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Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils

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ABSTRACT

Hydro-distilled volatile oils from the aerial parts of *Satureja montana* L., and *Satureja subspicata* Bartl. ex Vis., growing wild in Bosnia and Herzegovina, were analyzed by GC/MS. More than one hundred compounds were identified in both plant oils, representing 92.4–98.1% of the total oil. The major constituents of essential oils obtained from the plant material of *S. montana,* collected from two different localities, were thymol (31.7%), and geraniol (22.3%), respectively. The most abundant compounds in essential oils of *S. subspicata,* collected at two different stages of development, were thymol (28.6%), and spathulenol (37.6%), respectively. The screening of antimicrobial activity of essential oil samples was individually evaluated against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* using a paper disc diffusion method. All tested microorganisms were inhibited by essential oil samples. Antioxidant activity was tested using the DPPH radical-scavenging method. All samples showed activity comparable to thymol, which was used as a positive probe.

1. Introduction

Since ancient times, herbs and spices have been added to different types of food to improve the flavour and organoleptic properties. Especially popular today is the concept of food that combines nutritional and medicinal benefits. Many natural compounds isolated from plants have demonstrated a wide spectrum of biological activities. Among these various kinds of natural substances, essential oils from aromatic and medicinal plants receive particular attention as potential natural agents for food preservation. Moreover, essential oils are proven to have various pharmacological effects, such as spasmolytic, carminative, hepatoprotective, antiviral and anticarcinogenic effects (Bowles, 2004; Lahlou, 2004).

The genus *Satureja* belongs to the Lamiaceae family, and comprises over 30 species whose centre of distribution is located in the eastern part of the Mediterranean area (Šilić, 1984). These are annual or perennial semi-bushy aromatic plants that inhabit arid, sunny, stony and rocky regions. 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 different plant activities. The essential oil isolated from various *Satureja* species has certain biological properties, such as antimicrobial (Azaz, Demirci, Satil, Kurkcuoglu, & Baser, 2002; Azaz, Kurkcuoglu, Satil, Baser, & Tumen, 2005; Bezbradica, Tomovic, Vukasinovic, Siler-Marinkovic, & Ristic, 2005) and anti-HIV-1 (Yamasaki et al., 1998).

The *Satureja montana* L., known as winter savory, is frequently used in local spices and as a traditional medicinal plant. Due to the strong phenolic character of its essential oil, it is reminiscent of the taste and fragrance of commercial oregano and thyme oils. With regard to the presence of phenolic compounds, *S. montana* is known to posses a few pharmacological activities (Bezić, Skočibušić, & Dunkić, 2005; Skočibušić & Bezić, 2003). 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, Jancic, Bojovic, Milosavljevic, & Djoković, 2001; Šilić, 1979). *Satureja subspicata* Bartl. ex Vis. is a rare, endemic Dinaric species distributed in the eastern Mediterranean area (Šilić, 2005). Until now, we have found only one published report on phytochemical composition and antimicrobial activity of this species (Skočibušić, Bezić, & Dunkić, 2006). Thus, there is a considerable research interest in the assay of composition and/or biological properties of various *Satureja* essential oils. A survey of the literature reveals a few reports on

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the antioxidant activity of essential oil of *S. montana* (Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002; Radonic & Milos, 2003), and none for *S. subspicata*.

In the present work, we investigated the essential oil composition of *S. montana* and *S. subspicata* collected from two different localities in Bosnia and Herzegovina. In addition, the aim of this study was to determine the antioxidant and antimicrobial activities of the isolated essential oils that have not been reported to date.

2. Materials and methods

2.1. Plant material and reagents

Plant material of *S. montana* L. and *S. subspicata* Bartl. ex Vis. was collected in 2005 from two different localities in Bosnia and Herzegovina. A voucher specimen of each plant is deposited at the Faculty of Science, University of Sarajevo.

All applied reagents were of the highest purity available and purchased from the Sigma–Aldrich Chemical Company.

2.2. Sample preparation

Air-dried plant material of each individual was subjected to hydro-distillation for 2 h. The essential oils were extracted with dichloromethane and dried over anhydrous sodium sulphate. For the GC/MS analysis, samples of essential oils were dissolved in dichloromethane and, for antioxidant and antimicrobial assays, samples were dissolved in dimethylsulfoxide in concentrations of 3.0 mg/ml. Thymol was used as a positive probe for antioxidant and antimicrobial assays, and it was prepared in the same way as tested samples.

2.3. Microbial strains

The antimicrobial activity of essential oils was evaluated using a panel, which included laboratory control strains obtained from the American Type Culture Collection: three Gram-positive bacteria, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermdlis* ATCC 12228, *Bacillus subtilis* ATCC 6633, and two Gram-negative bacteria *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027.

2.4. Gas chromatography/mass spectrometry analysis of essential oil

Volatile compounds from the aerial parts of the plants were analyzed by GC/MS using Hewlett-Packard GC/MS system (GC 6890 series II; MSD 6890 series II). The GC conditions were: fused silica HP-5 column, carrier gas He (1.1 ml/min), temperature programme: 3 °C/min from 60 °C to 240 °C; the injection port temperature was 250 °C; detector temperature was 280 °C. Ionisation 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 C_8–C_{26} *n*-alkanes (Van Del Dool & Kratz, 1963). The identification of essential oil constituents was accomplished by visual interpretation, comparing their retention indices and mass spectra with literature data (Adams, 2001), by computer library search (HP Chemstation computer library NBS75K.L, NIST/EPA/NIH Mass Spectral Library 2.0 and Mass Finder 3 Computer Software and Terpenoids Library), and by the laboratory database.

2.5. 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging activity

The ability of the essential oils 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, Cuvelier, and Berset (1995). The concentrations of the tested samples ranged from 0.3 to 3.0 mg/ml. A portion of sample solution (200 μl) was mixed with 3 ml of 5.25×10^{-5} M DPPH· in absolute ethanol. Decreasing of absorbance of tested mixtures was monitored every 1 min for 30 min at 517 nm using a Perkin–Elmer Lambda 25 UV/Vis spectrophotometer. Absolute ethanol was used to zero the spectrophotometer; DPPH· solution was used as blank sample, and thymol was used as positive probe. The DPPH·solution was freshly prepared daily, stored in a flask covered with aluminium foil, and kept in the dark at 4 °C between measurements. All experiments were carried out in triplicate. The radical-scavenging activities of the tested samples, expressed as percentage inhibition of DPPH, were calculated according to the formula IC (%) = $[(A_0 - A_t)/A_0] \times 100$ (Yen & Duh, 1994), where A_0 and A_t are the absorbance values of the blank sample and the test sample, at particular times, respectively. Percent inhibition after 30 min was plotted against concentration, and the equation for the line was used to obtain the IC_{50} value. A lower IC_{50} value indicates greater antioxidant activity.

2.6. Antimicrobial screening

Antibacterial activity of essential oils was tested by the paper disc diffusion method according to the slightly modified National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 2001) using 100 μl of suspension of the tested microorganisms, containing 2.0×10^6 colony forming units (cfu/ml). Mueller-Hinton agar (15 ml), sterilized in a flask and cooled to 45–50 °C, was distributed to sterilized Petri dishes with a diameter of 9 cm. The filter paper discs (6 mm in diameter, Whatman No. 1) were individually impregnated with 10 μl of the sample dissolved in dimethylsulfoxide (DMSO), which was subsequently placed on the surface of the inoculated Petri dishes. The essential oils and thymol concentration in DMSO were adjusted to 3.0 mg/ml. The Petri dishes were kept at 4 °C for 2 h, and then incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimetres. Controls were set up with equivalent quantities of DMSO. Studies were performed in duplicate, and the developing inhibition zones were compared with those of reference discs. Antibiotic ampicillin (10 μg) was used as reference.

3. Results and discussion

3.1. GC/MS analysis

The essential oils of *S. montana* and *S. subspicata* were subjected to detailed GC/MS analysis in order to determine the impact of the locality and seasonal variations on their volatile constituents. The yield of oils ranged from 0.5 to 1.7%. Exactly 112 compounds were identified in four samples (Table 1).

In Sample 1 (*S. montana* from Trebinje), 57 compounds were identified, representing 93.5% of the total oil. The most abundant components were aromatic compounds (61.2%) and oxygenated sesquiterpenes (22.7%). The high percentages of thymol (31.7%) and carvacrol (23.3%) proved that this savory essential oil clearly belongs to the phenolic chemotype.

In contrast, the essential oil obtained form plant material of *S. montana*, collected near Konjic (Sample 2), was characterized by a high content of oxygenated monoterpenes (52.8%), with alcohols geraniol (22.3%) and terpinen-4-ol (10.3%) as the main constituents. Seventy-eight compounds were identified, representing the 98.1% of the total oil content.

In Sample 3 (*S. subspicata* from Konjic; collected at summer season), 79 compounds were identified, which represent 94.3% of total

Table 1
Chemical constituents of the essential oils of two *Satureja* species

RI	Compound	*S. montana*		*S. subspicata*	
		Sample 1 RA%	Sample 2 RA%	Sample 3 RA%	Sample 4 RA%
976	1-Octen-3-ol	–	0.6	0.4	–
995	3-Octanol	–	0.1	t	–
1030	1,8-Cineole	–	–	0.1	–
1066	*cis*-Sabinene hydrate	0.1	3.7	1.1	–
1072	*trans*-Linalool oxide (furanoid)	t	0.2	–	–
1088	*cis*-Linalool oxide (furanoid)	t	0.2	t	–
1097	*trans*-Sabinene hydrate	0.2	2.5	t	–
1098	Linalool	0.1	1.1	0.2	–
1112	1-Octen-3-yl acetate	–	–	0.1	–
1121	*cis*-*p*-Menth-2-en-1-ol	0.1	0.7	0.3	–
1126	2-Ethylhexanoic acid	0.1	–	–	–
1138	*cis*-*p*-Mentha-2,8-dien-1-ol	–	t	0.1	–
1143	*trans*-*p*-Menth-2-en-1-ol	t	0.5	0.2	–
1143	*trans*-Verbenol	0.2	0.2	–	–
1168	Borneol	2.9	4.8	3.2	t
1169	*cis*-Linalool oxide (pyranoid)	0.2	–	–	–
1173	*trans*-Linalool oxide (pyranoid)	0.2	–	–	–
1177	Terpinen-4-ol	0.8	10.3	1.1	–
1184	*p*-Cymen-8-ol	1.8	1.4	0.1	t
1190	α-Terpineol	1.9	1.5	2.0	–
1196	*cis*-Piperitol	–	0.2	t	–
1198	Myrtenol	–	0.1	–	–
1208	*trans*-Piperitol	0.1	0.4	0.2	–
1226	Piperiton epoxide	–	–	0.3	–
1228	*cis*-Carveol	–	–	0.1	–
1229	Nerol	–	2.0	–	–
1235	Thymol methyl ether	–	0.5	0.1	–
1238	Pulegone	–	–	0.1	–
1238	*trans*-Chrysanthenyl acetate	–	0.1	–	–
1239	Cumin aldehyde	t	0.2	–	–
1241	Neral	–	0.5	t	–
1244	Carvacrol methyl ether	–	1.1	0.3	–
1251	Thymoquinone	2.8	0.1	–	–
1251	*cis*-Piperitenone oxide	–	–	0.2	–
1258	Geraniol	0.1	22.3	–	–
1266	Geranial	–	1.1	–	–
1273	*n*-Nonanoic acid	–	–	–	0.1
1282	2-Ethylmenthone	0.2	–	–	–
1286	Bornyl acetate	–	0.1	–	–
1292	Thymol	31.7	3.8	28.6	0.1
1294	*o*-Acetanisole	–	0.4	–	–
1300	*p*-Cymen-7-ol	–	0.2	–	t
1306	Carvacrol	23.3	10.6	27.9	0.3
1335	*cis*-Piperitol acetate	–	0.1	–	–
1348	Piperitenone	–	–	3.5	–
1350	α-Terpinyl acetate	–	0.1	–	–
1354	Thymol acetate	0.1	–	0.6	–
1360	Eugenol	0.1	t	t	–
1370	Piperitenone oxide	–	t	1.3	–
1372	α-Copaene	–	0.1	–	–
1375	Carvacrol acetate	–	–	0.4	–
1384	β-Bourbonene	–	0.4	0.1	–
1387	Geranyl acetate	–	0.1	–	–
1390	β-Elemene	–	0.1	–	–
1400	(*Z*)-Jasmone	–	–	0.1	–
1419	β-Caryophyllene	–	2.9	1.1	–
1428	β-Copaene	–	0.1	t	–
1441	Aromadendrene	–	0.1	0.4	–
1443	4-*t*-Butylcatehol	1.1	–	–	–
1451	α-Humulene	–	0.1	0.2	
1453	Geranylacetone	–	–	0.1	t
1459	*allo*-Aromadendrene	–	0.1	–	–
1475	γ-Muurolene	–	0.1	0.4	–
1478	Germacrene D	–	1.9	0.4	t
1493	*epi*-Cubebol	–	–	0.3	0.3
1495	Bicyclogermacrene	–	1.0	–	–
1506	β-Bisabolene	–	0.7	0.4	–
1512	γ-Cadinene	–	0.1	0.2	–
1513	BHT	0.1	0.1	–	–
1513	Cubebol	–	–	0.1	0.6
1523	δ-Cadinene	t	0.2	0.5	t
1526	Dihydroactinidiolide	0.4	0.1	0.6	0.9
1536	α-Cadinene	–	0.1	0.1	t

(*continued on next page*)

Table 1 (*continued*)

RI	Compound	*S. montana*		*S. subspicata*	
		Sample 1 RA%	Sample 2 RA%	Sample 3 RA%	Sample 4 RA%
1541	α-Calacorene	t	t	0.1	t
1554	Salviadienol	–	0.1	t	0.7
1564	(*E*)-Nerolidol	0.3	–	–	4.2
1564	β-Calacorene	–	–	0.1	–
1577	Spathulenol	3.0	3.1	9.0	37.6
1583	Caryophyllene oxide	7.7	5.2	2.4	6.8
1589	Viridiflorol	0.3	–	–	3.1
1590	β-Copaene-4-α-ol	–	–	0.2	–
1593	Salvial-4(14)-en-1-one	t	0.2	0.6	t
1608	Humulene epoxide II	0.3	0.3	–	2.0
1608	Guaia-6,10(14)-diene-4β-ol	–	0.1	–	–
1610	Torilenol	0.2	0.3	0.3	1.2
1628	1-*epi*-Cubenol	t	–	0.2	0.7
1631	Caryophylla-3(15),7(14)-dien-6-α-ol	0.5	0.1	0.1	1.5
1635	Caryophylla-3(15),7(14)-dien-6-β-ol	2.2	0.4	0.5	3.0
1638	Isospathulenol	t	0.2	0.6	0.6
1641	epi-α-Cadinol	t	–	t	3.3
1644	3-*iso*-Thujopsanone	0.2	0.2	0.3	–
1647	Cubenol	–	–	–	1.7
1649	β-Eudesmol	0.2	–	–	–
1654	α-Cadinol	0.5	–	0.4	6.1
1657	14-Hydroxy-β-caryophyllene	2.6	0.4	0.3	1.0
1671	14-Hydroxy-9-*epi*-β-caryophyllene	4.1	0.8	0.2	2.7
1682	Khusinol	0.5	0.4	t	–
1684	6α-Hydroxygermacra-1(10),4-diene	–	0.1	–	–
1685	Eudesma-4(15),7-dien-1-β-ol	t	0.1	0.1	8.3
1691	Junicedranol	–	–	0.1	–
1715	14-Hydroxy-α-humulene	–	t	0.3	0.9
1735	Oplopanone	0.1	–	0.2	–
1761	Benzyl benzoate	0.2	–	0.2	0.5
1765	*n*-Tetradecanoic acid	–	–	t	0.2
1775	14-Hydroxy-α-muurolene	–	–	t	0.1
1970	*n*-Hexadecanoic acid	1.0	–	t	2.6
2099	*n*-Heneicosane	t	0.1	0.1	t
2199	*n*-Docosane	t	0.2	t	t
2299	*n*-Tricosane	0.1	0.6	t	t
2399	*n*-Tetracosane	0.3	1.2	0.1	0.3
2500	*n*-Pentacosane	0.3	1.6	0.2	0.5
2600	*n*-Hexacosane	0.3	2.4	0.2	0.5
	Alkanes	2.1	6.8	1.1	4.2
	Aromatic compounds	61.2	18.4	58.2	0.9
	Monoterpene hydrocarbons	–	–	0.1	–
	Oxygenated monoterpenes	7.1	52.8	14.0	t
	Sesquiterpene hydrocarbons	0.4	8.1	4.6	0.9
	Oxygenated sesquiterpenes	22.7	12.0	16.3	86.4
	Total identified	93.5	98.1	94.3	92.4

RI, Retention index on HP-5 column; RA, relative area; t, traces (<0.1%), –, not detected. Sample 1 – collection at Trebinje; Sample 2 – collection at Konjic; Sample 3 – collection at Konjic (summer season); Sample 4 – collection at Konjic (autumn season).

oil content. Aromatic compounds (58.2%) dominated in this oil, with thymol (28.6%), and carvacrol (27.9%), as the most abundant.

Sample 4 (*S. subspicata*. from Konjic; collected at autumn season) showed significant differences in qualitative and quantitative composition from Sample 3. Forty-three compounds were identified in this sample, representing the 92.4% of the total content of oil. Oxygenated sesquiterpenes were dominant in this sample, as 86.4% of total identified constituents. The amount of the major constituent, spathulenol, in the oil obtained from the plant material after flowering, was high (37.6%), in comparison to the next most abundant compounds, eudesma-4(15),7-dien-1-β-ol (8.3%), and caryophyllene oxide (6.8%).

As indicated above, essential oils obtained from *Satureja* species showed significant variability in their chemical composition. Generally, *S. montana* essential oils were characterized by high percentages of the monoterpene phenols, such as thymol and carvacrol. However, literature review showed variation between chemical compositions, depending of location and stages of development. *S. montana* from the central part of the Balkan Peninsula (Slavkovska et al., 2001) is mostly linalool chemotype, with high content of *p*-cymene. The oxygen-containing phenolic monoterpenes, carvacrol and thymol, are main constituents in Croatian winter savory (Bezić et al., 2005; Mastelić & Jerković, 2003; Milos, Radonic, Bezic, & Dunkic, 2001; Radonic & Milos, 2003; Skočibušić & Bezić, 2003, 2004), but *S. montana,* from Serbia and Montenegro, contains thymol and *p*-cymene as major components of its essential oil (Bezbradica et al., 2005). The high percentages of the phenols thymol and carvacrol in oil of our *S. montana* from Trebinje are, as expected, in agreement with results published earlier, while volatile constituents of *S. montana* from Konjic constitute the first report on savory essential oil of the geraniol chemotype.

According to the presence and quantity of dominant compounds, the essential oil of investigated populations of *S. subspicata* significantly differs from previously published data (Skočibušić et al., 2006). Croatian *S. subspicata* essential oil has carvacrol and *p*-cymene as the most abundant compounds, while volatile oil obtained form the species of the Bosnia and Herzegovina origin, collected in the summer season, contains almost the same amounts of

thymol and carvacrol (56.5% in total). Surprisingly, the composition of *S. subspicata* essential oil, obtained form plant material collected after flowering, was greatly changed and was oil rich in oxygenated sesquiterpenes (86.4%). The observed differences between those two *S. subspicata* essential oils confirm the influence of the stage of development on the nature of plant chemical composition.

In conclusion, chemical differentiation of Satureja essential oils might be correlated with the geographic origin of the populations and ecological conditions in which they grow and even suggest existence of a new chemotype.

3.2. 1,1-Diphenyl-2-picrylhydrazyl radical-scavenging activity

The antioxidant activity of the *S. montana* and *S. subspicata* essential oils has been evaluated by DPPH radical-scavenging test (Fig. 1). Assessed samples were able to reduce the stable violet DPPH radical to the yellow DPPH-H, reaching 50% of reduction with IC_{50} values ranging from 5.49 ± 0.26 mg/ml, for Sample 1, to 21.0 ± 0.21 mg/ml, for Sample 4 (Table 2). Those values are comparable to thymol (IC_{50} = 13.3 ± 0.19 mg/ml), whose antioxidant properties are already known (Moure et al., 2001; Yanishlieva, Marinova, Gordon, & Raneva, 1999).

Phenolic constituents in *S. montana* essential oil (Sample 1), thymol and carvacrol (55.0% in total) are responsible for the overall reactivity of the savory oil towards DPPH radical. Our findings are similar to those reported earlier by Radonic and Milos (2003) who examined *S. montana* from Croatia, using the β-carotene bleaching method and thiobarbituric acid method.

Despite the fact that *S. subspicata* oil (Sample 3) has a high percentage of phenolic compounds (56.6%), significant contributors to the high antiradical effect, it showed considerably lower activity than did Sample 1. Those unexpected results might be explained by the presence of some minor oxygen-containing terpenoids, known as pro-oxidants, e.g. nerolidol (4.2%) (Ruberto & Baratta, 2000).

In addition to thymol and carvacrol (14.4% in total), the effectiveness (in reducing of stable DPPH radical) of savory essential oil of the geraniol chemotype (Sample 2), is probably due to the high content of this allylic alcohol (22.3%), previously identified as a potential antioxidant (Choi, Song, Ukeda, & Sawamura, 2000).

S. subspicata oil (Sample 4), rich in sesquiterpenes (87.3%), exhibited the poorest antioxidant activity. Observed results are the consequence of very low amounts of phenolics (0.4%), which have a crucial role in the radical-scavenging activity in the DPPH· test.

To the best of our knowledge, no data have been published on antioxidant activity, using the DPPH· method, on *S. montana* and *S. subspicata* essential oils. The literature outlines different approaches for determination of the antioxidant activities of the plant extracts. Therefore, different methodological approaches lead to scattered results, which are hardly comparable and often conflicting. We chose the DPPH radical-scavenging method due to its simplicity, rapidity, sensitivity and reproducibility (Koleva et al., 2002). This method is also very convenient for the screening of large numbers of samples with different polarity.

3.3. Antimicrobial screening

The antimicrobial activities of *S. montana* and *S. subspicata* essential oils were evaluated by a paper disc diffusion method against Gram-positive, and Gram-negative bacteria. Essential oils exhibited antimicrobial activity against the tested strains, but in variable degree. Results are comparable to the antibiotic ampicillin, used as a positive probe (Table 3). Thymol was also tested, due to its content of essential oils, and its known antibacterial properties (Helander et al., 1998). The data indicated that Gram-positive *B. subtilis* was the most sensitive strain tested to the oils of *S. montana* and *S. subspicata*. Gram-negative *P. aeruginosa* is known to have a high level of intrinsic resistance to virtually all known antimicrobials and antibiotics, due to a very restrictive outer membrane barrier, highly resistant even to synthetic drugs (Mann, Cox, & Markham, 2000). However, both samples of *S. montana*, and Sample 3 (*S. subspicata*) inhibit growth of this bacterium. Confirming previous reports, it was found that the strength and spectrum of activity varied between investigated *Satureja* species and Gram-positive bacteria were generally more sensitive to the effects of the oils.

Table 2
Free radical-scavenging activity of *S. montana* and *S. subspicata* essential oils

	S. montana		*S. subspicata*		Thymol
	Sample 1	Sample 2	Sample 3	Sample 4	
IC_{50} (mg/ml)	5.49 ± 0.26	18.9 ± 0.19	15.6 ± 0.15	21.0 ± 0.21	13.3 ± 0.19

IC_{50}, the concentration required to inhibit radical formation by 50%. Sample 1 – collection at Trebinje; Sample 2 – collection at Konjic; Sample 3 – collection at Konjic (summer season); Sample 4 – collection at Konjic (autumn season).

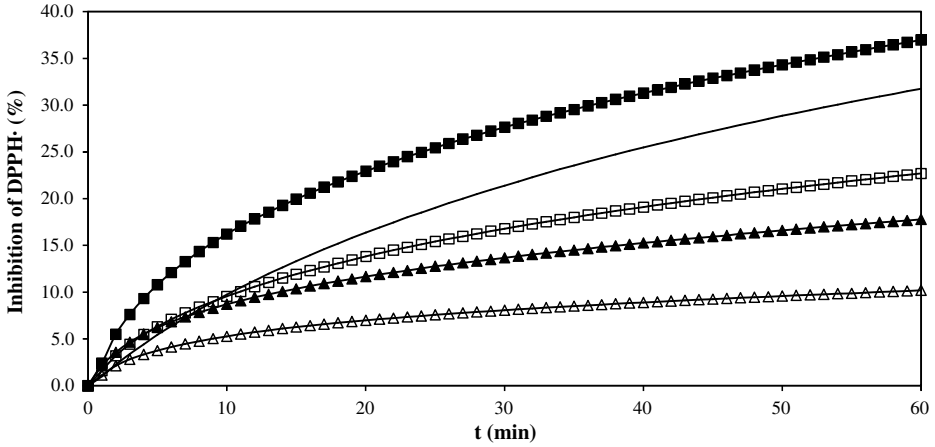

Fig. 1. Antioxidant activity of *S. montana* and *S. subspicata* essential oils (3 mg/ml) (■, *S. montana* (Sample 1); ♦, *S. montana* (Sample 2); □, *S. subspicata* (Sample 3); ◇, *S. subspicata* (Sample 4), –, thymol).

Table 3
Inhibition zones (mm) of the *S. montana* and *S. subspicata* essential oils

	S. montana		*S. subspicata*		Thymol	Ampicillin
	Sample 1 (10 μl/disc)	Sample 2 (10 μl/disc)	Sample 3 (10 μl/disc)	Sample 4 (10 μl/disc)		
Staphylococcus aureus ATCC 6538	15	11	8	10	11	41
Staphylococcus epidermidis ATCC 12228	10	11	0	13	10	22
Bacillus subtilis ATCC 6633	15	13	12	15	11	33
Escherichia coli ATCC 8739	11	9	0	11	12	16
Pseudomonas aeruginosa ATCC 9027	10	10	10	0	11	0

Sample 1 – collection at Trebinje; Sample 2 – collection at Konjic; Sample 3 – collection at Konjic (summer season); Sample 4 – collection at Konjic (autumn season).

However, the presence of the hydroxyl group seems to be more important for the antimicrobial activities of these compounds than the ability to expand and consequently to destabilize the bacterial membrane. Thymol and *p*-cymene have almost the same structures, although *p*-cymene lacks the hydroxyl group present in thymol that results in an increase of the antibacterial activity. In addition, it has to be considered that the other components, oxygenated monoterpenes and sesquiterpenes, such as α-terpineol, geraniol, and spathulenol, contribute to the antimicrobial activity of the oil.

4. Conclusion

Although essential oils of *S. montana* and *S. subspicata* have significant differences in their chemical compositions, all samples showed very effective and similar antioxidant and antibacterial activities. The results of this study suggest the possibility of using the essential oil of these two *Satureja* species as natural food preservatives, and potential sources of antibacterial ingredients for the food and pharmaceutical industry. Our results suggest that the essential oils of those species may warrant further investigation for their potential therapeutic efficacy.

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