Bioactivity of essential oils from cultivated winter savory, sage and hyssop

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Species of the Lamiaceae family have enjoyed a rich tradition of use for flavoring, food preservation, and medicinal purposes, due to their curative and preventive properties. Cultivated winter savory (*Satureja montana* L.), sage (*Salvia officinalis* L.) and hyssop (*Hyssopus officinalis* L.) are produced for seed, herb, and essential oil. Dominant compounds in *S. montana* essential oil were carvacrol (43.2%) and thymol (28.4%), while *cis*-thujone (27.1%) and camphor (19.3%), followed by *trans*-thujone and 1,8-cineole were the major compounds in *S. officinalis* essential oil. As for *H. officinalis* essential oil, *cis*- and *trans*-pinocamphone (41.1% and 20.5%, respectively) were the most abundant compounds, followed by β-pinene. *S. montana* essential oil exhibit the highest antimicrobial properties, as well as antioxidant capacity, compared to other tested essential oils. Furthermore, *H. officinalis* essential oils showed higher antioxidant activity than that of *S. officinalis*. The aim of this investigation was to determine the composition and bioactivity of essential oils of mentioned varieties. Presented results show that *S. montana* essential oil could be proposed as a valuable source of natural preservatives.

Key words: *Satureja montana; Salvia officinalis; Hyssopus officinalis; antibacterial; antioxidant*

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1. INTRODUCTION

Species of the family Lamiaceae have a long and a rich tradition of use for flavoring, food preservation, and medicinal purposes, due to their curative and preventive properties (Carović-Stanko et al., 2016). The majority of aromatic species belong to the Lamiaceae family, which is one of the largest families among the dicotyledons (Venkateshappa and Sreenath, 2013).

Winter savory (*Satureja montana*) is well known as a medicinal herb, mainly as a muscle pain reliever, tonic, and carminative agent in order to treat stomach and intestinal disorders such as cramps, nausea, indigestion, and diarrhea (Tepe and Cilkiz, 2016). It has a strong and spicy taste, and therefore it is used as a flavoring agent in salads, soups, sauces, stews, and lentil dishes (Wesołowska et al., 2014).

Sage (*Salvia officinalis*) has been used for culinary purposes as spice and preservative throughout history, but today it is commonly used to flavor meat, seafood, and cheese (Mapes and Xu, 2014). In traditional medicine, sage has been used to treat mild dyspepsia (such as heartburn and bloating), excessive sweating, age-related cognitive disorders, and inflammations in the throat and skin (Ghorbani and Esmaeilizadeh, 2017). Sage leaf (*Salviae officinalis folium*) has been listed in European Pharmacopoeia and many others (Ph. Eur. 7.0., 2010).

Despite its slightly bitter taste and minty flavor, hyssop (*Hyssopus officinalis*) is commonly used for centuries to produce flavors and fragrances in food, mainly sauces, and seasonings, and in bitters and liqueurs (Dehghanzadeh et al., 2012). Apart from this, it is used in folk medicine as a carminative, tonic, antiseptic, expectorant and cough reliever (Fathiazad et al., 2011).

Because of bioactive components in their essential oils, characterized by specific taste and fragrance, mentioned plants are popular today in the concept of functional food. In recent years, many research studies have been conducted to find new biological effects of plants including antioxidant, antimicrobial, anticancer, hypoglycemic and hypolipidemic effects. Sage represents a most common medicinal plant that is cultivated and collected from natural habitats. However, *S. montana* and especially, *H. officinalis*, are rarely cultivated and their natural habitats are constricted to sparse population area. Due to the extensive harvest of these plants in their natural habitats, comparison of quality and biological activity of essential oil between cultivated and wild plants is important to

all parties which use these species.
Bearing in mind numerous properties of savory, sage and hyssop, the aim of this paper was to determine chemical composition and content, as well as biological activity of essential oils of these plants grown in our collection and to compare these findings to available literature data.

2. MATERIALS AND METHODS

2.1. Plant material

As a part of medicinal plants collection of the Institute of Field and Vegetable Crops in Novi Sad, located in Bački Petrovac, at the Department for Alternative Crops and Organic Production, winter savory (*S. montana* L., variety "Domaći"), sage (*S. officinalis* L., variety "Primorska") and hyssop (*H. officinalis* L., variety "Domaći ljubičasti") are produced for essential oil extraction. Above-ground parts of selected plants were collected in July 2017. Voucher specimens were confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), Faculty of Sciences, University of Novi Sad. Voucher specimens were referenced as 2-1561 (*S. montana*), 2-1548 (*S. officinalis*) and 2-1567 (*H. officinalis*).

2.2. Essential oil extraction

Dried samples of winter savory, sage and hyssop were subjected to hydro-distillation using an all-glass Clevenger-type apparatus to extract essential oils according to the method outlined by the European Pharmacopoeia (Ph. Eur. 7.0., 2010). In order to extract the essential oils, 100 g of the plant material was placed in 1 L conical flask and connected to the Clevenger apparatus. Distilled water was added to the flask (500 mL) and heated to the boiling point. The steam in combination with the essential oils was distilled into a graduated cylinder for 4 h and then separated from the aqueous layer. Essential oils were kept refrigerated until further analysis.

2.3. GC and GC-MS analysis

The gas chromatographic-mass spectrometric analysis was performed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 Network transmission quadrupole mass spectrometer (Agilent, Santa Clara, USA), in positive ion-electron impact (EI) mode. The separation of individual compounds was achieved using non-polar HP-5 fused silica capillary column with 30 m × 0.25 mm i.d., 0.25 µm film thickness. The GC oven temperature was programmed from 60 °C to 285 °C at a rate of 3 °C/min. Helium was used as carrier gas; inlet pressure was 20.3 kPa; linear velocity was 1 mL/min at 210 °C. Injector temperature: 250 °C; injection mode: splitless. MS scan conditions: MS source temperature, 230 °C; MS Quad temperature, 150 °C; energy, 70 eV; mass scan range, 40–550 amu. The identification of components was carried out on the basis of retention indices followed by comparison with reference spectra (Wiley and NIST databases) and literature data.

2.4. Antibacterial activity

The antimicrobial activity was evaluated using control strains obtained from the American Type Culture Collection. Four Gram-positive bacteria: *Bacillus cereus* (ATCC 11778), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228), and four Gram-negative bacteria: *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enteritidis* (ATCC 13076), and *Proteus hauseri* (ATCC 13315). The activity of essential oils was tested by a modified broth microdilution method according to the National Committee for Clinical Laboratory Standards (CLSI, 2012). A serial doubling dilution of the tested essential oils was prepared in a 96-well microtiter plates over

Table 1. The essential oil composition of winter savory (*S. montana*).

#	Compound name[a]	RI[a]	%m/m
1	α-thujene	924	0.6
2	α-pinene	932	0.6
3	n.i.	942	tr
4	camphene	946	0.3
5	1-octen-3-ol	975	0.4
6	β-pinene	975	0.2
7	myrcene	989	1.1
8	α-phellandrene	1004	0.2
9	δ-3-carene	1010	0.1
10	α-terpinene	1015	1.7
11	p-cymene	1024	8.9
12	limonene	1027	0.5
13	1,8-cineole	1029	0.3
14	*cis*-β-ocimene	1036	tr
15	γ-terpinene	1057	7.5
16	*cis*-sabinene hydrate	1065	0.5
17	terpinolene	1088	tr
18	n.i.	1099	0.1
19	linalool	1100	0.5
20	*cis*-thujone	1106	tr
21	camphor	1143	tr
22	*trans*-pinocamphone	1159	tr
23	borneol	1163	0.4
24	*cis*-pinocamphone	1172	0.1
25	terpinen-4-ol	1175	0.5
26	α-terpineol	1189	0.1
27	carvacrol, methyl ether	1242	0.4
28	n.i.	1282	tr
29	thymol	1292	28.4
30	carvacrol	1301	43.2
31	α-copaene	1375	tr
32	β-bourbonene	1384	tr
33	*trans*-caryophyllene	1419	1.4
34	β-copaene	1429	tr
35	aromadendrene	1439	tr
36	α-humulene	1453	tr
37	γ-muurolene	1477	0.1
38	viridiflorene	1496	0.1
39	n.i.	1501	tr
40	β-bisabolene	1510	0.7
41	γ-cadinene	1515	0.1
42	δ-cadinene	1524	0.2
43	n.i.	1578	tr
44	caryophyllene oxide	1583	0.3
45	n.i.	1904	tr
46	n.i.	1931	tr
47	n.i.	2147	tr
48	n.i.	2164	tr
	Monoterpene hydrocarbons		21.1
	Oxigenatedmonoterpenes		75.0
	Sesquiterpene hydrocarbons		2.6
	Oxigenatedsesquiterpenes		0.3
	Other		0.4
	Total identified		99.5

[a] n.i. stands for not identified compounds; tr - traces.
[b] RI, retention indices as determined on HP-5 column using homologous series of C_8-C_{30} alkanes.

Table 2. The essential oil composition of sage *S. officinalis*.

#	Compound name[a]	RI[a]	%m/m
1	*cis*-salvene	846	0.4
2	*trans*-salvene	856	tr
3	n.i.	918	tr
4	tricyclene	922	0.1
5	α-thujene	925	0.1
6	α-pinene	932	3
7	camphene	947	4.6
8	sabinene	972	0.1
9	β-pinene	976	1.6
10	myrcene	989	0.8
11	α-phellandrene	1005	tr
12	α-terpinene	1016	0.2
13	*p*-cymene	1024	0.4
14	limonene	1028	4.4
15	1,8-cineole	1031	11.5
16	γ-terpinene	1057	0.4
17	*cis*-sabinene hydrate	1066	0.1
18	terpinolene	1088	0.2
19	*trans*-sabinene hydrate	1103	0.1
20	linalool	1108	0.3
21	*cis*-thujone	1110	27.1
22	*trans*-thujone	1119	12.3
23	α-campholenal	1126	tr
24	*iso*-3-thujanol	1138	0.1
25	n.i.	1143	tr
26	camphor	1147	19.3
27	*trans*-pinocamphone	1159	tr
28	borneol	1164	0.9
29	terpinen-4-ol	1175	0.4
30	α-terpineol	1188	tr
31	n.i.	1196	tr
32	bornyl acetate	1284	0.5
33	*trans*-sabinylacetate	1291	0.1
34	*trans*-carvyl acetate	1337	tr
35	*trans*-caryophyllene	1419	1.7
36	n.i.	1438	tr
37	α-humulene	1454	2.4
38	9-*epi*-*trans*-caryophyllene	1461	tr
39	viridiflorene	1496	tr
40	caryophyllene oxide	1582	0.3
41	viridiflorol	1591	3.4
42	n.i.	1598	0.1
43	humulene epoxide II	1609	0.7
44	n.i.	1630	tr
45	n.i.	1673	0.1
46	n.i.	1781	tr
47	n.i.	1806	tr
48	isopimara-9(11),15-diene	1913	tr
49	n.i.	1932	tr
50	n.i.	2001	tr
51	manool	2061	1.9
52	n.i.	2094	tr
	Norterpenes		0.4
	Monoterpene hydrocarbons		15.9
	Oxigenated monoterpenes		72.7
	Sesquiterpene hydrocarbons		7.5
	Oxigenated sesquiterpenes		1
	Diterpene hydrocarbons		tr
	Oxigenated diterpenes		1.9
	Total identified		99.6

[a] n.i. stands for not identified compounds; tr - traces.
[b] RI, retention indices as determined on HP-5 column using homologous series of C_8-C_{30} alkanes.

the range of 454.4-0.22 µL/mL in inoculated Mueller-Hinton broth (MHB, HiMedia). The mixture was discharged from the last well in a row (100 µL). The test was performed in a total volume of 110 µL/mL with a final microbial concentration of 106 CFU/mL per well. The plate was incubated for 24 h at 37 °C. The same tests were performed simultaneously for growth control (MHB + test organism), sterility control (MHB + test oil), and positive control (MHB + gentamicin+ test organism). Gentamicin was prepared in sterile water and diluted in MHB to obtain concentrations in a range of 16 to 0.016 µg/mL. Additionally, susceptibility to gentamicin was confirmed using a quantitative assay for determining the MIC (gentamicin Test Strip Liofilchem®) according to the manufacturers instructions. Microbial growth was determined by adding 10 µL of 0.01% resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide, HiMedia) aqueous solution. The MIC was defined as the lowest concentration of the samples inhibiting visible growth (blue colored pellet on the bottom of the wells after the addition of resazurin). To determine the MBC, the broth was taken from each well without visible growth and inoculated in Mueller-Hinton agar (MHA) for 24 h at 37 °C. The MBC was defined as the lowest sample concentration killing 99.9% of bacterial cells.

2.5. Antioxidant activity

Total potential antioxidant activity of tested essential oils was assessed based on their scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH-test) free radicals (Panda 2012). Reaction medium for DPPH-test was 3 mL aliquot of DPPH solution (24 mg DPPH in 100 mL methanol diluted to reach absorbance 0.980±0.02 at 517 nm) and 100 µL of essential oil of varying dilutions in methanol. The solution in the test tubes was shaken and incubated in the dark for 30 min at room temperature. Calculations were done using ascorbic acid calibration curve and results were expressed as µg ascorbic acid equivalents/mL essential oil required for scavenging 50% of tested radicals (IC_{50}). Lower values of IC_{50} indicate greater antioxidant activity.

3. RESULTS AND DISCUSSION

3.1. Chemical characterization of essential oils

Gas chromatographic and mass spectrometric analysis of the essential oil of winter savory (*S. montana*) showed 48 compounds, which represent 99.5% of the essential oil. The dominant compounds (higher than 5%) in the essential oil were carvacrol with 43.2% and thymol with 28.4%, followed by *p*-cymene (8.9%) and γ-terpinene (7.5%) (Table 1). Nevertheless, γ-terpinene and *p*-cymene are precursors of thymol and carvacrol and therefore some authors proposed that they are produced by a similar mechanism (Papadatou et al., 2015). Variety "Domaći" in general can be classified as phenol-rich chemotype since carvacrol and thymol are the most abundant compounds (Bezić et al., 2009).

Sage essential oil had 52 compounds, where the major compounds were *cis*-thujone with 27.1% and camphor with 19.3%, followed by *trans*-thujone with 12.3%, and 1,8-cineole with 11.5% (Table 2). Other abundant components presented in amount above 1% were: camphene (4.6%), limonene (4.4%), viridiflorol (3.4%), α-pinene (3.0%), α-humulene (2.4%), manool (1.9%), *trans*-caryophyllene (1.7%) and β-pinene (1.6%).
Qualitative analysis of the essential oil showed that *S. officinalis* variety "Primorska" belonged to Chemotype A, which is rich in *cis*-thujone and camphor (Cvetkovikj et al., 2015). However, the sum of toxic thujones, *cis*- and *trans*-thujone, was high (39.4%) according to the Perry et al. (1999) who classified in

Table 3. The essential oil composition of sage *S. officinalis*.

#	Compound name[a]	RI[a]	%m/m
1	n.i.	881	tr
2	n.i.	919	tr
3	α-thujene	925	0.3
4	α-pinene	932	0.7
5	camphene	947	0.1
6	thuja-2,4(10)-diene	952	tr
7	sabinene	972	1.7
8	β-pinene	975	12
9	myrcene	990	1.5
10	α-terpinene	1016	0.1
11	*p*-cymene	1023	0.1
12	β-phellandrene	1028	4.1
13	1,8-cineole	1030	0.3
14	*cis*-β-ocimene	1036	tr
15	*trans*-β-ocimene	1046	0.1
16	γ-terpinene	1057	0.2
17	*cis*-sabinene hydrate	1066	0.1
18	linalool	1100	0.4
19	*cis*-thujone	1106	0.1
20	*trans*-thujone	1116	0.1
21	nopinone	1136	0.1
22	*trans*-pinocarveol	1140	0.1
23	n.i.	1157	1.8
24	*trans*-pinocamphone	1158	20.5
25	pinocarvone	1162	2.8
26	*cis*-pinocamphone	1174	41.1
27	terpinen-4-ol	1179	0.7
28	α-terpineol	1190	0.2
29	myrtenol	1195	1.1
30	*trans*-2-hydroxy-pinocamphone	1246	0.1
31	methyl myrtenate	1296	0.1
32	myrtenyl acetate	1324	0.1
33	n.i.	1336	tr
34	α-copaene	1375	tr
35	β-bourbonene	1384	0.6
36	α-gurjunene	1409	0.2
37	*trans*-caryophyllene	1419	1.5
38	β-copaene	1429	tr
39	n.i.	1444	tr
40	α-humulene	1453	0.2
41	9-*epi*-*trans*-caryophyllene	1461	0.7
42	*cis*-muurola-4(14),5-diene	1466	tr
43	germacrene D	1482	1.7
44	n.i.	1489	tr
45	bicyclogermacrene	1497	1.3
46	γ-cadinene	1515	tr
47	δ-cadinene	1523	0.2
48	elemol	1550	1
49	n.i.	1568	tr
50	spathulenol	1577	0.4
51	caryophyllene oxide	1583	0.2
52	veridiflorol	1601	0.1
53	10-epi-γ-eudesmol	1619	tr
54	γ-eudesmol	1632	0.2
55	τ-cadinol	1641	0.1
56	β-eudesmol	1650	0.2
57	α-eudesmol	1654	0.2
58	n.i.	1662	tr
59	n.i.	1669	tr
	Monoterpene hydrocarbons		20.9
	Oxigenatedmonoterpenes		67.8
	Sesquiterpene hydrocarbons		6.4
	Oxigenatedsesquiterpenes		2.4
	Other		0.1
	Total identified		99.4

[a] n.i. stands for not identified compounds; tr - traces.
[b] RI, retention indices as determined on HP-5 column using homologous series of C_8-C_{30} alkanes.

this group all essential oils with 39-44% of thujone. However, a high concentration of toxic thujones seems to be characteristic to sage leaves cultivated in some countries under specific conditions (Maksimović et al., 2007; Raal et al., 2007).

The essential oil of hyssop (*H. officinalis*) had 59 compounds, where the most abundant compounds were *cis*- and *trans*-pinocamphone with 41.1% and 20.5%, respectively, followed by β-pinene with 12.0% (Table 3). Other components presented in amounts above 1% were: β-phellandrene (4.1%), pinocarvone (2.8%), sabinene (1.7%), germacrene D (1.7%), myrcene (1.5%), *trans*-caryophyllene (1.5%), bicyclogermacrene (1.3%), myrtenol (1.1%) and elemol (1.0%). Some authors found that the main components of the *H. officinalis* essential oil (also from Serbia) were *cis*-pinocamphone (42.9%), *trans*-pinocamphone (14.1%), germacrene-D-11-ol (5.7%) and elemol (5.6%) (Mitić and Đorđević, 2000). However, the chemical composition of *H. officinalis* essential oil from Bulgaria had *cis*-pinocamphone (48.98%–50.77%), β-pinene (13.38%–13.54%), *trans*-pinocamphone (5.78%–5.94%) and β-phellandrene (4.44%–5.17%) as the major compounds (Hristova et al., 2015).

3.2. Antimicrobial properties

The highest antimicrobial properties showed *S. montana* essential oil. The MIC was between 1.77 and 3.55 µL/mL, while MBC was between 3.55 and 7.10 µL/mL (Table 4). *S. montana* essential oil was more effective than streptomycinin case of *E. coli*, *E. faecalis*, *P. aeruginosa*, *S. epidermidis* and *P. hauseri*, and more effective than gentamicin in case of *E. coli* and *E. faecalis*. However, other oils (*S. officinalis* and *H. officinalis*) showed lower efficiency than synthetic antibiotics and *S. montana* essential oil.

Obtained results show that *S. montana* essential oil contains mainly carvacrol (43.2%) and thymol (28.4%) (Table 1). Previous investigations confirmed that antimicrobial activity of essential oils was strongly correlated with the content of terpenoid phenols such as carvacrol and thymol (Benbelaïd et al., 2014), which can inhibit the growth of both Gram-positive and Gram-negative bacteria (Memar et al., 2017). Carvacrol and thymol have desired antimicrobial effect due to the change in permeability and depolarization of the cytoplasmic membranes of the bacteria (Xu et al., 2008).

Essential oil of *S. montana* from Macedonia showed high antimicrobial activity; MIC ranged from 12.5 µL/mL against *S. epidermidis* to 50 µL/mL against *P. aeruginosa* and *C. albicans* (Kundaković et al., 2014). However, *S. montana* essential oil from Croatia showed significant activity against fungi and Gram positive bacteria, especially *Bacillus subtilis*, *S. epidermidis*, and *Listeria innocua*, and among Gram negative bacteria extreme sensitivity was detected in *E. coli* (Marin et al., 2012). High antimicrobial potential classifies *S. montana* essential oil as a natural source of compounds that can be used in the treatment of foodborne, as well as wound and other infections, and for general health improvement, as well (Mihajilov-Krstev et al., 2014).

The results of antimicrobial activity of essential oil from the leaves of *S. officinalis* grown in Serbia confirmed the activity against *B. subtilis*, *S. aureus*, *E. coli* and *S. enteritidis* in two different concentrations; 1% and 2%, in comparison to ampicillin (Miladinović and Miladinović, 2000). While, the essential oil of *S. officinalis* from Portugal showed very weak antimicrobial activity (Miguel et al., 2011). The same was concluded for *S. officinalis* essential oil from Greece that was lacking a noticeable antibacterial action since the MIC values recorded against all pathogens (*Klebsiella oxytoca*, *K. pneumonia* and *E. coli*) were above 150 µg/mL (Fournomiti et al., 2015).

H. officinalis has been traditionally used for its antiseptic prop-

Table 4. Antimicrobial properties of essential oils, MIC and MBC [µL/mL].

	S. montana		*S. officinalis*		*H. officinalis*		Streptomycin	Gentamicin
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MIC
Bacillus cereus (ATCC 11778)	1.77	3.55	113.62	227.25	14.20	28.40	1	0.19
Escherichia coli (ATCC 8739)	1.77	3.55	56.81	113.62	227.25	227.25	4	2
Enterococcus faecalis (ATCC 29212)	1.77	7.10	454.50	454.50	454.50	454.50	96	8
Pseudomonas aeruginosa (ATCC 27853)	3.55	3.55	113.62	227.25	454.50	454.50	16	1
Salmonella enteritidis (ATCC 13076)	3.55	7.10	113.62	113.62	227.25	227.25	2	0.5
Staphylococcus aureus (ATCC 25923)	3.55	3.55	113.62	113.62	227.25	227.25	3	0.38
Staphylococcus epidermidis (ATCC 12228)	3.55	3.55	113.62	113.62	227.25	227.25	>1024.00	0.094
Proteus hauseri (ATCC 13315)	3.55	3.55	454.50	454.50	227.25	454.50	6	1

erties in the treatment of infectious disorders (Mahboubi et al., 2011). Some previous studies showed the significant activity of *H. officinalis* essential oil against Gram-positive bacteria (Baj et al., 2018; De Martino et al., 2009; Mahboubi et al., 2011). Other reported that *H. officinalis* essential oil MIC ranged from 15.625 to 250 µL/mL depending on bacterial strains (Özer et al., 2006).

3.3. Antioxidative activity

S. montana showed the highest antioxidant capacity when compared to other tested species (Table 5). *H. officinalis* and *S. officinalis* followed with 1.5 and 3-fold lower antioxidant activity, respectively. All available literature mostly performed DPPH-test on extracts of analyzed plants and only a few of them used essential oils, as it was the case in our research.

Table 5. Antioxidative activity of cultivated savory, sage and hyssop essential oils.

Species	IC_{50} [µg/mL]
Satureja montana	17.0±0.1
Salvia officinalis	50.0±0.4
Hyssopus officinalis	24.0±0.2

If compared with literature data, *S. montana* essential oil tested in this study has greater antioxidant capacity than other *S. montana* plants collected in nature (Ćavar et al., 2008; Coutinho de Oliveira et al., 2012), or compared with other Satureja species: 32 µg/mL in *S. cilicica* (Ozkan et al., 2007)nd 185.5 µg/mg in *S. cuneifolia* (Oke et al., 2007). DPPH-test of *S. officinalis* essential oil showed lower antioxidant capacity than those from published data which reported IC_{50} activity as 1.78 µg/L (Bozin et al., 2007). Results from other studies varied for *H. officinalis*: 16.37 µg/mL (Kizil et al., 2010) and 156.6 mg/mL (Džamić et al., 2013).

CONCLUSION

With on-going use of an artificial preservative in the food industry, in addition to the challenge of microbial resistance, there is growing concern over side effects of these compounds. Alternatives such as the use of herbal essential oils in food preservation that have no side effects and sometimes even positive effects have to be considered. According to the presented results, *S. montana* essential oils could be proposed as an invaluable source of natural preservatives. Despite cultivation practice, our results showed that essential oils obtained from commercially grown varieties have high biological activity and could be used instead of plants grown in nature. In this way, more raw materials could be produced without effect on natural gene pool and habitats of these species.

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