Bull. Chem. Soc. Ethiop. **2010**, 24(1), 67-76. ISSN 1011-3924
Printed in Ethiopia

VOLATILE CONSTITUENTS OF *GLECHOMA HIRSUTA* WALDST. & KIT. AND *G. HEDERACEA* L. (*LAMIACEAE*)

Niko Radulović[1,*], Nevenka Đorđević[1], Marija Marković[2] and Radosav Palić[1]

[1]Department of Chemistry, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia
[2]Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia

(Received March 13, 2009; revised August 4, 2009)

ABSTRACT. The essential oils of two *Glechoma* species from Serbia have been analyzed by GC and GC/MS. Eighty eight and two hundred thirty eight constituents identified accounted for 90.6 and 86.6% of the total oils of *G. hirsuta* Waldst. & Kit. and *G. hederacea* L., respectively. In both oils the dominant constituent class was the terpenoid one, 75.7% in *G. hirsuta* and 47.4% in *G. hederacea*. 1,8-Cineole (42.6%) and spathulenol (7.4%) were the main constituents of *G. hirsuta* oil while palmitic (13.3%) and linoleic acids (9.3%) alongside with germacrene D (7.3%) were the major ones of *G. hederacea* oil. The relative percentage of the sesquiterpene fraction (19.5%) and fatty acid derived compounds (7.6%) distinguished nicely *G. hirsuta* from *G. hederacea*. Additionally, oxygenated sesquiterpenes (16.9%) dominated the oil of *G. hirsuta*, while the reversed situation was noted for *G. hederacea* oil (the hydrocarbon sesquiterpenes amounted to only 2.6%). The results obtained provide a rationale for the parallel ethnopharmacological usage of *G. hirsuta* and *G. hederacea*. This is the first report on the composition of *G. hirsuta* oil.

KEY WORDS: *Glechoma hirsuta* Waldst. & Kit., *Glechoma hederacea* L., Lamiaceae, Essential oil composition, 1,8-Cineole, Spathulenol, Germacrene D, Linoleic acid, Palmitic acid, Ethnopharmacological use

INTRODUCTION

Three species of the genus *Glechoma* L. (*G. hederacea* L., *G. hirsuta* Waldst. & Kit. and *G. serbica* Halácsy & Wettst.), belonging to the family Lamiaceae, grow spontaneously in Serbia [1]. The first two species are widespread but that is not case with the third one (a questionable species). Aerial parts of *G. hederacea* (ground ivy) are traditionally used for healing different diseases and as a spice [2, 3]. The leaves and flowering stems are reported to be anodyne, antiphlogistic, appetizer, digestive, astringent, diuretic, febrifuge, pectoral, gently stimulant, tonic and vermifuge [4]. *Glechoma hederacea* extract was shown to possess an antihypertensive effect in spontaneously hypertensive rats [5] and hypoglycemic effect on the blood glucose level in rats [6]. The cytotoxicity of hederacines A and B, the tropane alkaloids isolated from the MeOH extract of the aerial parts of *G. hederacea*, was demonstrated by the cytotoxicity assay using colon cancer cell line (CaCo-2) [7]. Preliminary studies also indicated that the potent insecticidal lectin, gleheda, present in the leaves of *G. hederacea*, preferentially agglutinates human erythrocytes carrying the Tn (GalNAcα1-Ser/Thr) antigen [8]. Two further recent references that merit to mention on the biological activity of *G. hedereacea* deal with its anti-inflammatory [9] and antibacterial and free radical scavenger activity [10].

For a plant with such a wide spectrum of pharmacological activity only a scarce number of publications reporting the phytochemical details exists. A very few references concern the chemical composition of its essential oil, that, in fact, may be one of the active principals involved. The only published results dealing with the subject are: two recent papers on the chemotaxonomy of *G. hederacea* from Lithuania [11, 12], based on volatiles, as well as a review on the chemical composition of Labiatae essential oils that included some data on the oil

*Corresponding author. E-mail: vangelis0703@yahoo.com

of *G. hederacea*, growing in North America in New Brunswick [13]. The volatiles of *G. hirsuta* have not been investigated so far.

Although also widely utilized in the same way as *G. hederacea*, little or nothing is known about the properties of *Glechoma* species other than *G. hederacea*. The aim of this paper was to perform a detailed compositional study of the essential oils of *G. hederacea* and *G. hirsuta* from Serbia and to possibly provide, based on the volatile profiles of these two species, the justification of the parallel traditional use of *G. hirsuta* and *G. hederacea*.

EXPERIMENTAL

Plant material

The plant material of *G. hederacea* and *G. hirsuta* (inflorescences, leaves and stems) was collected in May 2005 from the mountain V'dlič (near Pirot, E. Serbia). The species were collected and identified by Marija Marković, Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, and the voucher specimens were deposited at the Herbarium of the Faculty of Science and Mathematics, under the acquisition numbers NR 127 and NR 128.

The plant material was allowed to air dry in a dark unheated room with no heat source. After two weeks, when the samples attained constant weight, the samples were then analyzed.

Isolation of essential oils

Samples of *G. hederacea* (80.0 g) and *G. hirsuta* (366.5 g) consisting of dry aerial parts were subjected to hydrodistillation for 2.5 h using a Clevenger-type apparatus and yielded yellowish semi-solid essential oils. The oils were separated by extraction with diethyl ether (Merck, Germany) and dried over anhydrous magnesium sulfate (Aldrich, USA). The solvent was evaporated under a gentle stream on nitrogen at room temperature, in order to exclude any loss of the essential oil, and analyzed immediately. When the oil yields were determined, after the bulk of ether was removed under a stream of N_2, the residue was exposed to vacuum at room temperature for a short period to eliminate the solvent completely. The pure oil was then measured on an analytical balance and multiple gravimetric measurements were taken during 24 h to ensure that all of the solvent had evaporated.

GC/MS and GC (FID)

The GC/MS and GC analyses (three repetitions each sample) were carried out using a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column HP-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 and 300 ^{o}C, respectively. Oven temperature was raised from 70 to 290 ^{o}C at a heating rate of 5 ^{o}C/min and then isothermally held for 10 min. As a carrier gas helium at 1.0 mL/min was used. The samples, 1 μL of the oil solutions in diethyl ether (1:100), were injected in a pulsed split mode (the flow rate was 1.5 mL/min for the first 0.5 min and then set to 1.0 mL/min throughout the remainder of the analysis; split ratio 40:1). MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 35-500, scan time 0.32 s. Oil constituents were identified by comparison of their linear retention indices (relative to C_7-C_{25} alkanes [14] on the HP-5MS column) with literature values [15] and their mass spectra with those of authentic standards, as well as those from Wiley 6, NIST02, MassFinder 2.3, and a homemade MS library with the spectra corresponding to pure substances and components of

known essential oils, and wherever possible, by co-injection with an authentic sample. GC (FID) analysis was carried out under the same experimental conditions using the same column as described for the GC/MS. The percentage composition of the oil was computed from the GC peak areas without any corrections.

RESULTS AND DISCUSSION

The yields of the fragrant essential oils of *G. hederacea* and *G. hirsuta* were 0.09% and 0.02% (w/w based on dry plant material weight), respectively. Chemical composition of the oils is presented in Table 1. The identified compounds, 238 for *G. hederacea* and 88 for *G. hirsuta*, amounted to 86.6% and 90.6% of the oils, respectively. The dominant constituent classes of the oil of *G. hederacea* were the terpenoids 47.4% (monoterpenes 18.9%, sesquiterpenes 26.1% and diterpenes 2.4%) and fatty acids with fatty acid derived compounds (FAD-27.3%). The main constituents were palmitic (13.3%) and linoleic acids (9.3%) which belong to FAD, however, the terpenoid compounds were qualitatively much more diverse and numerous. The monoterpene fraction was mostly made up of oxygenated compounds with monoterpene hydrocarbons present in only trace amounts. Almost all of the monoterpenes identified possessed the *p*-menthane skeleton (16.6%). Contrary to the monoterpene fraction hydrocarbon-oxygenated derivatives ratio, oxygenated sesquiterpenes made up only 9.7%, whereas the sesquiterpene hydrocarbons dominated the C_{15} compound class with 16.4%. Constituents with the germacrane carbon skeleton (10.6%) were the second most abundant, following the FAD class (27.3%), with germacrene D as their main contributor (7.3%).

Table 1. Percentage composition of the essential oils of *Glechoma hirsuta* Waldst. & Kit. (GH) and *Glechoma hederacea* L. (GHE).

No.	Compound	RI [§]	Class	GH	GHE
1	Pyridine	769	O	-	tr
2	Prenol (syn. 3-methyl-2-buten-1-ol)	781	HT	tr	tr
3	Prenal (syn. 3-methyl-2-butenal)	790	HT	tr	-
4	3-Hexanol	797	FAD	-	tr
5	3-Methylbutanoic acid	817	FAD	-	tr
6	Furfural	829	O	0.2	tr
7	2,2-Dimethyl-3(2*H*)-furanone	834	O	-	tr
8	Furfuryl alcohol	845	O	-	tr
9	(*Z*)-3-Hexen-1-ol	849	FAD	0.5	0.1
10	(*E*)-2-Hexen-1-ol	857	FAD	tr	tr
11	1-Hexanol	860	FAD	tr	tr
12	(*Z*)-4-hexen-1-ol	866	FAD	-	tr
13	5-Methylene-2(5*H*)-furanone (syn. protoanemonene)	880	O	-	tr
14	2-Butylfuran	885	FAD	-	tr
15	Heptanal	902	FAD	-	tr
16	(2*E*,4*E*)-2,4-Hexadienal	911	FAD	-	tr
17	2,5-Dimethylpyridine	928	O	tr	tr
18	2,4-Dimethylpyridine	930	O	-	tr
19	α-Pinene	936	MP	tr	-
20	5,5-Dimethyl-2(5*H*)-furanone	945	HT	-	tr
21	4-Methyl-2-heptanone	949	O	tr	tr
22	5-Methylfurfural	958	O	-	tr
23	Hexanoic acid	961	FAD	-	tr
24	Benzaldehyde	964	O	0.1	tr
25	Phenol	972	O	-	tr

26	1-Octen-3-one	975	FAD	-	0.2
27	1-Octen-3-ol	975	FAD	1.2	0.2
28	3-Octanone	982	FAD	0.2	tr
29	β-Pinene	983	MP	0.4	-
30	β-Myrcene	989	AM	-	tr
31	2-Pentylfuran	990	FAD	tr	-
32	3-Octanol	995	FAD	0.6	0.1
33	(*Z*)-2-(2-Pentenyl)-furan	1000	FAD	tr	-
34	Octanal	1003	FAD	-	tr
35	(*Z*)-3-Hexenyl acetate	1004	FAD	-	tr
36	(2*E*,4*E*)-2,4-Heptadienal	1012	FAD	-	tr
37	2-Ethyl-1-hexanol	1025	FAD	0.1	tr
38	*p*-Cymene	1027	MM	0.1	tr
39	Benzyl alcohol	1034	O	tr	tr
40	1,8-Cineole	1035	MM	42.6	4.6
41	(*Z*)-β-Ocimene	1044	AM	-	tr
42	Phenylacetaldehyde	1045	O	0.2	tr
43	Salicylaldehyde	1047	O	-	tr
44	2-Methylphenol	1054	O	-	tr
45	γ-Terpinene	1059	MM	tr	-
46	2-Acetylpyrrole	1059	O	-	tr
47	Heptanoic acid	1062	FAD	-	tr
48	1-Octanol	1067	FAD	-	tr
49	4-Methylphenol	1070	O	-	tr
50	*cis*-Linalool oxide (furanoid)	1072	AM	0.5	tr
51	*trans*-Linalool oxide (furanoid)	1089	AM	0.5	tr
52	Linalool	1101	AM	0.8	1.1
53	(3*Z*)-6-Methyl-3,5-heptadien-2-one	1104	CDC	0.2	tr
54	1-Octen-3-yl acetate	1106	FAD	3.1	0.2
55	2-Ethylhexanoic acid	1108	FAD	-	tr
56	(2*E*,4*E*)-2,4-Octadienal	1111	FAD	-	tr
57	Dehydrosabina ketone	1113	MT	-	tr
58	2,6-Dimethylcyclohexanol	1114	O	-	0.1
59	3-Octyl acetate	1117	FAD	0.7	tr
60	α-Isophorone	1124	CDC	0.1	tr
61	*cis*-*p*-Menth-2-en-1-ol	1127	MM	-	tr
62	α-Campholenal	1130	MB	0.2	tr
63	*trans*-*p*-Menth-2,8-dien-1-ol	1139	MM	-	tr
64	Nopinone	1143	MP	0.4	0.1
65	4-Oxo-isophorone	1145	CDC	-	0.2
66	*trans*-Pinocarveol	1145	MP	1.8	-
67	*trans*-Verbenol	1148	MP	-	tr
68	*cis*-Verbenol	1153	MP	1.4	-
69	(2*E*,6*Z*)-2,6-Nonadienal	1153	FAD	0.5	0.1
70	Menthone	1159	MM	-	tr
71	(*E*)-2-Nonenal	1160	FAD	-	tr
72	Sabina ketone	1160	MT	-	0.1
73	3,5-Dimethylphenol	1165	O	-	0.7
74	Pinocarvone	1167	MP	0.6	tr
75	*p*-Menth-1,5-dien-8-ol	1173	MM	0.8	-
76	δ-Terpineol	1173	MM	-	0.2
77	Borneol	1177	MB	-	0.1
78	2,4-Dimethylbenzaldehyde	1178	O	-	tr

79	Limonen-4-ol (syn. *p*-menth-1,8-dien-4-ol)	1181	MM	0.1	-
80	Isopinocamphone	1181	MP	-	tr
81	Terpinen-4-ol	1184	MM	0.5	0.2
82	*p*-Methylacetophenone	1188	O	-	0.2
83	*p*-Cymen-8-ol	1189	MM	-	tr
84	Cryptone	1191	MM	0.4	0.2
85	Methyl salicylate	1195	O	0.2	tr
86	α-Terpineol	1199	MM	2.5	1.1
87	Myrtenal	1200	MM	-	tr
88	Safranal	1203	CDC	tr	tr
89	Decanal	1207	FAD	tr	tr
90	*trans*-Dihydrocarvone	1208	MM	-	tr
91	Verbenone	1211	MP	0.5	0.2
92	2-Oxocineole	1217	MM	-	tr
93	*trans*-Carveol	1221	MM	0.5	-
94	*cis*-Carveol	1222	MM	-	0.1
95	β-Cyclocitral	1223	CDC	-	tr
96	Phenyl hexanoate	1223	FAD	-	tr
97	Thymol methylether	1225	MM	-	0.6
98	*exo*-2-Hydroxy-1,8-cineole	1230	MM	-	0.5
99	(*Z*)-3-Hexenyl isovalerate	1236	FAD	0.1	tr
100	Hexyl isovalerate	1241	FAD	0.1	-
101	Pulegone	1242	MM	-	tr
102	(*E*)-2-Hexenyl isovalerate	1244	FAD	-	tr
103	Cuminal	1246	MM	0.6	0.2
104	Linalyl acetate	1248	AM	-	tr
105	Carvone	1247	MM	-	tr
106	(*Z*)-2-Decenal	1249	FAD	-	1.4
107	Geraniol	1250	AM	-	tr
108	*cis*-Piperitone oxide	1254	MM	-	0.2
109	Nonanoic acid	1264	FAD	-	0.1
110	1-Decanol	1272	FAD	-	tr
111	4,8-Dimethyl-1,7-nonadien-4-ol	1274	CDC	-	tr
112	Phellandral	1281	MM	0.1	-
113	Thymol	1281	MM	-	1.5
114	Carvacrol	1290	MM	-	0.8
115	α-Terpinen-7-al	1290	MM	0.1	-
116	Dihydroedulan IA	1293	CDC	0.8	tr
117	2-Methylnaphthalene	1301	O	-	tr
118	4-Vinyl-2-methoxyphenol (syn. 4-vinylguaiacol)	1311	O	-	0.4
119	1-Methylnaphthalene	1315	O	tr	tr
120	(2*E*,4*E*)-2,4-Decadienal	1319	FAD	-	0.1
121	*p*-Menth-1,4-dien-7-ol	1330	MM	tr	-
122	δ-Elemene	1338	ST	-	0.2
123	*exo*-2-Hydroxycineole acetate	1341	MM	0.6	-
124	α-Cubebene	1350	ST	-	tr
125	Eugenol	1353	O	0.5	1.9
126	α-Ionene (syn. 1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene)	1357	CDC	-	tr
127	Neryl acetate	1359	AM	-	0.5
128	Decanoic acid	1362	FAD	-	tr
129	1,2-Dimethoxy-4-ethenylbenzene (syn. 3,4-dimethoxystyrene; 4-vinylveratrole)	1365	O	-	tr
130	(4*Z*,6*Z*,8*E*)-Megastigma-4,6,8-triene	1373	CDC	-	tr

131	α-Ylangene	1374	ST	-	tr
132	Geranyl acetate	1378	AM	-	0.2
133	α-Copaene	1379	ST	0.6	0.6
134	(*E*)-β-Damascenone	1380	CDC	-	tr
135	(*Z*)-3-Hexenyl hexanoate	1384	FAD	0.4	tr
136	β-Bourbonene	1388	ST	0.8	2.6
137	β-Elemene	1393	ST	0.2	1.3
138	Vanillin	1395	O	-	tr
139	Benzyl isovalerate	1396	O	tr	-
140	2-Ethylnaphthalene	1398	O	-	tr
141	1,5-Di-*epi*-β-bourbonene	1399	ST	-	tr
142	2,6-Dimethylnaphthalene	1408	O	-	tr
143	(*E*)-β-Damascone	1411	CDC	0.2	0.2
144	1,3-Dimethylnaphthalene	1421	O	0.1	tr
145	β-Ylangene	1422	ST	-	0.2
146	β-Caryophyllene	1424	ST	-	0.2
147	1,6-Dimethylnaphthalene	1427	O	-	tr
148	γ-Elemene	1432	ST	-	tr
149	β-Gurjunene	1432	ST	-	0.4
150	Methyl 4-methoxysalicylate	1439	O	-	0.2
151	Aromadendrene	1440	ST	-	tr
152	2,3-Dimethylnaphthalene	1443	O	-	tr
153	β-Copaene	1449	ST	-	0.2
154	Ethyl vanillin	1453	O	-	0.2
155	Humulene	1460	ST	-	0.2
156	4-Methyltetradecane	1462	FAD	-	0.2
157	γ-Muurolene	1473	ST	-	tr
158	1-Dodecanol	1475	FAD	-	tr
159	Thymol isobutyrate	1476	MM	-	5.9
160	(*E*)-β-Ionone	1481	CDC	0.6	0.4
161	(*E*)-5,6-Epoxy-β-ionone	1484	CDC	0.6	tr
162	Germacrene D	1486	ST	0.9	7.3
163	(*Z*,*E*)-α-Farnesene	1491	ST	-	0.2
164	2-Propylnaphthalene	1493	O	-	tr
165	α-Selinene	1494	ST	-	0.1
166	α-Muurolene	1496	ST	-	0.1
167	Bicyclogermacrene	1499	ST	-	0.4
168	Pentadecane	1500	FAD	-	tr
169	(*E*,*E*)-α-Farnesene	1506	ST	-	0.6
170	Germacrene A	1509	ST	-	0.1
171	3,4-Dimethyl-5-pentyl-2(5*H*)-furanone	1511	FAD	0.2	-
172	Helminthogermacrene	1512	ST	-	0.8
173	o-Hydroxybiphenyl	1515	O	-	tr
174	δ-Amorphene	1516	ST	-	tr
175	γ-Cadinene	1517	ST	-	tr
176	Benzofuran	1520	O	-	tr
177	δ-Cadinene	1521	ST	0.1	0.4
178	*trans*-Calamenene	1524	ST	-	tr
179	Trimethylnaphthalene isomer*	1527	O	-	tr
180	(*E*)-γ-Bisabolene	1529	ST	-	0.1
181	Dihydroactinidiolide	1531	CDC	tr	0.2
182	Trimethylnaphthalene isomer*	1534	O	-	tr
183	(*Z*)-Nerolidol	1540	ST	-	0.4

184	α-Calacorene	1544	ST	tr	tr
185	Trimethylnaphthalene isomer*	1548	O	-	tr
186	Elemol	1551	ST	-	tr
187	2,3,6-Trimethylnaphthalene	1553	O	-	tr
188	Dodecanoid acid	1561	FAD	-	tr
189	15-*nor*-4-Bourbonanone**	1563	ST	0.7	tr
190	Germacrene B	1564	ST	-	2.3
191	2,3,5-Trimethylnaphthalene	1565	O	-	tr
192	Thymol isovalerate	1569	MM	-	0.5
193	Mint oxide	1573	ST	2.7	1.2
194	Spathulenol	1581	ST	7.4	2.1
195	Caryophyllene oxide	1586	ST	2.5	1.1
196	Mint ketone	1596	ST	0.2	-
197	*trans*-β-Elemenone	1597	ST	-	1.1
198	Hexadecane	1600	FAD	-	0.2
199	*cis*-Dihydromajurone	1610	ST	1.3	-
200	Humulene epoxide II	1614	ST	0.5	tr
201	4-Methylbenzofuran	1623	O	-	tr
202	2-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal*	1628	CDC	-	0.6
203	Guaia-6,10(14)-dien-4β-ol	1631	ST	0.4	-
204	Benzophenone	1631	O	-	tr
205	Methylanthracene isomer*	1633	O	-	tr
206	Longipinocarvone	1639	ST	-	0.6
207	(*Z*)-Methyl jasmonate	1638	FAD	-	0.4
208	α-Cadinol	1648	ST	-	0.5
209	Diisopropylnaphthalene isomer*	1669	O	-	tr
210	Tetramethylnaphthalene isomer*	1671	O	-	tr
211	Methyldibenzothiophene isomer*	1675	O	1.6	-
212	Mustakone	1676	ST	-	-
213	Cadalene	1676	ST	-	tr
214	Diisopropylnaphthalene isomer*	1677	O	-	1.2
215	Longiverbenone	1678	ST	-	-
216	*cis*-14-*nor*-Muurol-5-en-4-one	1688	ST	0.7	-
217	Isoeugenyl propanoate	1689	O	-	1.4
218	Heptadecane	1700	FAD	-	tr
219	1-Methyl-(9*H*)-fluorene	1706	O	-	0.1
220	Ethyldibenzothiophene isomers*	1716	O	0.8	tr
221	Ethyldibenzothiophene isomers*	1721	O	0.4	0.5
222	Ethyldibenzothiophene isomers*	1727	O	0.4	0.9
223	Oplopanone	1737	ST	-	tr
224	Isobicyclogermacrenal	1741	ST	0.5	-
225	Mint sulfide	1744	ST	-	tr
226	Tetramethylbiphenyl isomer*	1751	O	-	0.2
227	1-oxo-4α,5α-Epoxyeudesm-2-en-11β*H*-12,6α-olid	1757	ST	-	0.4
228	Tetradecanoic acid	1760	FAD	-	0.7
229	Phenanthrene	1784	O	tr	0.5
230	Octadecane	1800	FAD	-	0.1
231	Hexahydrofarnesyl acetone	1843	CDC	0.4	1.2
232	Methyldibenzothiophene isomer*	1855	O	-	tr
233	Platambin (syn. 5,10-di-*epi*-eudesma-4(15)-en-1β,6β-diol)	1867	ST	-	0.4
234	Benzyl salicylate	1872	O	0.1	0.2
235	1-Hexadecanol	1881	FAD	-	tr

236	*o*-Terphenyl	1886	O	-	0.1
237	Methylanthracene isomer*	1902	O	-	0.1
238	(*E,E*)-Farnesyl acetone	1911	CDC	-	0.1
239	Methyl hexadecanoate	1927	FAD	-	tr
240	Methylanthracene isomer*	1927	O	-	tr
241	(*Z*)-9-Hexadecenoic acid (syn. palmitoleic acid)	1940	FAD	-	0.1
242	Hexadecanoic acid (syn. palmitic acid)	1968	FAD	0.4	13.3
243	Eicosane	2000	FAD	-	tr
244	(*E,E*)-Geranyl linalool	2024	DT	-	tr
245	Farnesoic acid	2029	ST	-	tr
246	Benzyl dihydroxybenzoate isomer*	2043	O	-	0.1
247	Thunbergol	2047	DT	-	0.1
248	Methyl linoleate	2098	FAD	-	tr
249	Heneicosane	2100	FAD	-	0.1
250	(*E*)-Phytol	2109	DT	0.2	2.3
251	(*Z,Z*)-9,12-Octadecadienoic acid (syn. linoleic acid)	2133	FAD	-	9.3
252	Octadecanoic acid (syn. stearic acid)	2161	FAD	-	0.4
253	Docosane	2200	FAD	-	tr
254	Tricosane	2300	FAD	-	tr
255	Tetracosane	2400	FAD	-	tr
256	Pentacosane	2500	FAD	-	0.1
	Total identified			90.6	86.6
	Monoterpenes (M)			56.0	18.9
	hydrocarbons			2.4	tr
	oxygenated			53.6	18.9
	acyclic skeleton (AM)			1.8	1.8
	bornane and related (MB)			0.2	0.1
	p-menthane and related (MM)			48.9	16.6
	pinane and related (MP)			5.1	0.3
	thujane and related (MT)			-	0.1
	Sesquiterpenes (ST)			19.5	26.1
	hydrocarbons			2.6	16.4
	oxygenated			16.9	9.7
	Diterpenes (DT)			0.2	2.4
	Carotenoid derived compounds (CDC)			2.9	2.9
	Fatty acids and fatty acid derived compounds (FAD)			7.6	27.3
	Other (O)			4.4	9.0

§ Compounds listed in order of elution on HP-5MS column (RI- experimentally determined retention indices on the mentioned column by co-injection of a homologous series of n-alkanes C_7-C_{25}); GH- percentage composition of the essential oil of *G. hirsuta* Waldst. & Kit.; GHE- percentage composition of the essential oil of *G. hederacea* L.; *correct isomer not determined; **a guaiane numbering system is used; tr- trace (< 0.05%); syn. – synonym; M-monoterpenes; AM-acyclic monoterpenes; MB-monoterpenes with a bornane skeleton; MM-monoterpenes with a *p*-menthane skeleton; MP-monoterpenes with a pinane skeleton; MT-monoterpenes with a thujane skeleton; ST-sesquiterpenes; DT-diterpenes; CDC-carotenoid derived compounds; FAD-fatty acids and fatty acid derived compounds.

In the case of *G. hirsuta* oil, the terpenoids (total 75.7%, monoterpenes 56.0%, sesquiterpenes 19.5% and diterpenes 0.2%) were the most dominant class of compounds. The monoterpenoid fraction was unevenly distributed among hydrocarbons (2.4%) and their oxygenated counterparts (53.6%). *p*-Menthanes (48.9%) were by far the most predominant compound class identified in this oil. The main constituent was 1,8-cineole (42.6%) alongside with spathulenol (7.4%), both oxygenated compounds. In this work we found that FAD compounds made up only 7.6% of *G. hirsuta* oil, while being one of the major contributors of

G. hederacea oil. The relative percentage of the sesquiterpene fraction (19.5%) and FAD (7.6%) compounds distinguished nicely *G. hirsuta* from *G. hederacea* (Table 1). Additionally, oxygenated sesquiterpenes (16.9%) dominated the oil of *G. hirsuta*, while the reversed situation was noted for *G. hederacea* oil (the hydrocarbon sesquiterpenes amounted to only 2.6%).

Previous investigations [11, 12] of the essential oil of *G. hederacea* from Lithuania showed that the terpenes represented the main portion of the oil, with a prominent sesquiterpene fraction (57.5%-71.0%) and marked by lower monoterpene content (7.2-24.6%, out of which 1.9-3.5% corresponded to *p*-menthanes). The major constituents were mostly sesquiterpene hydrocarbons which comprised 55.5-66.2% of the oils while the oxygenated sesquiterpenes reached only 2.5-4.6% [11, 12]. The constituents with a germacrane skeleton comprised 19.4-23.5% of the Lithuanian oil samples. Germacrene D (15.6-18.8%) dominated all of the oils from the Vilnius district [11, 12]. This quite characteristic, large amount of sesquiterpene hydrocarbons with a germacrene-type skeleton, and germacrene D as the major constituent in that class, is in agreement with the results on *G. hederacea* oil investigated in this work. The essential oil of *G. hederacea* from N. America likewise had a high percentage of germacrene D (19.4%), but was additionally characterized by a rather high relative amount of 1,8-cineole (6.2%) [13]. Although the relative content of 1,8-cineole was markedly lower than that found in the oil of *G. hirsuta* (42.6%), it could represent a very important link between these two species regarding their ethnopharmacological usage. 1,8-Cineole, also known as eucalyptol, is well known for its antiseptic and expectorant activity. It is used in syrups, lozenges and inhalants to ease the discomfort of colds and to treat inflamed membranes of the upper respiratory tract. It also has myorelaxant, antispasmodic and rubefacient properties [16, 17]. All of the mentioned pharmacological properties of 1,8-cineole, the major constituent of *G. hirsuta* oil (42.6%) and one of the major contributors of *G. hederacea* oil (4.6%), might provide a rationale for the possible parallel use of *G. hirsuta* and *G. hederacea* in folk medicine.

Given the variability that the essential oil of each species may show, nowadays one should consider the analysis of multiple plant samples, or populations, for the complete characterization of the species essential oil. Additional analyses of these species are being performed at this moment by us with the aim of describing their natural variation or of determining the presence of different chemotypes. The preliminary results that are not to be included in this publication, since its aim was the comparison of volatiles and not the chemotypification of the two species, suggest that the chemical composition of these two species seems to be quite constant for the general area from which the plant material was collected. It then follows that all of the conclusions reached based on the currently presented data stand on solid ground and that the two *Glechoma* sp. in Serbia do not show any natural variability in neither their essential oil composition nor content.

CONCLUSIONS

Although *G. hederacea* and *G. hirsuta* have easily distinguishable essential oil chemical compositions (the relative content of terpenoids and fatty acids, as well as different major constituents), perhaps a number of the noted important similarities between the two compositions (mainly the content of 1,8-cineole) can suggestively justify their mutually identical ethnopharmacological usage. Since these findings are the first ones reported for *G. hirsuta*, future research in the field of non-volatile metabolites of *G. hirsuta* seem promising.

AKNOWLEDGEMENTS

The financial support of this work by the Ministry of Science and Technological Development of Serbia is gratefully acknowledged (project No. 142054B).

REFERENCES

1. Janković, M.M. Rod *Glechoma* L. in *Flora SR Srbije*, Josifović, M. (Ed.), SANU: Beograd; **1974**; pp 376-377.
2. Mills, S.; Bone, K. *Principles and Practice of Phytotherapy*, Churchill Livingstone: London; **2000**; p 216.
3. Seidemann, J. *World Spice Plants*, Springer-Verlag: Berlin; **2005**; p 165.
4. Mills, S.Y. *The Dictionary of Modern Herbalism*, Thorsons: Wellingborough; **1985**; pp 14, 54, 61, 108, 112, 114, 156.
5. Watanabe, T.; Kawata, A.; Inoue, M.; Ishihara, S.; Tsuji, K. *Nippon Sho. Kag. Kog. Kaish.* **2007**, 54, 415.
6. Ishihara, S.; Kawata, A.; Inoue, M.; Watanabe, T.; Tsuji, K. *Nippon Sho. Kag. Kog. Kaish.* **2007**, 54, 412.
7. Kumarasamy, Y.; Nahar, L.; Kong-Thu-Lin, P.; Jaspars, M.; Sarker, S.D. *Nat. Prod. Comm.* **2006**, 1, 33.
8. Singh, T.; Wu, H.J.; Peumans, J.W.; Rouge, P.; Van Damme, J.M.E.; Alvarez, R.A.; Blixt, O.; Wu, M.A. *Biochem. J.* **2006**, 393, 331.
9. An, H.-J.; Jeong, H.-J.; Um, J.-Y.; Kim, H.-M.; Hong, S.-H. *J. Ethnopharmacol.* **2006**, 106, 418.
10. Kumarasamy, Y.; Cox, P.J.; Jaspars, M.; Nahar, L.; Sarker, S.D. *Fitoterapia* **2002**, 73, 721.
11. Mockute, D.; Bernotiene, G.; Judzentiene A. *Chemija* **2005**, 16, 47.
12. Mockute D.; Bernotiene G.; Judzentiene, A. *J. Essent. Oil Res.* **2007**, 19, 449.
13. Lawrence, B.M.; *Essential oils 1988-1991*, Allured: Carol Strem; **1992**; p 194.
14. Van den Dool, H.; Kratz, P.D. *J. Chromatogr.* **1963**, 11, 463.
15. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed., Allured Publishing Corporation: Carol Stream; **2007**.
16. Magalhäes, J.C.P.; Criddle, D.N.; Tavares, R.A.; Melo, M.E.; Mota, L.T.; Leal-Cardoso, J.H. *Phytother. Res.* **1998**, 12, 172.
17. Schulz V.; Hänsel R.; Tyler V. E.; *Rational Phytotherapy*, 4th ed., Springer-Verlag: Berlin; **2001**; pp 205, 345.