α-copaene (9.15%) and bicyclogermacrene (29.54%). In another study from the Eastern Anatolian region, it was reported that main components of *S. aethiopis* were β-cubebene (9.9%), germacrene D (29%), α-copaene (19.8%) (Güllüce et al., 2006; Coisini et al., 2012) copaene as main constituents.

Table 1. The chemical compositions of *Salvia* essential oils

LRI [a]	**LRI** (lit)	**Constituents**	***S. aethiopis***	***S. blepharochlaena***	***S. euphratica***
939	939	α-pinene[b]	0.75	0.82	0.78
954	953	camphene[b]	-	5.57	1.35
975	976	sabinene[b]	-	4.29	0.99
978	978	β-pinene[c]	-	7.89	1.95
979	980	1-octen-3-one[b]	-	-	0.38
990	991	β-myrcene[b]	-	2.47	-
995	997	3-octanol[b]	-	-	0.31
1027	1025	β-phellandral[b] limonene	-	1.15	-
1029	1031	1,8-cineole[b]	7.17	0.35	0.55
1031	1032	*cis*-β-ocimene[c]	2.35	26.81	9.48
1044	1040	*trans*-β-ocimene[b]	-	15.34	-
1051	1050	γ-terpinene[b]	-	0.45	-
1060	1062	*m*-cymene[b]	-	0.65	-
1080	1082	α-terpinolene[b]	-	-	0.55
1089	1088	α-linalool	-	0.78	5.28
1096	1099	thujyl alcohol[c]	-	-	0.68
1102	1100	α-campholene aldehyde[b]	-	1.10	-
1105	1108	myrcenol[b]	-	1.19	-
1119	1123	pulegone[b]	-	0.71	2.95
1126	1129	citronellal[b]	-	-	1.25
1135	1137	*trans*-pinocarveol[b]	-	0.48	1.22
1136	1139	*cis*-sabinol[b]	3.67	1.88	3.27
1138	1140	camphor[b]	-	-	21.85
1146	1146	isoborneol[b]	-	5.59	1.25
1155	1156	borneol[b]	1.45	-	
1165	1165	Isopinocamphone[b]	-	3.04	-
1170	1173	*p*-allyl anisole[c] terpineol[b]	-	-	0.58
1176	1180	verbenone[b]	-	-	0.95
1185	1189	*trans*-carveol[b]	-	-	0.98
1205	1204	myrtenyl acetate[b]	-	-	0.64
1215	1217	isolinalyl acetate[c]	-	1.93	0.75
1230	1235		0.76	-	
1236	1238		-	-	1.63

1240	1242	myrcenyl acetate[c]	-	-	17.47
1280	1285	bornyl acetate[b]	-	-	1.89
1295	1299	carvacrol[b]	-	2.14	0.28
1350	1351	α-cubebene[c]	12.36	-	-
1358	1362	*cis*-carvyl acetate[b]	-	-	0.48
1360	1363	geranyl acetate[b]	0.95	1.15	-
1375	1376	α-copaene[b]	18.21	-	-
1390	1393	β-elemene[b]	1.75	-	0.58
1392	1394	*cis*-jasmone[b]	-	-	1.22
1435	1439	aromadendrene[b]	2.98	0.95	1.87
1465	1467	β-caryophyllene	-	1.48	1.87
1480	1485	germacrene D	8.21	-	-
1498	1500	α-farnesene	-	-	0.95
1530	1534	*cis*-nerolidol[b]	3.35	3.51	-
1540	1543	γ-cadinene	4.45	-	-
1562	1565	ledol[b]	7.7	-	-
1586	1585	unknown 1	0.35	-	-
1611	1616	epicedrol[c]	-	1.62	-
1620	1619	spathulenol[b]	12.25	0.35	-
1622	1618	unknown 2	3.87	2.03	0.75
1635	1636	delta-cadinol[c]	2.98	-	4.58
1718	1725	farnesol[b]	-	0.69	0.85
1725	1723	benzyl benzoate	-	-	0.35
1925	1928	unknown 3	2.67	-	-
		Total	98,23%	99.38%	92.76%

[a] LRI: Linear Retention Indices (OV-5 column), [b] Tentative identification, [c] Identification based on standards

The results of our study were in accordance to previous reports (Chalchat et al., 2001; Coisini et al., 2012; Güllüce et al., 2006; Morteza-Semnani et al., 2005; Rustajyan et al., 1999; Velickovi et al., 2002; Velickovic et al., 2003). However, there are significant variations in terms of the relative percentages of the components. For instance, in the present study components such as α-cubebene, spathulenole were relatively more when compared with the previous (Chalchat et al, 2001; Morteza-Semnani et al,2005; Rustajyan et al., 1999). *S*ome constituents including β-caryophyllene, α-copaene, germacrene-D were lower than in other reports (Chalchat et al., 2001; Güllüce et al., 2006; Morteza-Semnani et al., 2005; Rustajyan et al., 1999; Velickovic et al., 2003; Velickovi et al., 2002) compared. Such differences may be associated to geographic and climate differences and variations. As seen in Table 1., in this study the main constituents of the *S. blepharochlaena* oil were 1,8-cineol (26.8%), *cis*-β-ocimene (15.3%), β-pinene (7.9%), respectively. The main components of *S. euphraticta* were *cis*-sabinol (21.9%), myrcenyl acetate (17.5%), and 1,8-cineol (9.5%). The chemical composition of the *S. euphratica* oil from Turkey were subject to various reports (Başer et al., 1998; Baser et al., 2005; Giannouli and Kintzios., 2000). Başer et al., (1998), where *trans*-pinocarveol (11.7%), myrcenyl acetate (14%) were elucidated as the major components. While another study findings reported myrcenyl acetate (15.9%), and 1,8-cineole (13.8%) as main compounds (Başer et al., 2005). Yumrutaş et al. (2012), determined eucalyptol, syn. 1,8-cineole (18.4%), *trans*-pinocarveol (24.9%) as the major components, supporting the similarity of our present composition.

A literature survey showed that β-caryophyllene is one of the most prominent constituents of the volatile fraction of aerial parts of *S. limbata*, *S. virgata, S. hypoleuca, S. aethiopis, S. nemorosa, S. atropatana, S.*

verticillata. The volatile fraction of *S. hedgena and S. huberi* constituted β-pinene, 1,8-cineole, α-pinene as major constituents. As previously reported, β-caryophyllene, β-pinene and spathulenol are common constituents of *Salvia* essential oils (Demirci et al., 2005; Mirza and Sefidkon, 1999; Sefidkon and Mirza, 1999; Sefidkon and Khajavi, 1999).

In an earlier study from Eastern Anatolian samples it was reported that main components of *S. blepharochlaena* were 1,8-cineole (14.4%), camphor (18.3%), and α-terpineol (7.7%) (Tanker et al. 1993). After a decade Demirci et al., (2003), reported that α-pinene (10.1%), limonene (3%), α-copaene (8.6%), β-pinene (4%), camphor (8.5%), β-phellandrene (3.7%), spathulenol (7.3%) and 1,8-cineole (3%) were identified as the major constituents.

The Table 2. presents the free radical scavenging capacities of the essential oil samples which were determined by DPPH and β-carotene-linoleic acid assays, respectively. No inhibitory activity of essential oil was observed at the tested concentrations by the DPPH method; however, on the other hand a relatively weak inhibition ratio of 19% was observed via the β-carotene-linoleic acid method.

To the best of our knowledge there was no study reported on the antioxidant effects of the essential oils and extracts of S*. aethiopis, S. blepharochlaena* and *S. euphratica.*

Table 2. in vitro Effects of *Salvia* essential oils on the free radical DPPH and β-carotene-linoleic acid system

Sample	**DPPH** IC_{50} **(µg/mL)**	**β-carotene-linoleic acid (Inhibition %)**
S. aethiopis	-	19
S. blepharochlaena	-	22
S. euphratica	-	21
BHT	0,0105	100

- : No activity

In a study performed with *S. aethiopis* extract it was reported that the non-polar fraction showed a relatively weak antioxidant effect (Tepe et al, 2006), supporting our test results, where we used the oil. It is well known that *Salvia* sp. are used in Turkey for the treatment of flu and cold as infusion, where our *Salvia* sp. have also applications.

As a conclusion, there is still a need to investigate the phytochemical composition of *Salvia* sp. not only for the volatiles, but also on the non-volatile fractions in various biological evolutions, too.

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