

Composition of the essential oil of two *Salvia* taxa (*Salvia sclarea* and *Salvia verticillata* subsp. *verticillata*) from Turkey

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Abstract

Objective: The essential oil composition of two *Salvia* taxa (*Salvia sclarea* and *Salvia verticillata* subsp. *verticillata*) analysed and yield of compositions were analysed

Material and Methods: The essential oil was extracted by hydro distillation using a modified Clevenger apparatus coupled to a 2 L round-bottom flask. A total of 100 g of fresh plant material (aerial parts) and 1 L of water were used for the extraction. Gas chromatography / Mass spectrometry (GC-MS) analysis were applied to extracts.

Results: The essential oil yields of *Salvia sclarea* and *Salvia verticillata* L. subsp. *verticillata* were found as 0.4 and 0.3 %v/w, respectively. Overall, thirty seven compounds which accounted for 97.9% in *Salvia sclarea* and seventy four constituents, which accounted for 98.6% of the total compositions of each oil are determined in *Salvia verticillata* L. subsp. *verticillata*. The spathulenol (19%), caryophyllene oxide (15.5%), linolyl acetate (11.3%) and linalool L (8.5%) were the major compounds of *Salvia sclarea* and the germacrene D (13.8%), spathulenol (10%) and limonene (4.5%), 1,8- cineole (4.5%) were the main compounds of the *Salvia verticillata* L. subsp. *verticillata*

Conclusion: spathulenol was found as major compound for both *Salvia sclarea* and *Salvia verticillata* subsp. *verticillata* while, the other main components were not showed similarity

Keywords: *Salvia sclarea*, *Salvia verticillata*, GC-MS, Essential oil, Spathulenol

Introduction

The genus *Salvia* L. represents nearly 1000 species displaying a remarkable diversity in growth forms, secondary compounds, floral morphology, and pollination biology. The genus has distributed extensively in 3 regions of the world: Central and South America (500 spp.), western Asia (200 spp.), and eastern Asia (100 spp.) (1). Anatolia is a major diversity center for *Salvia* in Asia (2). Turkey is home to 95 *Salvia* species, 49 (52%) of which are endemic (3).

The first revision of *Salvia* in Turkey was made by Hedge (1982), who recognized 86 species, 1 hybrid and 1 doubtful species. There, he grouped the species by stamen characters and other morphological similarities. Since the publication of the Flora of Turkey, nine species have been added to the genus as new species (4-9) or new records were reported (10-12). The number of species now reaches 95, showing that Turkey is a major centre of diversity for the genus in Asia.

Salvia sclarea L. also known as muscat sage is one of the highly demanded Mediterranean species for its aromatic properties (13-14).

S. sclarea or clary sage is widely used in cosmetic industry as well (15). Extracts of the aerial part of clary sage have a broad spectrum of effects: analgesic, antiinflammatory (16), antifungal (17) and antibacterial (18).

Salvia species have been used in folk medicine for wound healing and in alleviating stomach, liver, and rheumatism pains and for treating the common cold in the form of infusion and decoction in various parts of the world (19,20). They and their essential oils are used in food flavoring, pharmaceuticals, and in perfumery. *Salvia* species mainly contain essential oil and phenolics (21). Some of the phenolic compounds of plants belonging to this genus have also shown excellent antimicrobial activity, as well as scavenging activity of active oxygen, inhibiting lipid peroxidation and antioxidant activity (22-24).

Recently, the essential oils and various extracts of plants have gained special interest as sources of natural antimicrobial and antioxidant agents because of the resistance to antibiotics that some microorganisms have acquired and the possible toxicities of the synthetic antioxidants (25,26).

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In the context of essential oil study in our laboratory in the same family (27-29) it is aimed that to evaluate the composition of the essential oils obtained from the aerial parts of *Salvia sclarea* and *Salvia verticillata* subsp. *verticillata* growing wild in Turkey. The results were discussed with the *Salvia* genus pattern in means of chemotaxonomy, natural products and renewable resources.

Materials and Methods

Plant material

Salvia sclarea L. specimens were collected during to flowering stage in May, 2010, at an altitude of 1400 m, Ovacik (Tunceli-Turkey) and *Salvia verticillata* L. subsp. *verticillata* specimens were collected during to flowering stage in June, 2009, at an altitude of 1380 m, Baskil (Elazığ-Turkey). Voucher specimens are kept at the Firat University Herbarium (FUH).

Extraction of the essential oil

The essential oil was extracted by hydrodistillation using a modified Clevenger apparatus coupled to a 2 L round-bottom flask. A total of 100 g of fresh plant material (aerial parts) and 1 L of water were used for the extraction. The extraction was performed over 3 hour period. Subsequently, the hydrolate was collected and centrifuged at 10,000 rpm for 10 minutes. The organic phase was removed with the aid of a Pasteur pipette, and subsequently transferred to an black coloured vials, wrapped in parafilm and aluminum foil and 4°C under refrigeration until analysis. The yields of oils were calculated on the basis of the dry mass.

Gas chromatography (GC) analysis

The essential oil was analysed using HP 6890 GC equipped with FID detector and HP- 5 MS (30 m x 0.25 mm *i.d.*, film tickness 0.25 µm) capillary column was used. The column and analysis conditions were the same as in GC-MS expressed as below. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors.

Gas chromatography / Mass spectrometry (GC-MS) analysis

GC-MS analyses of the oils were performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with Hewlett Packard 5973 mass spectrometer system equipped with a HP 5-MS capillary column (30 m x 0.25 mm id, film thickness 0.25 µm). The oven temperature was programmed from 70-240°C at the rate of 5°C/ min. The ion source was set at 240°C and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 mL/min. Scanning range was 35 to 425 amu. Diluted oil in *n*-hexane (1.0 µL) was injected into the GC-MS.

The identification of constituents was performed on the basis of retention indices (RI) determined by co-injection with reference to a homologous series of *n*-alkanes, under identical experimental conditions.

Further identification was performed by comparison of their mass spectra with those from NIST 98 Libraries (on ChemStation HP) and Wiley 7th Version. The relative amounts of individual components were calculated based on the GC (HP-5MS column) peak area (FID response) without using correction factors. The identified constituents of the essential oils are listed in Table 1.

Results and Discussion

The essential oils of the aerial parts of two *Salvia* species (*Salvia sclarea* and *Salvia verticillata* L. subsp. *verticillata*) collected from the Turkey were obtained by hydrodistillation, in 0.4% and 0.3 (v/w) oil yields respectively. Thirty seven and seventy four components were identified representing 97.9% and 98.6% of the oils, respectively. The major constituents of *S. sclarea* were spathulenol (19%), caryophyllene oxide (15.5%), linolyl acetate (11.3%) and linalool L (8.5%), whereas those *S. verticillata* subsp. *verticillata* were germacrene D (13.8%), spathulenol (10%) and limonene (4.5%), 1,8- cineole (4.5%) (Table 1). The oils were complex mixtures of non-terpenes, monoterpenes and sesquiterpenes: Totally, eighty nine components were identified in essential oils in the study.

The hydrodistilled essential oils of the aerial parts of wild-growing *Salvia sclarea* originated from two localities in Greece were analyzed by GC-MS. Sixty-six compounds, representing 93.26–98.19% of the oils, were identified. Linalyl acetate (19.75–31.05%), linalool (18.46–30.43%), geranyl acetate (4.45–12.1%), and α -terpineol (5.08–7.56%) were the main components (17). In general, we can said that there are some similarities between in Greece samples and Turkey specimens.

Twenty-eight components were identified for *S. verticillata*, constituting 98.2% of the total oil. *S. verticillata* oil was dominated by monoterpenes (64.5%). Among these monoterpenes hydrocarbons such as β -pinene (30.7%), *p*-cymene (23.0%) and α -pinene (7.6%) were reported by Pitarokili et al (30). It is interesting that although major components (β -pinene, *p*-cymene and α -pinene) of essential oils were reported by Pitarokili et al (30), those major components were detected trace amounts in our samples. On the other hand, the β -caryophyllene (13.3%), γ -muurolene (10.3%) and trans-chrysanthenol (6.1%) were the major compounds of essential oil of *Salvia verticillata* from Yugoslavia (31).

However, two dominant components in Iran, (E)-caryophyllene and α -humulene were also dominant components in all three Serbian populations, suggesting that these are generally present in *S. verticillata*. Additionally, germacrene D was the main component (48.0% and 24.6% of oil, respectively) in two populations collected from in Serbia (32).

Table 1: Constituents of the essential oils of *S. sclarea* and *S. verticillata* subsp. *Verticillata*.

RI: Retention Indices

No	Compounds	RI	<i>S. sclarea</i> (%)	<i>S. verticillata</i> subsp. <i>vert.</i> (%)
1	α -Thujone	1016	--	0.3
2	α -Pinene	1021	0.4	2.7
3	Camphene	1035	--	0.2
4	Sabinene	1052	--	0.9
5	β -Pinene	1056	--	2.8
6	β -Myrcene	1064	0.7	0.9
7	Mentha-1 (7) 8 diene	1075	--	0.2
8	δ -3-Carene	1079	--	1.3
9	α -Terpinene	1085	--	0.1
10	Benzene, 1-methyl-2	1087	--	0.2
11	<i>p</i> -Cymene	1092	--	1.1
12	Limonene	1094	--	4.5
13	Sabinen	1096	--	2.1
14	1.8-Cineole	1098	--	4.0
15	<i>cis</i> -Ocimene	1100	0.4	--
16	γ -Terpinene	1118	0.6	0.2
17	<i>cis</i> -Sabinenehydrate	1126	--	0.2
18	2-methyl 1-propenyl	1133	--	0.1
19	Benzene, 1-methyl-4	1140	--	0.1
20	Linalool-L	1148	8.5	--
21	Trans-verbenol	1180	--	0.4
22	Pinocarvone	1192	--	0.2
23	Borneol-L	1199	--	0.6
24	3-Cyclohexen-1-ol	1205	--	0.2
25	α -Terpineol	1215	4.5	0.4
26	Trans-carveol	1231	--	0.2
27	Nerol	1234	0.7	--
28	Propanol, 2-methyl-3-phenyl	1248	--	0.2
29	Linalyl acetate	1252	11.3	--
30	Benzeneacetaldehyde	1268	--	0.3
31	Bornyl acetate	1282	--	0.2
32	α -Cubebene	1337	--	1.9
33	Lavandulyl isobutanoate	1345	1.5	--
34	α -Ylangene	1355	--	0.4
35	α -Copaene	1360	--	1.7
36	Lavandulyl acetate	1361	3.8	--
37	β -Bourbonene	1366	--	2.0
38	β -Cubebene	1369	--	1.7
39	β -Caryophyllene	1393	1.8	1.8
40	β -Copaene	1400	--	3.8
41	Aromadendrene	1406	--	0.6
42	α -Amorphene	1410	--	0.4
43	5,9-Undecadien	1411	--	0.6
44	Trans- β -farnesene	1415	--	0.8
45	(+)-Epi-bicyclosesquiphallendrene	1422	--	1.2
46	Naphthalane	1431	--	3.1
47	Germacrene D	1435	0.7	13.8
48	β -Selinene	1441	--	0.4
49	Methyl isoeugenol	1442	--	1.3
50	Bicyclogermacrene	1445	0.5	3.3
51	γ -Cadinene	1455	--	1.3
52	δ -Cadinene	1458	0.6	2.9
53	<i>cis</i> -Calamenene	1461	--	1.3
54	3,5-diene muurola	1467	--	0.5

No	Compounds	RI	<i>S. sclarea</i> (%)	<i>S. verticillata</i> subsp. <i>vert.</i> (%)
55	α -Cadinene	1470	--	0.3
56	α -Calacorene	1473	--	0.5
57	Valencene	1479	0.6	--
58	Nerolidol	1485	--	0.6
59	1,5-Epoxisalvial-4 [14]-ene	1490	2.0	--
60	Spathulenol	1495	19.0	10.0
61	Caryophyllene oxide	1498	15.5	1.7
62	Salvial-4 (14)-en-1-one	1504	1.0	1.5
63	Jasmone	1512	--	0.8
64	Humulene epoxide II	1514	0.7	0.2
65	Isolongifolene	1517	0.5	0.8
66	Vulgarol-B	1521	--	0.3
67	Isospathulenol	1526	1.2	--
68	t-Muurolol	1531	0.8	--
69	Epi- α -cadinol	1532	--	0.7
70	δ -Selinene	1535	1.1	0.9
71	α -Eudesmol	1540	2.2	1.7
72	Eudesma-4 [15], 7-dien-1-beta-ol	1541	0.8	1.2
73	γ -Gurjunene	1544	--	0.6
74	Cadalene	1548	1.6	0.7
75	Valeranone	1550	1.5	2.5
76	Ethanone	1555	--	0.7
77	Cyercene	1558	0.7	1.7
78	Ledene	1575	--	0.1
79	2-Heptanone	1586	0.7	--
80	Benzylbenzoate	1595	0.6	--
81	γ -Muurolene	1608	--	0.1
82	2-Pentadecanone	1631	--	1.5
83	1H-Naphtol [2,1-b] pyran	1655	7.0	--
84	Farnesyl acetone	1664	--	0.1
85	Nerolidyl acetate	1667	0.5	--
86	Pimaradiene	1695	0.6	--
87	Manoyl oxide	1716	0.6	--
88	Abietetrane	1756	0.7	--
89	Abietal	1861	1.5	--
	Total		97.9	98.6

It is possible to say that Turkey and Serbian *S. verticillata* samples has same major essential oil compounds generally.

The analysis of the essential oil composition of several *Salvia* species indicates that 1,8-cineole (eucalyptol), and borneol are its main constituents. However, several authors have documented significant species specific variations in the concentration of these compounds and/or presence of others in high concentrations (33-41). Moreover, the essential oil composition of *Salvia* species, as occurs with other medicinal and aromatic plants, is highly influenced by genetic and environmental factors (42-43).

Some species of *Salvia* from Turkey were dominated in germacrene D (27%), bicyclogermacrene (11.3%), spathulenol (10%) in *S. ceratophylla* oil; germacrene D (26.3%), bicyclogermacrene (24.1%), α -copaene (21.1%), β -cubenene (8.1%) and δ -cadinene (5%) were in *S. aethiopis* oil (27); 1,8-cineole (30.5%), camphor (21.3%) and borneol (8.50%) in *S. aucheri* var. *aucheri* oil; β -pinene (10.3%), 1,8 cineole (46.0%) and camphor (8.7%) in *S. aramiensis* oil; α -thujene (36.1%) and α -pinene (13.8%) *S. pilifera* oil (44); β -caryophyllene (18%), germacrene D (16.5%), linalool L (9.2%), caryophyllene oxide (7.3%), sclaraeol (6.6%) and linalyl acetate (6%) in *S. palaestina*; α -pinene (33.7%), germacrene D (7.5%), β -pinene (6.8%), α -humulene (6%), and viridiflorol (3.8%) in *S. tomentosa* (29) were reported essential oil from Turkey. Limonene (11.7%), 2-cyclohexen-1-ol (9.2%), trans-verbenol (7.7%) and trans-(+)- carveol (6.7%) were found to be major components in *S. kronenburgii* (45).

The major components in each of the seven species were as follows: *S. coccinea* (Z)-3-hexenal (31%), viridiflorol (19%); *S. farinacea* 1-octen-3-ol (30%) and (Z)-3-hexenal (23%); *S. greggii* 1,8-cineole (22%), borneol (17%), camphene (11%) and α -pinene (10%); *S. leucantha* limonene (35%) and α -pinene (17%); *S. longispicata x farinacea* 1-octen-3-ol (50%) and (Z)-3-hexenal (24%); *S. madrensis* (Z)-3-hexenal (53%); *S. roemeriana* limonene (49%) and α -pinene (20%); and *S. splendens* (Z)-3-hexenal (36%), 2,5-dimethoxy-p-cymene (19%) and linalool (11%) (46).

It is said that, in the comparison of major compounds of two *Salvia* essential oil with the other *Salvia* species; we can say that α/β -pinene characteristic group in *S. syriaca* (12.6% - 7.3%) (47), *S. caespitosa* (6.8% - 22%) (48), *S. blepharochlaena* (10.1% - 4%) (48), *S. pilifera* (11.2% - 1%) (48), *S. hypoleuca* (5.9% - 7.2%) (49), *S. officinalis* (3.1% - 9.8%) (50), *S. tomentosa* (33.7% - 6.8%) (29), *S. verticillata* (30.7% - 7.6%) (30) and *S. bracteata* (9.4% - 10.5%); β -caryophyllene dominated group are; *S. triloba* (11.8%) (51), *S. longipedicellata* (16.1%) (48), *S. hypoleuca* (14.6%) (49), *S. palaestina* (18%) (28), *S. verticillata* (13.3%) (30), *S. russellii* and *S. bracteata* (4.8% - 16.7%) (52); caryophyllene oxide dominated group are; *S. hypergeia* (10.7%), *S. longipedicellata* (23.3%) (48), *S. palaestina* (7.3%) (29), *S. trichoclada* (7.0%) (28); *Salvia sclarea* (15.5%) (Table 1); camphor dominated group are; *Salvia multicaulis* (13.2%) (28), *S. trichoclada* (11.3%) (28), *S. verbenaca* (7.0%) (30) and *S. bracteata* (17.8%) (52).

Conclusion

The main conclusion from the above data, particularly infraspecific differences means, might be explain that genetic and environmental factors both play a role in determining the composition of essential oils of the *Salvia* species studied. In addition, spathulenol was found as major compound for both *Salvia sclarea* and *Salvia verticillata* subsp. *verticillata* while, the other main components were not showed similarity. In addition, the results were discussed with the *Salvia* genus pattern in means of chemotaxonomy, natural products and renewable resources.

Conflict of interests: We declare that we have no conflict of interests.

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