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COMPARATIVE STUDY OF CHEMICAL COMPOSITION OF THE ESSENTIAL OILS FROM *SATUREJA CUNEIFOLIA* TEN. AND *SATUREJA MONTANA* L., *LAMIACEAE* COLLECTED AT NATIONAL PARK LOVĆEN, MONTENEGRO

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ABSTRACT. The main purpose of this study was an investigation of the chemical composition of essential oils obtained from *Satureja cuneifolia* Ten. and *S. montana* L. collected at the National park Lovćen, Montenegro. The qualitative and quantitative analysis of the essential oils, performed by GC/MS and GC/FID, indicated that the most abundant compound in *S. cuneifolia* essential oil was oxygenated monoterpene linalool (20.3%). Within the sesquiterpene compounds, *trans*-(E)-caryophyllene (6.1%), germacrene D (5.8%), nerolidol (5.2%) and spathulenol (5.0%) were present in relatively high quantities. Conversely, *S. montana* essential oil was abundant in monoterpenes, with *p*-cymene being the main constituent (16.6%). Besides, limonene (10.8%), thymol (6.5%), *α*-pinene (6.1%) and borneol (5.5%) were present in a high percentage. The results indicated that investigated *Satureja* species essential oils possessed different chemical composition, but both might represent an interesting resource of pharmacologically active compounds.

Keywords: *Satureja cuneifolia; Satureja montana; essential oil; terpenes; Montenegro.*

INTRODUCTION

The medicinal plants of the genus *Satureja* (*Lamiaceae* family), commonly used herbs and shrubs, have been localised in the area of the Mediterranean region to Europe, Middle East, West Asia, North America

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and Africa. As annual or perennial semi-bushy, these plants inhabit arid, stony, sunny and rocky habitats along the coast of the Adriatic Sea [1, 2, 3].

Satureja species have been traditionally used in the treatment of various diseases such as nausea, indigestion, cramps, diarrhoea, infectious diseases and muscle pains [3, 4]. Up to now, numerous literature data stated that their essential oils possess antimicrobial activity against a wide range of multidrug-resistant pathogens [4]. In line with this statement, the essential oils obtained from *S. cuneifolia* and *S. montana* showed antimicrobial activity *in vitro* against various multidrug-resistant pathogens. The maximum activity of both essential oils was observed against methicillin-resistant *Staphylococcus aureus* and Gram (-) bacteria *Escherichia coli*. Both of these oils showed fungicidal activity against *Candida albicans* and *Saccharomyces cerevisiae* [5]. The considerable antibacterial and antifungal activities of essential oils from different *Satureja* species (*S. boissieri*, *S. coerulea*, *S. icarica*, *S. pilosa* and *S. intermedia*) also were documented in recently published papers [6, 7]. Furthermore, the aqueous extracts of *S. montana* showed antiviral activities against HIV [8].

Several *Satureja* species have been well-studied and documented from various aspects of their secondary metabolites. It was found that these plants have glands on the leaf surface that produce and secrete essential oils, like other aromatic plants belonging to the mint family. As documented in recently published literature, essential oil obtained from *S. montana ssp. montana* consisted mainly of linalool, borneol and *p*-cymene, while that from *S. montana ssp. variegata* contained monoterpene phenols such as carvacrol and thymol as dominant compounds. *S. cuneifolia* essential oils are rich in limonene, linalool, *α*-pinene, *β*-cubebene, *γ*-terpinene and carvacrol [9].

Different extraction methods might be employed in order to obtain volatile extracts and essential oils from *S. montana and S. cuneifolia*, conventional extraction techniques (hydrodistillation, HD, and Soxhlet extraction, SE) being mostly used [4, 10, 11, 12]. But, these methods have been recognosed to possess many disadvantages, such as a longer period of extraction, less extraction efficiency and solvent residues in the obtained extracts. Non-conventional extractions methods (supercritical fluid extraction, SFE and supercritical water extraction, SWE) might be considered the better option for obtaining the high quality and high active extracts [10, 13].

In our study, the analysis of essential oils obtained from two *Satureja* species, *S. cuneifolia* Ten. (wild savory) and *S. montana* L. (winter savory) were investigated in order to compare their chemical composition, taking into account that traditionally both species and their essential oils had application in the treatment of different health impairments. Winter savory honey is a very frequent ingredient in folk remedies known for their

beneficial effects in the treatment of bronchitis. In addition, it is used as an antiseptic in gastrointestinal complaints, might be used as choleretic, digestive remedy, and in the treatment of premature ejaculation [14]. Wild savory is a medicinal and aromatic plant which essential oil commonly has application in the preparation of the aromatic water, especially in the mountainous areas of Turkey and in the Mediterranean region. In addition, this plant has application as a spice and tea due to its carminative, tonic and stimulant effects [15]. The presence of phenolic compounds in these plants' essential oil might be responsible for their taste and fragrance. Winter savory has been known for its antimicrobial activity, probably due to the presence of monoterpene, alcohols and phenolic compounds, as stated in available literature data [14, 15]. The use of wild savory in the Montenegro has been less frequent in comparison to above-mentioned areas where traditionally this plant has been well recognised.

The objective of the present study was to investigate the chemical composition of essential oils of two *Satureja* species, *S. cuneifolia* and *S. montana* collected from different locations at National park Lovćen in the south-western part of Montenegro. Further, the evaluated difference in their chemical composition was discussed in order to compare the obtained results to the data revealed in up-to-now literature regarding these two *Satureja* species' essential oils chemistry profile. The presented results might be of importance for the direction of further biological investigations.

RESULTS AND DISCUSSION

The obtained essential oils from dried aerial parts of *S. cuneifolia* and *S. montana* were yellow liquids. The yields of essential oils amounted to 0.2 and 0.9 % (v/w), respectively. Identification of chemical composition of the essential oil was performed using the gas chromatographic techniques. Based on applied techniques, more than 100 compounds were identified in both investigated essential oils, which made 98.6-98.9% of the total chemical compounds (Table 1).

GC chromatograms of analysed essential oils and representative examples of MS spectra of compounds identified in both of analysed essential oils were presented at figures 1 - 3.

Table 1. Phytochemical analysis of essential oil profiles of *S. cuneifolia* and *S. montana*

N⁰	Compound§	RT	KI[a]/KI[b]	*S. cuneifolia* (%)	*S. montana* (%)
1.	tricyclene	6.45	921/912	-	0.3
2.	*α*-thujene	6.62	924/918	-	0.2
3.	*α*-pinene	6.70	932/923	0.7	6.1
4.	camphene	7.13	946/937	0.2	4.5
5.	thuja-2,4(10)-diene	7.38	953/944	-	0.1
6.	sabinene	7.92	969/965	0.3	0.1
7.	*β*-pinene	8.00	974/966	-	1.1
8.	myrcene	8.10	988/984	0.4	1.1
9.	*α*-phellandrene	8.98	1002/996	-	1.2
10.	*δ*-3-carene	9.15	1008/1002	-	0.4
11.	*α*-terpinene	9.39	1018/1008	-	0.5
12.	*p*-cymene	9.59	1020/1017	0.1	16.6
13.	limonene	9.73	1024/1020	1.1	10.8
14.	1,8-cineole	9.80	1026/1022	0.6	-
15.	(*Z*)-*β*-ocimene	10.10	1032/1031	0.6	1.5
16.	(E)-*β*-ocimene	10.40	1044/1041	0.3	0.6
17.	*γ*-terpinene	10.75	1054/1050	0.1	1.4
18.	*cis*-sabinene hydrate	11.09	1065/1060	0.5	1.3
19.	camphenilone	11.13	1083/1076	-	0.1
20.	terpinolene	11.85	1086/1080	-	0.1
21.	linalool	12.33	1095/1098	20.3	1.5
22.	*trans*-sabinene hydrate	12.42	1098/1099	0.1	0.4
23.	*cis*-thujone	12.45	1101/1101	-	0.1
24.	isopentyl isovalerate	12.51	1102/1102	0.3	-
25.	2-methylbutyl isovalerate	12.68	1103/1103	0.2	-
26.	*trans*-pinene hydrate	12.81	1110/1107	0.2	-
27.	(*Z*)-*p*-menth-2-en-1-ol	12.96	1118/1115	0.1	0.2
28.	*α*–campholenal	13.08	1122/1118	0.3	0.6
29.	*trans*-pinocarveol	13.17	1135/1130	-	0.6
30.	*trans*-verbenol	13.52	1140/1139	0.1	0.1
31.	camphor	13.65	1141/1139	0.4	4.5
32.	myrcenone	13.83	1148/1142	-	t
33.	nerol oxide	14.13	1154/1148	0.3	-
34.	sabina ketone	14.28	1154/1154	0.1	-
35.	pinocarvone	14.41	1160/1154	-	t
36.	borneol	14.48	1165/1160	3.6	5.5
37.	terpinene-4-ol	14.87	1174/1170	1.7	1.5
38.	*p*-cymen-8-ol	15.35	1183/1182	-	0.3
39.	*α*–terpineol	15.40	1186/1186	3.8	1.1
40.	*cis*-dihydrocarvone	15.63	1193/1190	-	0.4
41.	myrtenol	15.88	1194/1192	0.2	0.6
42.	*trans*-dihydrocarvone	16.00	1200/1197	-	t
43.	verbenone	16.10	1204/1202	-	0.3

N⁰	Compound§	RT	KI[a]/KI[b]	*S. cuneifolia* (%)	*S. montana* (%)
44.	*trans*-carveole	16.50	1215/1215	-	0.6
45.	isobornyl formate	16.62	1223/1218	-	0.5
46.	nerol	16.72	1227/1225	2.2	-
47.	hexyl-2-methyl-butanoate	16.92	1233/1230	0.2	-
48.	pulegone	17.00	1235/1230	0.1	-
49.	neral	17.10	1235/1232	1.2	-
50.	carvacrol methyl ether	17.21	1241/1236	-	3.6
51.	geraniol	17.67	1249/1252	1.4	-
52.	geranial	18.13	1264/1264	0.1	-
53.	bornyl acetate	18.52	1287/1284	0.1	0.1
54.	thymol	19.04	1289/1289	-	6.5
55.	carvacrol	19.42	1298/1302	-	1.1
56.	*cis*-pinocarcyl acetat	19.84	1309/1317	-	0.1
57.	myrtenyl acetate	19.84	1324/1324	0.7	-
58.	*γ*-nonalactone	20.03	1358/1358	0.2	-
59.	neoiso-dihydrocarveol acetate	20.20	1358/1358	0.1	-
60.	piperitone oxide	20.46	1366/1366	0.8	-
61.	linalool isobutanoate	21.17	1373/1373	1.6	-
62.	*α*-copaene	21.39	1374/1373	4.5	0.1
63.	geranyl acetate	21.67	1379/1377	0.5	-
64.	*β*-bourbonene	21.80	1387/1374	0.8	0.8
65.	*β*-cubebene	21.87	1387/1387	0.5	-
66.	*β*-elemene	21.94	1389/1389	0.5	0.2
67.	*β*-longipinene	22.43	1400/1398	-	0.1
68.	*trans*-(E)-caryophyllene	22.75	1417/1407	6.1	1.6
69.	*β*-gurjunene	23.14	1431/1414	-	0.2
70.	*χ*-elemene	23.32	1434/1424	-	t
71.	aromadendrene	23.44	1439/1433	-	0.1
72.	*α*-humulene	23.81	1452/1442	0.3	0.1
73.	*β*-(E)-farnesene	24.02	1454/1448	0.1	-
74.	*cis*-cadina-1(6),4-diene	24.12	1461/1461	0.2	-
75.	4,5-di-epi-aristolochene	24.21	1471/1469	-	0.1
76.	*χ*-muurolene	24.53	1478/1466	-	0.1
77.	germacrene D	24.68	1484/1470	5.8	0.7
78.	*cis*-*β*-guaiene	24.03	1490/1472	0.3	-
79.	*χ*-amorphene	25.05	1495/1477	-	t
80.	bicyclogermacrene	25.16	1500/1485	2.9	0.4
81.	*α*-muurolene	25.27	1500/1490	0.5	t
82.	*β*-bisabolene	25.39	1505/1500	0.5	0.2
83.	*α*-bulnesene	25.56	1509/1509	0.2	-
84.	*γ*-cadinene	25.68	1513/1513	1.4	0.2
85.	*β*-cubebol	25.77	1514/1514	0.1	-
86.	endo-1-bourbonanol	25.86	1515/1514	-	t
87.	*δ*-cadinene	25.97	1522/1520	1.1	0.3
88.	*cis*-sesquisabinene hydrate	26.63	1542/1542	0.8	-
89.	hedycaryol	26.81	1546/1544	0.8	0.7

N⁰	Compound§	RT	KI[a]/KI[b]	*S. cuneifolia* (%)	*S. montana* (%)
90.	germacrene B	26.98	1559/1556	-	0.4
91.	nerolidol	27.24	1561/1561	5.2	-
92.	1-nor-bourbonanone	27.17	1561/1561	-	t
93.	caryophyllene alcohol	27.30	1570/1565	-	t
94.	germacrene D–4-ol	27.45	1574/1567	-	0.1
95.	spathulenol	27.61	1577/1567	5.0	1.4
96.	caryophyllene oxide	27.69	1582/1571	3.1	4.5
97.	globulol	27.99	1590/1580	1.8	-
98.	viridiflorol	28.31	1592/1581	0.2	5.4
99.	ledol	28.34	1602/1592	-	0.2
100.	*β*-oplopenone	28.50	1607/1599	-	0.5
101.	humulene epoxide II	28.50	1608/1598	0.6	-
102.	heliofolen-12-ol C	28.64	1619/1617	-	0.7
103.	1-*epi*-cubenol	29.01	1622/1622	0.3	0.1
104.	*γ*-desmol	29.12	1630/1630	0.9	-
105.	*epi*-*α*-cadinol	29.10	1638/1631	0.3	-
106.	caryophylla-4(12),8(13)-dien-5*α*-ol	29.41	1639/1631	1.6	0.8
107.	*τ*-cadinol	29.45	1640/1631	-	t
108.	*α*-muurolol	29.58	1645/1632	-	t
109.	*β*-eudesmol	29.64	1649/1640	4.5	t
110.	*α*-eudesmol	29.74	1652/1643	t	t
111.	*α*-cadinol	29.80	1653/1645	-	t
112.	14-hydroxy-9-epi-(E)-caryophyllene	29.91	1664/1648	-	0.5
113.	14-hydroxy-(E)-caryophyllene	30.10	1666/1662	t	0.1
114.	*α*-bisabolol	30.28	1683/1677	0.4	-
115.	germacra-4(15),5,10(14)-trien-1-*α*-ol	30.65	1684/1678	1.3	0.1
116.	27(14)-bisaboladien-12-ol	30.72	1760/1755	-	t
117.	*β*-costol	31.65	1765/1765	0.2	-
118.	*α*-costol	32.80	1773/1773	t	-
	The percent of the total chemical compounds			**98.6**	**98.9**

* § - The minimum acceptable match factor of experimental MS spectra with those from the libraries was specified to be 80 or more.
* KI[a] = Kovats index, literature data;
* KI[b] = Kovats index, experimentally determined;
* RT = Retention time;
* % = Percentage of chemical compounds;
* t = Chemical compounds with percentage less of 0.05 %;
* - = Chemical compounds which are not detected in the analysed sample.

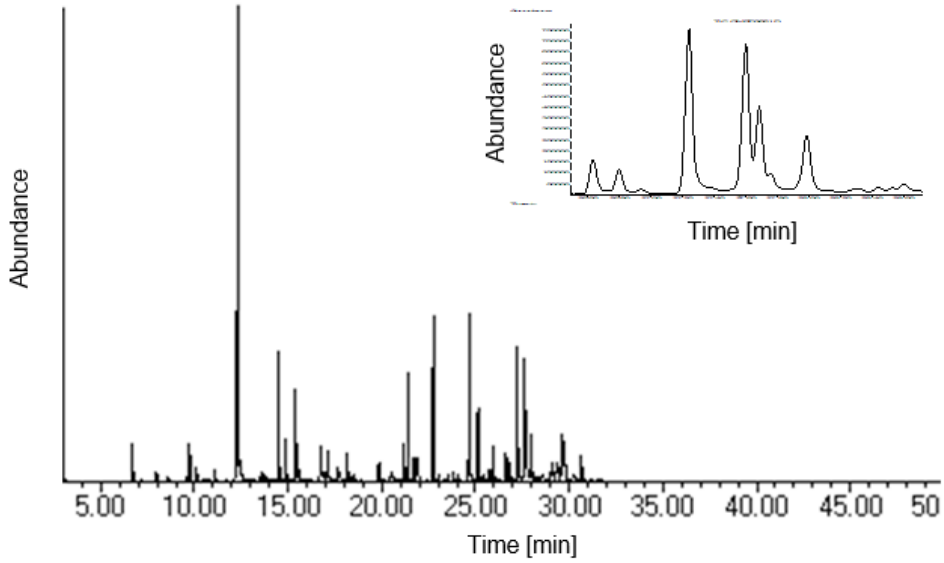

Figure 1. GC/MS chromatogram of *S. cuneifolia* essential oil

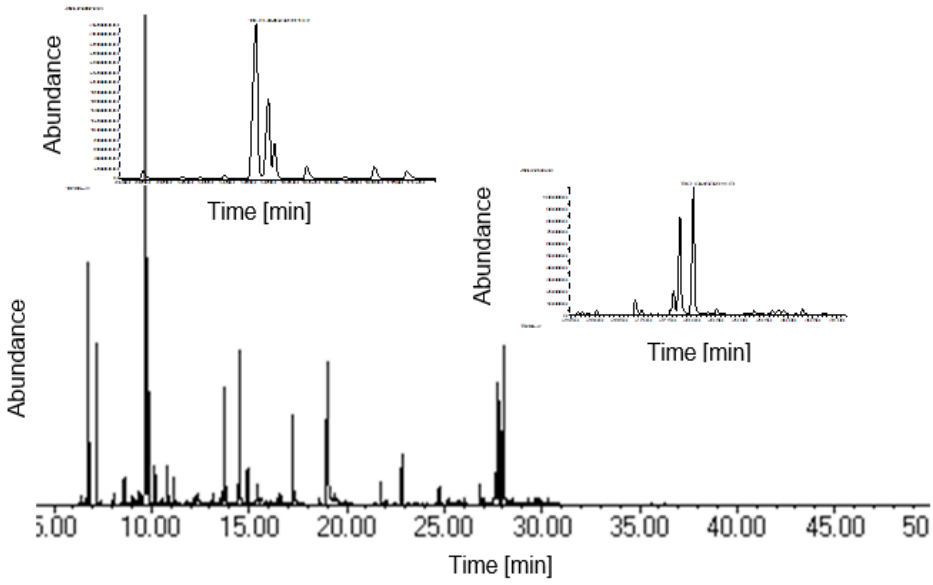

Figure 2. GC/MS chromatogram of *S. montana* essential oil

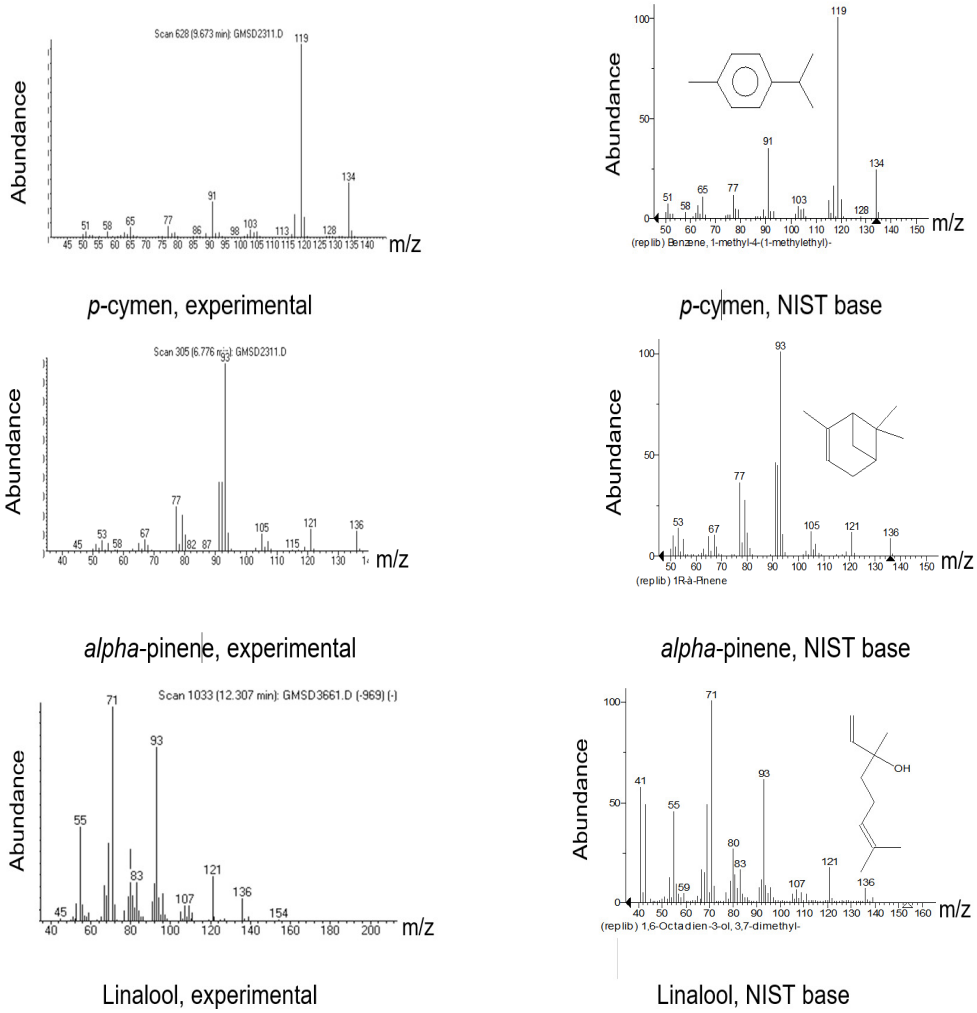

Figure 3. Some of MS spectra of compounds identified in both of analysed essential oils

The performed analysis of *S. cuneifolia* essential oil showed that the oxygenated monoterpenes (41.1%) were the predominant group of compounds, with linalool as the most abundant compound representing 20.3% of the oil. Besides, *α*-terpineol (3.8%) and borneol (3.6%) were present in relatively high concentrations. Within the sesquiterpene hydrocarbons, constituting 25.7% of the analysed sample, *trans*-(E)-caryophyllene (6.1%), germacrene D (5.8%) and *α*-copaene (4.2%) were

present in significant quantities. Oxygenated sesquiterpenes (27.1%) were represented mostly by nerolidol (5.2%), spathulenol (5.0%), *β*-eudesmol (4.5%) and caryophyllene oxide (3.1%). The group of monoterpene hydrocarbons were represented with only 3.8% with limonene (1.1%) and *α*-pinene (0.7%) as the main constituents.

Conversely, the oil of *S. montana* showed that the group of monoterpene hydrocarbons (46.6%) were the predominant group of compounds. The most abundant compound in the group was *p*-cymene representing 16.6%. Limonene (10.8%) and *α*-pinene (6.1%) were present in a high percentage, as well. The group of oxygenated monoterpenes constituted 31.6% of the oil. Within this group thymol (6.5%), borneol (5.5%) and camphor (4.5%) were determined to be in significant amounts. Oxygenated sesquiterpenes (15.2%) and hydrocarbon sesquiterpenes (5.5%) were present in a lower percentage in comparison to *S. cuneifolia*. Within the group of oxygenated sesquiterpenes viridiflorol (5.4%), caryophyllene oxide (4.5%) and spathulenol (1.4%) were the main constituents. Furthermore, the most abundant compounds in the group of sesquiterpenes hydrocarbons were *trans*-(E)-caryophyllene (1.6%) and *β*-bourbonene (0.7%).

The different classes of the chemical compounds identified in the investigated essential oils were presented in Figure 4.

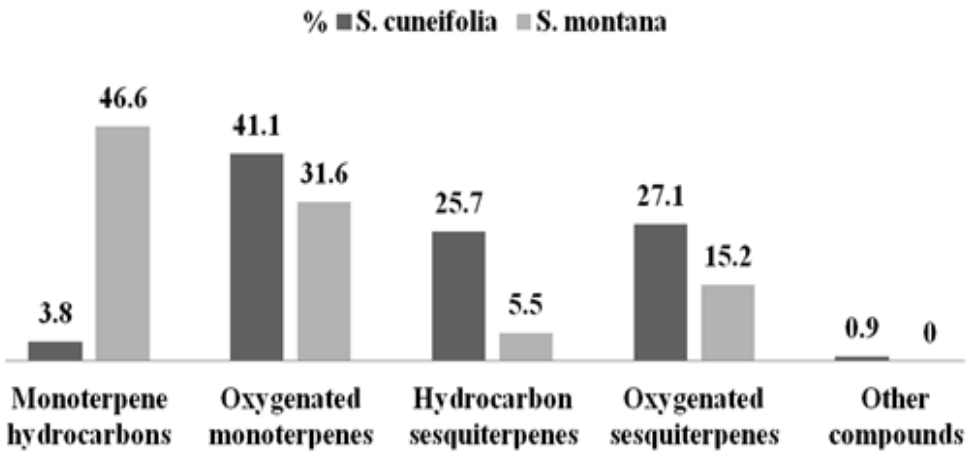

Figure 4. The comparison of the different classes of the chemical compounds of essential oils obtained from two *Satureja* species

The presented data revealed the significant difference in the qualitative and quantitative composition of essential oils obtained from *S. cuneifolia* and *S montana*. Generally, the presented chemical profiles of the essential oils and comparison to the data presented in available literature might be important in order to evaluate the quality of the investigated plant materials and their possible applications for different therapeutic purposes.

S. cuneifolia essential oil contained linalool (20.3%), *trans*-(E)-caryophyllene (6.1%), germacrene D (5.8%), nerolidol (5.2%) and spathulenol (5.0%) as the main constituents with the concentration greater than five percent. The oil obtained from *S. montana* showed that *p*-cymene (16.6%), limonene (10.8%), thymol (6.5%), *α*-pinene (6.1%), borneol (5.5%) and viridiflorol (5.4%) were the main constituents (Table 2).

The search of the up to now published papers, revealed quite different chemical profile regarding the investigated essential oils. Namely, according to Bezić *et al*. [4], the main constituent of *S. cuneifolia* essential oil was carvacrol, which representing 17.7% of the oil. In addition, *γ*-terpinene (14.8%), *p*-cymene (9.8%), linalool (6.6%) and limonene (6.2%) were present in a high percentage. Similar results were obtained for species *S. montana*, the major compounds in the essential oil were carvacrol (13.7%), *p*-cymene (11.8%), *γ*-terpinene (10.6%), limonene (9.5%) and borneol (5.8%) [4]. The summary of the comparison of the main constituents determined in investigated essential oils to the literature data were presented in Table 2.

Besides Bezic *et al*. [4], Tommasi *et al*. [17] investigated the chemical composition of *S. cuneifolia* essential oil obtained from Mediterranean area, determined linalool (9.6–32.7%), borneol (12.9–24.0%) and *α*-pinene (9.5–11.7%) to be the main constituents [17]. The essential oil of *S. montana* originating from Albania, according to De Oliveira *et al*. [18] was characterised by a high content of thymol (28.9%), *p*-cymene (12.0%), linalool (11.0%) and carvacrol (10.7%) [18].

Based on recently published data and results presented in this study, it is evident that a significant difference existed in the chemical composition of investigated oils. The chemical composition variability of essential oils among the two *Satureja* species probably could be attributed to ecological conditions, the stages of development, life cycle and/or some genetically influenced factors [4, 18].

Table 2. Comparison of the main constituents (percentage more than 5) in the analysed essential oils of *S. cuneifolia* and *S. montana* to the results available in the recently published papers

N⁰	**Compound**	**Investigated sample** *S. cuneifolia*	**Previous studies*** *S .cuneifolia*	**Ref.***	**Investigated sample** *S. montana*	**Previous studies**** *S. montana*	**Ref.****
1.	*α*-pinene	<5%	5.8-20.7%	5, 15	6.1%	<5%	14, 15
2.	*p*-cymene	<5%	8.7-14.8%	4, 15	16.6%	6.61-12.6%	4, 5, 14
3.	limonene	<5%	6.2-17.4%	4, 5, 15	10.8%	9.5%	4
4.	*γ*-terpinene	<5%	5.6-14.8%	4, 15	<5%	8.1-13.24%	4, 5
5.	linalool	20.3%	6.6-18.2%	4, 15	<5%	15.38-32.58%	14, 15
6.	borneol	<5%	5.8-12.2%	15	5.5%	5.8-11.5%	4, 15
7.	thymol	-	<5%	4, 15, 16	6.5%	5.4-24.69%	14, 15, 16
8.	carvacrol	-	5.0-17.7%	4, 15, 16	<5%	15.19-63.4%	14, 16
9.	*trans*-(E)-caryophyllene	6.1%	5.2-9.3%	15	<5%	<5%	14, 15
10.	germacrene D	5.8%	-	4, 5, 15	<5%	<5%	14
11.	nerolidol	5.2%	-	4, 5, 15	-	9.36%	14
12.	spathulenol	5.0%	5.3-13.2%	15, 16	<5%	<5%	14, 15
13.	viridiflorol	<5%	<5%	15	5.4%	-	4, 15

*, ** Ref. = Reference;
*** <5% = Compounds with percentage less than five percent;
**** - = Data not detected or not available in this research.

CONCLUSIONS

The presented data revealed the significant difference in the qualitative and quantitative composition of essential oils obtained from *S. cuneifolia* and *S. montana*. Based on applied techniques, more than 100 compounds were identified in both investigated essential oils, which made 98.6-98.9% of the total chemical compounds. The results also showed that investigated essential oils of *Satureja* species have authentic terpenoid composition in comparison to other published studies. Moreover, *Satureja* species from different geographical origins showed different chemical profiles. Hence, chemical composition variability of essential oils among *Satureja* species most probably depended on the genotype of the plant, ecological conditions and the stage of plant ontogenetic development.

EXPERIMENTAL SECTION

Plant material

The plant material was collected at the end of August 2014, at the National park Lovćen, Montenegro (Figure 5). The aerial parts of two *Lamiaceae* species, wild savory (*Satureja cuneifolia* Ten.) and winter savory (*Satureja montana* L.) were air-dried in a shade at room temperature and afterwards stored in paper bags in a cool and dry place. Determination of plant species was performed by Prof. Danijela Stešević and voucher specimens kept at the Department of Biology, Faculty of Natural Science and Mathematics, University of Montenegro.

Figure 5. Map of the samples origins from National park Lovćen (A) is indicated at the map of Montenegro

Isolation procedure

The dried, powdered plant material was subjected to hydrodistillation for 3 hours by using glass Clevenger type apparatus, according to the method described by the European Pharmacopoeia and by the Yugoslav Pharmacopoeia [19, 20]. The obtained essential oils were dried over anhydrous sodium sulfate (Na_2SO_4), filtered and stored in an airtight container in a freeze until gas chromatography analyses.

Chemical analysis of essential oil profiles (GC-FID and GC-MS)

Gas chromatography (GC-FID). Gas chromatography analysis of the essential oils were carried out on an HP-5890 Series II GC apparatus [Hewlett-Packard, Waldbronn (Germany)], equipped with the split–splitless injector and automatic liquid sampler (ALS), attached to HP-5 column (25 m

× 0.32 mm i.d. and 0.52 μm film thickness) and fitted with a flame ionization detector (FID). Carrier gas was H_2 (1 ml/min), with a split ratio of 1:30, injector temperature was 250°C, detector temperature 300°C, while column temperature was linearly programmed from 40 to 260°C with a rate of change of the 4°C/min, and then kept isothermally at 260°C for 10 min. Solutions of essential oil in alcohol (10 mg/ml) were consecutively injected in an amount of 1μl. Area percent reports, obtained as result of standard processing of chromatograms, were used as a base for the quantification analysis.

Gas chromatography / mass spectrometry (GC-MS). The same analytical conditions as those mentioned for GC-FID were employed for GC/MS analysis, along with column HP-5MS (30 m × 0.25 mm i.d. and 0.25 μm film thickness), using HP G 1800C Series II GCD system [Hewlett-Packard, Palo Alto, CA (USA)]. As the carrier gas used helium. The transfer line was heated to 260°C. The mass spectra were obtained in EI mode, with an ionisation voltage of 70 electron volt (eV); in the range from 40 to 450 m/z. The amount of the injected sample, dissolved in alcohol (10 mg/ml) was 0.2 μl. The components of the essential oil were identified by comparison of their mass spectra to those from Wiley 275 and NIST/NBS (NIST–National Institute of Standards and Technology / NBS-National Bureau of Standards) libraries, using different search engines. Identification of the compounds was achieved by comparing their Kovats' retention indices and mass spectra with those reported in the literature [21] and supplemented by the Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver. 2.1), GC-MS library [22]. The experimental values for Kovats' retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System Software (AMDIS ver. 2.1), GC-MS library [22], and also compared to those from available literature (Adams 2007) [21] and used as additional tool to approve MS findings. The relative proportion of the essential oil constituents were expressed as percentages obtained by peak area normalisation, all the relative response factors were entered as one.

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