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Essential oil and its systematic significance in species of *Micromeria* Bentham from Serbia & Montenegro

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Abstract. The composition and quantity of the essential oil of *Micromeria* allowed us to clearly distinguish between sections *Pseudomelissa* and *Eumicromeria*. According to our results the species of section *Pseudomelissa* (*M. thymifolia*, *M. albanica*, *M. dalmatica* and *M. pulegium*) contained a large quantity of oil (>0.5%) dominated by oxygenated monoterpenes of the menthane type, while the species of section *Eumicromeria* (*M. croatica*, *M. juliana*, *M. cristata* and *M. parviflora*) had a small quantity of essential oil (<0.5 %) dominated by various terpene compounds. 0.5% of essential oil is defined like boundary value on the basis of the lowest quantity of essential oil measured in populations of species of section *Pseudomelissa*.

Key words: *Micromeria*, *Pseudomelissa*, *Eumicromeria*, Lamiaceae, chemotaxonomy, essential oils.

The genus *Micromeria* Benth. includes about 130 species widespread in the Mediterranean region (Diklic 1974). In the flora of Serbia and Montenegro this genus is represented by ten species, seven of which are endemic (Silic 1979).

On the basis of their morphological characteristics and phylogenetic relationships, the species of the genus *Micromeria* are grouped in three sections (Boissier 1879): *Cymularia*, *Eumicromeria* and *Pseudomelissa*. Boissier used the term section for classification of species in the genus *Micromeria* (Flora Orientalis, 1879, pp. 568–575). The species of *Micromeria* inhabiting the territory of Serbia and Montenegro belong to sections *Pseudomelissa* and *Eumicromeria*.

The species of *Micromeria* are well known as aromatic species because they contain considerable quantities of essential oil. The quantity of essential oil (≥ 0.5%) is one of the characteristics on the basis of which the species of this genus have been classified into subfamily *Nepetoideae* (El-Gazzar and Watson 1970). It is well known that the following species possess this characteristic: *Micromeria thymifolia* (Scop.) Fritsch (Savarda et al. 1979; Pavlovic et al. 1983; Kalodjera et al. 1990, 1994; Marinkovic et al. 2001; Vladimir-Knezevic et al. 2001), *M. dalmatica* Benth. (Savarda et al. 1979, Pavlovic et al. 1983, Karuza-Stojakovic et al. 1989), *M. pulegium* (Rochel.) Benth. (Pavlovic et al. 1983), *M. albanica* (Griseb. ex K. Maly) Silic (Stojanovic et al. 1999, Marinkovic et al. 2001), *Micromeria fruticosa* (L.) Druce (Fleisher and Fleisher 1991; Kirimer 1992; Kirimer et al. 1993a, 1993b; Putievsky et al. 1995; Baser et al. 1996, 1998) and *M. dolichodonta* P. H. Davis (Baser et al. 1997a). However, by examining the chemical composition of the species of the genus *Micromeria* as well as according to data gathered from literature (Pavlovic et al. 1983; Stanic et al. 1988; Özek et al. 1992; Baser et al. 1995, 1997b; Pérez-Alonso et al. 1996; Mastelic et al. 1998) we have established that certain species contain only small quantities or even merely traces of essential oils. These differences in quantity prompted us to make a detailed examination of the essential oil characteristics of chosen populations of different species of the genus *Micromeria* on the territory of Serbia and Montenegro and compare them to the existing literature data. The number of examined populations is directly commensurate to areal size and width of ecological species valence, in the examined area. The results will be the basis for determining the interpopulation, interspecies and intersection variability of quantity and composition of essential oil which will point to the adaptive, and possibly, systematic importance of these characters.

Material and methods

Plant material. We have investigated the following plant material: *M. thymifolia* from five different localities, Derventa canyon, Beli Rzav gorge, Moraca canyon, Semolj and mount Orjen; *M. dalmatica* from the area of Kotor; *M. pulegium* from Beli Rzav gorge, *M. albanica* from Prizren area, *M. croatica* from Beli Rzav gorge, *M. juliana* from three localities, Moraca canyon, Cijevna canyon and mount Orjen; *M. cristata* from Jerma gorge and *M. parviflora* from three localities, Moraca canyon, Cijevna canyon and Rijeka Crnojevica (Fig. 1). The basic characteristics of the 16 mentioned localities are given in Table 1. The samples were gathered in the flowering period.

Voucher specimens are kept at the Herbarium of the Institute of Botany, Faculty of Pharmacy, University of Belgrade, Serbia and Montenegro.

Steam distillation. Air-dried aerial parts of the plant were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus.

Combined GC-MS. GC-MS analysis was carried out using the Hewlett Packard 5973–6890 GC-MS system operating in EI mode at 70 eV, equipped with a HP 5MS capillary column (30 m $\times$ 0.25 mm; film thickness 0.25 μm). The initial temperature of the column was 60°C and was heated to 280°C with a rate of 3°C/min. Carrier gas He; flow rate 1 ml/min; split ratio, 1:10. The injection volume was 1 μl. Relative percentage amounts were calculated from total ion chromatograms (TICs).

The identification of the compounds was based on the comparison of their Kovats indices (KI), their retention times (RT) and mass spectra with those obtained from authentic samples and/or the MS library (Adams 1995).

Statistical analysis. Cluster analysis represents the most appropriate method to illustrate the similarities and dissimilarities of the data matrix. The dendrogram acquired by Optimal clustering (Chord distance) emphasizes intergroup heterogeneity which is, according to Orloci (1966) and Pielou (1984), primary characteristic of this clustering method. Cluster analyses were carried out using FLORA software (Karadzic et al. 1998). In total, 120 characteristics (quantity of essential oils and chemical compounds) were analyzed in 16 populations.

Results

The composition and content of essential oils of the studied species are given in Table 2. The qualitative composition of the essential oils of *M. thymifolia* populations was always similar. Oxygenated terpenes of the menthane type dominated in all the oils ($>73\%$). However, the contribution of certain major components

Fig. 1. Locations of the analyzed populations of species of *Micromeria*. For explanation of numbers compare Table 1

Table 1. Basic characteristics of the studied populations of *Micromeria* spp.

Species	Code	Locality	H[a] (m)	Coordinates	Substrate	Climate
M. thymifolia	1	Derventa canyon	500	43°9N 19°4E	limestone	sub-mediterranean
M. thymifolia	2	Beli Rzav gorge	600	43°7N 19°3E	limestone	sub-mediterranean
M. thymifolia	3	Moraca canyon	500	42°5N 19°4E	limestone	sub-mediterranean
M. thymifolia	4	Semolj	700	42°6N 19°2E	limestone	sub-mediterranean
M. thymifolia	5	Mt Orjen	1540	42°4N 18°5E	limestone	sub-med. montane
M. dalmatica		Kotor area	800	42°3N 18°8E	limestone	sub-mediterranean
M. pulegium		Beli Rzav gorge	600	43°7N 19°3E	limestone	sub-mediterranean
M. albanica		Prizren area	500	42°1N 20°7E	limestone	sub-med. montane
M. croatica		Beli Rzav gorge	600	43°7N 19°3E	limestone	sub-mediterranean
M. juliana	1	Moraca canyon	300	42°5N 19°4E	limestone	sub-mediterranean
M. juliana	2	Cijevna canyon	45	42°3N 19°5E	limestone	sub-mediterranean
M. juliana	3	Mt Orjen	900	42°4N 18°5E	limestone	sub-med. montane
M. cristata		Jerma gorge	900	42°9N 22°6E	limestone	sub-mediterranean
M. parviflora	1	Moraca canyon	300	42°5N 19°4E	limestone	sub-mediterranean
M. parviflora	2	Cijevna canyon	45	42°3N 19°5E	limestone	sub-mediterranean
M. parviflora	3	Rijeka Crnojevica	15	42°3N 19°1E	limestone	sub-mediterranean

[a] H = height above sea level

was varying. In the essential oil from the Derventa canyon population there was a considerable presence of piperitone oxide (63.8%). The proportion of piperitenone oxide was 9.2%. Piperitenone was present in a very small quantity (0.4%), while pulegone was

Table 2. The composition (%) of essential oils of the studied populations of *Micromeria* spp

	Populations		*M. thymifolia*					*M. dalmatica*	*M. pulegium*	*M. albanica*	*M. croatica*	*M. juliana*			*M. cristata*	*M. parviflora*		
			1	2	3	4	5					1	2	3		1	2	3
	(%)		0.5	0.9	0.5	1.3	0.6	1.7	1.0	0.9	0.12	0.08	0.09	0.04	0.13	< 0.05	< 0.05	< 0.05
	Compounds																	
	MONO-TERPENES	KI																
	Hydrocarbons																	
1.	Tricyclene	922	t [c]	t	t	–	t	t	t	t	–	–	–	–	–	–	–	–
2.	α-Thujene	923	–	–	–	t	–	–	–	–	–	–	–	–	–	–	–	–
3.	α-Pinene	929	1.7	1.9	1.0	1.0	1.2	1.8	1.2	1.4	0.6	t	t	t	t	t	t	t
4.	Camphene	944	t	t	t	–	–	t	t	t	1.4	–	–	–	t	t	t	t
5.	Verbenene	960	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	–
6.	Sabinene	967	0.6	t	t	0.4	0.1	0.4	t	t	–	–	–	–	–	–	–	–
7.	β-Pinene	973	3.0	2.7	4.3	2.2	1.7	2.7	2.4	2.6	1.3	t	1.9	t	t	t	t	t
8.	Myrcene	988	0.9	0.4	0.9	0.4	0.3	0.8	0.9	0.5	–	–	–	–	–	–	–	–
9.	p-Mentha-1(7), 8-diene	999	–	–	–	t	–	–	–	–	–	–	–	–	–	–	–	–
10.	δ-3-carene	1011	–	–	–	–	–	–	–	–	t	–	–	–	–	–	–	–
11.	α-terpinene	1015	t	–	–	t	–	t	t	t	t	–	–	–	–	–	–	–
12.	*o*-Cymene	1020	–	–	–	–	–	–	–	–	0.6	**10.8**	–	–	–	–	–	–
13.	ρ-Cymene	1021	t	t	t	t	t	t	t	t	1.6	5.3	2.1	1.6	1.1	**11.5**	t	**14.6**
14.	Limonene	1029	11.7	4.5	8.9	3.0	4.4	8.3	6.8	7.0	0.9	–	t	0.8	–	1.3	t	t
15.	(*Z*)-β-Ocimene	1035	t	–	t	t	t	t	0.7	t	–	–	–	–	–	–	–	–
16.	(*E*)-β-Ocimene	1044	t	–	–	t	t	t	t	t	–	–	–	–	–	–	–	–
17.	γ-terpinene	1056	t	t	t	t	t	t	1.2	t	0.6	t	t	–	–	t	t	t
18.	Terpinolene	1089	–	–	–	t	–	t	t	t	t	–	–	–	–	–	–	–
	Oxygenated monoterpenes																	
	ALDEHYDES																	
19.	α-Campholenal	1126	–	–	–	–	–	–	–	–	0.6	t	t	–	0.7	–	–	–
20.	Myrtenal	1195	–	–	–	–	–	–	–	–	0.9	t	4.3	–	1.1	–	–	–
	ALCOHOLS																	
21.	*cis*-Sabinene hydrate	1068	t	–	t	–	t	t	6.4	t	–	–	–	–	–	–	–	–
22.	*trans*-Sabinene hydrate	1098	–	–	–	–	–	t	t	t	–	–	–	–	–	–	–	–

23.	Linalool	1099	t	–	–	–	t	–	–	–	t	1.4	1.9	t	t	2.0	**14.3**	t
24.	endo-Fenchol	1114	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	–
25.	*trans*-Pino-carveol	1139	–	–	–	–	–	–	–	–	t	t	2.6	t	–	–	–	–
26.	*trans*-Verbenol	1146	–	–	–	–	–	–	–	–	–	t	4.8	–	–	–	–	–
27.	Borneol	1165	–	–	–	–	–	–	–	–	**10.8**	3.8	6.3	t	5.7	2.3	t	–
28.	Terpinen-4-ol	1183	t	–	0.5	–	–	–	2.5	–	0.8	1.5	t	t	t	0.7	t	–
29.	ρ-Cymen-8-ol	1187	–	–	–	–	–	–	–	–	–	t	t	t	t	–	–	–
30.	*Neo*-*iso*-men-thol	1189	–	–	–	–	–	–	2.6	–	–	–	–	–	–	–	–	–
31.	α-terpineol	1194	0.2	t	t	–	0.3	0.6	0.2	–	t	–	–	–	0.8	0.9	3.0	t
32.	Myrtenol	1197	–	–	–	–	–	–	–	–	t	–	–	–	–	–	–	–
33.	*trans*-Carveol	1220	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	–
34.	Geraniol	1260	–	–	–	–	–	–	–	–	–	–	–	–	–	–	t	–
35.	Thymol	1294	0.8	0.6	0.2	–	0.2	–	–	–	–	3.4	t	7.3	–	–	t	t
36.	Carvacrol	1303	–	–	–	–	–	–	3.5	–	–	**18.1**	t	t	t	–	**10.6**	t
	ETHERS																	
37.	1,8 Cineole	1031	–	–	–	–	–	–	–	–	–	–	–	–	5.0	t	t	t
38.	methyl-ether Carvacrol	1245	–	–	–	–	–	–	–	–	–	t	–	t	–	0.7	t	–
	OXIDES																	
39.	*cis*-Linalool oxide	1072	–	–	–	–	–	–	–	–	–	–	–	–	–	t	t	–
40.	*trans*-Linalool oxide	1088	–	–	–	–	–	–	–	–	–	–	–	–	–	t	t	–
41.	Piperitone oxide *	1259	**63.8**	**20.0**	1.8	–	t	–	7.4	**36.9**	–	–	–	–	–	1.2	–	t
42.	Piperitenone oxide	1366	9.2	9.8	**16.0**	2.4	2.8	0.9	3.6	**21.9**	0.7	–	–	1.2	t	–	–	–
	KETONES																	
43.	*cis*-Thujone	1105	–	–	–	–	–	–	–	–	–	–	–	–	3.1	–	–	–
44.	*trans*-Thujone	1117	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	–
45.	Camphor	1145	–	–	–	–	–	–	–	–	2.2	–	–	–	7.5	–	–	–
46.	Menthone	1156	t 1	0.5 2	2.7 3	1.1 4	8.7 5	t	4.3	4.5	–	t 1	t 2	1.3 3	–	t 1	t 2	t 3
47.	Pinocarvone	1161	–	–	–	–	–	–	–	–	–	t	1.8	t	t	–	–	–
48.	Isomenthone	1164	t	6.9	t	5.0	8.3	0.6	**27.2**	t	–	t	t	**10.1**	–	–	–	7.9
49.	*cis*-Isopulegone	1173	–	t	t	2.1	2.2	0.5	–	–	–	–	–	–	–	–	–	–

Table 2. (Continued)

	Populations		*M. thymifolia*					*M. dalmatica*	*M. pulegium*	*M. albanica*	*M. croatica*	*M. juliana*			*M. cristata*	*M. parviflora*		
			1	2	3	4	5					1	2	3		1	2	3
50.	Verbenone	1209	–	–	–	–	–	–	–	–	–	–	–	–	0.6	–	–	–
51.	Pulegone	1240	t	**37.2**	6.2	**72.3**	**48.0**	**12.1**	4.0	7.8	t	t	t	8.1	t	t	t	t
52.	Carvone	1244	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	–
53.	Piperitone	1257	–	1.7	**24.0**	0.9	2.8	3.3	–	–	–	–	–	–	–	–	–	–
54.	Piperitenone	1340	0.4	6.3	**28.7**	4.3	**13.9**	**56.7**	1.2	**10.0**	1.0	t	t	0.8	t	–	–	–
	ESTERS																	
55.	Bornyl acetate	1285	–	–	–	–	–	–	–	–	–	–	–	–	4.1	1.1	–	t
56.	Isobornyl acetate	1285	–	–	–	–	–	–	–	–	2.9	–	–	–	–	–	–	–
57.	Isomenthyl acetate	1318	–	–	–	–	–	–	3.0	–	–	–	–	–	–	–	–	–
58.	*Neo-iso*-Isopulegol acetate	1321	–	–	–	–	–	–	1.1	–	–	–	–	–	–	–	–	–
59.	Isobornyl formate	1233	–	–	–	–	–	–	–	–	1.1	–	–	–	t	–	–	–
	SESQUITERPENES Hydrocarbons																	
60.	α-Cubebene	1351	–	–	–	–	–	–	–	–	0.5	–	–	–	–	–	–	–
61.	α-Copaene	1373	0.2	–	–	0.1	0.3	–	t	–	0.5	t	t	–	–	–	–	–
62.	β-Bourbonene	1382	0.5	–	0.9	0.2	0.3	–	1.2	–	2.4	1.5	2.1	1.1	–	4.9	t	t
63.	Isolongifolene	1386	–	–	–	–	–	–	–	–	–	–	–	–	1.3	–	–	–
64.	β-Cubebene	1391	–	–	–	–	–	–	–	–	t	–	–	–	–	–	–	–
65.	β-Elemene	1392	t	t	t	t	–	t	t	–	–	t	–	t	t	t	–	–
66.	1,7-di-epi-α-Cedrene	1399	–	–	–	–	–	–	–	–	0.5	–	–	–	–	–	–	–
67.	β-Longipinene	1400	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	–
68.	(*Z*)-Car- yophyllene	1405	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	–
69.	α-Cedrene	1408	–	–	–	–	–	–	–	–	t	–	–	–	–	–	–	–
70.	α-Gurjunene	1414	–	–	–	–	–	–	–	–	–	–	–	–	1.2	–	–	–
71.	β-Cedrene	1417	–	–	–	–	–	–	–	–	0.5	–	–	–	–	–	–	–
72.	(*E*)-Caryophyllene	1423	–	–	–	–	–	–	4.7	t	–	3.7	7.1	5.6	1.9	1.5	t	t
73.	β-Gurjunene	1427	t	t	t	–	–	0.2	t	t	t	t	t	t	–	t	–	t

74.	α-Guaiene	1440	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	–
75.	α-Humulene	1452	–	–	–	–	–	–	1.2	–	–	t	t	0.8	t	–	–	–
76.	(*E*)-β-Farnesene	1458	–	–	–	–	–	–	0.6	–	–	–	–	–	–	–	–	–
77.	allo-Aromadendrene	1460	–	t	t	–	t	–	–	–	–	1.1	3.5	5.3	3.7	–	–	–
78.	*cis*-Muurola-4(15)5-diene	1462	–	–	–	–	–	–	–	–	1.1	–	–	–	–	–	–	–
79.	β-Acoradiene	1465	–	–	–	–	–	–	–	–	t	–	–	–	–	–	–	–
80.	γ-Muurolene	1475	t	t	t	–	t	–	–	–	t	t	t	t	t		–	–
81.	Germacrene D	1480	1.1	0.5	t	1.0	0.2	3.0	2.7	2.1	0.7	1.1	2.9	5.2	–	2.5	t	t
82.	ar-Curcumene	1483	–	–	–	–	–	–	–	–	1.3	t	t	1.3	–	–	–	–
83.	*cis*-β-Guaiene	1487	–	–	–	–	–	–	–	–	t	–	–	–	–	–	–	–
84.	Bicyclogermacrene	1489	0.4	–	–	0.5	–	0.6	0.5	0.5	–	–	–	–	–	2.6	t	t
85.	α-Muurolene	1496	–	–	–	–	–	–	–	–	–	t	t	t	–	–	–	–
86.	Germacrene A	1501	–	–	–	–	–	–	–	–	t	–	–	–	–	–	–	–
87.	β-Bisabolene	1502	–	–	–	–	–	–	–	–	–	–	–	–	–	t	t	t
88.	β-Curcumene	1506	–	–	–	–	–	–	–	–	–	t	t	t	–	–	–	–
89.	γ-Cadinene	1518	–	–	–	–	–	t	t	t	**10.9**	t	t	t	1.3	–	–	–
90.	δ-Cadinene	1523	0.1	0.2	t	t	t	0.3	t	0.4	–	3.8	3.0	3.7	t	1.8	t	t
91.	*trans*-Calamenene	1526	–	–	–	–	–	–	–	–	3.9	–	–	–	–	–	–	–
92.	α-Cadinene	1536	–	–	–	–	–	–	–	–	0.4	t	t	t	–	–	–	–
93.	Unknown	1554	–	–	–	–	–	–	–	–	–	–	–	–	**17.7**	–	–	–
	Oxygenated sesquiterpenes																	
	ALCOHOLS																	
94.	endo-1-Bourbonanol	1522	–	–	–	–	–	–	–	–	4.3	2.9	2.3	0.9	–	–	–	–
95.	(*E*)-Nerolidol	1565	–	–	–	–	–	–	–	–	–	–	–	–	3.9	–	–	–
96.	Spathulenol	1572	1.9	2.3	2.0	1.1	1.9	1.3	0.9	2.3	–	t	t	1.5	**11.7**	**46.7**	**12.7**	**19.0**
97.	Germacrene D-4-ol	1574	–	–	–	–	–	–	–	–	–	4.1	4.8	1.4	–	–	–	–
98.	Globulol	1591	–	–	–	–	–	–	–	–	–	–	–	–	6.0	–	–	–
99.	Viridiflorol	1595	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	–
100.	1,10-di-epi-Cubenol	1616	–	–	–	–	–	–	–	–	1.1	–	–	–	2.9	–	–	–
101.	Caryophylla-3,8(13)-dien-5-β-ol	1635	–	–	–	–	–	–	–	–	–	–	–	–	1.0	–	–	–

Table 2. (Continued)

Populations		*M. thymifolia*					*M. dalmatica*	*M. pulegium*	*M. albanica*	*M. croatica*	*M. juliana*			*M. cristata*	*M. parviflora*		
		1	2	3	4	5					1	2	3		1	2	3
102. Isospathulenol	1641	0.1	0.6	0.5	–	0.2	0.5	t	t	–	–	–	–	–	–	–	–
103. epi-α-Muralol	1642	–	–	–	–	–	–	–	–	–	3.9	3.4	1.6	–	–	–	–
104. α-Muurolol	1647	–	–	–	–	–	–	–	–	8.1	–	–	–	–	–	–	–
105. α-Cadinol	1658	t	0.5	t	–	t	t	t	t	1.6	4.4	6.6	1.9	4.3	–	–	–
106. Caryophyllenol II	1674	–	–	–	–	–	–	–	–	1.4	–	–	–	0.6	–	–	–
OXIDES																	
107 Caryophyllene oxide	1583	–	–	–	–	–	–	1.5	t	**24.4**	**18.1**	**20.4**	**15.9**	–	–	–	–
KETONES																	
108. 1-nor Bourbonanone	1560	–	–	–	–	–	–	–	–	–	1.4	1.9	2.2	–	–	–	–
109. β-Oplopenone	1611	–	–	–	–	–	–	–	–	2.9	–	–	–	–	–	–	–
110. Hexahydro-farnesyl acetone	1846	–	–	–	–	–	–	–	–	0.4	1.6	1.2	2.6	0.5	1.9	5.0	4.4
FATTY ACIDS																	
111. Hexadecanoic acid	–	–	–	–	–	–	–	–	–	–	t	t	1.0	–	t	3.9	t
ESTERS																	
112. Ethyl linoleate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7.9	t
HYDROCARBONS																	
ALDEHYDES																	
113. (*E*)-2-Hexenal	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–	–	–	–
114. (*E,E*)-2,4-hepta-dienal	1006	–	–	–	t	–	–	–	–	–	–	–	–	–	–	–	–
ALCOHOLS																	
115. 3-Octanol	993	t	t	0.4	0.3	0.6	0.3	1.6	t	–	–	–	–	–	–	–	–
PHENYLPRO-PANOIDS																	
116. Eugenol	1362	–	–	–	–	–	–	t	–	–	–	–	–	–	–	–	–

OTHERS																	
117. Benzaldehyde	956	–	–	–	–	–	t	t	–	–	–	–	–	–	–	–	–
118. Dihydroedullan II	1285	–	–	–	–	–	–	–	–	–	2.9	4.2	1.3	–	–	–	–
119. *β*-(*E*)-Ionone	1485	t	t	t	t	–	–	t	t	–	–	–	–	t	–	–	–
Monoterpene compounds		92.3	92.5	95.2	95.1	94.9	88.7	80.2	92.6	28.0	**44.3**	25.7	31.2	29.7	21.7	**27.9**	**22.5**
Monoterpene hydrocarbons		17.9	9.5	15.1	7.0	7.7	14.0	13.2	11.5	7.0	16.1	4.0	2.4	1.1	12.8	t	14.6
Oxygenated monoterpenes		74.4	83.0	80.1	88.1	87.2	74.7	67.0	81.1	21.0	28.2	21.7	28.8	28.6	8.9	**27.9**	7.9
Oxygenated monoterpenes of the menthane type		**73.4**	**82.4**	**79.4**	**88.1**	**86.7**	**74.1**	**51.8**	**81.1**	1.7	t	t	21.5	t	1.2	t	7.9
Sesquiterpene compounds		4.3	4.1	3.4	2.9	2.9	5.9	13.3	5.3	**66.9**	**47.6**	**59.2**	**52.0**	**58.0**	**61.9**	17.7	**23.4**
Sesquiterpene hydrocarbons		2.3	0.7	0.9	1.8	0.8	4.1	10.9	3.0	22.7	11.2	18.6	23.0	27.1	13.3	t	t
Oxygenated sesquiterpenes		2.0	3.4	2.5	1.1	2.1	1.8	2.4	2.3	44.2	36.4	40.6	29.0	30.9	48.6	17.7	23.4
Other compounds		t	t	0.4	0.5	0.6	0.3	1.6	t	–	2.9	4.2	1.3	–	t	11.8	t
Total		96.6	96.6	99.0	98.5	98.4	94.9	95.1	97.9	94.9	94.8	89.1	84.5	87.7	83.6	57.4	45.9

% percentage calculated from TIC data
t – traces
*correct isomer not identified

found in traces. Pulegone (37.2%) and piperitone oxide (20.0%) were the main compounds in the essential oil of *M. thymifolia* from Beli Rzav gorge. The quantities of piperitenone oxide and piperitenone corresponded to 9.8% and 6.3% of total content of essential oil, respectively. The population from Moraca canyon had a high content of piperitenone (28.7%), piperitone (24.0%) and piperitenone oxide (16.0%), while the concentration of pulegone amounted to 6.2%. The essential oil of the Semolj populations contained 72.3% of pulegone, 4.3% piperitenone, 12.4% piperitenone oxide and 0.9% of piperitone. There was no piperitone oxide. The mount Orjen population contained essential oil rich in pulegone (48.0%) and piperitenone (13.9%). Piperitone and piperitenone oxide were ascertained in smaller quantities (2.8%). Piperitone oxide was found in traces. The proportion of sesquiterpenes in all *M. thymifolia* populations varied between 2.9 and 4.3 %.

Oxygenated terpenes of the menthane type were dominant (74.1%) in the oil of *M. dalmaatica*. The oil was rich in piperitenone (56.7%) and pulegone (12.1%). The proportion of sesquiterpenes was 5.9%.

Oxygenated terpenes of the menthane type were dominant in the oils of *M. pulegium* (51.8%). The main compound in the essential oil was isomenthone (27.2%). Sesquiterpene compounds were found to contribute 13.3% to the essential oil content.

The oil of *M. albanica* contained 81.1% of oxygenated terpenes of the menthane type. Piperitone oxide (36.9%), piperitenone oxide (21.9%) and piperitenone (10.0%) were dominant in the oil. The proportion of sesquiterpenes was 5.3%.

All four species of sect. *Pseudomelissa* were characterized by a higher quantity of essential oils (0.8%–1.7%) in which monoterpene compounds dominated (even higher than 80.2%). Among the monoterpenes oxygenated monoterpenes of the menthane type had the highest presence (51.8%–82.0%). They shared an increased piperitenone, pulegone, piperitenone oxide content, as well as the absence of borneol. Sesquiterpene compounds appeared in a smaller quantity (3.5%–13.3%).

The main components of the *M. croatica* essential oil were sesquiterpenes (66.9%). Caryophyllene oxide (24.4%) and γ-cadinene (10.9%) were dominant among them. The bicyclic monoterpene, borneol, was also one of the dominant components (10.8%). Oxygenated terpenes of the menthane type were ascertained in the concentration of 1.7%.

The content of monoterpene (44.3%) and sesquiterpene (47.6%) compounds in the oil of the *M. juliana* population from Moraca canyon was approximately the same, while sesquiterpenes dominated in the oils of the other populations (59.2% - Cijevna canyon population; 52.0% - Mt Orjen population). The largest quantity of oxygenated terpenes of the menthane type was present in the oil of the Orjen population (21.5%), while in the other populations these components were present in traces. Caryophyllene oxide was the main component of all the oils. The Moraca canyon population had a high content of caryophyllene oxide (18.1%), carvacrol (18.1%) and *o*-cymene (10.8%). Traces of isomenthone were found. In the essential oil from the Cijevna canyon population there was a considerable presence of caryophyllene oxide (20.4%). Isomenthone and carvacrol were found in traces, while no *o*-cymene was detected in the essential oil of this population. The Mt. Orjen population contained essential oil rich in caryophyllene oxide (15.9%) and isomenthone (10.1%). There were traces of carvacrol, while there was no presence of *o*-cymene.

An unidentified sesquiterpene (KI 1554) was dominant (17.7%) in the oil of *M. cristata*. Spathulenol was also found in a large quantity (11.7%). A considerable quantity of sesquiterpenes (58.0%) was also found in this oil, while oxygenated menthane monoterpenes were found in traces.

The essential oil of *M. parviflora* from Rijeka Crnojevica had approximately the same content of monoterpene (22.5%) and sesquiterpene (23.4%) compounds, while sesquiterpenes dominated in the oil of the Moraca canyon populations (61.9%), and monoterpenes dominated (27.9%) in the oil of the Cijevna canyon population. In the essential oil from the Cijevna canyon population there was a considerable presence (11.8%) of other compounds (fatty acids and fatty acid esters). These compounds were found in traces in the Rijeka Crnojevica and Moraca canyon populations.

High content of spathulenol (46.7%) and *p*-cymene (11.5%) was found in the oil of the Moraca canyon population. Linalool (2.0%) was ascertained in a smaller quantity. Carvacrol was not present. Linalool (14.3%), spathulenol (12.7%), and carvacrol (10.6%) were the dominant compounds of the oil from the Cijevna canyon population. *p*-Cymene was found in traces. The Rijeka Crnojevica population contained essential oil rich in spathulenol (19.0%) and *p*-cymene (14.6%). Linalool and carvacrol were found in traces.

The higher content of borneol, *p*-cymene, hexahydrofarnesyl acetone and lower content of piperitone oxide was what the species of the *Eumicromeria* section had in common. Also, they were characterised by a small quantity of essential oil (0.05–0.13%) in which sesquiterpenes predominated (34.3%–66.9%), while the proportion of monoterpene compounds of the menthane type varied from 0.1%–7.2%.

The optimal clustering (Chord distance) dendrogram based on a matrix of total character differences is shown in Fig. 2. The dendogram Fig. 2a represents the interrelation of all examined populations. The mountain populations of the *M. thymifolia* species from Orjen and Semolj were the most alike, and the Beli Rzav gorge population was the one closest to them. All three populations were characterized by pulegone dominance in the oil. The Derventa Canyon *M. thymifolia* was the most similar to the *M. albanica* species because of the piperitone oxide domination, while the Moraca Canyon *M. thymifolia* was similar to the *M. dalmatica* species because of the piperitenone predominance. The Rijeka Crnojevica and Moraca canyon *M. parviflora* populations were closer to the *M. cristata* species according to their quantity of spathulenol. The Cijevna canyon *M. parviflora* stood apart from the previous group because of its quantity of linalool and carvacrol. According to its *β*-pinene and myrtenal content, *M. juliana* from the Cijevna canyon was similar to *M. croatica*. *M. juliana* from the Moraca canyon was prominent for its *o*-cimene and carvacrol domination, and the Orjen population for its quantity of isomenthone and pulegone.

The dendogram (Fig. 2b) shows a distinct division of the species into three groups, the first of which is clearly separated from the others. The first group consists of representatives of section *Pseudomelissa* (*M. thymifolia*, *M. albanica*, *M. dalmatica* and *M. pulegium*). The *M. thymifolia* populations, although heterogeneous, were closest to *M. albanica* because of their domination of piperitone oxide. *M. dalmatica* contained somewhat more α-pinene and the largest quantity of piperitenone. *M. pulegium* especially distinguished itself from the other species of the section *Pseudomelissa* by its higher content of isomenthone, cis-sabinene hydrate, (*E*) caryophyllene, carvacrol and isomenthyl acetate.

The representatives of section *Eumicromeria* are divided in two groups. Because of the domination of caryophyllene oxide and presence and content of endo-1-bourbonanol the species *M. croatica* and *M. juliana* are the most similar. *M. cristata* and *M. parviflora* are between the species of section *Pseudomelissa* section and *M. croatica* and *M. juliana*, and they are characterized by the domination of spathulenol.

Discussion

The essential oil of *Micromeria thymifolia* has been analyzed quite frequently. Our results referring to the quantity of essential oil agree with previously published results (Savarda et al. 1979, Pavlovic et al. 1983, Kalodjera et al. 1990, Marinkovic et al. 2001, Vladimir-Knezevic et al. 2001). In works published so far, in the essential oil of *M. thymifolia,*

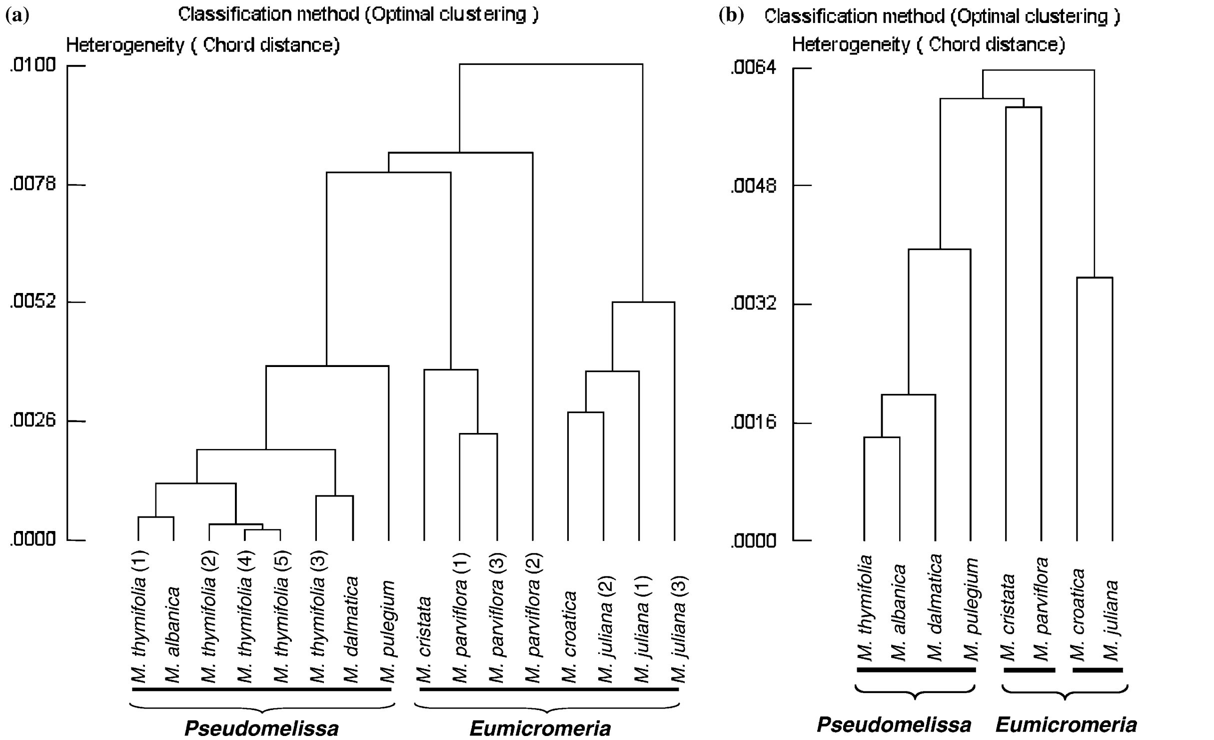

Fig. 2. Dendrogram based on Optimal cluster analysis (Chord distance) of 16 populations (**a**) and 8 species (**b**) of *Micromeria* sp. essential oils

pulegone was found in the largest quantity (Kalodjera et al. 1993, 1994; Vladimir et al. 1994; Duletic-Lausevic et al. 2001; Marinkovic et al. 2001). However, in our researches pulegone appeared as dominant in the Beli Rzav gorge, Semolj and Orjen populations, while piperitone, piperitenone and their epoxides were the main components in the rest of the populations.

M. dalmatica had the highest quantity of essential oil among all the *Micromeria* species investigated in this work. Previously published works confirm the large quantity of oil, although piperitenone oxide, established as dominant in earlier researches (Savarda et al. 1979, Karuza-Stojakovic et al. 1989), was discovered in a very small quantity (0.9%) in our examination.

In earlier works concerning the quantity of essential oils of the species *M. pulegium,* the same approximate value was found as in our researches (Pavlovic et al. 1983). In our work, for the first time the qualitative composition of the oil of this species was determined as well.

In the works of Stojanovic et al. (1999), and Marinkovic et al. (2001) epoxyketones were determined as dominant components in the *M. albanica* oil, which was also confirmed by our research. The antibacterial activity of the oil is ascribed to the presence of these compounds, too. However, the first of the listed studies points out the absence of sesquiterpenes which has not been confirmed by our research.

Earlier publications point out a very small quantity of oil in *M. croatica*, in which sesquiterpenes were dominant while the quantity of terpene hydrocarbons and ketones was very small (Stanic et al. 1988), which agrees with our results.

If we compare our results about the oil of *M. juliana* with those published previously (Mastelic et al. 1998) we can conclude that they are similar in the quantities of caryophyllene and borneol and that they differ in the presence of caryophyllene oxide and the quantity of α-pinene, β-pinene, limonene, β-gurjunene and ar-curcumene. Considerable quantities of α- and β-pinene as well as bisabolol were determined in the oil of *M. juliana* from Greece (Skaltsa et al. 1998).

Previously published results about the essential oil of *M. cristata* (Tabanca et al. 2001) differ from ours in the presence of caryophyllene oxide and trans-verbenol, as well as in the quantity of dominant components. In the work of Tabanca et al. (2001) borneol was found in the quantity of 27.0%–29.0%, whereas its quantity in our research was 5.7%.

The small quantity of essential oil in the species *M. parviflora* was also established by Pavlovic et al. (1983). It is in our work that the qualitative composition of the oil of this endemic plant species was determined for the first time.

The species of section *Pseudomelissa* that cannot be found on the territory of Serbia and Montenegro, such as *Micromeria fruticosa* (Fleisher and Fleisher 1991; Kirimer 1992; Kirimer et al. 1993a, 1993b; Baser et al. 1996), *M. dolichodonta* (Baser et al. 1997a) and *M. capitellata* Benth. (Baser et al. 1998) were characterized by a large quantity of oil (0.6%–4%) and domination of oxygenated terpenes of the menthane type (78%–91%), especially pulegone (15%–80%).

Representatives of section *Eumicromeria* that do not inhabit our territory contained a small quantity of oil (0.02%–0.4%), however, the oils differed in the quantity of dominant components. *M. biflora* (D. Don.) Benth. was rich in the oxygenated monoterpenes neral and geranial (25.3%–41.3%) (Mallavarapu et al. 1997). The species *M. carminea* P. H. Davis (Baser et al. 1995), *M. varia* Benth., *M. herpyllomorpha* Webb. and Berth., *M. lachnophylla* Webb. and Berth. (Pedro et al. 1995, Pérez-Alonso et al. 1996) also abounded in oxygenated monoterpenes but of the bornane (5.0%–26.0%) and pinane type (8.3%–13.9%). *M. myrtifolia* Boiss. et Hohen. (Özek et al. 1992), *M. cremnophila* Boiss. et Heldr. (Baser et al. 1997b) and *M. graeca* (L.) Benth. et Rchb. (Tzakou and Couladis 2001) were the most similar to the representatives of our flora of this section in their content of sesquiterpene compounds, β-caryophyllene (22.6%–42.6%), caryophyllene oxide (9.9%–17.9%) and germacrene D (7.0%–24.0%).

If one takes into consideration the results of our research, literature data about the oil of the selected species and the species that do not live on the territory of Serbia and Montenegro it is clear that the variability in quantity and quality is less among populations of the same species and that it increases with the taxon rank. It is higher among the species of the same section and it is highest among different sections.

The oil composition showed a greater variability than the quantity, noting that the variability is less prominent within section *Pseudomelissa*. In the oils of the representatives of this section different oxygenated monoterpenes dominated, but they were all of the menthane type. Heterogeneity was more pronounced in section *Eumicromeria*, since several different chemical types were observed within it.

The quantity of oil is a less variable characteristic. All representatives of section *Pseudomelissa* had a large quantity of essential oil (>0.5%), and section *Eumicromeria* had less than 0.5%.

The relative stability of the quantity and quality of essential oil within each taxon of section *Pseudomelissa* means that these characteristics are probably under a relatively strict control of natural selection and that they are of adaptive importance. Consequently we can say that the characteristics of the essential oils can also be used as additional characters when defining the species of this section.

The species of section *Eumicromeria* as well as their undoubtable relatives from the same genus, contain essential oil, but in a considerably lesser quantity. The composition of their essential oil is variable, so the adaptive value of this characteristic is small as well. Therefore, in the case of the *Eumi-*

cromeria species, only the small quantity of essential oil can be taken into consideration as a taxonomic character.

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