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Chemical Composition and Biological Activity of *Salvia verbenaca* Essential Oil

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Salvia verbenaca L. (syn. *S. minore*) is a perennial herb known in the traditional medicine of Sicily as "spaccapetri" and is used to resolve cases of kidney stones, chewing the fresh leaves or in decoction. The chemical composition of the essential oil obtained from aerial parts of *S. verbenaca* collected in Piano Battaglia (Sicily) on July 2009, was analyzed by GC and GC-MS. The oil was strongly characterized by fatty acids (39.5%) and carbonylic compounds (21.2%), with hexadecanoic acid (23.1%), (*Z*)-9-octadecenoic acid (11.1%) and benzaldehyde (7.3%) as the main constituents. The *in vitro* activity of the essential oil against some microorganisms in comparison with chloramphenicol by the broth dilution method was determined. The oil exhibited a good activity as inhibitor of growth of Gram + bacteria.

Keywords: *Salvia verbenaca*, Lamiaceae, volatile components, hexadecanoic acid, (*Z*)-9-octadecenoic acid, benzaldehyde, β-phellandrene, antibacterial activity.

The genus *Salvia* (sage) is one of the largest and the most important aromatic and medicinal genus of the Lamiaceae family, comprising about 900 species widespread throughout the world [1]. *Salvia* species are used in folk medicine all around the world for their antibacterial, antitumor [2], antidiabetic [3], and antioxidant [4] activities. Members of this genus produce many useful secondary metabolites including terpenes and phenolics and their derivatives that have been in the center of pharmacopoeias of many countries [5].

Salvia verbenaca L. is known in Italy as *Salvia minore* and it's a tall herbaceous perennial plant, 20-50 cm high, with bluish purple flowers of about 1 cm length arranged in verticillasters (each of which generally contains six flowers). The calyx (green, 4-8 mm long) encloses a 6-10 mm long corolla. Nutlet fruits contain 1-4 seeds. The verticillasters are close together on the stern at flowering, but move further apart by fruit set. Flowering commences in mid-April and finishes towards the end of May [6]. The species is distributed in the Mediterranean area and in Italy is found frequently throughout the territory with the exclusion of the Alps. In Sicily, the plant is spread in scrublands and grasslands throughout all the island, from sea level to 1.200 m. above sea level [7]. In the Sicilian traditional medicine is known as "*spaccapetri*" and its leaves and flowering aerial parts are used to resolve cases of kidney stones, chewing the fresh leaves or in decoction. The plant is also known as bactericide against respiratory ailments, as healing in wounds and ulcers, and above all as eyedrops, because fruits or seeds when applied on the eyes remove impurities or dust particles.

As part of our extensive screening program of *Salvia* species from Mediterranean Area [2,4,8,9], we report in this paper the qualitative and quantitative analyses of the essential oil of wild population of *S. verbenaca* collected in Sicily and compare it with those previously reported.

A total of 76 constituents, representing 91.8% of the total oil, have been identified from the essential oil extracted from the aerial parts of *S. verbenaca*. In Table 1 the

Table 1: Volatile components of aerial parts of *Salvia verbenaca*.

COMPONENT	LRI [a]	LRI [b]	% [c]	Identification[d]
Hydrocarbons			**4.7**	
Nonane	900	900	1.2	R_i, MS
α-Ionene	1208		0.2	R_i, MS
Tricosane	2300	2300	0.9	Ri, MS, Co-GC
Tetracosane	2400	2400	0.4	Ri, MS, Co-GC
Pentacosane	2500	2500	0.8	R_i, MS, Co-GC
Heptacosane	2700	2700	0.7	R_i, MS, Co-GC
Nonacosane	2900	2900	0.5	R_i, MS, Co-GC
Carbonylic compounds			**21.2**	
(*E*)-2-Hexenal	854	1209	1.5	R_i, MS
Heptanal	903	1188	0.4	R_i, MS
Benzaldehyde	963	1543	7.3	R_i, MS, Co-GC
1-Octen-3-one	975	1308	0.3	R_i, MS
Phenyl acetaldehyde	1044	1663	1.5	R_i, MS, Co-GC
Acetophenone	1058	1657	0.2	R_i, MS, Co-GC
Nonanal	1102	1401	1.1	R_i, MS
(*E,E*)-2,4-Octadienal	1125		0.3	R_i, MS
(*E,Z*)-2,6-Nonadienal	1154	1572	0.5	R_i, MS
Decanal	1204	1508	0.3	R_i, MS
(E)-2-Decenal	1260	1655	0.5	R_i, MS
(*E,E*)-2,4-Decadienal	1315	1827	0.3	R_i, MS
β-Damascenone	1380	1835	0.8	R_i, MS
(*E*)-Geranylacetone	1453	1867	0.5	R_i, MS
(*E*)-β-Ionone	1484	1958	1.9	Ri, MS, Co-GC
Tetradecanal	1619	1934	0.2	R_i, MS
Hexahydrofarnesyl acetone	1845	2131	0.7	R_i, MS
9,12,15-Octadecatrienal	2111		2.9	R_i, MS, Co-GC
Terpenoids				
Monoterpene hydrocarbons			**11.5**	
α-Pinene	936	1075	0.5	R_i, MS, Co-GC
Sabinene	973	1132	0.5	R_i, MS, Co-GC
β-Pinene	978	1118	0.5	R_i, MS, Co-GC
α-Terpinene	1012	1189	0.9	R_i, MS, Co-GC
p-Cymene	1025	1278	0.4	R_i, MS, Co-GC
β-Phellandrene	1029	1218	5.9	R_i, MS, Co-GC
Limonene	1030	1203	2.0	R_i, MS, Co-GC
γ-Terpinene	1057	1256	0.8	R_i, MS, Co-GC
Sesquiterpene hydrocarbons			**2.2**	
(*E*)-Caryophyllene	1418	1612	1.2	R_i, MS, Co-GC
(*E*)-β-Farnesene	1452	1673	0.3	R_i, MS
α-Humulene	1455	1689	0.2	R_i, MS
allo-Aromadendrene	1463	1661	0.1	R_i, MS
α-Amorphene	1475	1679	t	R_i, MS
β-Selinene	1475	1715	0.1	R_i, MS
Germacrene D	1477	1726	t	R_i, MS
γ-Cadinene	1515	1776	0.3	R_i, MS
α-Calacorene	1542	1918	t	R_i, MS
Oxygenated monoterpenes			**3.3**	
1,8-Cineole	1034	1213	0.2	R_i, MS, Co-GC
Linalool	1098	1553	0.7	R_i, MS, Co-GC
Camphor	1143	1532	t	R_i, MS, Co-GC
Pinocarvone	1154	1587	1.1	R_i, MS
Borneol	1167	1719	0.1	R_i, MS, Co-GC
Terpinen-4-ol	1176	1611	t	R_i, MS, Co-GC
p-Cymen-8-ol	1185	1856	0.3	R_i, MS
α-Terpineol	1187	1706	0.5	R_i, MS, Co-GC
Safranal	1201	1618	0.2	R_i, MS
Carvone	1242	1750	0.2	R_i, MS
Oxygenated sesquiterpenes			**3.9**	
Isocaryophyllene oxide	1527	2001	t	R_i, MS
Germacrene D 4-ol	1575	2065	0.3	R_i, MS
Spathulenol	1577	2148	1.7	R_i, MS
Caryophyllene oxide	1581	2208	1.9	R_i, MS, Co-GC
Caryophylladienol I	1640	2316	t	R_i, MS
Fatty acids and esters			**39.5**	
Bornyl angelate	1566		t	Ri, MS
Dodecanoic acid	1566	2503	0.4	Ri, MS, Co-GC
Tetradecanoic acid	1769	2713	0.9	Ri, MS, Co-GC
Pentadecanoic acid	1873	2740	0.5	Ri, MS, Co-GC
Methyl hexadecanoate	1925	2208	0.7	Ri, MS, Co-GC
Hexadecanoic acid	1972	2931	23.1	Ri, MS, Co-GC
Ethyl hexadecanoate	1994	2245	2.6	Ri, MS
(*Z*)-9-Octadecenoic acid	2117	3157	11.1	Ri, MS
(*Z,Z*)-9,12-Octadecadienoic acid ethyl ester	2162	2532	0.2	Ri, MS

Table 1 (*contd.*)

Phenolic compounds			**2.3**	
Thymol methyl ether	1239	1611	0.2	R_i, MS
Thymol	1290	2198	0.8	R_i, MS, Co-GC
Carvacrol	1297	2239	0.6	R_i, MS, Co-GC
Eugenol	1353	2186	0.7	R_i, MS, Co-GC
Others			**3.2**	
1-Octen-3-ol	977	1452	0.4	R_i, MS
2-Pentylfuran	1002	1243	0.9	R_i, MS
2-Phenylethanol	1115	1934	0.4	R_i, MS
(*Z*)-Phytol	1949	2622	1.5	R_i, MS
(*E*)-Phytol	2132	2625	T	R_i, MS
Squalene	2828	3408	t	R_i, MS
Total amount of compounds			**91.8**	

[a]Linear Retention Index on a HP-5 MS column, [b]Linear Retention Index: retention index on an Innowax column, [c]t: trace, less than 0,05%, [d] R_i: retention index matches with bibliography; MS: identification based on comparison of mass spectra; Co-GC: comparison of retention time of authentic compounds.

retention indices, percentage composition and identification methods are given; the components, grouped in class of substances, are listed in order of elution on a HP 5MS column. Carbonylic compounds (21.2%) and fatty acids (39.5%) were the main fractions of the oil, while the terpenoidic fraction of the oil amounted to 20.9%, with monoterpenes accounting to 14.8% and sesquiterpenes to 6.1%. The most abundant compound was hexadecanoic acid (23.1%), followed by (*Z*)-9-octadecenoic acid (11.1%), benzaldehyde (7.3%) and the monoterpene hydrocarbon β-phellandrene (5.9%).

The essential oil of *S. verbenaca* from Sicily presented noticeably different qualitative and quantitative results compared with the other studied oils. Pitarokili *et al*., (Greece) [10] detected as main compounds β-phellandrene (30.3%) and (*E*)-caryophyllene (16.1%), whereas Al-Howiriny (Saudi Arabia) [11] reported sabinene (16.0%), cadinene (7.9%), 4-terpineol (7.4%) and pinene (7.3%) as the dominating compounds. *S. verbenaca* essential oil from Morocco presents terpineol (19.2%), camphor (6.6%) and β-thujone (6.1%) as main compounds [12], while Ben Taarit *et al*. [13,14] showed that *S. verbenaca* wild-growing in different locations in Tunisia is particularly rich in oxygenated sesquiterpenes and monoterpene hydrocarbons, particularly viridiflorol (21.8%), tricyclene (18.8%), (Z)-β-ocimene (29.5%) for the samples collected in Sabelet Ben Ammar, Somaa and Sers respectively and β-caryophyllene (23.1%) and caryophyllene oxide (15.9%) for the samples collected in the northeast region of Tunisia. Previous papers [15, 16] showed that many factors affect the yield and the composition of essential oils of *Salvia* species, including plant source, individual plant chemotypes, time of harvest, the environmental conditions and the proportion of plant parts distilled.

The *in vitro* antibacterial activity of the essential oil of *S. verbenaca* against eight bacterial strains was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using the broth method. The oil appeared to be not active against Gram – bacteria (MICs >100 μg/mL), while it showed antibacterial activity against the Gram + bacteria tested. In particular, *Staphylococcus aureus* and *Streptococcus faecalis* were slightly sensitive to the action of the oil (MIC = 100 μg/mL), while the oil exerted an appreciable activity against *Bacillus subtilis* and *Staphylococcus epidermidis* (MIC = 50 μg/mL for both)

Experimental

Plant material: Aerial parts of *S. verbenaca* were collected at the full flowering stage on July 2009 from plants growing in Piano Battaglia (Sicily). Voucher specimens were deposited at the Herbarium of the Botanical Gardens of Palermo (Italy) under the number PAL 09-876.

Isolation of the volatile components: The fresh samples were cut into small pieces, then subjected to hydrodistillation according to the standard procedure described in the European Pharmacopoeia [17]. The yield (w/w) was 0.18%. The oil was dried over anhydrous sodium sulphate and then stored in sealed vials, at - 20°C, ready for the GC and GC-MS analyses.

Gas chromatography: Analytical gas chromatography was carried out on a Perkin-Elmer Sigma 115 gas chromatograph fitted with a HP-5 MS capillary column (30 m x 0.25 mm i.d.; 0.25 μm film thickness). Column temperature was initially kept at 40°C for 5 min, then gradually increased to 250°C at 2°C min^{-1}, held for 15 min and finally raised to 270°C at 10°C min^{-1}. Diluted samples (1/100 v/v, in *n*-pentane) of 1 μL were injected manually at 250°C, and in the splitless mode with a 1 minute purge-off due to the small amount of oil partially utilized for biological tests. Flame ionization detection (FID) was performed at 280°C. Analysis was also run by using a fused silica HP Innowax polyethylenglycol capillary column (50 m x 0.20 mm i.d.; 0.20 μm film thickness). Helium was the carrier gas (1 mL min^{-1}) in both cases.

Gas chromatography - mass spectrometry: GC-MS analysis was performed on an Agilent 6850 Ser. II apparatus, fitted with a fused silica HP-1 capillary column (30 m x 0.25 mm i.d.; 0.33 μm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization energy voltage 70 eV; electron multiplier voltage energy 2000 V. Mass spectra were scanned in the range 35-450 amu, scan time 5 scans/s. Gas chromatographic conditions were as reported above; transfer line temperature, 295°C.

Identification of components: Most constituents were identified by gas chromatography by comparison of their retention indices (LRI) with either those of the literature [18, 19] or with those of authentic compounds available in our laboratories. The retention indices were determined by GC-FID mode in relation to a homologous series of *n*-alkanes (C_8-C_{28}) under the same operating conditions on both columns. Further identification was made by comparison of their mass spectra on both columns with either those stored in NIST 02 and Wiley 275 libraries or with mass spectra from the literature [19, 20] and our home made library. Component relative concentrations were calculated based on GC-FID peak areas without using correction factors.

Antimicrobial activity: The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using the broth dilution method as previously described [2]. Eight bacteria species, selected as representative of the class of Gram positive and Gram negative, were tested: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATTC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Proteus vulgaris* (ATCC 13315) and *Pseudomonas aeruginosa* (ATCC 27853).

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