



Chemical composition and antioxidant activity of two *Satureja* species from Mt. Biokovo

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ABSTRACT: Hydrodistilled volatile oils, as well as the extracts from waste water after hydrodistillation from the aerial parts of *Satureja montana* and *Satureja cuneifolia*, growing wild at Mt. Biokovo (Croatia), were analyzed by GC-MS. Sixty compounds were identified in four samples. The major constituents of essential oil obtained from the plant material of *S. montana* were carvacrol (63.4%) and thymol (19.4%), while thymoquinone (38.7%) was the major component of extracts from waste water after hydrodistillation. The most abundant compounds in essential oil of *S. cuneifolia* were carvacrol (17.7%) and spathulenol (13.2%), respectively. The extract from waste water after hydrodistillation of this plant species was rich in (*E*)-coniferyl alcohol (18.1%). Antioxidant activity was tested using two spectrophotometric methods. Isolated extracts revealed activity in reducing stable radical and transition metals, comparable to thymol, carvacrol, and thymoquinone, which were used as positive probes.

KEY WORDS: *Satureja montana*, *Satureja cuneifolia*, GC-MS, antioxidant activity.

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INTRODUCTION

The genus *Satureja* L. (savory) belonging to the family Lamiaceae, contains about 200 species of aromatic herbs and shrubs that grow in the Middle East, Mediterranean region to Europe, West Asia, North Africa, the Canary Islands and South America. Over 30 species of this genus are distributed in eastern parts of the Mediterranean area (ŠLIĆ 1979, 1984). A characteristic of the subfamily Nepetoideae is that its representatives contain more than 0.5% of essential oil. Many members of this genus are well known for their aromatic and medicinal character. They are used as culinary herbs and in folk medicine to treat various ailments based on the plants' various activities. Moreover, considerable differences between and within

the chemical composition of the essential oils of *Satureja* subspecies have been found (SLAVKOVSKA *et al.* 2001). Phytochemical studies have revealed volatile oils, tannins, phenolic compounds, sterols, acids, gum, mucilage and pyrocatechol as the main components of *Satureja* species. Because of the presence of high amounts of thymol and carvacrol in *Satureja* species, good aromas and simple cultivation, they are used as a flavoring compound in food, pharmaceutical and cosmetic industries (MOMTAZ & ABDOLLAHI 2010).

Satureja montana L. and *Satureja cuneifolia* Ten. are frequently used in local spices and as traditional medicinal plants. Due to the strong phenolic character of their essential oils, they are reminiscent of the taste and

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fragrance of commercial oregano and thyme oils. The genus *Satureja* is known to possess high variability, even within a single population polymorphism and chemotype, and especially in populations coming from distant habitats (SLAVKOVSKA et al., 2001; ŠILIC, 1979). In the present study we investigated the composition of volatile constituents of *S. montana* and *S. cuneifolia* growing wild at Mt. Biokovo, Croatia. In addition, this study present determined the antioxidant activity of *Satureja* essential oils that has not been reported to date.

MATERIAL AND METHODS

Plant material and reagents. Plant material of *S. montana* and *S. cuneifolia* was collected in August 2007 from its natural habitat in Mt. Biokovo, Croatia. A voucher specimen of each plant is deposited at the Faculty of Science, University of Sarajevo. All reagents were of the highest purity available and purchased from the Sigma-Aldrich Chemical Company.

Sample preparation. Air-dried plant material was subjected to hydrodistillation for 2 h. The essential oil was extracted with dichloromethane and dried over anhydrous sodium sulphate. Remaining volatiles in waste water after hydrodistillation were also extracted with dichloromethane. For the GC-MS analysis, samples of essential oils were dissolved in dichloromethane, while for antioxidant assays, samples were dissolved in dimethylsulfoxide. Thymol, carvacrol, and thymoquinone were used as positive probes for antioxidant assays, and were prepared in the same way as plant samples. All determinations were carried out in triplicate.

Gas chromatography-mass spectrometry. Volatile compounds from the aerial parts of plants were analyzed by GC/MS using a Hewlett-Packard GC/MS system (GC 6890 series II; MSD 6890 series II). The GC conditions were a fused silica HP-5 column, carrier gas He (1.1 mL/min), with temperature program 3°C/min from 60°C to 240°C; the injection port temperature was 250°C and detector temperature 280°C. Ionization of the sample components was performed in the EI mode (70 eV). The linear retention indices, RI, for all compounds were determined by co-injection of the sample with a solution containing the homologous series of C₈-C₂₆ *n*-alkanes (VAN DEN DOOL & KRATZ 1963). The identification of the essential oil constituents was accomplished by visual interpretation, comparing their retention indices and mass spectra with 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 using

the laboratory's own database.

DPPH radical scavenging activity. The ability of the samples to donate a hydrogen atom or electron and scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by the slightly modified method of BRAND-WILLIAMS et al. (1995). The concentrations of samples ranged from 0.1 to 30.0 mg/mL. A portion of sample solution (100 µL) was mixed with 1 mL of 5.25 x 10⁻⁵ M DPPH in ethanol. Reduction of absorbance of the mixtures was monitored every minute for 30 min at 517 nm using a Perkin-Elmer Lambda 25 UV/Vis spectrophotometer. Ethanol was used to zero the spectrophotometer, DPPH solution was used as a sample blank, and thymol, carvacrol, and thymoquinone were used as positive probes. The DPPH solution was freshly prepared daily, stored in a flask covered with aluminum foil, and kept in the dark at 4°C between measurements. All experiments were carried out in triplicate. The radical-scavenging activity of the tested samples, expressed as percentage inhibition of DPPH, was calculated according to the formula $IC (%) = [(A_0 - A_t)/A_0] \times 100$ (YEN & DUH, 1994), where A_t is the absorbance of the tested sample and A_0 is the absorbance of the sample blank, at a particular time. Percent inhibition after 30 min was plotted against concentration, and the equation for the line was used to obtain the IC₅₀ value. A lower IC₅₀ value indicates greater antioxidant activity.

Total antioxidant activity by the phosphomolybdenum method. The total antioxidant activity of samples was evaluated by the phosphomolybdenum complex formation method (PRIETO et al. 1999). Briefly, 600 mL of each sample in different concentrations was mixed with 2400 µL of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in sample vials. The blank solution contained 600 mL of DMSO and 2400 µL of reagent. Vitamin C (1 mg/mL) was used as the control. The vials were capped and incubated at 95°C for 90 minutes. After the samples had been cooled to room temperature, the absorbance of the mixture was measured at 695 nm. Total antioxidant activity of the samples, expressed as percentage reduction of the phosphomolybdenum complex, was calculated according to the formula $IC (%) = \frac{A_{\text{sample}} - A_{\text{blank sample}}}{A_{\text{blank}}}$, where A_{sample} is the absorbance value of the tested sample, and $A_{\text{blank sample}}$ is the absorbance value of the vial containing 600 mL of sample and 2400 mL of water, and A_{blank} is the sample blank. The vial containing vitamin C was used as 100% of antioxidant activity. Percent reduction was plotted against concentration, and the equation for the line was used to obtain the IC₅₀ value. A lower IC₅₀ value indicates greater antioxidant activity. All samples were assayed in triplicate and means calculated.

RESULTS AND DISCUSSION

Gas chromatography-mass spectrometry. The essential oils of *S. montana* and *S. cuneifolia* were subjected to detailed GC/MS analysis to determine their chemical composition. The yield of oils was 0.97% for *S. montana*, and 0.07% for *S. cuneifolia*. Sixty compounds were identified among four samples (Table 1).

In sample SM-1 (essential oil of *S. montana*) twenty-nine compounds were identified, representing 97.9% of the total oil. The most abundant components were aromatic compounds (86.7%) and monoterpene hydrocarbons (7.1%). The high percentage of thymol (19.4%) and carvacrol (63.4%) showed that this savory essential oil clearly belonged to the phenolic – chemotype. The extract obtained from waste water after hydrodistillation of the same plant material (SM-2) was also very rich in aromatic compounds (69.1%), with thymoquinone (38.7%) and thymohydroquinone (11.8%) as the main representatives.

Generally, *S. montana* essential oils were characterized by a high percentage of monoterpene phenols, such as thymol and carvacrol. However, variation between chemical compositions depending on location and stages of development has previously been found (MILOŠ *et al.* 2001, SKOČIBUŠIĆ & BEZIĆ 2004). For example, essential oil of *S. montana* from Bosnia and Herzegovina has been characterized as a thymol-chemotype, as well as a geraniol-chemotype (ČAVAR *et al.* 2008). Winter savory of Croatian origin has carvacrol as the main constituent, but its content is highly variable, depending on the environment and phenological stage of the plant (BEZIĆ *et al.* 2005, 2009; MARIN *et al.* 2012). *Satureja montana* from Pelješac is one of the richest sources of carvacrol and its isomer thymol with a total phenolic terpenoid content of 79.2% (VIDIĆ *et al.* 2009). Plant material originating from Serbia (SLAVKOVSKA *et al.* 2001; BEZBRADICA *et al.* 2005), Montenegro (BEZBRADICA *et al.* 2005; DAMJANOVIĆ-VRATNICA *et al.* 2011), Slovenia (STOILOVA *et al.* 2008), and Albania (IBRALIU *et al.* 2011)

Table 1. Volatile constituents of two *Satureja* plant species.

#	RI	Compound	SM-1 RA (%)	SM-2 RA (%)	SC-1 RA (%)	SC-2 RA (%)
964	1-Octen-3-ol		0.5			
1022	1,8-Cineole		0.2			
1053	γ-Terpinene		tr			
1062	<i>cis</i> -Sabinene hydrate		0.4			
1100	<i>trans</i> -Sabinene hydrate		0.1			
1101	Linalool		0.5			
1105	2-Methyl butyl-2-methyl butyrate		0.1			
1120	<i>cis-p</i> -Menth-2-en-1-ol		tr			
1136	<i>trans-p</i> -Menth-2-en-1ol		tr			
1141	Camphor		tr			
1156	Borneol		4.2		0.3	
1167	Terpinen-4-ol		1.0			
1178	<i>p</i> -Cymen-8-ol		0.1			
1182	α-Terpineol		0.5		0.1	
1223	Thymol methyl ether		1.0			
1231	Carvacrol methyl ether		2.0			
1237	Carvone		0.1			
1240	Thymoquinone		0.2	38.7		
1283	Thymol		19.4	0.9	2.0	
1294	Carvacrol		63.4	7.0	17.7	0.6
1328	Myrtenyl acetate				0.1	
1343	Thymol acetate		0.1			
1360	Carvacrol acetate		0.5			
1399	(<i>Z</i>)-Caryophyllene		1.4			
1418	(<i>E</i>)-Caryophyllene		0.1			

#	RI	Compound	SM-1 RA (%)	SM-2 RA (%)	SC-1 RA (%)	SC-2 RA (%)
1456		2-Hydroxy-4-methoxyacetophenone		2.2		
1457		<i>cis</i> -Cadina-1(6),4-diene	0.1			
1464		Geranyl acetone			0.1	
1483		γ -Muuroolene			0.2	
1486		(<i>E</i>)-Methyl isoeugenol	0.1			
1487		Amorpha-4,7(11)-diene			0.4	
1491		β -Bisabolene	1.6			
1494		<i>trans</i> -Muuroola-4(14),5-diene	0.1			
1504		δ -Amorphene	0.2			
1509		α -Muuroolene			0.3	
1519		β -Sesquiphellandrene			0.3	
1524		10- <i>epi</i> -Cubebol			1.4	
1533		δ -Cadinene			0.9	
1540		Dihydroactinidiolide				0.7
1547		α -Cadinene			0.2	
1576		Thymohydroquinone		11.8		
1579		Neryl isovalerate			4.4	
1589		Spathulenol			13.2	
1593		Caryophyllene oxide			9.5	
1604		Salvia-10(14)-en-1-one			1.9	
1615		Isospathulenol			0.8	
1621		Humulene epoxide II			1.8	
1625		Torilenol			2.6	
1641		1- <i>epi</i> -Cubenol			1.1	
1645		Caryophylla-3(15),7(14)-dien-6-ol			0.5	
1652		Guaia-6,10(14)-dien-4b-ol			0.9	
1655		α -Muurolol			4.6	
1669		α -Cadinol			7.1	
1675		<i>cis</i> -Calamene-10-ol			0.5	
1701		Amorpha-4,9-dien-2-ol			6.7	
1755		Oplopanone			0.5	4.3
1756		4-Hydroxy-3-methoxycinnamaldehyde		1.4		
1759		(<i>E</i>)- Coniferyl alcohol		7.1		18.1
1796		14-Hydroxy- α -muuroolene			0.3	
2090		Abietatriene			0.4	
		Aliphatic compounds	1.5			
		Aromatic compounds	86.7	69.1	19.7	18.7
		Monoterpene hydrocarbons	7.1		5.0	
		Sesquiterpene hydrocarbons	3.5		2.3	
		Oxygenated sesquiterpenes			53.4	5.0
		Diterpene hydrocarbons			0.4	
		Total identified	97.9	69.1	80.8	23.7

RI – Retention index on HP-5 column; RA – relative area; t – traces (<0.1%), SM-1 – essential oil of *S. montana*; SM-2 – extract of waste water after hydrodistillation of *S. montana*; SC-1 – essential oil of *S. cuneifolia*; SC-2 – extract of waste water after hydrodistillation of *S. cuneifolia*.

Table 2. Antioxidant activity of volatiles of two *Satureja* plant species.

Sample	DPPH IC ₅₀ (mg/mL)	R-Mo IC ₅₀ (mg/mL)
SM-1	28.90±1.14	5.55±0.00
SM-2	10.24±0.12	2.25±0.00
SC-1	19.00±1.25	0.48±0.00
SC-2	7.04±0.55	0.80±0.00
T	10.97±0.84	27.54±0.43
C	14.99±0.65	20.39±0.09
TQ	9.69±0.54	22.73±0.26

T – thymol; C – carvacrol; TQ – thymoquinone.

has also been characterized as a phenol-chemotype with differences in the content of thymol and carvacrol.

The essential oil obtained from plant material of *S. cuneifolia* (SC-1) had a lower content of carvacrol (17.7%), but was characterized by a high content of oxygenated sesquiterpenes (53.4%), with spathulenol (13.2%) and caryophyllene oxide (9.5%) as the main constituents. Thirty compounds were identified, representing 80.8% of the total oil content. The extract obtained from waste water after hydrodistillation of the same plant material (SC-2) revealed a high concentration of (*E*)-coniferyl alcohol (18.1%). Similarly, according to the literature data *S. cuneifolia* also shows high variability in the chemical composition of its essential oil, in some cases even higher than *S. montana*. BEZIĆ *et al.* (2005) also investigated *S. cuneifolia* from Dalmatia, though their results significantly differ from those presented above. The major constituents of this wild savory oil were sesquiterpenes β -cubebene (8.7%), spathulenol, and β -caryophyllene (BEZIĆ *et al.* 2005), as well as linalool (18.2-17.2%) and carvacrol (16.0-5.0%) (SKOČIBUŠIĆ *et al.* 2004). Moreover, linalool (19.9%) and α -pinene (12.3%) were found to be the dominant compounds in essential oil of *S. cuneifolia* from Serbia (ŠAVIKIN *et al.* 2010).

As indicated above, essential oils obtained from *Satureja* species showed significant variability in their chemical composition. Our results support the fact of highly complex chemical polymorphism of this genus. Differences in the essential oil profiles of these two species reflect influences of the origin of the plant material, as well as the environmental conditions, on the nature of plant chemical composition.

Antioxidant activity. The antioxidant activity of samples of *S. montana* and *S. cuneifolia* was evaluated by two methods, DPPH and total antioxidant activity using the phosphomolybdenum method (Table 2). The samples were able to reduce the stable violet DPPH radical to the yellow DPPH-H, reaching a 50% reduction with IC₅₀ values from 7.04±0.55 mg/mL, for the extract of the waste water

after hydrodistillation of *S. cuneifolia* (SC-2), to 28.90±1.14 mg/mL for essential oil of *S. montana* (SM-1). Extracts of waste water after hydrodistillation of both plant materials showed better ability to reduce stable DPPH radical than the standards thymol, carvacrol, and thymoquinone. This is probably due to the high concentration of other active compounds, such as (*E*)-coniferyl alcohol. To compare these results, total antioxidant activity of these two *Satureja* species was also tested using the phosphomolybdenum method. This test is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. Total antioxidant activity of the phosphomolybdenum model evaluates both water-soluble and fat-soluble antioxidants, i. e. total antioxidant capacity. Samples were able to reduce the molybdenum(VI) ions reaching 50% of reduction with IC₅₀ values from 0.48±0.00 mg/mL for essential oil of *S. cuneifolia*, to 5.55±0.00 mg/mL for essential oil of *S. montana*. Using this method, all samples showed better total antioxidant activity than thymol, carvacrol, and thymoquinone that were used as positive probes.

Different methodologies used to determine the antioxidant activities of plant extracts have led to diverse results, difficult to compare and often conflicting. These two methods were chosen because of their simplicity, rapidity, sensitivity and reproducibility (PRIETO *et al.* 1999). They are also very convenient for screening large numbers of samples with different polarity. To the best of our knowledge, this paper present the first investigation of total antioxidant activity of *S. montana* and *S. cuneifolia* volatiles by the phosphomolybdenum method. Moreover, there are only a few data results of scavenging DPPH radical by essential oil of these two *Satureja* species (EMINAGAOGLU *et al.* 2007, ČAVAR *et al.* 2008, KOŠAR *et al.* 2008, OKE *et al.* 2009), though none of them originated from Croatia. As expected from the differences in chemical composition, our results differ from those published earlier. IC₅₀ values from Turkish *S. cuneifolia* were lower than those from Croatia (EMINAGAOGLU *et al.*

2007). This could be because Turkish wild savory contains much higher levels of carvacrol than the Croatian species. Similarly, *S. montana* from Bosnia contained higher amounts of thymol and carvacrol (ĆAVAR *et al.* 2008), thereby demonstrating better scavenging activity against DPPH radical.

CONCLUSION

In conclusion, chemical differentiation of the volatiles of two *Satureja* species growing wild on Mt. Biokovo, Croatia, might be correlated to the ecological conditions in which they grow. Thereby, the results of antioxidant activity of isolated samples showed significant differences with those published earlier. The results of total antioxidant activity showed very high reducing power of these two species that could be interesting from a pharmaceutical and food-engineering standpoint.

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Botanica SERBICA



REZIME

Hemijski sastav i antioksidativna aktivnost dve vrste roda *Satureja* sa planine Biokovo, Hrvatska

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Hidrodestilovana volatilna ulja i ekstrakt vodenog ostatka posle hidrodestilacije nadzemnog dela *Satureja montana* i *Satureja cuneifolia*, sa planine Biokovo (Hrvatska), analizirani su GC-MS metodom. U četiri uzorka određeno je 60 jedinjenja. Glavni sastojak etarskog ulja *S. montana* je karvakrol (63.4%) i timol (19.4%), dok je timokinon (38.7%) glavni sastojak u ekstraktu vodenog ostatka posle hidrodestilacije. Najčešći sastojak kod *S. cuneifolia* su karvakol (17.7%) i spatulenol (13.2%). Ekstrakt vodenog ostatka posle hidrodestilacije ove vrste sadržao je (*E*)-koniferil alkohol (18.1%). Antioksidativna aktivnost testirana je uz pomoć dve spektrofotometrijske metode. Izolovani ekstrakti pokazuju aktivnost u redukciji stabilnih radikala i prelaznih metala, sličnu antioksidativnoj aktivnosti tinola, karvakrola i timokinona koji bu bili korišćeni kao pozitivne probe.

Ključne reči: *Satureja montana*, *Satureja cuneifolia*, GC-MS, antioksidativna aktivnost.

