Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 64 (2003) 743–752
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Essential oil analysis and antimicrobial activity of eight *Stachys* species from Greece

Helen D. Skaltsa[a,*], Costas Demetzos[a], Diamanto Lazari[b], Marina Sokovic[c]

[a]*Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou, GR-157 71 Athens, Greece*
[b]*Laboratory of Pharmacognosy, Department of Pharmacy, School of Health Science, Aristotle University of Thessaloniki, GR-541 24, Thessaloniki, Greece*
[c]*Department of Plant Physiology, Mycological Laboratory, Institute for Biological Research, 29 November 142, 11 000 Belgrade, Yugoslavia*

Received 31 March 2003; received in revised form 21 May 2003

Abstract

The volatile composition of eight *Stachys* species has been studied. The investigated taxa were *St. alopecuros* (L.) Bentham., *St. scardica* (Griseb.) Hayek, *St. cretica* L. ssp. *cretica*, *St. germanica* L. ssp. *heldreichii* (Boiss.) Hayek, *St. recta* L., *St. spinulosa* L., *St. euboica* Rech. and *St. menthifolia* Vis., growing wild in Greece. The essential oils were obtained by hydrodistillation in a modified Clevenger-type apparatus, and their analyses were performed by GC and GC–MS. Identification of the substances was made by comparison of mass spectra and retention indices with literature records. Sesquiterpene hydrocarbons were shown to be the main group of constituents of all taxa. Furthermore, the obtained essential oils were tested against the following six bacteria: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35210), *Bacillus subtilis* (ATCC 10907), *Bacillus cereus* (clinical isolates), *Micrococcus flavus* (ATCC 10240), *Staphylococcus epidermidis* (ATCC 2228), as well as against the following five fungi: *Aspergillus niger* (ATCC 6275), *Penicillium ochrochloron* (ATCC 9112), *Epidermophyton floccosum* (clinical isolates), *Candida albicans* (clinical isolates) and *Trichophyton mentagrophytes* (clinical isolates). The tested essential oils showed better activity against bacterial species than against fungi. *Pseudomonas aeruginosa* was the most resistant strain, as none of the essential oils was active against this strain. The essential oil of *St. scardica* has been proven most active against both bacteria and fungi.

Keywords: *Stachys*; Labiatae; Volatile constituents; Chemotaxonomy; Antimicrobial activity

1. Introduction

The subcosmopolitan genus *Stachys* L. comprises more than 270 species (Mabberley, 1997) and is justifiably considered as one of the largest genera of the Labiatae. Greece is certainly an area particularly rich in taxa. More than 50 species and subspecies are distributed in the mainland and/or the islands (Greuter et al., 1986); a significant proportion of them are considered endemic. In previous studies, we have investigated the composition of the essential oils of two taxa belonging to sectio Candida (Skaltsa et al., 1999) and of all taxa belonging to subsect. Swainsonianeae (Skaltsa et al., 2001).

The present study aims at investigating the volatile compounds of eight different previously unknown taxa: *St. alopecuros* and *St. scardica* belonging to the sect. Betonica; *St. cretica* ssp. *cretica* and *St. germanica* ssp. *heldreichii*, both belonging to the group *St. germanica* of the sect. Eriostomum; *St. recta*, belonging to the sect. Olisia; *St. menthifolia* and *St. euboica* belonging to the sect. Swainsoniana; *St. spinulosa* belonging to the sect. Campanistrum. Some of them are used in folk medicine to treat many disorders (Hartwell, 1982; Duke, 1986), therefore, it seemed interesting to investigate their antimicrobial potential. The results are compared with the biology and taxonomy of the taxa, to gather information on possible chemotaxonomic significance.

* Corresponding author. Tel.: +30-1-727-4593; fax: +30-1-861-3965.
E-mail address: skaltsa@pharm.uoa.gr (H.D. Skaltsa).

0031-9422/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00386-8

2. Results and discussion

2.1. Chemical composition of the essential oils

All the investigated *Stachys* taxa contain essential oils that range from 0.01 to 6.09% based on dry weight (Table 1). The highest oil contents were found in *St. menthifolia* (0.14%), *St. germanica* ssp. *heldreichii* [hel$_1$] (0.24%), *St. scardica* (0.53%), and *St. alopecuros* (6.09%). In the other five taxa, the oil content ranged between 0.01 and 0.04%.

The optical rotation of the oils were: *St. alopecuros* $[\alpha]_D^{25} = -15.00^\circ$ (pentane, *c* 0.07), *St. scardica* $[\alpha]_D^{25} = -1.85^\circ$ (pentane, *c* 1.35); *St. cretica* ssp. *cretica* $[\alpha]_D^{25} = -17.75^\circ$ (pentane, *c* 2.37); *St. germanica* ssp. *heldreichii* [hel$_1$] $[\alpha]_D^{25} = -0.74^\circ$ (pentane, *c* 0.54), [hel$_2$] $[\alpha]_D^{25} = -9.45^\circ$ (pentane, *c* 0.55); *St. recta* $[\alpha]_D^{25} = +4.92^\circ$ (pentane, *c* 0.51); *St. euboica* $[\alpha]_D^{25} = -8.40^\circ$ (pentane, *c* 0.17); *St. menthifolia* $[\alpha]_D^{25} = +3.26^\circ$ (pentane, *c* 3.93); *St. spinulosa* $[\alpha]_D^{25} = -5{,}15^\circ$ (pentane, *c* 0.25).

The main constituents of the investigated essential oils of *Stachys* are the following: in *St. alopecuros* (+)-caryophyllene oxide (8.9%), (−)-(*E*)-caryophyllene (7.6%) and α-calacorene (13.4%); in *St. scardica* germacrene D (19.9%), (+)-α-pinene (19.7%), δ-cadinene (10.5%) and γ-muurolene (10.3%); in *St. cretica* ssp. *cretica* germacrene D (33.5%) and pimaradiene (18.6%); in both populations of *St. germanica* ssp. *heldreichii* germacrene D (21.3% and 16.3% for hel$_1$ and hel$_2$, respectively), (−)-(*E*)-caryophyllene (18.4% and 15.1% for hel$_1$ and hel$_2$, respectively), (+)-caryophyllene oxide (5.9 and 5.4% for hel$_1$ and hel$_2$, respectively); in *St. recta* (−)-linalool (33.9%) and dihydroedulan I (15.9%); in *St. spinulosa* spathulenol (10.8%); in *St. euboica* (−)-α-copaene 12.5%), (−)-(*E*)-caryophyllene (9.8%), δ-cadinene (8.7%) and (+)-caryophyllene oxide (6.7%); in *St. menthifolia* abietatriene (13.7%), kaurene (9.0%) and 13-*epi*-manoyl oxide (7.5%).

As shown in the analytical Table 1, all the essential oils are complex mixtures.

Among them, the terpenoids consist the main portion of all investigated essential oils. Sesquiterpene hydrocarbons were shown to be the main group of constituents of almost all taxa (69.3% in *St. scardica*, 67.7% and 55.6% for hel$_1$ and hel$_2$, respectively, in *St. germanica* ssp. *heldreichii*; 49.9% in *St. cretica* ssp. *cretica*; 27.4% in *St. spinulosa*, 50.4% in *St. euboica* and 47.6% in *St. alopecuros*).

Generally, all taxa have rather low amounts of aliphatics and phenylpropanoids. Apart from *St. germanica* ssp. *heldreichii* [hel$_1$] and *St. spinulosa*, which have a significant aldehyde fraction (7.3% and 7.3%, respectively), and *St. spinulosa* and *St. euboica*, which contain relatively high amounts of fatty acids and aliphatic esters (6.4% and 5.6%, respectively), all other taxa have a much lower level of such compounds (< 5.1%).

The use of a chiral column allowed the determination of enantiomers in several main compounds. In most cases only one isomer is present in the essential oil, the other being absent or present in trace amounts.

In spite of the large size of *Stachys*, the composition of volatile compounds is known in only a small number of species. Concerning the taxa belonging to the same sections with the members of the present study, the following investigations have been carried out: *St. betonica* Benth. [= *St. officinalis* (L.) Trevisan] (Malý, 1985) of sect. Betonica; *St. balansae* Boiss. (Çakir et al., 1997) and *St. obliqua* Waldst. and Kit. (Harmandar et al., 1997), two species of sect. Eriostomum; *St. annua* L. (Malý, 1988) and *St. recta* L. (Malý, 1985; Çakir et al., 1997) two species of sect. Olisia; and all members of *Stachys* subsect. Swainsonianeae, with the exception of *St. euboica* (Skaltsa et al., 2001). The vulnerable *St. euboica* Rech. fil. is localized to the SE part of Evvia island (Constantinidis, 1995), and its subsectional placement appears problematic: although originally described as an ally of *St. mollisima* Willd., it was not placed together with it in subsect. Decumbentes by Bhattacharjee (1980) but in subsect. Swainsonianeae. Because of its problematic placement, the chemical analysis of its volatile constituents was not included in our previous paper (Skaltsa et al., 2001).

The essential oil of *St. betonica* Benth. (Malý, 1985) is totally different compared to those of *St. alopecuros* and *St. scardica*, all belonging to the sect. Betonica.

Concerning the allover investigated members of section Eriostomum, it is noteworthy that germacrene D present in large amount in *St. germanica* ssp. *heldreichii* is also present in *St. obliqua* (Harmandar et al., 1997), while α-pinene, one of the main constituents of *St. scardica* is also present in high proportions in *St. balansae* (Çakir et al., 1997). The essential oil of *St. cretica* ssp. *anatolica* from Turkey is carvacrol-rich (Kirimer et al., 1995), while carvacrol is totally absent in the essential oil of *St. cretica* ssp. *cretica*. The previously investigated essential oils of *St. recta* and of *St. annua* (Malý, 1985) revealed a different composition compared to our members of sect. Olisia.

The composition of the essential oil of *St. recta* from Serbia (Chalchat et al., 2000) was similar to the oil from Turkey (Çakir et al., 1997) by the preponderance of 1-octen-3-ol. In contrast to our oil and to the oil from Turkey, in the oil from Serbia, linalool was present in very low percentage only.

Abietatriene and 13-*epi*-manoyl oxide, present in high amounts in *St. menthifolia*, are also present in high amounts in *St. candida* (Skaltsa et al., 1999), a member of *St.* sect. Candida most closely related to sect. Swainsoniana, as well as in some taxa of the subsect. Swainsonianeae (Skaltsa et al., 2001). Similarly, (+)(*E*)-caryophyllene and δ-cadinene were found to be present

Table 1
Qualitative and quantitative composition (% v/v) of volatile compounds in *Stachys* sp

	Compounds	RI[a]	RI[b]	**alo**	**sca**	**spi**	**cre**	**hel$_1$**	**hel$_2$**	**rec**	**eub**	**men**
1	(*E*)-2-Hexenal	854		0.2	–	–	–	–	–	–	–	–
2	Heptanal	899	1186	tr	–	–	–	–	–	0.3	–	–
3	α-Thujene	929	1025	0.3	–	–	–	–	–	–	–	0.1
4	(+)-α-Pinene	936	1030	1.8	19.7	–	–	–	–	–	–	0.8
5	Benzaldeyde	959		–	1.6	–	–	–	–	0.2	–	–
6	Verbenene	967		tr	–	–	–	–	–	–	–	–
7	Sabinene	1020	974	0.1	–	–	–	–	–	–	0.3	0.4
8	1-Octen-3-ol	977	1453	1.3	–	–	–	0.3	–	–	–	0.1
9	(+)-β-Pinene	978	1105	–	–	–	–	0.2	–	–	–	–
10	Isolimonene	983		tr	–	–	–	–	–	–	–	–
11	6-Methyl-5-hepten-2-one	985		0.1	–	–	–	–	–	–	–	–
12	Myrcene	988	1167	0.1	–	–	–	–	–	–	–	tr
13	3-Octanol	993		–	–	–	–	tr	–	–	–	–
14	α-Phellandrene	1003	1160	0.1	–	–	–	–	–	–	–	–
15	(*E*, *E*)-2,4-Heptadienal	1008		tr	–	–	–	–	–	–	–	–
16	α-Terpinene	1018	1179	0.1	–	–	–	–	–	–	0.2	0.1
17	*p*-Cymene	1024	1024	0.5	–	–	–	–	–	–	–	0.1
18	(+)-Limonene	1027	1193	0.1	–	–	–	–	–	–	6.1	2.6
19	1,8-Cineole	1031		–	–	–	–	–	–	–	1.5	–
20	*cis*-Ocimene	1038		tr	–	–	–	–	–	0.5	–	–
21	Phenylacetaldehyde	1041		tr	–	–	–	tr	–	0.5	–	–
22	*trans*-Ocimene	1048		tr	–	–	–	–	tr	–	–	–
23	γ-Terpinene	1059	1231	0.1	–	–	–	–	–	–	0.7	0.1
24	2-Octen-1-ol	1060		tr	–	–	–	–	–	–	–	–
25	*cis*-Sabinene hydrate	1064		–	–	–	–	–	–	–	0.4	–
26	*n*-Octanol	1068	1558	–	–	–	–	0.1	–	–	–	–
27	Terpinolene	1085	1278	0.2	–	–	–	–	–	1.1	0.2	0.1
28	(−)-β-Linalool	1098	1554	1.1	–	1.2	–	1.1	–	33.9	3.6	0.2
29	*n*-Nonanal	1102	1387	0.1	–	0.4	–	0.4	–	1.5	0.8	–
30	*p*-Mentha-1,3,8-triene	1108		tr	–	–	–	–	–	–	–	–
31	*cis*-*p*-2-Menthen-1-ol	1117		tr	–	–	–	–	–	–	–	–
32	α-Campholenal	1125		tr	–	–	–	–	–	–	0.4	–
33	*trans*-Pinocarveol	1134		–	–	–	–	0.1	–	–	–	–
34	*trans*-Sabinol	1139		–	–	–	–	–	–	–	0.4	–
35	*trans*-*p*-2-Menthen-1-ol	1140		tr	–	–	–	–	–	–	–	–
36	*trans*-Verbenol	1140		–	–	0.6	–	–	–	–	0.5	–
37	Camphor	1143	1515	–	–	–	–	tr	–	–	–	–
38	2,6-Nonadienal	1151		–	–	–	–	0.1	–	–	–	–
39	(*E*)-2-Nonenal	1158		tr	–	–	–	0.2	–	–	–	–
40	Benzyl acetate	1162		–	–	–	–	–	–	–	0.3	–
41	*p*-Mentha-1,5-dien-8-ol	1166		tr	–	–	–	–	–	–	–	–
42	Nonanol	1171		–	–	–	–	0.1	–	–	–	–
43	(+)-Terpinen-4-ol	1175	1594	0.2	–	–	–	0.1	–	–	1.8	0.2
44	Dill ether	1183		–	–	–	–	–	–	0.1	–	–
45	*p*-Cymen-8-ol	1182		0.1	–	–	–	–	–	–	–	–
46	(+)-α-Terpineol	1187	1718	0.2	–	0.3	–	0.2	–	4.6	0.3	0.1
47	Methyl salicylate	1190		0.2	–	–	–	–	tr	1.2	–	–
48	Myrtenal	1193		–	–	–	–	–	–	–	0.4	–
49	Myrtenol	1194		–	–	–	–	0.1	–	–	–	–
50	Decanal	1203		–	–	0.9	–	0.4	–	0.6	0.2	–
51	Verbanone	1204		tr	–	–	–	–	–	–	–	–
52	Fragranol	1212		–	–	–	–	–	–	2.1	–	–
53	*trans*-Carveol	1217		–	–	–	–	0.1	–	–	–	–
54	β-Cyclocitral	1218		tr	–	0.6	–	0.1	0.1	0.6	0.3	–
55	Nerol	1225	1817	–	–	–	–	–	–	0.4	–	–
56	Geraniol	1255	1856	–	–	0.4	–	–	tr	0.2	–	–
57	(+)-Linaloyl acetate	1258		–	–	–	–	0.3	–	–	–	–
58	(*E*)-2-Decenal	1260		–	–	–	–	–	–	–	0.5	–
59	*cis*-Chrysanthenyl acetate	1262		–	–	3.1	–	–	–	–	–	–
60	*n*-Decanol	1272		–	–	0.8	–	0.2	–	–	–	–

(*continued on next page*)

Table 1 (*continued*)

	Compounds	RI[a]	RI[b]	alo	sca	spi	cre	hel$_1$	hel$_2$	rec	eub	men
61	Dihydroedulan II	1284		–	–	–	–	–	0.1	1.1	–	–
62	Dihydroedulan I	1289		tr	–	0.3	–	–	–	15.9	–	–
63	Thymol	1290		0.1	–	–	–	–	–	–	–	–
64	Thymol acetate	1294		0.1	–	–	–	–	–	–	–	–
65	Undecanal	1305		–	–	0.4	–	0.7	–	–	–	–
66	(*E,E*)-2,4-Decadienal	1315		–	–	0.5	–	0.1	–	–	0.2	–
67	Hexyl tiglate	1331		–	–	–	–	tr	–	–	–	–
68	Methyl phenyl isovalerate	1334		–	–	0.3	–	–	–	–	–	–
69	α-Cubebene	1348	1457	0.1	1.4	–	–	0.1	0.1	–	–	0.1
70	α-Longipinene	1351		–	–	2.9	–	–	0.1	–	–	–
71	Eugenol	1353		–	–	–	–	–	0.1	0.6	–	–
72	Cyclosativene	1364		0.1	–	–	–	0.3	–	–	2.4	–
73	α-Ylangene	1368		–	–	–	–	–	–	0.2	–	–
74	Undecanol	1370		–	–	–	–	–	–	–	–	–
75	Longicyclene	1376		0.7	0.8	–	–	0.3	–	–	–	–
76	(−)-α-Copaene	1375	1489	0.1	2.4	3.7	0.5	6.6	1.0	–	12.5	1.4
77	β-Bourbonene	1382	1586	0.2	5.6	–	1.6	1.9	1.7	–	0.5	0.1
78	β-Damascone	1383		–	–	2.5	–	–	–	–	–	–
79	β-Cubebene	1388		–	–	–	0.3	1.0	0.2	–	–	0.2
80	β-Elemene	1391	1585	–	–	0.8	1.1	2.8	2.9	1.5	1.7	0.1
81	Italicene	1397		0.1	–	–	–	–	–	–	–	–
82	β-Longipinene	1398		–	–	–	–	–	1.0	1.4	–	–
83	Methyl eugenol	1399		–	–	–	–	–	–	1.1	–	–
84	Tetradecane	1400		–	–	0.7	–	0.1	–	–	–	–
85	Longifolene	1402	1557	–	–	–	–	–	–	1.9	–	–
86	(*Z*)-Caryophyllene	1404		–	–	–	–	0.1	–	–	–	–
87	Dodecanal	1407		–	–	0.7	–	5.3	–	–	–	–
88	α-Cedrene	1408		1.7	–	–	–	–	tr	–	–	–
89	α-Gurjunene	1409		–	–	–	–	–	–	–	0.6	0.6
90	(*E*)-β-Damascone	1412		–	–	0.5	–	–	–	–	–	–
91	(−)-(*E*)-Caryophyllene	1418	1598	7.6	3.2	–	–	18.4	15.1	–	9.8	1.7
92	(+)-(*E*)-Caryophyllene	1418		–	–	2.5	–	–	–	–	–	–
93	*cis*-Thujopsene	1426		–	–	0.4	–	–	–	–	–	–
94	Calarene	1428		–	2.6	–	1.0	0.4	–	–	–	–
95	*trans*-α-Bergamotene	1434		1.3	–	1.8	0.4	–	0.7	–	–	–
96	(*Z*)-β-Farnesene	1448		tr	0.5	2.1	3.5	–	3.1	–	1.2	–
97	α-Humulene	1452	1664	3.2	–	–	–	1.6	2.5	–	–	0.2
98	(*E*)-β-Farnesene	1457	1668	1.6	–	2.1	0.6	–	3.0	–	6.3	–
99	*allo*-Aromadendrene	1458	1637	–	–	–	–	0.4	–	–	–	–
100	α-Acoradiene	1463		0.6	–	–	–	–	–	–	–	–
101	β-Acoradiene	1466		0.1	–	–	–	–	–	–	–	–
102	α-Amorphene	1470		0.1	–	0.7	–	–	–	–	0.3	–
103	γ-Gurjunene	1473		1.2	–	–	–	–	0.1	–	–	–
104	γ-Muurolene	1477		–	10.3	–	–	0.4	0.4	0.1	–	–
105	γ-Curcumene	1478		3.1	–	–	–	–	–	–	–	–
106	ar-Curcumene	1479	1789	3.9	–	–	–	–	–	–	–	1.2
107	Germacrene D	1480	1727	0.1	19.9	2.4	33.5	21.3	16.3	1.0	1.4	0.8
108	*epi*-Bicyclosesquiphellandrene	1482		–	–	–	–	–	0.2	–	–	0.2
109	(*E*)-β-Ionone	1484		–	–	3.2	–	0.4	–	1.6	0.8	–
110	β-Selinene	1485	1743	1.9	–	–	–	–	–	–	–	–
111	1-Pentadecene	1489		–	–	–	0.4	–	–	–	–	–
112	Valencene	1491		–	–	–	–	0.2	–	–	1.0	–
113	α-Selinene	1494	1748	–	3.2	–	–	–	–	–	–	–
114	Bicyclogermacrene	1494	1730	0.1	2.1	3.7	4.2	2.4	0.8	0.1	–	0.2
115	α-Zingiberene	1495		–	–	–	–	–	1.6	–	–	–
116	Cuparene	1498		–	–	0.3	–	–	–	–	–	–
117	*trans*-β-Guaiene	1499		–	–	0.4	–	–	–	–	–	–
118	α-Muurolene	1499	1724	–	1.8	–	0.3	0.8	0.8	–	0.4	0.8
119	β-Himachalene	1499		–	–	–	–	–	0.1	–	–	–
120	Germacrene A	1503		–	–	–	–	0.7	–	–	0.3	–
121	Eremophilene	1503		0.1	–	–	–	–	–	–	–	–

(*continued on next page*)

Table 1 (*continued*)

	Compounds	RI[a]	RI[b]	alo	sca	spi	cre	hel_1	hel_2	rec	eub	men
122	β-Bisabolene	1506		–	–	–	0.7	–	–	–	0.5	–
123	(*E*,*E*)-α-Farnesene	1508	1723	–	–	–	0.3	–	1.4	–	–	–
124	β-Curcumene	1512		4.5	–	–	–	–	–	–	–	–
125	Tridecanal	1512		–	–	0.9	–	0.1	–	–	–	–
126	γ-Cadinene	1513		1.1	5.0	–	0.4	0.8	–	–	0.5	2.3
127	(*Z*)-γ-Bisabolene	1515		0.3	–	–	–	–	–	–	–	–
128	β-Cadinene	1518		0.3	–	–	–	–	–	–	–	–
129	δ-Cadinene	1524	1765	0.1	10.5	1.7	1.6	6.8	2.0	0.4	8.7	4.9
130	*trans*-Calamenene	1529		–	–	1.9	–	–	–	–	–	–
131	Cadina-1,4-diene	1531		–	–	–	–	0.1	0.2	–	–	0.2
132	(*E*)-γ-Bisabolene	1535		–	–	–	–	–	0.3	–	0.3	–
133	α-Cadinene	1537	1785	–	–	–	–	0.1	–	–	–	0.1
134	α-Calacorene	1540	1914	13.4	–	–	–	0.2	–	–	1.0	0.2
135	Elemol	1550		–	–	–	–	–	–	–	–	0.3
136	11-Norbourbonan-1-one	1558		–	–	–	–	0.1	–	–	–	–
137	β-Calacorene	1561		–	–	–	–	–	–	–	1.0	–
138	(*E*)-Nerolidol	1564	2053	–	2.5	0.2	3.4	0.7	–	–	–	0.7
139	Dodecanoic acid	1566		–	–	1.6	–	–	–	–	–	–
140	Germacrene-D-4-ol	1574		1.1	–	–	1.2	0.8	–	–	–	1.4
141	Spathulenol	1577	2121	–	–	10.8	0.4	0.3	–	–	0.5	–
142	β-Copaen-4-α-ol	1579		–	–	–	–	–	–	–	0.5	–
143	*trans*-Sesquisabinene hydrate	1581		2.0	–	1.9	–	–	0.2	–	–	–
144	(−)-Caryophyllene oxide	1581	1966	–	–	–	–	–	–	–	–	2.5
145	(+)-Caryophyllene oxide	1581	1966	8.9	1.6	3.7	–	5.9	5.4	–	6.7	–
146	Viridiflorol	1589	2098	–	–	0.5	0.8	0.4	–	–	0.3	1.0
147	Hexadecene	1590		–	–	1.0	–	–	–	–	–	–
148	Ethyl dodecanoate	1591		–	–	1.9	–	–	–	–	–	–
149	Carotol	1594		–	–	–	–	–	0.1	–	–	–
150	α-Cedrol	1599		1.3	–	–	–	1.6	–	–	–	–
151	Hexadecane	1600		–	–	–	–	1.1	–	–	–	–
152	Oplopenone	1608		–	–	–	–	–	–	–	–	1.0
153	Tetradecanal	1609		–	–	0.6	–	–	–	–	–	–
154	*epi*-Cedrol	1611		5.0	–	–	–	–	–	–	–	–
155	γ-Eudesmol	1631		–	–	–	–	–	–	–	0.2	–
156	Di-*epi*-cedrenoxide	1631		6.0	–	–	–	–	–	–	–	–
157	T-Cadinol	1635	2166	–	–	0.6	–	–	1.4	–	–	–
158	Torreyol	1643	2180	–	–	–	–	1.2	0.4	–	0.4	0.4
159	Vulgarone	1647		0.4	–	–	–	–	–	–	–	–
160	β-Eudesmol	1650	2220	–	–	1.5	–	–	–	–	–	0.2
161	α-Cadinol	1656	2225	–	1.2	–	1.3	1.4	2.0	–	2.1	3.4
162	Valeranone	1672		–	–	–	–	–	–	–	–	3.1
163	β-Bisabolol	1673	2162	6.8	–	–	2.1	–	1.3	–	–	–
164	Tetradecanol	1676		–	–	–	–	–	–	–	0.7	–
165	α-Bisabolol	1682	2022	1.8	–	–	–	–	1.2	–	2.6	0.2
166	Heptedecanal	1682		–	–	2.9	–	–	–	–	–	–
167	Heptadecane	1700		–	–	1.5	–	–	–	–	–	–
168	(*Z*,*Z*)-Farnesol	1713		–	–	–	1.1	1.5	–	–	–	–
169	(*E*,*E*)-Farnesol	1722		0.1	–	–	4.4	–	–	–	–	–
170	(*E*,*Z*)-Farnesol	1742		0.1	–	–	–	–	–	–	–	–
171	Benzyl benzoate	1762		0.1	–	–	–	–	–	–	–	–
172	1-Octadecene	1793		–	–	0.7	–	–	–	–	–	–
173	Octadecane	1799		–	–	1.2	–	–	–	–	–	–
174	Nootkatone	1800		tr	–	–	–	–	–	–	–	–
175	Isopropyl myristate	1827		–	–	2.9	–	tr	–	–	–	–
176	*E*,*E*-Farnesyl acetate	1843		tr	–	–	–	–	–	–	–	–
177	6,10,14-Trimethyl pentadecan-2-one	1845		0.1	–	–	0.8	0.4	–	–	–	–
178	13*R*,*S*-14,15-Dinorlabdane-8,13-diol	1873		0.6	–	–	–	–	–	–	–	–
179	Hexadecanol	1882		tr	–	–	–	0.1	–	–	–	–
180	7-Ethenyl-1,2,3,4,4a,5,6,7,8,9,10,10a-dodecahydro-1,1,4a,7-tetramethyl phenanthrene	1908		–	–	–	–	–	–	–	–	1.5

(*continued on next page*)

Table 1 (*continued*)

	Compounds	RI[a]	RI[b]	alo	sca	spi	cre	hel$_1$	hel$_2$	rec	eub	men
181	Farnesyl acetone	1914		tr	–	–	–	–	–	–	–	–
182	Pimaradiene	1942		–	–	–	18.6	–	–	–	–	–
183	13-*epi*-Manool	1961		–	–	–	–	–	–	–	–	0.8
184	Isophyllocladene	1963		–	–	–	tr	–	14.2	–	–	–
185	Hexadecanoic acid	1972		–	–	–	–	0.3	–	–	4.5	–
186	13-*epi*-Manoyl oxide	2012		tr	–	–	–	0.4	–	–	–	7.5
187	Kaurene	2036		–	–	–	–	–	–	–	–	9.0
188	Abietatriene	2058		–	–	–	–	–	–	–	–	13.7
189	Octadecanol	2082		–	–	–	–	tr	–	–	–	–
190	13(16),14-Labdien-8-ol	2101		1.5	–	–	–	–	–	–	–	–
191	*cis*-Phytol	2114		–	–	–	–	–	–	0.1	–	1.0
192	*trans*-Phytol	2135		–	–	–	–	0.7	–	–	–	–
193	(*Z*)-9-Octadecanoic acid = linoleic acid	2152		–	–	–	–	–	–	–	1.1	–
194	3β-Hydroxy-13-*epi*-manoyl oxide	2239		–	–	–	–	–	–	–	–	0.6
195	Tricosane	2300		0.1	–	–	–	–	–	–	–	–
196	Abieta-8,11,13-trien-7-one	2315		–	–	–	–	–	–	–	–	2.0
197	3β-Acetoxy-13-*epi*-manoyl oxide	2370		–	–	–	–	–	–	–	–	tr
198	Tetracosane	2400		tr	–	–	–	–	–	–	–	0.1
199	Labd-13-ene-8,15-diol	2409		–	–	–	–	–	–	–	–	0.1
200	Pentacosane	2500		tr	–	–	–	–	–	–	–	0.1
201	Hexacosane	2600		tr	–	–	–	–	–	–	–	0.1
Total				**90.6**	**95.9**	**79.2**	**84.5**	**95.4**	**82.1**	**74.8**	**89.9**	**70.9**
Yield (% v/dry wt)				6.09	0.53	0.01	0.04	0.24	0.03	0.01	0.01	0.14

[a] Kovàts index on HP-5 column.
[b] Kovàts index on HP-Innowax column. Concentrations below 0.01% are marked as –; those between 0.01 and 0.05 as tr (traces).

in high values in *St. euboica*, as well as in *St. candida* and *St. chrysantha* (Skaltsa et al., 1999) and in all taxa of subsect. Swainsonianeae (Skaltsa et al., 2001). Comparing the chemical composition of the essential oil of *St. euboica* with the six taxa of subsect. Swainsonianeae (Skaltsa et al., 2001), many similarities are obvious, with the exception of the essential oil of *St. ionica*, a taxon confined to the Ionian islands and, therefore, well-isolated from the rest of the group. (−)-Linalool and (+)-caryophyllene oxide are abundant in the essential oils of *St. euboica* and *St. swainsonii* ssp. *argolica*. The essential oil of *St. euboica* is as rich in (−)-α-copaene, germacrene D and δ-cadinene, as the oil of *St. swainsonii* ssp. *melangavica* and in hexadecanoic acid as the oil of *St. swainsonii* ssp. *swainsonii*. (−)-*E*-Caryophyllene was found in large amount in the essential oil of *St. euboica*, as in all studied taxa of subsect. Swainsonianeae.

At present, it is unknown in which way the composition of volatile oils truly reflects taxonomic relationships in *Stachys*, since many of its ca. 270 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 (Stahl-Biskup, 1991; Kokkini, 1992; Skoula et al., 1999; Sanz et al., 2000; Skaltsa et al., 2001).

2.2. Antimicrobial activity

The essential oils investigated showed better activity against bacterial species than against fungi. *Pseudomonas aeruginosa* was found to be the most resistant strain, as none of the essential oils was active against this strain. The essential oil of *St. scardica* was the most active one against both bacteria and fungi.

Knobloch et al. (1987) showed that terpene alcohols such as linalool exhibit strong antimicrobial activity, especially pronounced on whole cells, while hydrocarbon derivatives possess lower antifungal properties, as their low water solubility limits their diffusion through the medium. Griffin et al. (2000) have shown that hydrocarbons tend to be relatively inactive regardless of their structural type, and this inactivity is closely related to their limited hydrogen capacity and water solubility. Ketones, aldehydes and alcohols are active but with differing specificity and levels of activity, which is related to the present functional group but also associated with hydrogen-bounding parameters in all cases. Previous results showed that greater antifungal potential could be ascribed to the oxygenated terpenes (Knobloch et al., 1987; Jansen et al., 1987; Panizzi et al., 1993; Adam et al., 1998).

Table 2
Grouped components (% v/v) in *Stachys* sp. essential oils

	alo	sca	spi	cre	hel_1	hel_2	rec	eub	men
Aliphatics									
Alkanes. Alkenes	0.1	–	5.1	0.4	1.2	–	–	–	0.3
Alcohols	1.3	–	0.8	–	0.8	–	–	0.7	0.1
Aldehydes	0.3	1.6	7.3	–	7.3	–	3.1	1.7	–
Ketones	0.2	–	–	0.8	0.4	–	–	–	–
Fatty acids and aliphatic esters	–	–	6.4	–	0.3	–	–	5.6	–
Esters	0.3	–	0.3	–	–	–	1.2	0.3	–
Terpenoids									
Monoterpene hydrocarbons	3.4	19.7	–	–	0.2	–	1.6	7.5	4.3
Oxygenated monoterpenes	1.8	–	6.2	0.3	2.1	0.1	41.9	9.6	0.5
Sesquiterpenes hydrocarbons	47.6	69.3	27.4	49.9	67.7	55.6	6.6	50.4	15.3
Oxygenated sesquiterpenes	33.5	5.3	19.2	14.5	13.9	12.0	–	13.3	14.2
Diterpenoids	2.1	–	–	18.6	1.1	14.2	0.1	–	36.2
Compounds with 13 carbons	–	–	6.5	–	0.4	0.1	18.6	0.8	–
Phenylpropanoids	–	–	–	–	–	0.1	1.7	–	–

3. Experimental

3.1. Plant material

All investigated taxa were collected from natural populations growing on calcareous rocks or stony slopes. Details of the collection localities are presented in Table 3. Voucher specimens of each population were determined by Dr Th. Constaninidis and deposited in the Herbarium of the Institute of Botany, University of Patras (UPA) and in the Herbarium of the Agricultural University of Athens (ACA).

3.2. Analysis of volatile compounds

Fifty grams of air-dried plant material from each taxon were cut in small pieces, and the essential oils were obtained by steam distillation in 500 ml H_2O for 1 h in a modified Clevenger apparatus with a water-cooled oil receiver to reduce artifacts produced during hydrodistillation by over-heating. The oils, taken in 2 ml of capillary GC grade *n*-pentane and dried over anhydrous sodium sulphate, were subsequently analyzed by GC–MS and stored at −20 °C. The composition of the volatile constituents was established by GC–MS analyses. GC–MS analyses were performed on a Hewlett-Packard 5973–6890 system operating in EI mode (70 eV) equipped with a split/splitless injector (220 °C), a split ratio 1/10, using three different columns: a fused silica HP-5 MS capillary column [30 m×0.25 mm (i.d.), film thickness: 0.25 μm]; a HP-Innowax capillary column [30 m×0.25 mm (i.d.), film thickness: 0.50 μm] and a chiral Cydex B (SGE) capillary column [50 m×0.22 mm (i.d.), film thickness: 0.25 μm]. The temperature program for the HP-5 MS column was from 60 °C (5 min) to 280 °C at a rate of 4 °C/min; for the HP-Innowax column from 60 to 260 °C at a rate of 3 °C/min and for the Cydex B column from 50 to 130 °C (2 min) at a rate of 2 °C/min and from 130 to 250 °C at a rate of 4 °C/min. Helium was used as a carrier gas at a flow rate of 0.8 ml/min. Injection volumes of each sample were 2 μl. Retention indices for all compounds were determined according to the Van den Dool approach (Van den Dool and Kratz, 1963), using *n*-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of Wiley and NBS Libraries (Massada, 1976) and those described by Adams (2001), as well as by comparison of their retention indices with literature data (Adams, 2001; Bisio et al., 1998; Davies, 1990). In many cases, the essential oils were subject to co-chromatography with authentic compounds (Fluka, Sigma). The recognition of the optical isomers was made by comparison with authentic samples and according to reported elution order for the particular column (Ravid et al., 1994, 1995; SGE Scientific Ltd data). Polarimeter: Perkin-Elmer 341. Optical rotation values were determined at 25 °C at 589 nm in *n*-pentane (Tables 2–5).

3.3. Tests for antibacterial activity

Each sample was dissolved at 10 mg/ml with dimethyl sulfoxide (DMSO) and diluted with the appropriate medium for each case. Final concentrations were 1000, 500, 250, 125 μg/ml in the medium. Final concentration of DMSO was 1% in the medium (Mitscher et al., 1972).

The following bacteria were used: *P. aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35210), *Bacillus subtilis* (ATCC 10907), *Bacillus cereus* (clinical isolates), *Micrococcus flavus* (ATCC 10240), *Staphylococcus epidermidis* (ATCC 2228).

Table 3
List of the *Stachys* taxa investigated with provenance, abbreviations used and voucher specimens

Name	Abbreviation	Sectio	Locality	Voucher specimen (UPA)
St. alopecuros (L.) Bentham.	alo	Betonica	Nomos Fthiotidos, Eparchia Fthiotidos, Mt. Iti. The middle and upper parts of Greveno summit. Alt. 1900–2114 m.; Lat. 38°49′ N Long. 22°17′ E	Constantinidis & Mavrakis 9723 (ACA, UPA)
St. scardica (Griseb.) Hayek	sca	Betonica	Nomos Fthiotidos, Eparchia Domokou. C. 2.3 km after the village of Ano Agoriani towards Panagia. Alt. 540–650 m.; Lat. 39°06′ N Long. 22°11′ E	Constantinidis & Iliadis 7887 (UPA).
St. spinulosa Sm.	spi	Campanistrum	Nomos Zakinthou, Eparchia Zakinthou, Zakinthos island. Just SW Keri village. Alt. 120 m.; Lat. 37°40′ N Long. 20°50′ E	Phitos et al. 25482 (UPA)
St. cretica L. ssp. *cretica*	cre	Eriostomum	Nomos Attikis, Eparchia Megaridos. The lower, NE parts of Mt. Gerania Alt. 300 m.; Lat. 38°03′ N Long. 23°12′ E	Constantinidis 6724 (UPA)
St. germanica L. ssp. *heldreichii* (Boiss.) Hayek	hel_1	Eriostomum	Nomos Karditsis, Eparchia Karditsis, Mt. Katachloron. Close to Loutra Smokovou village. Alt. 280–340 m.; Lat. 39°09′ N Long. 22°02′ E	Constantinidis & Iliadis 7799 (UPA)
St. gemanica L. ssp. *heldreichii* (Boiss.) Hayek	hel_2	Eriostomum	Nomos Ioanninon/Trikalon, Eparchia Metsovou/Kalambakas. C. 2.1 km from Rachi Kataras to the village of Anthousa. Alt. 1660 m.; Lat. 39°46′ N Long. 21°13′ E	Constantinidis 8641 (ACA)
St. recta L.	rec	Olisia	Nomos Kastorias, Eparchia Kastorias. C. 5.0–5.8 km S. of Eptachori. Alt. 840 m.; Lat 40°13′ N Long. 20°58′ E	Constantinidis 8685 (ACA)
St. euboica Rech.	eub	Swainsoniana	Nomos Evvias, Eparchia Karistias, Evvia island. NE the village of Evangelismos. Alt. 2–100 m.; Lat. 38°05′ N Long. 24°34′ E	Constantinidis & Karabourniotis 9999 (ACA, UPA)
St. menthifolia Vis.	men	Swainsoniana	Nomos Ioanninon, Eparchia Konitsis. C. 1.5 km NE Konitsa. Alt. 950 m.; Lat. 40°03′ N Long. 20°46′ E	Constantinidis & Garofalo 8100 (UPA).

In order to obtain quantitative data, a modified microdilution technique was used (Hanel and Raether, 1988; Daouk et al., 1995). Bacterial species were cultured overnight at 37 °C in LB medium (Lutria Broth). Suspensions containing $\sim 10^9$ cells/ml.

The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 μl per well. The inocula were stored at +4 °C for further use. Dilutions of the inocula were cultured on solid MH (Mueller Hinton) to verify the absence of contamination and to check the validity of the inoculum.

Minimum inhibitory concentrations (MICs) determination was performed by a serial dilution technique using 96-well microtitre plates. The plates were incubated for 48 h at 37 °C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations, which completely inhib-

Table 4
Antibacterial activities of the investigated essential oils and their main compounds (mg/ml)

Compound	*E. coli*	*M. luteus*	*B. subtilis*	*B. cereus*	*S. epidermidis*	*P. aeruginosa*
Streptomycine	0.02	0.01	0.01	0.01	0.04	0.05
sca	0.1	0.1	0.1	0.1	0.5	–
cre	0.6	0.3	0.3	0.3	0.1	–
hel_1	0.7	0.35	0.7	0.7	0.7	–
hel_2	0.7	0.35	0.7	0.7	0.7	–
rec	0.6	0.3	0.3	0.3	0.6	–
eub	0.4	0.1	0.1	0.1	0.4	–
men	0.8	0.4	0.4	0.4	0.8	–
β-Caryophyllene	0.2	0.1	0.1	0.1	0.1	–
β-Caryophyllene oxide	0.2	0.1	0.1	0.1	0.2	–
α-Pinene	0.2	0.1	0.1	0.1	0.2	–
Cadinene	0.05	0.05	0.05	0.05	0.1	–
Linalool	0.1	0.05	0.05	0.1	0.1	–

Table 5
Antifungal activities of the investigated essential oils and their main compounds (mg/ml)

Compounds	*A. niger*	*P. ochrochloron*	*T. mentagrophytes*	*E. floccosum*	*C. albicans*
Bifonazole	0.01	0.02	0.01	0.01	0.05
sca	0.05	0.5	0.025	0.05	1.0
cre	0.03	0.3	0.015	0.03	0.6
hel_1	0.035	0.35	0.035	0.035	0.7
hel_2	0.035	0.35	0.035	0.035	0.7
rec	0.03	0.3	0.03	0.03	0.6
eub	0.04	0.4	0.04	0.04	0.8
men	0.04	0.4	0.04	0.04	0.8
β-Caryophyllene	0.09	0.15	0.09	0.1	0.3
β-Caryophyllene oxide	0.09	0.1	0.06	0.1	0.3
α-Pinene	0.09	0.15	0.09	0.1	0.3
Cadinene	0.09	0.1	0.06	0.1	0.3
Linalool	0.03	0.07	0.03	0.05	0.1

ited bacterial growth (MICs). DMSO was used as a control while streptomycin was used as a positive control.

3.4. Test for antifungal activity

For the bioassays the following fungi were used: *Aspergillus niger* (ATCC 6275), *Penicillium ochrochloron* (ATCC 9112), *Trichophyton mentagrophytes, Epidermophyton floccosum* and *Candida albicans*. The last three micromycetes were directly isolated from patients at the Centre for Preventive Medicine, Military Medicinal Academy, Department of Microbiology, Belgrade, Yugoslavia.

The micromycetes were maintained on malt agar (MA), dermatomycetes on Sabouraud Agar (SDA), and the cultures were stored at +4 °C and subcultured once a month (Booth, 1971).

In order to investigate the antifungal activity of essential oils and their main components, the modified microdilution technique was used (Hanel and Raether, 1988; Daouk et al., 1995). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (vol/vol). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 μl per well. The inocula were stored at +4 °C for further use. Dilutions of the inocula were cultured on solid MA to verify the absence of contamination and to check the validity of the inoculum. The plates were incubated for 72 h at 28 °C. The lowest concentration without visible growth was defined as the MFC, indicating ca. 99.5% killing of the original inoculum. DMSO was used as a control while bifonazole was used as a positive control.

Acknowledgements

The authors wish to thank Dr. T. Constantinidis (Institute of Systematic Botany, Department of Biotechnology, Agricultural University of Athens) for the collection and the identification of the plant material.

References

Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., Arsenakis, M., 1998. Antifungal activities of *Origanum vulgare* subsp. *hirtum, Mentha spicata, Lavandula angustifolia* and *Salvia fruticosa* essential

oils against human pathogenic fungi. J. Agric. Food Chem. 46, 1738–1745.

Adams, R., 2001. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing, Carol Stream, IL.

Bhattacharjee, R., 1980. Taxonomic studies in *Stachys*: II. A new infrageneric classification of *Stachys* L. Notes from the Royal Botanic Garden Edinburgh 38, 65–96.

Bisio, A., Ciarallo, G., Romussi, G., Fontana, N., Mascolo, N., Capasso, R., Biscardi, D., 1998. Chemical composition of essential oils from some *Salvia* species. Phytotherapy Res. 12, S117–S120.

Booth, C., 1971. Fungal culture media. In: Norris, J.R., Ribbons, D.W. (Eds.), Methods in Microbiology, 4th ed. Academic Press, London, New York, pp. 49–94.

Çakir, A., Duru, M.E., Harmandar, M., Izumi, S., Hirata, T., 1997. The volatile constituents of *Stachys recta* L. and *Stachys balansae* L. from Turkey. Flavour Fragr. J. 12, 215–218.

Chalchat, J.-C., Petrovic, S.D., Maksimovic, Z.A., Gorunovic, M.S., 2000. Essential oil of the herb of *Stachys recta* L., Lamiaceae from Serbia. J. Ess. Oil Res. 12, 455–458.

Constantinidis, T., 1995. *Stachys euboica* Rech. fil. In: Phitos, D., Strid, A., Snogerup, S., Greuter, W. (Eds.), The Red Data Book of Rare, Threatened Plants of Greece. Michalas K., Athens, pp. 478–479.

Daouk, K.D., Dagher, M.S., Sattout, J.E., 1995. Antifungal activity of the essential oil of *Origanum syriacum* L. J. Food Protect. 58, 1147–1149.

Davies, N.N., 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. J. Chromatogr. 503, 1–24.

Duke, J.A., 1986. Handbook of Medicinal Herbs. CRC Press, FL, p. 457.

Greuter, W., Burdet, H.M., Long, G., 1986. Med-Checklist, Vol. 3. Editions des Conservatoire et Jardin Botaniques de la Ville de Genève, Genève.

Griffin, G.S., Markham, L.J., Leach, N.D., 2000. An agar dilution method for the determination of the minimum inhibitory concentration of essential oils. J. Ess. Oil Res. 12, 149–255.

Hanel, H., Raether, W., 1988. A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example. Mycoses 31, 148–154.

Harmandar, M., Duru, M.E., Çakir, A., Hirata, T., Izumi, S., 1997. Volatile constituents of *Stachys obliqua* L. (Lamiaceae) from Turkey. Flavour Fragr. J. 12, 211–213.

Hartwell, J.L., 1982. Plants Used Against Cancer. A Survey. Quarterman Publications, Lawrence, MA, pp. 274–275..

Jansen, A.M., Scheffer, J.J.C., Svendsen, A.B., 1987. Antimicrobial activities of essential oils. A 1976–1986 literature review on possible applications. Pharm. Week. Sci. Ed. 9, 193–197.

Kirimer, N., Baser, K.H.C., Tumen, G., 1995. Carvacrol rich plants in Turkey. Khim. Prir. Soedin. 1, 49-54. CHEMABS 124, 226510.

Kokkini, S., 1992. Essential oils as taxonomic markers in *Mentha*. In: Harley, R.M., Reynolds, T. (Eds.), Advances in Labiatae Science. Kew Royal Botanic Gardens, Kew, pp. 325–334.

Knobloch, E., Pauli, A., Iberl, B., Wies, N., Weigand, H., 1987. Mode of action of essential oil components on whole cells of bacteria and fungi in plate tests. Bioflavour 287–299.

Mabberley, D.J., 1997. The Plant-Book. Cambridge University Press, Cambridge, New York, Melbourne.

Malý, E., 1985. Paper chromatography of the essential oils occurring in the genus *Stachys*. J. Chromatogr. 333, 288–289.

Malý, E., 1988. A sesquiterpene enol and hydrocarbon from *Stachys annua* L. Herba Hungarica 27, 107–116.

Massada, Y., 1976. Analysis of Essential Oil by Gas Chromatography and Spectrometry. John Wiley & Sons, New York.

Mitscher, L.A., Leu, R.P., Bathala, M.S., Wu, W.N., Beal, J.L., White, R., 1972. Antimicrobial agents from higher plants. I: Introduction, rationale and methodology. Lloydia 35, 157.

Panizzi, L., Flamini, G., Cioni, P.L., Morelli, I., 1993. Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae. J. Ethnopharmacol. 39, 167–170.

Ravid, U., Putievsky, E., Katzir, I., 1994. Chiral GC analysis of enantiomerically pure (*R*)(−)-linalyl acetate in some Lamiaceae, myrtle and petigrain oils. Flavour Fragr. J. 9, 275–276.

Ravid, U., Putievsky, E., Katzir, I., 1995. Determination of the enantiomeric composition of α-terpineol in essential oils. Flavour Fragr. J. 10, 281–284.

Sanz, J., Mus, M., Rosselló, J., 2000. Volatile components variation in the *Teucrium marum* complex (Lamiaceae) from the Balearic Islands. Botanical Journal of the Linnean Society 132, 253–261.

SGE Scientific Ltd. Data; available from <http://www.sge.com.au/capillary>.

Skaltsa, H.D., Lazari, D.M., Chinou, I.B., Loukis, A.E., 1999. Composition and antimicrobial activity of the essential oils of *Stachys candida* and *S. chrysantha* from southern Greece. Planta Med. 65, 255–256.

Skaltsa, H.D., Mavrommati, A., Constantinidis, T., 2001. A chemotaxonomic investigation of volatile constituents in *Stachys* subsect. Swainsonianeae (Labiatae). Phytochemistry 57, 235–244.

Skoula, M., Gotsiou, P., Naxakis, G., Johnson, C.B., 1999. A chemosystematic investigation on the mono- and sesquiterpenoids in the genus *Origanum* (Labiatae). Phytochemistry 52, 649–657.

Stahl-Biskup, E., 1991. The chemical composition of *Thymus* oils: a review of the literature 1960–1989. J. Ess. Oil Res. 3, 61–82.

Van den Dool, H., Kratz, P.D., 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. 11, 463–471.