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Chemical Composition of the Essential Oil from Aerial Parts of *Stachys palustris* L. (Lamiaceae) Growing Wild in Southern Italy

Felice Senatore,[a,*] Carmen Formisano,[a] Daniela Rigano,[a] Franco Piozzi,[b] and Sergio Rosselli[b]

[a]*Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli »Federico II«, Via D. Montesano, 49, I-80131 Napoli, Italy*

[b]*Dipartimento di Chimica Organica, Università degli Studi di Palermo, Viale delle Scienze, Parco d'Orleans II, I-90128 Palermo, Italy*

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The paper reports the composition of the essential oil from aerial parts of *Stachys palustris* L. (Lamiaceae) from Southern Italy. The essential oil was extracted by hydrodistillation from selected plants and its chemical composition was determined by the GC-MS system on two fused-silica capillary columns of different polarity. The mass fraction of oil was 0.21 % on a dry weight basis. Altogether, 92 compounds were identified accounting for 93.6 % of the total oil, which was characterized mainly by carbonylic compounds (25.4 %), fatty acids and their esters (24.2 %), along with sesquiterpenoidic compounds (16.0 %) and phenols (11.2 %). The major components of the sample were caryophyllene oxide (7.8 %), hexahydrofarnesyl acetone (7.4 %), hexadecanoic acid (6.8 %), (*Z*,*Z*,*Z*)-9,12,15-octadecatrienoic acid (6.7 %), (*Z*)-phytol (6.4 %), thymol (5.8 %), *p*-methoxyacetophenone (5.1 %), 4-vinylguiacole (3. %), tetradecanoic acid (3.8 %), (*E*)-caryophyllene (3.6 %), β-ionone (3.3 %) and β-damascenone (3.0 %).

Keywords
Stachys palustris
Lamiaceae
essential oil
caryophyllene oxide
hexahydrofarnesyl acetone
GC-MS

INTRODUCTION

Stachys L. is a subcosmopolitan genus of herbs and shrubs that comprises more than 270 species[1] and is one of the largest genera of the Lamiaceae. This genus is distributed in temperate and tropical regions of the world, with the exception of Australasia, and is widespread in mountainous and moist places all over the Italian peninsula.[2] The taxonomy of the genus is complicated as there is a wide range of variability between some species, and many infraspecific taxa have been described. At present, it is unknown in which way the composition of volatile oils truly reflects taxonomic relationships in *Stachys*, since many of its members remain to be investigated; however, the chemistry of volatile compounds has been proven particularly helpful in assessing taxonomic relationships of several genera in Labiatae.[3] For this reason, we have analyzed the essential oil of *Stachys palustris* L. (common Italian name *erba strega o scabbiosa*), a common herbaceous creeping perennial of marshy ground. The plant is in flower from July to September, with rather pale purplish flowers in spikes, growing up to about 90 cm.[4] All parts of the plant emit an unpleasant smell when bruised.[5] *S. palustris* is considered a wholesome and nutritious food; edible parts of the plant are leaves, roots and seeds. Tubers are consumed raw or cooked, and they have a pleasant mild nutty flavour. The tubers, harvested in autumn, can be dried and ground into a powder that is used in making

* Author to whom correspondence should be addressed. (E-mail: fesenato@unina.it)

bread. Young shoots can be used as an asparagus substitute, since they have a pleasant taste despite the disagreeable smell.[6–8] In folk medicine, *S. palustris* flowered aerial parts harvested in spring or summer, when just coming into flower, are known as antiseptic, antispasmodic, emetic, emmenagogue, expectorant, haemostatic, nervine, sedative, tonic, vulnerary agents.[9,10] The plant is highly valued for its wound-healing activity, being effective against both internal and external bleeding, and is also used in the treatment of gout, cramps and pains in the joints.[9,10] The active compounds of the plant are iridoids such as harpagide, acetylharpagide and aucubin,[11] flavonoids, phenolic acids,[12,13] tannins,[14] triterpenoids and steroids.[15] In a previous study,[16] the essential oil from *S. palustris* was analyzed by paper chromatography, and two main components, stachynone and stachynene, were determined in the oil. To extend the knowledge about the volatile compounds of *S. palustris*, here we report for the first time the GC/MS determination of the essential oil composition of *S. palustris*. Many *Stachys* species have been investigated for their essential oil to date.[17–23] In the more recent works, Javidnia *et al.*[17] analyzed the essential oil obtained from the aerial parts of *S. obtusicrena* finding spathulenol (11.5 %) and 10-epi-γ-eudesmol (6.8 %) as the main components. The major components of essential oils of dried flowering aerial parts of *S. byzantina*, *S. inflata*, *S. lavandulifolia* and *S. laxa*, collected in the north of Iran, were piperitenone (9.9 %) for *S. byzantina*, hexadecanoic acid (9.1 %) and germacrene D (8.9 %) for *S. inflata*, 4-hydroxy-4-methyl-2-pentanone (9.3 %) and α-pinene (7.9 %) for *S. lavandulifolia*, germacrene D (17.1 %) and 4-hydroxy-4-methyl-2-pentanone (12.3 %) for *S. laxa*.[18] The essential oils from aerial parts of *S. schtschegleevii* and *S. balansae* were both rich in sesquiterpenes (54.2 % and 37.2 %, resp.) with germacrene D (25.8 % and 16.4 %, resp.) as the major component.[19] Skaltsa *et al.* analyzed different *Stachys* species endemic to Greece and made a chemotaxonomic investigation of volatile constituents of this genus;[3,20] sesquiterpene hydrocarbons were shown to be the main group of constituents of all taxa. Grujic-Jovanovic *et al.* analyzed different *Stachys* species from Serbia,[21] finding that sesquiterpene hydrocarbons were the major components of all samples except that of *S. plumosa*, which was rich in monoterpene hydrocarbons. A species from Turkey, *S. aleurites*, was studied by Flamini *et al.* and was found to be rich in sesquiterpene hydrocarbons[22] while germacrene D was the main component of *S. sylvatica*, an Italian species.[23]

EXPERIMENTAL

Plant

S. palustris aerial parts were collected at full flowering in Campania (Southern Italy) in June 2005. A voucher specimen (NAP # SP022) has been deposited in the Herbarium Neapolitanum (NAP), Dipartimento di Biologia Vegetale, Università degli Studi di Napoli »Federico II«, Italy.

Isolation of the Essential Oil

Dried aerial parts of the plants (lots of 25 g) were cut in small pieces and then subjected to hydrodistillation for 3 h with n-hexane as solvent, using a Clevenger apparatus according to the standard procedure reported in the *European Pharmacopoeia*.[24] The dry material gave a pale yellowish oil in a yield of 0.21 % (mass fraction, *w*).

Gas Chromatography

GC analyses were performed on a Perkin-Elmer Sigma-115 gas chromatograph equipped with a FID and a data handling processor. Separation was achieved using a HP-5MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Column temperature: 40 °C, with 5 min initial hold, and then to 260 °C at 2 °C/min, 260 °C (20 min) using He as carrier gas (1.0 μL/min); injection mode splitless (1 μL of a 1:1000 n-hexane solution). Injector and detector temperatures were 250 °C and 290 °C, respectively. Analysis was also run using a fused silica HP Innowax polyethylenglycol capillary column (50 m × 0.20 mm), 0.20 μm film thickness. In both cases, helium was used as carrier gas. Retention indices (R_i) were determined in relation to a homologous series of n-alkanes (C_8–C_{24}) under the same conditions. Relative concentrations of the components were obtained by peak area normalization. No response factors were calculated.

Gas Chromatography – Mass Spectrometry

GC-MS analyses were performed using an Agilent 6850 Series II gas chromatograph linked on-line with an Agilent Mass Selective Detector MSD 5973Network. The column was a HP-5 fused-silica capillary column (30 m × 0.25 mm i.d.; 0.33 μm film thickness). Temperature conditions were the same as used for GC analysis. Interface temperature was 295 °C; mass range 29–350 *m/z*, ionization energy 70 eV, multiplier energy 2000 V, scan time 1 s. Helium was used as carrier gas at 1.0 mL/min. Peak identification was accomplished by comparison of their mass spectra with those stored in the GC-MS data bases (NIST 98 and Wiley 5) and reported in literature.[25,26] Identification of the oil components was also possible by comparison of their linear retention indices with those from literature.[25–27] Whenever possible, co-injection with authentic substances was also performed.

RESULTS AND DISCUSSION

The essential oil from *S. palustris* was a complex mixture of ninety-two constituents, representing 93.6 % of the oil. The components are listed in Table I according to their elution order on a HP-5 MS column. Twenty-two compounds were present in traces while mass fractions of other 24 components were between 0.1 % and 0.2 %.

TABLE I. – Chemical composition of the essential oil from aerial parts of *Stachys palustris* I.

K_i[a]	K_i[b]	Component	Identification[c]	*w*/%[d]
854	1209	(*E*)-2-Hexenal	I, MS	0.2
900	1195	Heptanal	I, MS	T
906	1395	(*E,E*)-2,4-Hexadienal	I, MS	0.1
959	1541	Benzaldehyde	I, MS, Co-GC	0.1
936	1075	α-Pinene	I, MS, Co-GC	T
969	1294	1-Octen-3-one	I, MS	0.8
977	1254	1-Octen-3-ol	I, MS	0.5
978	1118	β-Pinene	I, MS, Co-GC	0.3
990	1244	2-Pentylfuran	I, MS	0.1
996	1233	Ethyl hexanoate	I, MS	T
1008	1506	(*E,E*)-2,4-Heptadienal	I, MS	T
1024	1278	*p*-Cymene	I, MS, Co-GC	0.2
1024	1893-	Benzyl alcohol	I, MS, Co-GC	0.3
1041	1663	Phenylacetaldehyde	I, MS, Co-GC	1.3
1088	1553	Linalool	I, MS, Co-GC	1.5
1113	1925	2-Phenylethanol	I, MS	0.1
1148		(*E,E*)-2,6-Nonadienal	I, MS	0.3
1187	1706	α-Terpineol	I, MS, Co-GC	0.6
1189	1798	Methyl salicylate	I, MS, Co-GC	0.2
1201		Safranal	I, MS	0.4
1232		*p*-Anisaldehyde	I, MS	0.2
1234	1662	Pulegone	I, MS	0.3
1241		(*Z*)-3-Hexyl-2-methyl-butanoate	I, MS	T
1255	1857	Geraniol	I, MS, Co-GC	0.4
1260	1655	(*E*)-2-Decenal	I, MS	T
1267		(*Z*)-Chrysanthenyl acetate	I, MS	T
1290	2198	Thymol	I, MS, Co-GC	5.8
1291	2471	Indole	I, MS, Co-GC	0.6
1297	2239	Carvacrol	I, MS, Co-GC	1.2
1302	1797	*p*-Methoxyacetophenone	I, MS, Co-GC	5.1
1313	2180	4-Vinylguaiacole	I, MS	3.8
1315	1827	(*E,E*)-2,4-Decadienal	I, MS	T
1348	1466	α-Cubebene	I, MS	T
1353	2186	Eugenol	I, MS, Co-GC	0.4
1380	1835	β-Damascenone	I, MS	3.0
1387	1600	β-Elemene	I, MS	0.5
1394	2050	Methyl cinnamate	I, MS, Co-GC	1.0
1404	1666	(*Z*)-Caryophyllene	I, MS	0.2
1410	1568	α-Cedrene	I, MS	T
1418	1612	(*E*)-Caryophyllene	I, MS, Co-GC	3.6
1440	1868	(*E*)-2-Dodecenal	I, MS	0.3
1453	1867	Geranyl acetone	I, MS	0.4
1472	1709	Dodecanol	I, MS, Co-GC	0.3
1484	1958	β-Ionone	I, MS, Co-GC	3.3
1486	2354	Dihydroactinidiolide	I, MS	2.0
1499		β-Himachalene	I, MS	1.0
1520	1839	Calamenene[e]	I, MS	0.1
1523		Megastigmatrienone[e]	I, MS	2.0
1560	2050	(*E*)-Nerolidol	I, MS,Co-GC	1.4
1566	2503	Dodecanoic acid	I, MS, Co-GC	T
1575		Longipinanol	I, MS	0.1
1581	2008	Caryophyllene oxide	I, MS, Co-GC	7.8
1599	2120	α-Cedrol	I, MS	0.4
1600		Widdrol	I, MS	0.3
1606	2133	Cedrenol	I, MS	0.2
1608	2098	β-Oplopenone	I, MS	0.1
1635	2158	T-Cadinol	I, MS	0.3
1640	2316	Caryophylladienol I	I, MS	T
1641	2209	T-Muurolol	I, MS	0.1
1646		Torreyol	I, MS	T
1672		Valeranone	I, MS	0.1
1762	2655	Benzyl benzoate	I, MS, Co-GC	0.5
1769	2713	Tetradecanoic acid	I, MS, Co-GC	3.8
1828	2099	Methyl pentadecanoate	I, MS, Co-GC	0.2
1843		(*E,E*)-Farnesyl acetate	I, MS	T
1845	2131	Hexahydrofarnesyl acetone	I, MS	7.4
1870	2822	Pentadecanoic acid	I, MS, Co-GC	0.3
1892		1-Nonadecene	I, MS	0.4
1925	2208	Methyl hexadecanoate	I, MS, Co-GC	0.2
1942		*ent*-Pimara-8,15-diene	I, MS	0.1
1949	2622	(*Z*)-Phytol	I, MS	6.4
1961		13-*epi*-Manool	I, MS	T
1972	2931	Hexadecanoic acid	I, MS, Co-GC	6.8
2011	2380	13-*epi*-Manoil oxide	I, MS	T
2074	2975	Heptadecanoic acid	I, MS, Co-GC	0.4
2122	3157	(*Z,Z*)-9,12-Octadeca-dienoic acid	I, MS, Co-GC	2.8
2132	2625	(*E*)-Phytol	I, MS	0.4
2140	3193	(*Z,Z,Z*)-9,12,15-Octadecatrienoic acid	I, MS, Co-GC	6.7
2172	3402	Octadecanoic acid	I, MS, Co-GC	1.2
2363		Docosanoic acid	I, MS, Co-GC	0.1
2460		Eicosanoic acid	I, MS	T
		Others[f]		2.6
		TOTAL		93.6

[a] HP-5 MS column. [b] HP Innowax column. [c] I, the retention index; MS, mass spectrum; Co-GC, co-injection with authentic compound. [d] *w*, mass fraction; T, trace (< 0.05 %). [e] correct isomer not identified. [f] long-chain alkanes (see in text).

The essential oil consisted mainly of carbonylic compounds (25.4 %), fatty acids and their esters (24.2 %), accompanied with sesquiterpenoidic compounds (16.0 %) and phenols (11.2 %). *S. palustris* oil showed a significant presence of carbonylic compounds and fatty acids in contrast to the other *Stachys* species.[20–23] Among the carbonylic compounds, ketones prevailed over aldehydes and in the first group hexahydrofarnesyl acetone (7.4 %) predominated along with *p*-methoxyacetophenone (5.1 %), β-ionone (3.3 %), β-damascenone (3.0 %), dihydroactinidiolide and a megastigmatrienone (2.0 %). Among the

aldehydes, phenylacetaldehyde (1.3 %) was the most abundant compound, since fractions of other compounds of the aldehyde group varied between 0.4 % (safranal) and trace amounts. Hexadecanoic and (*Z,Z*)-9,12-octadecadienoic acids, present in similar amounts in the oil (6.8 % and 6.7 %, respectively) prevailed among the seventeen carboxylic compounds. The presence of (*Z,Z*)-9,12-octadecadienoic acid is noteworthy because this acid, considered an essential fatty acid, is the precursor of prostaglandins PG_1 and PG_2. Other components of the oil were sesquiterpenoids and phenols. In the first group, oxygen containing sesquiterpenes predominated over sesquiterpene hydrocarbons both numerically and quantitatively. In fact, thirteen oxygen containing sesquiterpenes accounted for 10.6 % of the oil, with caryophyllene oxide (7.8 %) as the main compound of this group, while seven sesquiterpene hydrocarbons, with (*E*)-caryophyllene (3.6 %) as the most representative compound, accounted for 5.4 % of the oil. These sesquiterpenes were also identified as major components in some other *Stachys* essential oils, such as caryophyllene oxide in the oils of *S. swainsonii* ssp. *scyronica* and *S. swainsonii* ssp. *swainsonii*[3] and (*E*)-caryophyllene in the oils of *S. swainsonii* ssp. *argolica*[3] and *S. aleurites*.[22] Thymol (5.8 %) and 4-vinylguiacole (3.8 %) represented the almost total phenol content of the oil. The monoterpenoidic fraction was scarce: it constituted 3.6 % of the oil, with linalool (1.5 %) being the major constituent of this fraction. Long-chain alkanes amounted to 2.6 % of the oil and were present in low amounts, between 0.8 % (nonacosane) and traces (octadecane, heptacosane and octacosane). The other hydrocarbons were: pentacosane (0.6 %), hentriacontane (0.5 %), tricosane and tritriacontane (0.2 %), tetracosane, triacontane and dotriacontane (0.1 %). Stachynone and stachynene, reported by Maly[16] in the essential oil of *S. palustris,* were not found in our sample; these compounds were not found in the essential oils from *S. recta*[20] and *S. sylvatica*[22] either, though Maly reported them as the main components of these oils as well. The significant carbonylic and fatty acids fractions in the essential oil of *S. palustris* could be helpful in the taxonomic characterization of other *Stachys* species.

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SAŽETAK

Kemijski sastav eteričnog ulja nadzemnih dijelova vrste *Stachys palustris* L. (Lamiaceae) koja samoniklo raste u južnoj Italiji

Felice Senatore, Carmen Formisano, Daniela Rigano, Franco Piozzi i Sergio Rosselli

U radu je prikazan sastav eteričnog ulja nadzemnih dijelova vrste *Stachys palustris* L. (Lamiaceae) iz južne Italije. Eterično ulje izolirano je hidrodestilacijom iz biljnog materijala, a kemijski je sastav određen vezanim sustavom plinska kromatografija-spektrometrija mase, GC-MS, na dvije kapilarne kolone različite polarnosti. Sadržaj ulja bio je 0.21 % (maseni udio, *w*) preračunato na suhu tvar. Identificirana su 92 spoja, što predstavlja 93.6 % ukupnog ulja. Ulje je karakterizirano uglavnom karbonilnim spojevima (25.4 %), masnim kiselinama i njihovim esterima (24.2 %), uz seskviterpenske spojeve (16.0 %) i fenole (11.2 %). Glavne komponente ulja su kariofilen oksid (7.8 %), heksahidrofarnezil aceton (7.4 %), heksadekanska kiselina (6.8 %), (*Z*,*Z*,*Z*)-9,12,15-oktadekatrienska kiselina (6.7 %), (*Z*)-fitol (6.4 %), timol (5.8 %), *p*-metoksiacetofenon (5.1 %), 4-vinilgvajakol (3.8 %), tetradekanska kiselina (3.8 %), (*E*)-kariofilen (3.6 %), ß-jonon (3.3 %) i ß-damascenon (3.0 %).