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RESEARCH ARTICLE

Characterisation of *Satureja montana* L. essential oil and headspace volatiles

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Abstract

Volatile constituents of *Satureja montana* L. collected from a natural habitat in the southern region of Bosnia and Herzegovina were isolated both by steam distillation and headspace sampling. The relative abundance of volatile compounds from the air-dried fragrant plant was analysed by GC-MS using two columns of different polarity. A total of 28 compounds were identified, constituting 93.5% of the essential oil composition. The GC pattern of headspace was different from that prepared by steam distillation. The former consisted of 14 compounds that made up 84.1% of the components identified. Both samples were dominated by oxygenated monoterpenes ranging from 55.2% for headspace of the plant material to 75.5% for the steam-distilled oil. Eleven alcohols represented the most diverse chemical class in the volatile oil (71.8%), as well as seven alcohols detected in the headspace (57.3%). GC-MS analysis of the volatiles indicates that *S. montana* belongs to linalool chemotype with its relative content of 38.7% for the essential oil and 23.7% for the headspace. With linalool as the most abundant constituent, the other major components were α-terpineol (14.7%) in the essential oil, and *cis*-sabinene hydrate (21.8%) and *p*-cymene (17.9%) in the headspace sample. The results prove that the combined approach in the extraction of volatile compounds is needed for the analysis of the aroma of complex herbal samples.

Keywords: *Satureja montana*, essential oil, headspace, GC-MS, linalool

Introduction

Satureja L. (savory) is a genus of the well-known plant family Lamiaceae, comprising about 200 species that are widespread in the warm areas of both hemispheres with a centre of distribution located in the eastern part of the Mediterranean region. Commonly, they are annual or perennial aromatic herbs and shrubs that inhabit arid, sunny, stony and rocky habitats. Over 30 species of this genus are growing wild in many species, subspecies and varieties. Nine species of this genus have been registered in the area of central and western Balkans (Šilić, 1979), some of which are widely used in traditional medicine as muscle pain relievers, tonic, and carminative agent for the treatment of stomach and intestinal disorders. Since ancient times, the genus *Satureja* L. has been well recognized for its therapeutic values. Antimicrobial, anti-inflammatory, stimulant, diuretic, mutagenic effects and other biological activities of *Satureja* species have been reported in the literature (Saeidnia et al., 2016). The aerial parts of these plants have distinctive tastes and can be added to food products as seasoning and a garnish.

The *Satureja* genus includes numerous aromatic and medicinal species with quite different botanical characteristics and a broad chemical heterogeneity. The ease of cross-pollination leads to a large number of subspecies, varieties, and forms. According to WCSP: World Checklist of Selected Plant Families Database, facilitated by the Royal Botanic Gardens, Kew, there are 741 records on *Satureja* species retrieved, of which only 64 have accepted names (Govaerts, 2003). Besides, the taxa *S. montana* is represented by 26 subspecies. Its high variability is evident, even within a single population polymorphism, and especially in populations coming from distant habitats. Of these, only four have accepted infraspecific name: subsp. *montana*, subsp. *variegata*, subsp. *macedonica,* and subsp. *pisidia*.

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Satureja montana L., known as winter savory, is an evergreen perennial shrub plant native to Mediterranean region where it grows as a wild plant on calcareous and rocky soils. Already two thousand years ago, this very fragrant herb was used fresh or dried as a flavouring agent for foods, and also in folk medicine as a remedy for digestive and intestinal ailments (Redzic, 2006). Among all the species of *Satureja* genus, S. *montana* has the most economic importance and is cultivated and utilized throughout the world.

Beside traditional use in folk medicine, up to now, many scientific studies confirmed *S. montana* 's significant pharmacological activity: antimicrobial activity against a wide range of multidrug-resistant pathogens (Skočibušić & Bezić, 2004, Panizzi et al., 1993, Ciani et al., 2000, Ćavar et al., 2008, Silva et al., 2009) diuretic activity (Stanic & Samaržija, 1993), antidiarrheal and antispasmodic (Skočibušić & Bezić, 2003), anti-HIV-1 activity (Yamasaki et al., 1998), antioxidant activity (Radonic & Milos, 2003, Ćavar et al., 2008, Vidović et al., 2014), anticholinesterase activity (Silva et al., 2009), cytotoxic activity (Miladi et al., 2013), and treatment of premature ejaculation (Zavatti et al., 2011).

The positive effects of this plant on human health have been attributed to a variety of biologically active ingredients, such as the constituents of its essential oil with a characteristic aroma. The chemical composition of *S. montana* essential oils of various origins has been the subject of many studies and hundreds of components have been identified to date. This leads to the existence of different chemotypes that distinguish essential oils of different origins. It is generally accepted that the variability of the chemical composition of *S. montana* essential oil depends on the plant origin and the stage of plant development. Thus, there is still a considerable research interest in the assay of the composition of essential oil of *S. montana* of different geographical origin.

The present paper is a continuation of our study on the essential oil profile of some species of *Satureja* genus that grow wild on the Balkan Peninsula, i.e. from Bosnia and Herzegovina (Ćavar et al., 2008), and from Croatia (Vidic et al., 2009, Ćavar et al., 2013). In the present work, the comparison of volatile compounds from the leaves and flowers of *S. montana* obtained by different extraction methods, conventional steam distillation, and headspace sampling technique, was investigated. The aim of this study was to extend knowledge on *S. montana* essential oil obtained so far, not only to provide more detailed information on the oil composition achieved by GC-MS, but also to assess the best option for obtaining the volatile composition characteristics responsible for the pleasant scent of this aromatic species. In order to detect the "true fragrance", the aroma fingerprint of the plant material was characterized by collecting volatiles trough dynamic headspace technique. According to the literature search, this is the first report on the headspace analysis of aerial parts of *S. montana*.

Materials and Methods

Plant material and chemicals

The plant material used in this study was randomly selected above-ground plant parts of the *S. montana* population from the natural habitat in Herzegovina, Glavatičevo, 43°29'44" N, 18°6'19" E, elevation 365 m.s.m. The aerial parts, leaves and flower heads, were collected at the end of August, during the full flowering stage. A voucher specimen of the plant was deposited at the Faculty of Science, University of Sarajevo, Bosnia and Herzegovina (voucher No. KU-2309). All applied reagents were of the highest purity available and purchased from the Sigma-Aldrich Chemical Company, Germany.

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Sample preparation - Isolation of volatiles

Once harvested, the plant material was dried at ambient temperature, in a shaded, well ventilated place to minimize losses of volatile constituents and changes in the profile of essential oil and headspace. The air-dried and finely ground aerial parts of the plant were pooled in a unique composite sample (50 g), and subjected to standard steam distillation during 3 h in order to isolate plant volatile components. The essential oil was extracted with dichloromethane, dried over anhydrous sodium sulphate, stored in a dark glass bottle, and kept at 4°C until analysis (Sample 1).

Floral and leaf aroma of dry plant material (10 g) was collected using dynamic headspace sorption on Dräger charcoal tubes #6728631 with sorption agent coconut shell charcoal; trapping time was 3 h, at ambient condition (Vidic et al., 2018). The desiccator was used as a sampling chamber to minimize the possibility of contamination from environment. The trapped volatiles were eluted with dichloromethane (Sample 2).

Gas chromatography-mass spectrometry (GC-MS)

The analysis of volatile compounds was performed using two GC-MS instruments and two columns of different polarity. First GC-MS analysis was carried out on the Perkin Elmer Mass, fitted with a fused-silica PE-5 (5% phenyl methyl siloxane) capillary column coupled to a Turbo Mass Auto System XL. The GC-MS analysis on the polar column (a fused silica HP-20M polyethylene glycol) was carried out using Hewlett Packard GC-MS system (GC 5890 series II; MSD 5971A, Hewlett Packard, Vienna, Austria). Working conditions were set as we described earlier (Vidic et al., 2018).

The linear retention indices for all compounds were determined by injection of the sample with a solution containing the homologous series of C_8-C_{24} *n*-alkanes under the above conditions in order to calculate the retention indices using generalized equation (Van den Dool & Kratz, 1963). The identification of the essential oil constituents was accomplished by comparing their retention indices and mass spectra from the literature data (Adams, 2007), by computer library search (HP Chemstation computer library NBS75K.L, NIST/ EPA/NIH Mass Spectral Library 2.0 and Mass Finder 4 Computer Software and Terpenoids Library), and by the laboratory database.

Results and Discussion

Chemical characterization of the volatile profile of *S. montana* was performed using two different isolation methods, steam distillation (SD) and headspace sampling (HS). Steam distillation was applied as one of the simplest conventional ways to separate essential oil from different parts of plants. However, the main restrictions of SD, are as long extraction times, the possibility of the decomposition, and/or loss of heat-sensitive compounds during extraction. In contrast, the HS method is a very facile, sensitive, and solvent-free sampling and concentration technique for determining volatile compounds without destroying plant material.

The oil isolated by SD from aerial parts of *S. montana* was found to be a pale yellow liquid of strong fragrance with an acrid, grassy, medicinal smell, and obtained in a yield of 0.3% based on the weight of dried biomass.

The chemical composition of steam-distilled essential oil and headspace sample was determined by detailed GC-MS analysis. The identification of the plant fragrance and other extractable plant components was performed using two GC columns, polar HP-20 and semi-polar PE-5. The distribution of volatile constituents, their RI indices, and relative amounts are presented in Table 1. Compounds are listed in order of elution from PE-5 capillary column and chemical class distribution is also reported. The relative amounts of the individual

components of the essential oil and headspace are expressed as a percentage of the peak area relative to the total peak area. The percentages of the five most dominant compounds in both samples are given in bold.

Table 1. Volatile constituents of *Satureja montana* L.

Compound	**Class**[a]	**RI-1**[b]	**RI-2**[c]	**RA**[d] **[%]**		**MOI**[g]
				Sample 1[e]	**Sample 2**[f]	
1-Octen-3-ol	other	971	1413	1.0	4.1	A[h], MF[i], MS[j]
β-Pinene	MH	977	1102	t	3.1	A, MF, MS
p-Cymene	MH	1019	1247	1.1	**17.9**	A, MF, MS
cis-Sabinene hydrate	MO	1061	1524	1.8	**21.8**	A, MF,
Linalool	MO	1089	1510	**38.7**	**23.7**	A, MF, MS
Camphor	MO	1149	1476	0.7	1.6	A, MF, MS
Borneol	MO	1160	1656	7.7	1.8	A, MF, MS
Terpinen-4-ol	MO	1170	1564	3.3	0.5	A, MF, MS
p-Cymen-8-ol	MO	1175	2032	1.6	-	A, MF
α-Terpineol	MO	1182	1654	**14.7**	2.5	A, MF, MS
Thymol methyl ether	MO	1229	1568	1.7	0.4	A, MF, MS
Cuminaldehyde	MO	1240	1725	0.2	-	A
Carvone	MO	1243	1691	0.8	-	A, MF
Carvacrol methyl ether	MO	1245	-	0.5	-	A, MF
Geraniol	MO	1246	1798	0.4	2.9	A, MF, MS
Thymol	MO	1293	2117	2.0	-	A, MF, MS
Carvacrol	MO	1311	2146	0.8	-	A, MF, MS
β-Bourbonene	SH	1394	1489	0.8	1.0	A, MF
β-Caryophyllene	SH	1426	1565	2.4	2.0	A, MF, MS
Germacrene D	SH	1490	1693	0.4	-	A, MF, MS
trans-β-Bergamotene	SH	1511	1690	1.2	-	MF
Dihydroactinidiolide	MO	1532	-	0.6	-	MF, MS
Spathulenol	SO	1585	2066	0.7		A, MF, MS
Caryophyllene oxide	SO	1590	1933	6.7	0.8	A, MF, MS
Caryophylla-4(12),8(13)-dien-5β-ol	SO	1643	-	1.0	-	A
14-Hydroxy-9-epi-(*E*)-caryophyllene	SO	1672	-	0.9	-	A
Oplopanone	SO	1726	-	1.0	-	A, MF
Hexahydrofarnesyl acetone	other	1841	-	0.8	-	MS
Monoterpene hydrocarbons (MH)				1.1	21.0	
Oxygenated monoterpenes (MO)				75.5	55.2	
Sesquiterpene hydrocarbons (SH)				4.8	3.0	
Oxygenated sesquiterpenes (SO)				10.3	0.8	
Non-terpenoids (NT)				1.8	4.1	
Total identified (%)				**93.5**	**84.1**	

[a] The abbreviations of the compound classes are given at the end of the table.
[b] RI-1: Linear retention indices determined experimentally on the PE-5 column relative to a series of *n*-alkanes (C_8-C_{26}).
[c] RI-2: Linear retention indices determined experimentally on HP-20 column relative to a series of *n*-alkanes (C_8-C_{26}).
[d] RA*:* relative area; t, traces (<0.1%)
[e] Sample 1: essential oil obtained by steam distillation
[f] Sample 2: headspace
[g] MOI: Mode of identification
[h] A: Reference (Adams, 2007).
[i] MF: Mass Finder database.
[j] MS: Mass spectra from the laboratory database.

In total, 28 compounds were identified in the essential oil, half of which occurred only in amount below 1%, constituting 93.5% of the essential oil composition (Sample 1). Only 14 compounds were identified in headspace sample comprising 84.1% of the total components detected (Sample 2). Although their

compositional patterns are similar, these compounds were found in different percentages in headspace and steam distilled samples.

When grouping the components into different terpenoid types, there were found six hydrocarbons, only one monoterpene (MH), one alkylbenzene related to a monoterpene, and four sesquiterpene hydrocarbons (SH), followed by 15 oxygenated monoterpenes (MO) and five oxygenated sesquiterpenes (SO). It is noticeable that the essential oil and headspace from *S. montana* almost entirely consist of oxygenated compounds (87.6% and 60.1%, respectively). The most abundant compounds that constituted the fragrance emitted from *S. montana* leaf and flowers, were MO ranged from 55.2% for headspace, up to 75.5% for steam-distilled oil.

Alcohols are the main contributors to the oxygenated fraction, representing 71.8% of all volatile constituents in essential oil and 57.3% in headspace. Eleven alcohols represented the most diverse chemical class in volatile oil as well as seven alcohols detected in the headspace. Considering the main components, the composition analysis of the volatiles indicates that *S. montana* clearly belongs to linalool-chemotype with its relative content of 38.7% for essential oil (Sample 1) and 23.7% for the headspace (Sample 2). With linalool as the most abundant constituent, the other major components were α-terpineol (14.7%) in the essential oil and *cis*-sabinene hydrate (21.8%) and *p*-cymene (17.9%) in the headspace.

The headspace composition of *S. montana* leaves and flowers gives a better overall representation of the main compounds responsible for the aroma as compared with that of the steam distillate. The study successfully isolated and characterized low temperature volatile aromatic compounds in *S. montana* using headspace sampling technique. For steam distillation, the increased temperatures and extended extraction time can cause chemical modifications of the oil components and loss of the most volatile constituents. Especially when grouping the compounds into monoterpene hydrocarbons, MH, and oxygenated monoterpenes, MO, the percentage concentrations drop from 75.5% (Sample 1) to 55.2% (Sample 2) for MO, while that of MH increase from 1.1% (Sample 1) to 21.0% (Sample 2). Among the components of the hydrocarbon fraction, the predominant compounds were found to be *p*-cymene (17.9%) and β-pinene (3.1%) in headspace, while the percentage of *p*-cymene in the essential oil was significantly lower (1.1%), and β-pinene was detected only in trace amount.

At the same time, it is possible that the thermal degradation of some unstable compounds during the SD procedure produced some artefacts not found by HS sampling. On the other hand, SD was suitable for sampling stable compounds not found by the HS extraction technique. As expected, the contribution of sesquiterpenes in headspace was lower than that of essential oil. Oxygenated sesquiterpene caryophyllene oxide was identified as the most abundant representative of this class (6.7% in Sample 1 and 0.8% in Sample 2). Similarly, three oxygenated sesquiterpenes participating in the alcoholic fraction of the essential oil: spathulenol (0.7%), caryophylla-4(12),8(13)-dien-5β-ol (1.0%), and 14-hydroxy-9-epi-(*E*)-caryophyllene (0.9%) were not detected in the headspace. These high boiling point sesquiterpene alcohols were extracted under high temperature (boiling point of water) compared to the extraction conditions during headspace trapping. Therefore, a combination of HS and conventional SD could provide better qualitative and semi-quantitative information on *S. montana* volatiles.

A review of the published literature indicates that *S. montana* essential oil exhibits large variations in the relative concentrations of its principal components, viz. carvacrol, thymol, linalool, γ-terpinene and *p*-cymene, from the existence of different chemotypes. Table 2. summarizes the results of chemical composition in terms of the major constituents of all essential oils of *S. montana* reported to date.

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Table 2. Chemical composition of *Satureja montana* L. essential oil found in the literature

Locality/ Origin	subspecies	Chemotype/Main Constituents %	Reference
Albania	-	carvacrol 56.8-61.9, thymol 12.4-27.3, *p*-cymene 16.2-17.4, γ-terpinene 13.1-13.8	Ibraliu et al., 2011.
Albania	-	carvacrol 2.21-55.95, *p*-cymene 1.13-16.22, γ-terpinene 0.31-8.86,	Ibraliu et al., 2013.
Albania	-	thymol 28.99, *p*-cymene 12.00, linalool 11.00, carvacrol 10.71	de Oliveira et al., 2011.
Albania	-	thymol 3.89-31.08, linalool 0.68-21.45, carvacrol 4.82-16.20, *p*-cymene 5.14-13.73	Hajdari et al., 2016.
Albania	-	carvacrol 23.9-29.0, thymol 14.5-16.5, linalool 16.0-16.4, γ-terpinene 12.3-15.0, *p*-cymene 9.4-12.3	Maccelli et al., 2020.
Bosnia and Herzegovina	-	carvacrol 23.3, thymol 31.7	Ćavar et al., 2008.
Bosnia and Herzegovina	-	geraniol 22.3, terpinen-4-ol 10.3	Ćavar et al., 2008.
Bosnia and Herzegovina	*montana*	carvacrol 57.24, 1,8-cineole 18.16	Kustrak et al., 1996.
Bosnia and Herzegovina	*montana*	*p*-cymene 13.3, *trans*-sabinene hydrate 9.3, *p*-cymen-8-o1, 8.6, linalool 8.1	Slavkovska et al., 2001.
Bosnia and Herzegovina,	*montana*	linalool 14.4-43.4, *p*-cymene 19.5-47.7, *trans*-sabinene hydrate 14.2-27.1, *p*-cymen-8-ol 11.3-27.0	Slavkovska et al., 1997.
Bosnia and Herzegovina	*variegata*	carvacrol 5.58-63.54, *p*-cymene 4.66-26.28, γ–terpinene 11.49-15.87, linalool 3.82-11.49	Kustrak et al., 1996.
Bulgaria	*kitaibelii*	limonene 15.7, *p*-cymene 13.1	Konakchiev & Tsankova, 2002.
Croatia	-	carvacrol 16.1-45.7, *p*-cymene 3.0-28.9, linalool 0.5-24.8, thymol 1.9-20.6	Milos et al., 2001.
Croatia	-	carvacrol 50.2, thymol 11.0, γ-terpinene 5.8, *p*-cymene 4.8	Skočibušić & Bezić, 2003.
Croatia	-	thymol 45.2, *p*-cymene 6.4, γ-terpinene 5.9, carvacrol 5.3	Radonic & Milos, 2003.
Croatia	-	carvacrol 16.1-52.4, *p*-cymene 3.8-25.6	Skočibušić & Bezić, 2004.
Croatia	-	carvacrol 45.7, *p*-cymene 12.6, carvacrol methyl ether 11.0	Bezić et al., 2005.
Croatia	-	carvacrol 59.1, thymol 20.1	Vidic et al., 2009.
Croatia	-	carvacrol 13.7, *p*-cymene 11.8, γ-terpinene 10.6	Bezić et al., 2009.
Croatia	-	carvacrol 44.5, *p*-cymene 16.9, γ-terpinene 8.7	Marin et al., 2012.
Croatia	-	carvacrol 23.6-37.9, thymol 30.8	Dunkić et al., 2012.
Croatia	-	carvacrol 63.4, thymol 19.4	Ćavar et al., 2013.
Croatia	*montana*	carvacrol 4.8-61.1, thymol 1.0-61.0, γ-terpinene 3.1-16.9	Stanic et al., 1991.
Croatia	*montana*	carvacrol 30.83-67.88, 1,8-cineole 0.89-19.45, linalool 1.42-6.61	Kustrak et al., 1996.
Croatia	*montana*	thymol 30.88-46.02, *p*-cymene 7.10-13.48, γ-terpinene 7.57-9.74, carvacrol 3.81-6.86	Mastelić & Jerković, 2003.
Croatia	*variegata*	carvacrol 83.84-84.19, *p*-cymene 5.54-7.36,	Kustrak et al., 1996.
Croatia	*variegata*	carvacrol 19.4, thymol 16.6, γ-terpinene 6.9, linalool 5.9	Dunkić et al., 2010.
Egypt	-	carvacrol 24.3	El-Hagrassi et al., 2018
France	-	linalool 0.4-71.9, carvacrol 6.1-67.8, *p*-cymene 6.7-57.7, γ-terpinene 0.7-24.0	Chizzola, 2003.
France	-	carvacrol 29.19, thymol 15.4, *p*-cymene 11.77, γ-terpinene 6.72	Djenane et al., 2011.
France		carvacrol 53.35, γ-terpinene 13.54, *p*-cymene 13.03 .	Miladi et al., 2013.
Greece	-	carvacrol 47.12, thymol 12.39, γ-terpinene 6.49, *p*-cymene 5.48	Michaelakis et al., 2007.
Iran	-	carvacrol 65.80, γ-terpinene 16.33, *p*-cymene 4.56	Omidbaigi et al., 2007.
Italy	-	carvacrol 26.38-41.23, *p*-cymene 11.00-16.32, γ-terpinene 1.40-6.16	Piccaglia et al., 1991.
Italy	-	carvacrol 56.82, γ-terpinene 10.03, *p*-cymene 9.83	Panizzi et al., 1993.
Italy	-	carvacrol 56.8, γ-terpinene 13.2, *p*-cymene 9.7	Angelini et al., 2003.

Italy	-	carvacrol 18.00, *p*-cymene 14.30, thymol 9.92	Fraternale et al., 2007.
Italy	-	p-cymene 41.4, carvacrol 37.0, γ-terpinene 3.0	Prieto et al., 2007.
Italy	*montana*	carvacrol 45.91-73.69, *p*-cymene 3.57-16.67, γ-terpinene 0.20-15.81	Bilia et al., 1992.
Italy	*montana*	carvacrol 22.4, thymol 18.8, *p*-cymene 18.9, carvacrol methyl ether 9.7	Benelli et al., 2017.
Italy	*montana*	carvacrol 61.9, *p*-cymene 9.9, γ-terpinene 8.2	Caprioli et al., 2018.
Italy	*variegata*	carvacrol 22.5, *p*-cymene 17.6, thymol 17.4	Caprioli et al., 2018.
Kosovo	-	linalool 11.20-50.42, *p*-cymene 12.16-29.58, myrcene 17.36-21.09	Hajdari et al., 2016.
North Macedonia	*pisidica*	*p*-cymene 22.4-75.5, carvacrol 26.2-70.5, linalool 22.5-68.0	Slavkovska et al., 1997.
North Macedonia	*pisidica*	*p*-cymene 29.3, linalool 24.0, carvacrol 18.3	Slavkovska et al., 2001.
North Macedonia	*pisidica*	carvacrol 37.6, thymol 24.5, γ-terpinene 8.2, *p*-cymene 6.8	Kundaković et al., 2014.
Montenegro	-	thymol 27.68-37.36, carvacrol 4.40-15.47, *p*-cymene 7.86-31.37, γ-terpinene 8.66-11.75	Damjanović-Vratnica et al., 2011.
Montenegro	-	*p*-cymene 26.14, carvacrol 17.92, thymol 14.97, linalool 5.71	Hajdari et al., 2016.
Montenegro	-	thymol 35.4, carvacrol 14.1, γ-terpinene 11.4, *p*-cymene 10.1	Damjanović-Vratnica et al., 2016.
Montenegro	-	*p*-cymene 16.6%, limonene 10.8%, thymol 6.5%	Bojović et al., 2018.
Montenegro	*montana*	thymol 33.4, *p*-cymene 28.8	Bezbradica et al., 2005.
Montenegro	*montana*	thymol 24.69, linalool 15.38, carvacrol 15.19	Mihajilov-Krstev et al., 2014.
Poland	-	carvacrol 44.82-79.90, *p*-cymene12.51-21.92, γ-terpinene 1.42-9.69	Rzepa et al., 2012.
Portugal	-	γ-terpinene 39.8, carvacrol 38.8, *p*-cymene 7.1	Barbosa et al., 2010.
Portugal	-	carvacrol 30.6, thymol 14.1, carvacrol methyl ether 6.3	Serrano et al., 2011
Portugal	-	carvacrol 81.46-94.61, borneol 0.48-0.90, 4-terpineol 0.38-0.65	Santos et al., 2019.
Serbia	-	*p*-cymene 20.46-48.27, carvacrol 2.73-31.73, limonene 7.64-12.94	Rzepa et al., 2012.
Serbia	-	carvacrol 67.58-86.29, *p*-cymene 1.88-7.12	Vidović et al., 2014.
Serbia	-	carvacrol 14.7-25.5, borneol 3.04-4.35	Vladić et al., 2017.
Serbia	-	carvacrol 57.10, γ-terpinene 5.22	Elgndi et al., 2017.
Serbia	-	carvacrol 59.16, *p*-cymene 6.48	Šojić et al., 2019.
Serbia	*kitaibelii*	*p*-cymene 20.9, limonene 16.0, borneol 9.8, *trans*-sabinene hydrate 8.2	Slavkovska et al., 2001.
Serbia	*montana*	*p*-cymene 14.5-47.2, borneol 2.6-28.8, linalool 0.5-17.5, *trans*-sabinene hydrate 2.7-16.0	Slavkovska et al., 1997.
Serbia	*montana*	linalool 22.8, *p*-cymene 18.8, borneol 10.6, *trans*-sabinene hydrate 7.2	Slavkovska et al., 2001.
Slovenia	-	carvacrol 41.5, *p*-cymene 11.0, thymol 8.6, γ-terpinene 6.2	Stoilova et al., 2008.
Spain	-	carvacrol 41.7-64.5, *p*-cymene 6.0-17.8, thymol 6.0-11.3, γ-terpinene 2.3-9.4	Grosso et al., 2009.
Spain	-	carvacrol 52.2-52.7, *p*-cymene 10.1-12.8, thymol 10.9-11.0, γ-terpinene 4.3-8.9	Silva et al., 2009.
Spain	-	carvacrol 58-41, *p*-cymene 18-33	Navarro-Rocha et al., 2020.

The common main characteristic of these oils is the highly oxygenated nature of their terpenoid secondary metabolites. The differences in the results provided by these studies revealed a high variability of *S. montana* essential oil profile, and several physiological forms or chemotypes of different geographical origin have been classified. Taking into account the main constituents identified in the previous studies, it is possible to differentiate the oils of *S. montana* into two chemotypes, namely A and B, depending on the prevalence of phenolic compounds or terpenic alcohols, respectively (Palic et al., 1983). These chemotypes are subdivided in A: thymol or carvacrol, and B: either linalool, α-terpineol, geraniol or terpinen-4-ol. After the extensive

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compilation on the essential oil diversity of species *S. montana* of various origins, it was found that chemotype A was the most prevalent among its essential oils studied to date. Thus, two isomeric monoterpene phenols - carvacrol and thymol along with two monoterpene hydrocarbons - γ-terpinen and *p*-cymene - appear as the main constituents in *S. montana* collected from different locations in Croatia as described by Milos et al. (2001), Radonic & Milos (2003), Vidic et al. (2009), Dunkić et al. (2012), Ćavar et al. (2013), as well as from Italy (Fraternale et al., 2007, Benelli et al., 2017, Caprioli et al. 2019); Albania (Ibrailu et al., 2011, Maccelli et al., 2020); Montenegro (Damjanović-Vratnica et al., 2011); Portugal (Santos et al., 2019); Serbia (Vidović et al., 2014), and Spain (Navarro-Rocha et al., 2020). Mediterranean and Sub Mediterranean bioclimates, moderate water stress, full flowering stage of the plant, and drying method can be critical factors that are able to enhance the percentage of carvacrol in the oil of the plants with chemotype A. On the other hand, several terpenic alcohols have been found to be the major constituents of essential oils of chemotype B obtained from *S. montana* growing in a Mediterranean climate or a continental one, viz. geraniol and terpinen-4-ol as described by Ćavar et al. (2008), and linalool reported by Slavkovska et al. (1997), Chizzola (2003), and Hajdari et al. (2016).

From the results of the detailed essential oil analysis in this study, it is clear that the examined sample differs from the typical and prevailing chemotype A, which is characterized by a high amount of carvacrol and/or thymol. With regard to these phenols known as the most represented constituents in the essential oils of the vast majority of *S. montana* so far published, they showed the peak area percentages of 2.0% (carvacrol) and 0.8% (thymol) in Sample 1, while these monoterpenic phenols were not detected in Sample 2.

The specific chemical composition of essential oil obtained from winter savory originating from Herzegovina in this work was characterized by a high content of linalool (75.5%). From a chemotaxonomic point of view, this is a less common chemotype B. Based on the data published so far, there are only a few papers on the occurrence of terpenic alcohols as major volatile constituents. In all of these works, *S. montana* plant material was collected from habitats located in the continental part of the Balkan Peninsula. Thus, linalool was found to be the main ingredient of essential oils from two distinct populations at the border of Bosnia (14.4-43.4%) and Serbia (16.6-74.05%), (Slavkovska et al., 1997, 2001). In addition, linalool exhibits the most frequent appearance among the monoterpenes in the herb from Kosovo (11.20-50.42%), (Hajdari et al., 2016), and from population of *S. montana* spp. *pisidica* (22.5-68%) from North Macedonia collected at an altitude of 1800 m above sea-level, with a sub-Mediterranean-montane climate (Slavkovska et al., 1997). Compared to these previous studies, it can be concluded that the essential oil which was the subject of research in this paper, matches to some extent with oils of different geographical origins. However, in contrast to the similarity in the exceptionally high linalool content, the amount of other major characteristic components and their relationship still differ significantly.

It is worth noting that variations in the content and chemical composition of volatile compounds, even between geographically close populations, have also been published previously, indicating the presence of different chemotypes. Based on data reported by Ćavar et al. (2008), it was found that essential oil of *S. montana* originated from Herzegovina belongs to the chemotype with a high content of terpenic alcohols, geraniol (22.3%) and terpinen-4-ol (10.3%). On the other hand, the population samples of winter savory used in this work were also collected in Herzegovina, but the chemical profile of its essential oil was distinctly different, and characterised by the presence of linalool (38.7%) and α-terpineol (14.7%) as the principal ingredients. It is interesting to note that both localities are exposed to the same temperate-continental climate, and lie at almost the same height above sea-level. This is not entirely surprising since species *S. montana* is known to exhibit a high variability even within a single population, not just in populations from

distant habitats. This variability is reflected in the morphological characteristics (Šilić, 1979), but also in the chemical composition. Thus, according to a study by Chizzola (2003), one population from southern France was found to be heterogeneous in which about third of the individuals were rich in linalool whereas the other displayed *p*-cymene or carvacrol as the main compound of essential oil. Therefore, a classification into linalool and carvacrol/*p*-cymene chemotype was suggested.

While the chemical composition of *S. montana* essential oil from different geographical localities has been studied in detail, this is the first report on its headspace volatile constituents responsible for the characteristic fragrance of the plant at room temperature. The only previous work related to the headspace of *S. montana* should be mentioned, but it cannot be taken for comparison since the sampling technique was different, and the sample analysed in this paper had a different chemical profile of carvacrol/*p*-cymene-chemotype (Rzepa, 2012). Moreover, it was performed at elevated temperatures (i.e., at 80 and 100°C), which cannot reflect the true aroma profile.

In conclusion, the results in this paper confirm the already observed that the content and composition of the essential oil is greatly influenced by different extraction methods and analytical protocols. The application of a simple non-destructive technique of headspace trapping proved crucial for a reliable insight into *S. montana* chemistry. Furthermore, this methodical approach, in addition to standard pharmacopoeial recommendations, i.e. hydro and/or steam distillation techniques, can be successfully applied to a true fingerprint of the volatile fraction of the plant.

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