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Constituents of the Essential Oil from the Flower, Leaf and Stem of *Salvia viridis* L. Grown in Turkey

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The essential oil of *Salvia viridis* L. (Lamiaceae) was prepared by hydrodistillation of flower, leaf and stem and characterized by GC and GC-MS. A total of 53, 66 and 35 compounds were identified, constituting over 96.5, 96.4 and 88.5 % of oil composition of the flower, leaf and stem of *S. viridis*, respectively. Sesquiterpene hydrocarbons were shown to be the main group of constituents (in flower: 52.8 %, in leaf: 50.2 %, in stem: 48.7 %). The major components of the oils of *S. viridis* were β-pinene (leaf, 26.4 %), *trans*-muurola-4(14),5-diene (flower, 18.5 %) and germacrene D (stem, 16.0 %). Flower and leaf parts of the oils were rich in terpenoid constituents in the total ratio of 96.3 % in flower, 94.9 % in leaf and stem oil of *S. viridis* had less terpenoids (72.6 %).

Key Words: *Salvia viridis*, Lamiaceae, Essential oil, GC-FID, GC-MS.

INTRODUCTION

Salvia, the largest genus of Lamiaceae, consists of about 900 species and widespread throughout the world[1-3]. *Salvia* L. is represented with 86 native species in Anatolia which is a major centre for this genera in Asia, 50 % of the 86 species are endemic to Turkey[1,2]. *S. viridis* L. is an annual plant and known as synonym of *S. horminum* L[3,4]. But some botanist still believes that they are two separate species[3,4]. *S. viridis* with lilac-purple to white flower is the only annual species of *Salvia* in Turkey and its flowering shoots are used in Anatolian folk medicine[5]. In "A Modern Herbal" mentioned that the seeds and leaves of *S. viridis* used for the fermenting vats to greatly increase the inebriating quality of the liquor. Powdered of the leaves was used for snuff and an infusion of the leaves for sore gums. It is also known as a good honey producing plant[3,4]. *S. viridis* is mainly distributed rocky slopes, waste ground and fields of Mediterranean area, Central and South East Anatolia, Caucasia and Iran[1,2]. Several species such as *S. officinarum* L., *S. fruticosa* Miller, *S. aethiopis* L., *S. dichroantha* Staof, *S. tomentosa* Miller and *S. sclarea* L.

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are used in Anatolian folk medicines against stomach pain and carminative[5]. *S. sclarea* is now cultivated in France and Russia for its essential oils[6] and the roots of *S. moorcroftiana* Wall ex Benth are used in colds and coughs against stomach pain in several countries[6]. The most important species of *Salvia* is *S. officinalis* L. known as sage. Sage is stated to poses carminative, antispasmodic, antiseptic and antihidrolitic properties and traditionally it has been used to treat flatulent dyspepsia, pharryngitis and stomatitis[7]. The herbs and/or their essential oils are widely used as herbal tea, cosmetics, perfumery, pharmaceuticals and food flavoring and possess significant biological activities, including antibacterial, antiviral, antitumor, antioxidant, antiinflammatory and antihydrotic activity[7-25].

Antibacterial, antioxidant activities and chemotaxonomic evaluation of *S. viridis* have mentioned and showed moderate activities[18-25]. Previous works on the essential oils from *Salvia* L.[8-17] genus included; *S. verticillata* L., *S. santolinifolia* Boiss., *S. palaestina* Bentham., *S. bertolonii* Vis., *S. pratensis* L., *S. spinosa* L., *S. bracteata* Bank et Sol., *S. rhytidea* Benth., *S. aethiopis* L., *S. nemorosa* L., *S. reuterana* Boiss., *S. fructicosa* Miller., *S. aramiensis* Rech. Fil., *S. cyanescens* Boiss. and Bal. and *S. horminum* L. The major constituents of *S. Verticillata*[8] were β-caryophyllene (24.7 %), γ-muurolene (22.8 %), limonene (8.9 %) and α-humulene (7.8 %). α-Pinene (59.4 %), β-pinene (12.4 %) and limonene (3.8 %) were the main components in the essential oil of *S. Santolinifolia*[8]. The minor components were sclareol (26.8 %), β-caryophyllene (16.9 %), linalool (7.8 %), guaiol (5.4 %) and 1,8-cineole (5.2 %) in the oil of *S. Palaestina*[9]. The major compound in the essential oil of *S. Pratensis*[10] was E-caryophyllene (26.4 %) while in *S. Berolonii*[10] essential oil caryophyllene oxide was the major component (35.1 %). (E)-β-Ocimene (12.3 %), β-caryophyllene (10.2 %) and isopentyl isovalerate (9.5 %) were the main constituents in the essential oil of *S. Spinosa*[11]. The oil of *S. bracteata*[12] had high amounts of monoterpene compounds with α-pinene, limonene, myrecene and β-pinene as major components in different growth stages. The main constituents were *p*-cymene-8-ol (11.9 %), spathulenol (7.3 %), pulegone (6.4 %), sabinene (5.8 %), terpinen-4-ol (5.5 %) and β-copaene (5.3 %) in *S. rhytidea*[13]. Germacrene D (29.0 %), α-copaene (19.8 %), β-cubebene + β-elemene (9.9 %), bicyclogermacrene (9.3 %), δ-cadinene (8.7 %) and β-caryophyllene (7.3 %) were the main components of *S. aethiopis*[14]. β-Caryophyllene (41.6 %), germacrene-D (21.3 %), caryophyllene oxide (6.8 %), *cis*-β-farnesene (6.0 %) and germacrene-D (5.6 %) as the major constituents in the oil of *S. nemorosa*[15]. The major constituents in the oil of *S. reuterana*[15] were (E)-β-ocimene (32.3 %), α-gurjunene (14.1 %), germacrene-D (11.2 %) and hexyl acetate (7.6 %). *S. fructicosa*[16] essential oil gave high contents of 1,8-cineole (47.48 %), R- and α-thujone (11.93 %) and camphor (9.04 %). The main constituents of the essential oils were obtained as 1,8-cineole (60.0 %) in *S. aramiensis*[17] and spathulenol (32.5 %) in *S. cyanescens*[17]. β-Pinene (32.5 %) and α-humulene (15.3 %) were the main components of *S. horminum*[26].

The literature survey showed the whole plant essential oil composition of *S. horminum*, but no reports on the essential oil composition from the flower, leaf and stem parts of the *S. viridis* are available in literature. The crude volatiles were investigated by GC-FID and GC-MS technique[27-36]. The identification of the substances was performed by comparison of retention indexes on HP-5 column (determined relatively to the retention times of a series of *n*-alkanes), authentic compounds and mass spectra with literature (Nist and Wiley)[27-36]. The present study was undertaken to verify the composition of the volatile compounds present in the flower, leaf and stem parts of the *S. viridis*.

EXPERIMENTAL

Salvia viridis L. was collected in Sebinkarahisar, Giresun-Turkey (at heights of 850 m) in the northeastern part of Turkey in June, 2008. The plant was authenticated by S. Terzioglu[1,2]. Voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO(KATO: 10482), Karadeniz Technical University, Turkey.

Isolation of the essential oils: The fresh plant materials were separated into flower, leaf and stem parts and then grounded into small pieces. The essential oils from fresh aerial parts (*ca.* 100 g, each) of *S. viridis* were isolated by hydrodistillation in a Clevenger-type apparatus[14,15] with cooling bath (-15 ºC) system (4 h) (yields: 0.28, 0.17 and 0.12 % (v/w), respectively). The obtained oils were extracted with HPLC grade *n*-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at 4-6 ºC in a sealed brown vial.

Gas chromatography and gas chromatography-mass spectrometry (GC-MS) analysis: GC-FID and GC-MS analyses were done as described previously[28,29].

Identification of constituents: Retention indices of all the components were determined by Kovats method using *n*-alkanes (C_5-C_{32}) as standards. The constituents of the oils were identified by comparison of their mass spectra with those of mass spectral libraries (NIST and Wiley), authentic compounds (α-pinene, camphene, β-pinene, myrcene α-terpinene, γ-terpinene, linalool, heneicosane, tricosane, tetracosane, pentacosane and hexacosane) and with data published in the literature[27-36].

RESULTS AND DISCUSSION

The chemical composition of the essential oils from the flower, leaf and stem of *S. viridis* are presented in Table-1. Ninety two essential compounds were identified by GC-FID and GC-MS with HP-5 column[27-36]. The flower oil showed the presence of 53 components, representing 96.5 % of the total oil. The major components in the essential oil of the flower were *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 %). Sixty two compounds were identified in the oil of leaf, accounting 96.4 % of the total oil. The main constituents of the leaf oil were β-pinene (26.4 %), β-copaene (13.3 %), *trans*-muurola-4(14),5-diene (9.0 %), zonarene (3.8 %) and α-humulene (3.6 %). On the other hands, 35 components representing for 88.5 % of constituents of the

TABLE-1
IDENTIFIED COMPONENTS IN THE ESSENTIAL OILS OF *S. viridis*[a,b]

Compounds	Flower area (%)	Leaf area (%)	Stem area (%)	Ex. RI	Lit. RI
Monoterpene hydrocarbons					
α-Thujene	0.1	0.2	–	930	930
α-Pinene	1.8	2.1	–	940	939
Camphene	–	0.2	–	952	954
β-Pinene	4.5	26.4	1.4	979	979
Myrcene	17.2	0.4	–	992	991
p-Mentha-1(7),8-diene	0.6	0.2	–	1004	1004
α-Terpinene	–	0.1	–	1015	1017
p-Cymene	–	0.1	–	1024	1025
β-Phellandrene	3.4	1.3	–	1030	1030
δ-3-Carene	5.1	–	–	1032	1031
(Z)-β-Ocimene	–	1.5	–	1037	1037
(E)-β-Ocimene	1.2	3.0	–	1051	1050
γ-Terpinene	-	0.2	–	1059	1060
Terpinolene	0.2	0.3	–	1087	1089
Allo-ocimene	0.1	–	–	1130	1132
Oxygenated monoterpenes					
cis-Sabinene hydrate	–	0.1	–	1067	1070
Linalool	0.7	0.1	–	1095	1097
trans-Pinocarveol	–	0.1	–	1138	1139
Pinocarvone	0.1	0.1	–	1165	1165
Terpinen-4-ol	0.2	0.3	–	1179	1177
Safranal	0.3	–	–	1198	1197
γ-Terpineol	–	0.2	–	1200	1199
β-Cyclocitral	–	0.3	–	1219	1217
Sesquiterpene hydrocarbons			–		
δ-Elemene	0.1	–	–	1340	1338
α-Cubebene	0.3	0.5	–	1354	1351
α-Ylangene	–	0.2	–	1378	1375
α-Copaene	0.1	1.1	0.4	1379	1377
β-Cubebene	1.1	0.3	–	1388	1388
β-Bourbonene	5.0	2.9	3.4	1390	1388
β-Elemene	1.2	0.5	–	1394	1391
(Z)-Caryophyllene	0.2	0.1	0.5	1410	1409
(E)-Caryophyllene	–	–	9.8	1421	1419
β-Copaene	12.6	13.3	0.8	1432	1432
γ-Elemene	1.7	–	–	1440	1437
Aromadendrene	–	0.1	0.5	1441	1441
cis-muurola-3,5-diene	0.8	0.2	–	1450	1450
α-Humulene	3.4	3.6	3.3	1456	1455
Sesquisabinen	–	–	1.0	1459	1460
Allo-aromadendrene	–	0.3	–	1463	1460
9-Epi-(*E*)-Caryophyllene	0.9	–	–	1466	1466
cis-Muurola-4(14),5-diene	–	–	0.3	1468	1467

γ-Muurolene	0.9	0.8	–	1477	1480
Germacrene D	–	7.9	16.0	1485	1485
trans-Muurola-4(14),5-diene	18.5	9.0	–	1493	1494
γ-Amorphene	1.3	–	1.5	1497	1496
Bicyclogermacrene	–	1.5	–	1502	1500
α-Muurolene	0.5	-	0.8	1501	1500
Epizonarene	–	0.8	–	1504	1502
δ-Amorphene	0.1	0.2	–	1513	1512
γ-Cadinene	1.4	–	2.8	1516	1514
δ-Cadinene	–	2.0	5.7	1522	1523
Zonarene	–	3.8	–	1531	1530
α-Cadinene	0.4	0.2	1.0	1540	1539
α-Calacorene	0.1	0.5	0.2	1545	1546
Selina-3,7(11)-diene	–	0.1	–	1550	1547
Germacrene-B	2.1	0.2	0.7	1562	1561
β-Calacorene	0.1	0.1	–	1568	1566
Oxygenated sesquiterpenes					
Germacrene D-4-ol	0.4	–	–	1579	1576
Caryophyllene oxide	2.3	2.0	7.3	1584	1583
Viridiflorol	–	0.1	–	1592	1593
Salvial-4(14)-en-1-one	–	–	0.8	1595	1595
Humulene epoxide II	0.4	0.7	1.5	1610	1608
1-Epi-cubenol	–	0.7	–	1629	1629
Alloaromadendrene epoxide	–	–	0.6	1643	1641
α-Muurolol	–	–	3.0	1648	1646
Cubenol	0.3	0.1	–	1649	1647
α-Cadinol	1.3	1.4	5.1	1655	1654
Valerianol	0.2	–	–	1656	1658
Neo-intermedeol	–	1.6	–	1659	1660
Intermedeol	1.9	–	–	1665	1667
trans-Calamenen-10-ol	–	0.1	0.2	1670	1669
(Z)-α-Santalol	0.1	–	–	1673	1675
Khusinol	0.1	–	–	1682	1680
α-Bisabolol	–	–	2.3	1684	1686
δ-Cedren-3-ol	0.4	–	–	1690	1689
Mint sulfide	0.1	0.1	–	1739	1741
14-Hydroxy-δ-Muurolene	–	0.1	–	1777	1780
14-Hydroxy-δ-Cadinene	0.1	0.2	–	1806	1804
Oxygenated diterpenes					
Epi-13-manoyl oxide	0.2	0.2	1.3	2014	2017
Terpene related compounds					
Cryptone	0.1	-	–	1189	1186
Hexahydro farnesylacetone	0.1	0.1	0.4	1848	1847
Farnesyl acetone	–	0.1	–	1920	1919
Others					
1-Octen-3-ol	–	–	1.5	982	979
Benzene acetaldehyde	–	0.2	0.3	1043	1042
Nonanal	–	0.1	1.3	1098	1101
Decanal	–	–	1.0	1205	1202

Palmitic acid	–	0.7	11.4	1986	1983
Heneicosane	–	0.1	0.2	2100	2100
Tricosane	0.1	0.2	–	2302	2300
Tetracosane	–	0.1	–	2401	2400
Pentacosane	–	0.1	–	2500	2500
Hexacosane	0.1	–	0.2	2601	2600

[a]RI calculated from retention times relative to that of *n*-alkanes (C_5-C_{32}) on the non-polar HP-5 column. [b]Percentages obtained by FID peak-area normalization. [c]Identified by authentic samples.

stem oil were identified and the major compounds were germacrene D (16.0 %), palmitic acid (11.4), (E)-caryophyllene (9.8 %), caryophyllene oxide (7.3 %) and δ-cadinene (5.7 %). A total of 17 compounds (five of them are traces) were mentioned, constituting over 70.7 % of oil composition of the *S. horminum*[26]. β-Pinene (32.5 %) and α-humulene (15.3 %) were the main constituents of *S. horminum*[26]. In present case, β-pinene was main component in the leaf oil, but α-humulene was minor constituent in all three parts of essential oil in *S. viridis*.

The chemical class distributions of the volatile constituents in the flower, leaf and stem of *S. viridis* are summarized in Table-2. The compounds were separated into two classes, which were terpenoids (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, oxygenated diterpene and terpene related compounds) and others (Table-2)[27-36]. The major constituents were sesquiterpene hydrocarbons (in flower: 52.8 %, in leaf: 50.2 %, in stem: 48.7 %), monoterpene hydrocarbons (in flower: 34.2 %, in leaf: 36.0 %, in stem: 1.4 %) and oxygenated sesquiterpenes (in flower: 7.6 %, in leaf: 7.1 %, in stem: 20.8 %) in the oils of *S. viridis*. The numbers of the identified terpenoids in the flower, leaf and stem of *S. viridis* were 49, 57 and 27 out of 53, 66 and 35 compounds, respectively. Fourteen components were common to all tree part of the plant with the total ratio of 32.4, 52.4 and 25.6 %, respectively.

TABLE-2
CHEMICAL CLASS DISTRIBUTION IN THE ESSENTIAL OILS OF *S. viridis*

Constituents	Flower		Leaf		Stem	
	Area (%)	NC[a]	Area (%)	NC[a]	Area (%)	NC[a]
Terpenoids						
Monoterpene hydrocarbons	34.2	10	36.0	13	1.4	1
Oxygenated monoterpenes	1.3	4	1.2	7	–	–
Sesquiterpene hydrocarbons	52.8	22	50.2	25	48.7	17
Oxygenated sesquiterpenes	7.6	12	7.1	11	20.8	8
Oxygenated diterpene	0.2	1	0.2	1	1.3	1
Terpene related compounds	0.2	2	0.2	2	0.4	1
Others	0.2	2	1.5	7	15.9	7
Total	96.5	53	96.4	66	88.5	35

[a]NC: Number of compounds.

Whole part of the essential oil composition of *S. horminum* has been demonstrated the presence of about 40 % monoterpene hydrocarbons, 20 % of oxygenated monoterpenes and 40 % of sesquiterpenes.

β-Pinene (26.4 %), *trans*-muurola-4(14),5-diene (18.5 %), myrcene (17.2 %) and germacrene D (16.0 %) were common in the essential oils of several *Salvia* species (*S. verticillata*, *S. bracteata* and *S. nemorosa*)[8,12,15] with different ratios. Comparison of volatiles with those mentioned in the literature indicates that identified compounds are in most cases similar in the sage essential oil[8-17]. The oil of the stem did not give the monoterpene hydrocarbons and oxygenated monoterpenes except β-pinene. Monoterpene and sesquiterpene type hydrocarbons; β-pinene, α-copaene, β-bourbonene, (Z)-caryophyllene, β-copaene, α-humulene, α-cadinene, α-calacorene, germacrene D, caryophyllene oxide, humulene epoxide II and α-cadinol are found in all three parts of the oils but in different percentages. Some components exist in just one of the oils, which can be seen in the Table-1.

A total of 17 compounds (5 of them are traces) were mentioned, constituting over 70.7 % of oil composition of the *S. horminum* and β-pinene (32.5 %) and α-humulene (15.3 %) were the main constituents of it[26]. Whole part of the essential oil composition of *S. horminum* has been demonstrated the presence of about 40 % monoterpene hydrocarbons, 20 % of oxygenated monoterpenes and 40 % of sesquiterpenes. Camphene, *p*-cymene, myrcene, β-phellandrene, pinene, terpinene, terpinolene, α-humulene, caryophyllene, linalool, terpinen-4-ol and terpineol were common to *S. horminum*[26] and *S. viridis* with different ratios. But, limonene, α-phellandrene, tricylene, camphor and 1,8-cineole were not found in the essential oils of *S. viridis*. In our case, we identified 75 more terpenoids and other constituents form *S. viridis* (Table-1). All parts of the oils were rich in terpenoid components in the total ratio of 96.3 % in flower, 94.9 % in leaf and 72.6 % in stem of *S. viridis*. Others were the minor constituents (flower: 0.2 %, leaf: 1.5 %, stem: 15.9 %) in the oils. It could be concluded that the compositions of the volatile oils extracted from the flower, leaf and stem were different as expected. The general chemical profile of the essential oils of *S. horminum* and *S. viridis* showed differences which can be explained by the environmental factors, the subspecies and the parts of the plant used.

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