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NATURAL DRUGS

GC-MS EVALUATION OF THE ESSENTIAL OILS FROM THE NEWLY DEVELOPED CULTIVARS OF *SATUREJA MONTANA* GROWN IN SOUTHERN UKRAINE

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Abstract: *Satureja montana* L. is widely used in traditional medicine and cooking. New varieties of this species are created and grown for different purposes. Essential oil (EsO) samples were obtained from the fresh flowering shoots of four new Ukrainian cultivars of *S. montana*: 'Krymski smaragd', 'Lunata', '697-1', and 'N3-18'. The EsO of cultivars 'Lunata' and 'Krymski smaragd' were studied in two following harvest years (2019 and 2020). The EsO samples were analyzed using gas chromatography-mass spectrometry (GC-MS). More than 60 components were identified in the studied EsO. Carvacrol was the principal component of all the samples (58.3-87.0% as GC-MS relative abundances). *p*-Cymene was the second predominant component (5.0-8.8%) in the EsO of all the cultivars collected in the year 2020, while cultivars 'Lunata' and 'Krymski smaragd' gathered in 2019 contained it in much fewer amounts (1.7% and 0.5%, respectively). The third main component in the EsO of the most studied varieties was γ-terpinene (0.9% to 6.6%). Only cultivar 'N697-1' contained carvacrol methyl ether at a significant level (11.7%). All the tested samples met the requirements of International Standard Organization (ISO) 79284:1991(E), supposing that the qualitative composition of the *S. montana* EsO should comprise the following main constituents: γ-terpinene, *p*-cymene, linalool, terpinen-4-ol, and carvacrol. It can be concluded that we dealt with the carvacrol chemotypes of *S. montana*. It was also revealed that growth year influenced the EsO composition of 'Krymski smaragd' and 'Lunata' cultivars. Our results allowed to compare the EsO of the *S. montana* new cultivars.

Keywords: winter savory, cultivars, essential oil, carvacrol chemotype, GC-MS analysis.

The *Lamiaceae* Martinov family is known as the family of plants useful for flavoring or medicinal properties (1, 2). This family covers 263 genera and over 7000 species (2). The Savory (*Satureja* L.) genus is one of the genera of the *Lamiaceae* family and comprises 64 recognized species, including subspecies and hybrids (3). These aromatic and medicinal plants grow spontaneously in the Mediterranean region, West Asia, the Middle East, and South America (2, 4). Summer savory (*S. hortensis* L.) and winter—or mountain—savory (*S. montana* L.) are the most popular medicinal and aromatic plants among the *Satureja* species (4, 5). In the Pubmed database in April 2022, more than 500 scientific papers were found using the search phrase '*Satureja*', and among them '*Satureja montana'* and '*Satureja hortensis'* dominated (114 and 133 results, respectively). In other words, almost half of the papers were focused on these two *Satureja* species.

As a perennial evergreen subshrub, winter savory is more preferred in cooking for flavoring food compared to summer savory, which is

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an annual plant and only available fresh during the summer (5). *S. montana* is native to warm arid regions in the Mediterranean area and Northern Africa. However, it is cultivated in subtropical to temperate climate zones, including Ukraine (5, 6). It grows up to 70 cm high, with linear leaves and verticillaster inflorescences composed of pale pink flowers. The height depends on the year of growth (2). The aerial part of *S. montana* produces many bioactive phytochemicals, such as mono-, di- or triterpenoids, phenolic acids, flavonoids, tannins, etc. (2, 4, 6-9). *S. montana* has a typically strong and pleasant odor similar to carvacrol (9). Many researchers study the herb and EsO of *S. montana* as possible sources of antibacterial and antioxidant components (6, 10, 11).

The significant amounts of such aromatic monoterpenoids as carvacrol, thymol, *p*-cymene or estragole can be considered the main contributors to the antimicrobial activity of *S. montana* EsO (4, 5, 12-15). The species of the *Satureja* genus and other species of the *Lamiaceae* family could be considered effective sources to overcoming the resistance of many pathogenic microorganisms to antibiotics (1, 16-19). The EsO of Italian *S. montana*—containing carvacrol and estragole as the main components—showed antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* alone or in combination with gentamicin (20). The EsO of the Croatian *S. montana*—containing 50.2% of carvacrol and 11.0% of thymol in GC-MS analysis—possessed anti-diarrheal activities by inhibiting the appropriate pathogenic bacteria (*Shigella flexneri, Salmonella enterica* serovar *typhimurium* and *Escherichia coli*) (21). The aerial part of *S. montana* also possesses antioxidant, expectorant, carminative, digestive, anti-helminthic, immunomodulatory, anti-inflammatory, analgesic and other valuable biological activities (5, 20, 22). Significant insecticidal, acaricidal, and nematicidal effects were also found for the EsO obtained from this species (22).

The existence of many chemotypes of *S. montana*, as well as other *Lamiaceae* representatives, was reported by many researchers (1, 2, 6, 13, 15, 21). The chemical composition of EsO also depends on the geographic location, growth conditions, isolation techniques, the stage of plant development, and other known and unknown factors (6, 21, 23, 24). Thus, Ibraliu et al. (24) found using GC-MS and GC-FID analyses that carvacrol (21.07-55.95%) was the main compound in five out of six samples of the *S. montana* EsO obtained from the plants growing in different natural locations in Albania. These five samples grew in the Mediterranean climatic subzone. One sample (P4), grown in the Continental climatic subzone, had a unique chemical composition, because it did not belong to either the carvacrol or thymol chemotype, but contained a significant fraction of borneol (9.64%) and sesquiterpenes such as *trans*-*β*-caryophyllene (10.79%) and germacrene D (10.44%). Chizzola described the carvacrol/*p*-cymene and linalool chemotypes of *S. montana* from different natural locations in Southern France (25). Damjanovic-Vratnica et al. monitored the effect of the vegetation stage on the EsO composition of the wild-growing *S. montana* from Montenegro (26). The increased content of thymoquinone, with important therapeutic effects, was reached using supercritical fluid extraction of the *S. montana* EsO instead of hydrodistillation (27).

The cultivation of aromatic medicinal plants could help solve such problems as variations in the amounts of active ingredients and the conservation of valuable species and varieties (2, 24, 28-33). The need to obtain certain active ingredients highlights the necessity of the experimental breeding and cultivation of varieties rich in bioactive compounds (1, 2, 6, 15, 34).

This study aimed to analyze the qualitative EsO composition of four new cultivars of *S. montana* grown in southern Ukraine and developed by the Institute of Rice of the Ukrainian National Academy of Agrarian Sciences (NAAS) in Ukraine. The results of the study will aid in plant selection with desirable characteristics of bioactive compounds for further large-scale cultivation and the identification of specimens for future selective breeding.

EXPERIMENTAL (MATERIALS AND METHODS)

Plant Material

The flowering shoots of *S. montana* cultivars were collected in the Kherson region (Ukraine) in 2019 and 2020 from plots of the Sector of Mobilization and Conservation of Plant Resources of the Rice Institute (Plodove, Kherson region, Ukraine, 46°44′11″ N. latitude, 33°24′09″ E. longitude). Fresh plant raw material was used for the hydrodistillation of EsO. The voucher specimens of the studied cultivars were deposited in the Sector of Mobilization and Conservation of Plant Resources of the Rice Institute. A total of 100 g of ground flowering shoots were used for each hydrodistillation. Detailed information about the studied cultivars is provided in Table 1.

The average temperatures and average precipitation were 13.2°C and 13.3°C and 307.7 mm and 280 mm, respectively, in 2019 and 2020; 2019 was slightly wetter (35, 36).

Isolation and GC-MS EsO Analyses

The EsO isolation procedure involved its hydrodistillation using a Clevenger-type apparatus and was described previously (6, 18, 19).

The Hewlett Packard HP 6890 GC chromatograph was used for the GC-MS analysis of EsO according to the method described in (18, 19). The experimental linear retention indices were calculated according to the definition of Van den Dool and Kratz using the *n*-alkanes standard obtained from Supelco (Bellefonte, US). The reference linear retention indice (LRI) values were sourced from the NIST Chemistry WebBook and the work of Babushok et al. (37, 38). The LRIs were used to confirm or reject GC-MS hits based on MS spectra similarity with the NIST 11 library. The presence of compounds like thymol, carvacrol, *p*-thymol (3-methyl-4-isopropylphenol), *cis*-sabinene hydrate, *α*-terpinene, terpinen-4-ol, α-terpineol, linalool, and linalyl acetate, was confirmed using reference materials purchased from Merck (Darmstadt, Germany). Each analysis was repeated three times and the results were expressed as a mean value. For semi-quantification, the normalized peak area abundances were employed.

RESULTS AND DISCUSSION

The EsO of the four new cultivars of *S. montana* bred at the Rice Institute of Ukraine are discussed in this paper. The names and characteristics of the studied cultivars are presented in Table 1.

In total, more than 60 compounds were identified in the studied samples (Table 2). An example of the total ion chromatogram is provided in Figure 1. The identified components were found in different relative abundancies (Tables 2 and 3, Figures 2 and 3).

The EsO of the newly studied *S. montana* cultivars were rich in some terpene compounds, which we discussed and described below. All of the tested samples met the requirements of ISO 79284:1991,

Table 1. Description of the tested cultivars of *Satureja montana.*

No.	Sample ID	Cultivar Name	Characteristics	Date of harvesting and EsO distillation	Vegetation phase of plant	EsO Yield [%]
S1	425	'Lunata'	Shrubs were of a compact shape, 40-42 cm tall and 60-70 cm in diameter. Leaves were 2.5-2.7 cm long and 0.4-0.5 cm wide. Flowers were large with broad blades, 1.3-1.5 cm to 1.0 cm in diameter, and white. Inflorescences were 17.8 cm tall, and 3.1 cm in diameter.	29.07.2019	Full flowering	0.40
S4	485	'Lunata'		10.08.2020	Full flowering	0.35
S2	426	'Krymski smaragd'	It is from the selection of the Nikitsky Botanical Garden. Shrubs of a compact shape, 50-55 cm tall, 80 cm in diameter. Leaves are 2.0-2.5 cm long and 0.50 cm wide. Flowers were 1.0-1.3 cm in diameter, white with purple dots on the blades of the lower lip and with a purple tinge on the edge of the upper lip, collected in corymbose paniculate inflorescences. Inflorescences were 22 cm long.	29.07.2019	Full flowering	0.43
S3	483	'Krymski smaragd'		10.08.2020	Full flowering	0.35
S5	486	№3-18	Shrubs were spreading, 45-50 cm tall and 80-90 cm in diameter. Leaves were 2.0-2.1 cm long and 0.3-0.4 cm wide. Flowers were small, 0.9-1.1 cm in diameter, white with purple dots on the blades of the lower lip, collected in corymbose paniculate inflorescences. Inflorescences were 27-29 cm long.	14.08.2020	Full flowering	0.33
S6	487	№697-1	Shrubs had a compact shape, 60-75 cm tall and 80-90 cm in diameter. Leaves were 2.0-2.5 cm long and 0.5 cm wide. Flowers were 1.0-1.2 cm in diameter, white-pink colour with purple dots on blades of the lower lip, collected in corymbose paniculate inflorescences. Inflorescences were 26-30 cm long.	17.08.2020	Full flowering	0.30

Table 2. Relative abundance (in%) of the components in the EsO from six samples of the new *Satureja montana* cultivars grown in 2019-2020. The sample description (S1-S6) is in Table 1.

	Component	Rt	LRI		Cultivar of *Satureja montana*											
					S1 'Lunata'-2019		S2 'Krymski smaragd'-2019		S3 'Krymski smaragd'-2020		S4 'Lunata'-2020		S5 N3-18		S6 697-1	
			exp.	lit.	avg.	SD	avg.	SD	avg.	SD	avg.	SD	avg.	SD	avg.	SD
1	*α*-thujene	4.37	923	928					0.38	0.00	0.34	0.01	0.70	0.02	0.69	0.01
2	*α*-pinene	4.49	929	936					0.17	0.00	0.15	0.01	0.36	0.01	0.43	0.01
3	Camphene	4.79	942	950					0.05	0.00	0.03	0.00	0.12	0.03	0.07	0.02
4	*β*-pinene	5.42	971	978					0.10	0.00	0.10	0.00	0.14	0.01	0.15	0.00
5	1-octen-3-ol	5.64	981	980	0.94	0.15	0.82	0.04	0.83	0.01	1.07	0.01	1.20	0.01	0.63	0.00
6	Myrcene	5.85	990	989	0.11	0.00	0.08	0.00	0.78	0.01	0.88	0.02	0.70	0.02	0.87	0.02
7	3-octanol	6.03	998	993	0.02	0.01	0.09	0.01	0.07	0.00	0.06	0.01	0.09	0.00	0.06	0.00
8	*α*-phellandrene	6.12	1002	1004					0.18	0.01	0.17	0.01	0.15	0.00	0.21	0.01
9	3-carene	6.25	1007	1011					0.06	0.01	0.06	0.01	0.06	0.00	0.06	0.00
10	*α*-terpinene	6.42	1014	1017	0.04	0.00	0.04	0.00	1.13	0.02	1.02	0.03	0.93	0.02	1.11	0.02
11	*p*-cymene	6.64	1022	1024	1.67	0.06	0.51	0.02	6.27	0.01	4.95	0.09	8.78	0.16	5.42	0.07
12	Limonene	6.64	1026	1030											1.71	0.03
13	Eucalyptol	6.74	1026	1032	0.37	0.01	0.29	0.01	0.79	0.08	0.82	0.03	1.35	0.02		
14	*Cis*-*β*-ocimene	7.09	1039	1038					0.05	0.02	0.09	0.03	0.07	0.01	0.43	0.02
15	*Trans*-*β*-ocimene	7.29	1047	1048							0.06	0.01	0.08	0.00	0.14	0.01
16	Unidentified	7.43	1052		0.03	0.00					0.03	0.01				
17	*γ*-terpinene	7.52	1056	1060	1.61	0.09	0.92	0.03	6.00	0.04	5.79	0.10	5.03	0.08	6.62	0.09
18	*Cis*-sabinene hydrate	7.75	1065	1067	0.52	0.05	0.85	0.02	0.83	0.04	0.90	0.02	1.24	0.03	0.93	0.06
19	Terpinolene	8.29	1085	1087					0.13	0.02	0.08	0.01	0.09	0.00	0.08	0.00
20	*Trans*-sabinene hydrate	8.58	1096	1098	0.15	0.01	0.24	0.00	0.19	0.01	0.20	0.00	0.28	0.00	0.19	0.00
21	Linalool	8.70	1101	1099	0.80	0.03	1.21	0.02	1.30	0.00	0.79	0.00	3.24	0.02	1.17	0.00
22	*Cis*-*p*-menth-2-en-1-ol	9.20	1120	1123	0.05	0.00	0.06	0.00	0.06	0.00			0.08	0.00	0.06	0.00

Table 2. Relative abundance (in%) of the components in the EsO from six samples of the new *Satureja montana* cultivars grown in 2019-2020. The sample description (S1-S6) is in Table 1 (cont.).

	Component	Rt	LRI		Cultivar of *Satureja montana*											
					S1 'Lunata'-2019		S2 'Krymski smaragd'-2019		S3 'Krymski smaragd'-2020		S4 'Lunata'-2020		S5 N3-18		S6 697-1	
			exp.	lit.	avg.	SD	avg.	SD	avg.	SD	avg.	SD	avg.	SD	avg.	SD
23	*Trans*-pinocarveol	9.62	1135	1140									0.03	0.01		
24	Camphor	9.72	1139	1143	0.03	0.02	0.09	0.00	0.22	0.01	0.03	0.00	0.51	0.01	0.07	0.01
25	Borneol	10.38	1163	1166	0.36	0.01	0.36	0.00	0.35	0.01	0.28	0.00	0.77	0.01	0.45	0.00
26	Unidentified	10.52	1168		0.03	0.03	0.05	0.00	0.05	0.00	0.00	0.00	0.15	0.01	0.06	0.00
27	Terpinen-4-ol	10.70	1175	1177	0.71	0.02	0.88	0.01	0.70	0.01	0.66	0.01	1.24	0.01	0.74	0.01
28	*α*-terpineol	11.11	1190	1190	0.18	0.02	0.16	0.01	0.13	0.00	0.14	0.01	0.36	0.01	0.20	0.01
29	Unidentified	11.22	1194	1195	0.09	0.02	0.13	0.03	0.13	0.01	0.13	0.01	0.27	0.00	0.15	0.01
30	*Trans*-dihydrocarvone*	11.40	1201	1201											0.12	0.04
31	Pulegone*	12.36	1236	1234					0.07	0.00						
32	Carvacrol methyl ether	12.53	1243	1243	0.08	0.00	0.09	0.00			0.06	0.00	0.15	0.00	11.70	0.31
33	Unidentified	12.57	1245						0.55	0.01	0.12	0.00				
34	Carvone (=*cis*-dihydrocarvone)	12.68	1249	1242	0.19	0.01	0.08	0.03	0.08	0.00	0.17	0.00	0.22	0.01	0.52	0.01
35	Thymoquinone	12.73	1251	1252					0.12	0.01			0.14	0.01	0.10	0.02
36	Linalool acetate	12.87	1256	1255					0.10	0.00			0.59	0.00	0.10	0.00
37	Geraniol	13.09	1264	1255									0.09	0.00		
38	*p*-cymen-7-ol*	13.90	1294	1288	0.08	0.01			0.02	0.00					0.03	0.00
39	Thymol	14.06	1300	1290	0.66	0.03	0.50	0.01	0.36	0.01	0.41	0.00	0.61	0.02	0.43	0.01
40	Carvacrol	14.47	1316	1300	83.70	2.81	87.03	0.59	69.52	0.90	76.31	0.17	65.39	0.22	58.26	0.34
41	3-methyl-4-isopropylphenol (=p-thymol)	15.03	1338	1334			0.46	0.38	0.21	0.01						
42	*α*-cubebene	15.23	1346	1351	0.13	0.18	0.22	0.19	0.47	0.01						
43	Carvacrol acetate	15.89	1372	1373	0.52	0.02	0.39	0.02	0.26	0.00	0.22	0.00	0.07	0.04	0.07	0.00

Table 2. Relative abundance (in%) of the components in the EsO from six samples of the new *Satureja montana* cultivars grown in 2019-2020. The sample description (S1-S6) is in Table 1 (cont.).

	Component	Rt	LRI		Cultivar of *Satureja montana*											
					S1 'Lunata'-2019		S2 'Krymski smaragd'-2019		S3 'Krymski smaragd'-2020		S4 'Lunata'-2020		S5 N3-18		S6 697-1	
			exp.	lit.	avg.	SD	avg.	SD	avg.	SD	avg.	SD	avg.	SD	avg.	SD
44	*β*-bourbonene	16.08	1379	1384	0.05	0.01	0.07	0.01	0.09	0.00	0.05	0.00	0.05	0.02	0.06	0.00
45	Geranyl acetate	16.21	1384	1380									0.12	0.02		
46	Caryophyllene	16.93	1413	1420	0.13	0.01	0.56	0.01	0.66	0.01	0.42	0.00	0.65	0.00	1.47	0.01
47	*β*-copaene	17.18	1423	1433	0.06	0.00	0.13	0.00	0.10	0.00	0.06	0.00	0.04	0.00	0.05	0.00
48	Aromadendrene	17.41	1433	1441	0.08	0.00	0.11	0.00	0.08	0.00	0.11	0.00	0.07	0.00	0.15	0.00
49	*α-h*umulene (=α-caryophyllene)	17.76	1447	1453					0.05	0.00			0.04	0.00	0.07	0.00
50	*Trans-β*-farnesene	17.97	1456	1446					0.06	0.00			0.05	0.00		
51	*γ*-muurolene	18.37	1472	1476	0.10	0.01	0.26	0.00	0.27	0.01	0.13	0.00	0.08	0.00	0.10	0.00
52	Germacrene D	18.45	1475	1481			0.37	0.00	1.32	0.03	0.39	0.00	0.17	0.00	0.70	0.00
53	Unidentified	18.57	1480				0.07	0.00	0.07	0.01						
54	Bicyclogermacrene	18.81	1490	1494					0.54	0.01					0.87	0.01
55	Viridiflorene (=leden)*	18.81	1490	1492							0.41	0.01				
56	Unidentified	18.81	1490	1492			0.55	0.01					0.22	0.01		
57	*α*-muurolene	18.91	1494	1498	0.10	0.01	0.08	0.00	0.11	0.00	0.04	0.00				
58	*β*-bisabolene	19.18	1506	1508	0.72	0.03	0.72	0.01	0.75	0.05	0.49	0.00	0.11	0.00	0.30	0.01
59	*γ*-cadinene	19.26	1509	1513					0.23	0.03	0.14	0.01	0.13	0.00	0.11	0.01
60	*δ*-cadinene	19.50	1520	1523	0.15	0.01	0.39	0.01	0.41	0.01	0.22	0.00	0.15	0.00	0.16	0.00
61	*Trans-α*-bisabolene	19.99	1541	1540					0.08	0.01			0.04	0.01		
62	Spathulenol	20.63	1566	1576	1.10	0.09	0.13	0.13	0.15	0.01	0.59	0.01	0.30	0.00	0.44	0.01
63	Caryophyllene oxide	20.80	1575	1581	0.62	0.03	0.24	0.02	0.16	0.01	0.22	0.02	0.52	0.00	0.40	0.01
64	*α*-cadinol*	21.38	1601	1601			0.16	0.09								

Legend: exp. – experimental, lit. – reference, avg. – average, SD – standard deviation, * – tentative identification

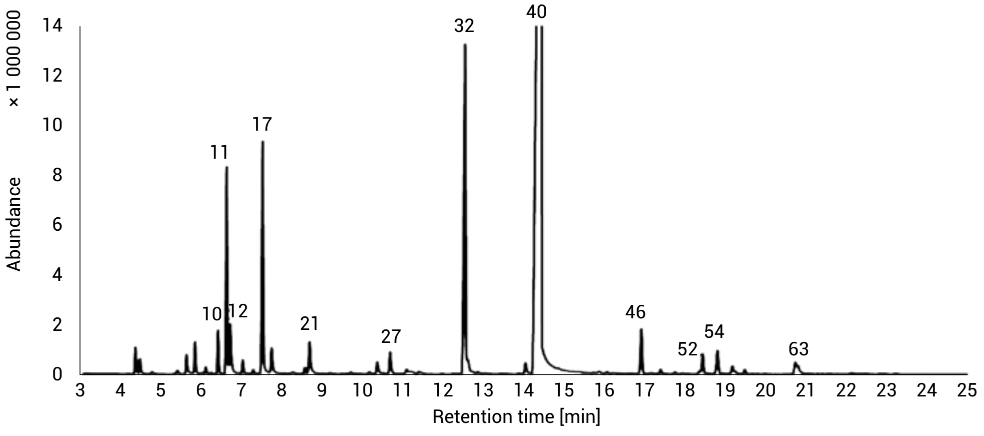


Figure 1. Chromatogram of the *S. montana* EsO (S6). The main peaks were numbered according to Table 2.

Table 3. Relative abundance (in%) of the components in the EsO from six samples of the *Satureja montana* cultivars. The compounds are ordered according to the total sum of% area for all of the cultivars. Numbers above peaks correspond to the order of components in Table 2; nd - not detected.

9	Component	Samples					
		S1	S2	S3	S4	S5	S6
1	Carvacrol	83.70	87.03	69.52	76.31	65.39	58.26
2	*p*-Cymene	1.67	0.51	6.27	4.95	8.78	5.42
3	*γ*-Terpinene	1.61	0.92	6.00	5.79	5.03	6.62
4	Carvacrol methyl ether	0.08	0.09	nd	0.06	0.15	11.70
5	Linalool	0.80	1.21	1.30	0.79	3.24	1.17
6	1-Octen-3-ol	0.94	0.82	0.83	1.07	1.20	0.63
7	*cis*-Sabinene hydrate	0.52	0.85	0.83	0.90	1.24	0.93
8	Terpinen-4-ol	0.71	0.88	0.70	0.66	1.24	0.74
9	*α*-Terpinene	0.04	0.04	1.13	1.02	0.93	1.11
10	Caryophyllene	0.13	0.56	0.66	0.42	0.65	1.47

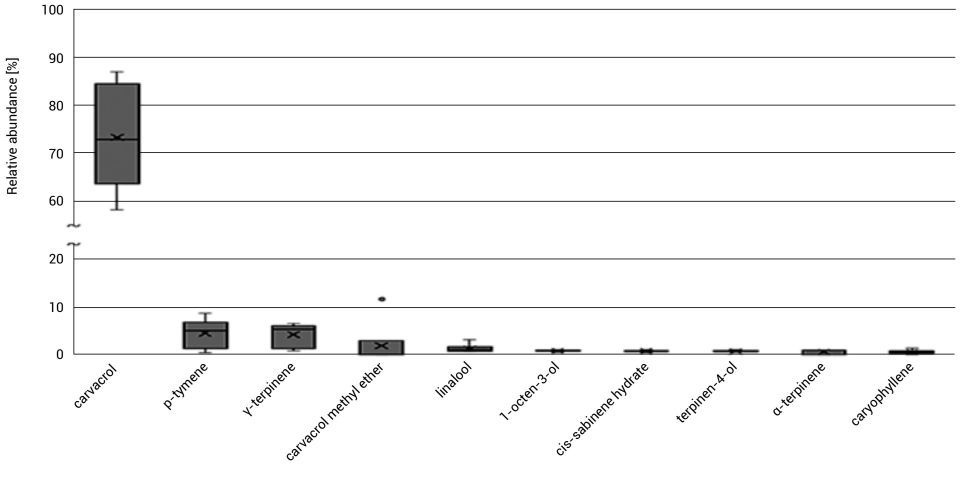
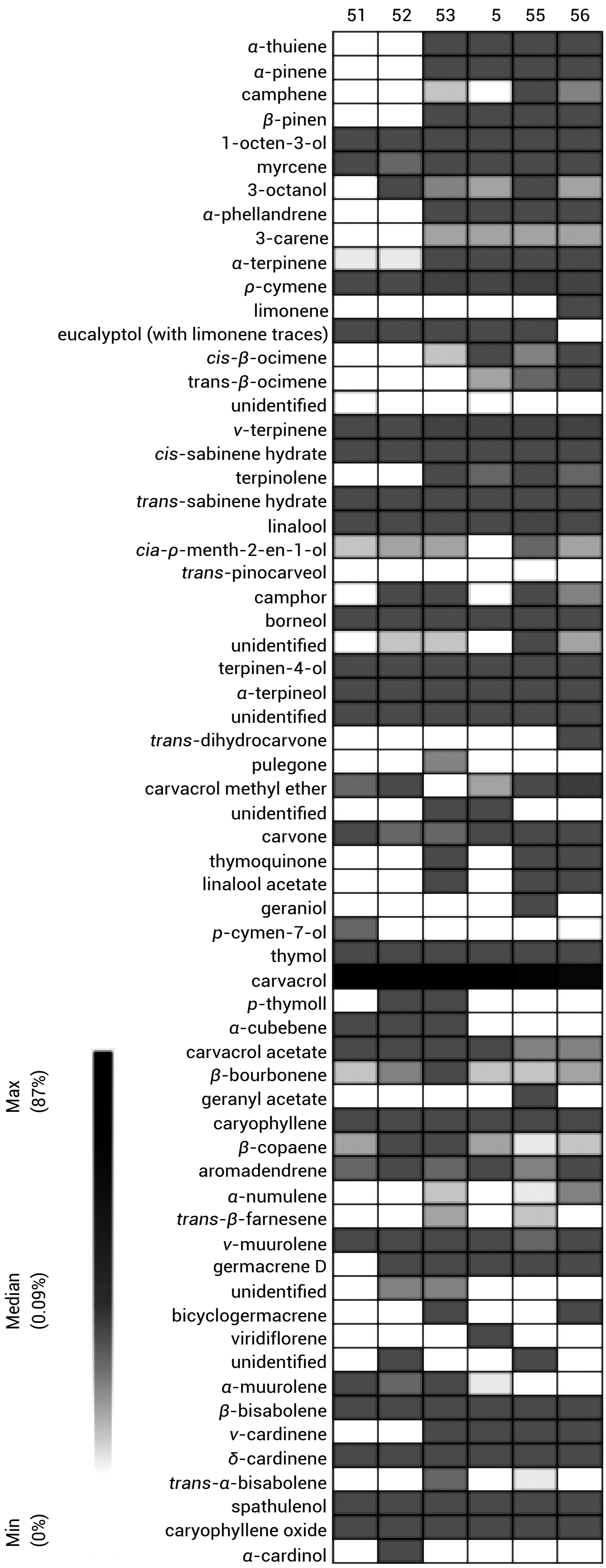


Figure 2. Box plots for the relative abundance of the main components in the studied *Satureja montana* EsO. The boxes represent the Q1–Q3 interquartile ranges, lines splitting the boxes indicate medians, crosses indicate the mean values, whiskers indicate the range, and dot indicates the outlying result.



commanding that the EsO of *S. montana* should comprise the following main constituents: γ-terpinene, *p*-cymene, linalool, terpinen-4-ol and carvacrol (9). Furthermore, we can conclude that the studied new cultivars belong to the carvacrol chemotype of *S. montana* with a high abundance of carvacrol which was more than 58%. According to one more classification (13, 32), these cultivars are related to chemotype A or phenolic chemotype, characterized by a high content of phenolic compounds (carvacrol and thymol).

The aromatic monoterpenoid carvacrol was the main constituent of the EsO of all the studied *S. montana* cultivars (Figure 4). Its relative abundance ranged from 58.3% to 87.0%. *p*-Cymene was the second predominant component (5.0-8.8%) in the EsO of all the four cultivars collected in the year 2020, while the cultivars 'Lunata' and 'Krymski smaragd' gathered in 2019 contained much less (1.7% and 0.5%, respectively). Another major component of EsO from the cultivars collected in 2020 was γ-Terpinene and ranged from 5.0% to 6.6%. The cultivars 'Lunata' (2019) and 'Krymski smaragd' (2019) accumulated only 1.6% and 0.9% of γ-terpinene, respectively. Therefore, firstly, we can suppose to a certain extent that the chemical composition of EsO of *S. montana* depends on the microclimatic peculiarities of the year of growth. However, this assumption needs further studies, especially for the pharmaceutical application of EsO. Secondly, our samples of EsO had similar percentages to full flowering samples from the south of France, which grew in the Mediterranean climate (31).

Figure 3. A heatmap of the studied *S. montana* EsO. The color black indicates that the compound was not detected in the sample. The color scale represents GC-MS relative abundance in%.

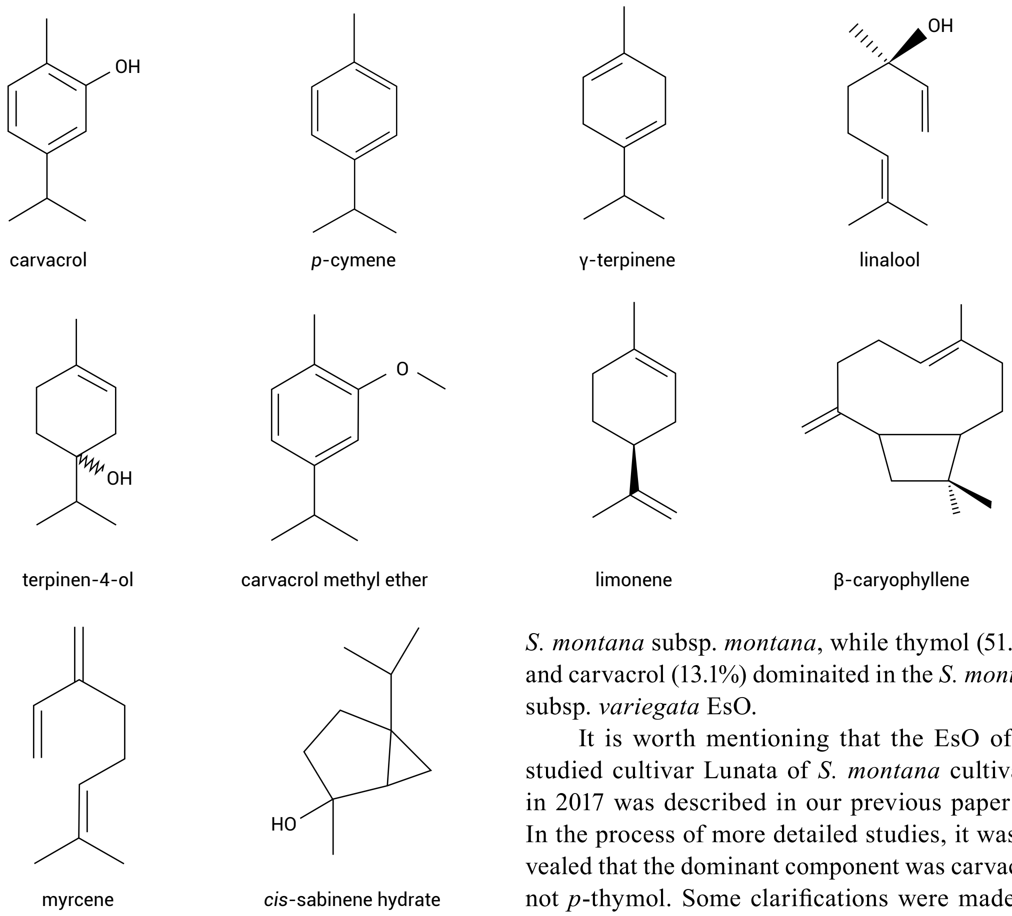


Figure 4. Structures of the main components of EsO of the different cultivars of *S. montana*.

It should be noted that Miladi et al. described the carvacrol/*p*-cymene/*γ*-terpinene chemotype grown in the south of France where the percentage of carvacrol, *γ*-terpinene, and *p*-cymene was 53.35%, 13.54%, and 13.03%, respectively, in GC-MS analysis (31). The Italian researchers Ebani et al. (39) revealed by GC-MS that carvacrol (60.0%) followed by thymol (9.5%) were the most prevalent compounds in *S. montana* EsO. Other Italian researchers (40) demonstrated that carvacrol (63.16%) and *p*-cymene (9.82%) were the major compounds in *S. montana* EsO from Verona region. However, recently it was found that the predominant compounds of EsO from *S. montana* harvested in Serbia were *p*-cymene (19.24%), linalool (11.91%), geranyl acetate (12.64%), and geraniol (10.15%) (41). Aćimović et al. (42) revealed by GC-MS using both GC-FID and GC-MS that EsO of two Serbian *S. montana* subspecies differed significantly in the EsO compositions. Thus, carvacrol (35.7%) and *p*-cymene (32.3%) were the main compounds of *S. montana* subsp. *montana*, while thymol (51.4%) and carvacrol (13.1%) dominaited in the *S. montana* subsp. *variegata* EsO.

It is worth mentioning that the EsO of the studied cultivar Lunata of *S. montana* cultivated in 2017 was described in our previous paper (6). In the process of more detailed studies, it was revealed that the dominant component was carvacrol, not *p*-thymol. Some clarifications were made regarding cymene isomers, i.e. *p*-cymene, not *o*-cymene, was identified in the studied EsO (Lunata of 2017). In addition, with the exception of *p*-thymol, the cultivar Lunata of 2017 was similar to 2019 in terms of a high abundance of carvacrol (81.8% and 83.70%) and a lower abundance of *p*-cymene (1.26% and 1.67%).

Carvacrol (2-methyl-5-(propan-2-yl)-benzenol, $C_{10}H_{14}O$), as the principal component of *S. montana* EsO, is an oxygenated aromatic monoterpenoid found in the EsO of plants from the genera *Thymus*, *Origanum*, *Satureja*, *Thymbra*, *Monarda*, etc. (*Lamiaceae* family) (1, 4, 13, 15, 21, 24, 31, 32, 43-45). Carvacrol was regarded as the main indicator of the antimicrobial activity of EsO in which it dominated (5, 21, 32, 45). It is widely used as a food preservative and flavoring ingredient (46, 47). Recently, Balahbib et al. described the prominent antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, and antitumor activities of the aromatic monoterpene *p*-cymene (1-isopropyl-4-methylbenzene) (48), which belongs to the major components of the studied *S. montana* EsO. The noticeable antimicrobial effect of *p*-cymene was proven by Marchese and co-authors (49).

Our studies revealed that among the investigated cultivars only 'N697-1' (S6) contained carvacrol methyl ether at a substantial level (11.7%). This cultivar also had the highest caryophyllene abundance among the tested samples. Ibraliu et al. (24) found that carvacrol methyl ether was identified in small amounts (0.07-0.2%) in the EsO of *S. montana* collected in Albania. A more significant level of carvacrol methyl ether (4.6%) was detected by GC-MS in the EsO of *S. montana* collected on the Croatian Brač island, but still much lower than in our cultivar 'N697-1' (21).

It should be mentioned that cultivar 'N697-1' (S6) also contained the highest limonene abundance among the studied cultivars (1.7%) and did not contain eucalyptol. Miladi et al. reported that limonene was found in small amounts (0.73%) in the EsO of *S. montana* collected in the south of France (31).

Summing up, our results are partly in line with the data reported by many researchers regarding the chemical composition of the EsO of *S. montana* subspecies and cultivars.

Generally, EsO containing many aromatic compounds are a promising source of antimicrobial medicines (1, 16, 20, 21, 32, 50-54). The synergistic effects of many components of natural EsO were reported by many researchers, especially in the context of their antibacterial and antioxidant properties (51, 53, 55, 56). Therefore, the analyzed EsO of the studied *S. montana* cultivars could be a promising active pharmaceutical ingredient of antimicrobial medicinal products.

CONCLUSIONS

The composition of EsO obtained from four new *S. montana* cultivars of Ukrainian origin was analyzed using GC-MS. It was found that carvacrol was the principal component of the EsO of all the cultivars and ranged from 58.3% to 87.0%. Therefore, we concluded that the new cultivars from the south of Ukraine represent the carvacrol chemotype of *S. montana*. All of the studied EsO conform to the requirements of ISO 79284:1991 regarding the chemical composition of the *S. montana* EsO. It was found that the chemical composition of EsO from 'Krymski smaragd' and 'Lunata' cultivars differed depending on the harvesting year. A significant inter-year variability for the abundance of carvacrol, γ-terpinene, and *p*-cymene was revealed for the EsO of these two cultivars. The obtained data can help discover the potential for future selective breeding of the studied four cultivars as well as pharmacological testing with the purpose of further application in the pharmaceutical development of various dosage forms. Future studies could be directed at in-depth investigations of different groups of bioactive phytochemicals of the tested cultivars as well as their therapeutic potential.

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Conflicts of Interest

The authors declare no conflicts of interest.

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