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Chemical composition of hyssop cv. "Domaći ljubičasti" essential oil and its antimicrobial activity

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Summary: Hyssop (*Hyssopus officinalis* L., Lamiaceae) is a perennial shrub or subshrub violet-blue flowers in verticillasters and spicy taste with a pungent flavour. Besides being used as a culinary herb for flavouring and food preservation, this plant is also an ornamental, bee attracting plant and a traditional remedy for respiratory diseases and digestive disturbances. Hyssop is an essential oil-bearing plant, and its essential oil (*Hyssopi aetheroleum*) is used in the pharmaceutical, perfume and cosmetics industries as well as in aromatherapy. The objective of this study was to determine the chemical composition of essential oil of hyssop cv. "Domaći ljubičasti", grown in Serbia, and investigate its antimicrobial activity against 16 bacteria, mainly pathogens in the food industry. A total of 61 compounds were detected in the hyssop essential oil. The bicyclic monoterpene ketones *cis*-pinocamphone (43.8%) and *trans*-pinocamphone (18.3%) were the most abundant, comprising 62.1%, followed by *β*-pinene (6.3%) and pinocarvone (6.1%). Hyssop essential oil expressed antibacterial activity against: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Proteus hauseri*, *Listeria monocytogenes*, *Rhodococcus equi*, *Listeria ivanovii*, *Salmonella* Enteritidis, *Enterococcus faecalis*, *Listeria innocua* and *Bacillus spizizenii*. Hyssop essential oil did not express antibacterial activity against *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, *Klebsiella aerogenes* and *Staphylococcus epidermidis*. Results of this study show that hyssop essential oil has potential for using as natural supplement for control of foodborne diseases of microbiological origin, as well as flavor compositions (herbaceous, camphor-like odour with warm and spicy undernotes), especially for meat products, sauces, soups and seasonings.
Key words: antibacterial activity, essential oils, hyssop, *Hyssopus officinalis*, pinocamphone

Introduction

Hyssop (*Hyssopus officinalis* L., Lamiaceae) is a polymorphous self-fertile plant species that grows as a perennial shrub or subshrub, up to 20-60 cm high. The root is strong branching, multi-headed and taproot. Stems are straight, woody and branched at the base. Leaves are opposite to each other, lanceolate, stalkless and toothless, dark green, covered with thin silky hairs, and bearing essential oil-producing glands. Flowers are hermaphrodite, tubular, two-lipped, clustered in upper leaf axils, usually 3-9 in the nodes, often forming a spike. Fruit is small (up to 2.5 mm in length), oblong trihedral achene, and dark brown (Judžentiene, 2016, Aćimović et al., 2019a).

Hyssop has quite a spicy taste with a pungent flavour (Jahantigh et al., 2016). Because of this, it is used as a culinary herb for flavouring and food preservation (Moro et al., 2011) of meat products, sauces, soups and seasonings, alcoholic beverages, bitters and liqueurs (Kizil et al., 2008, Jahantigh et al., 2016, Ozer et al., 2005, Baj et al., 2018). It is also an ornamental and bee attracting plant (Piccaglia et al., 1999). In Serbian folk medicine, hyssop is used in case of respiratory diseases (for chronic bronchitis and asthma relief) and digestive disturbances as a part of tea blends (Tucakov, 2006). The same use is also present in other traditional medicines such as Bulgarian (Nanova et al., 2007),

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Turkish (Said-Al Ahl et al., 2015), as well as Unani and Ayurveda (Fathiazad and Hamedeyazdan, 2011, Tahir et al., 2018). However, hyssop is mostly used for essential oil distillation. This essential oil is used in the pharmaceutical and perfume industries (perfumes, especially in *eau de cologne* and oriental bases) and cosmetics (for soaps) as well as in aromatherapy (Jahantigh et al., 2016, Judžentiene, 2016, Aćimović 2021).

Only *H. officinalis* ssp. *aristatus* grows wild on sunny and calcareous hillsides in Serbia as well as in Eastern Europe (Josifović, 1975, Piccaglia et al., 1999). Selected hyssop cultivars *H. officinalis* ssp. *officinalis* are commercially cultivated in France, Italy, Germany, Serbia, Montenegro, Slovenia, Croatia, Bosnia and Herzegovina, Bulgaria, Hungary, Holland, Poland, Moldova, Iran, China, India, Russia and USA (Gorunović et al., 1995, Kizil et al., 2008, Fraternale et al., 2004, Judžentiene, 2016, Zawislak, 2013). Several cultivars are being grown: Sophie, Erfurter Ysop, Blankyt, Hysop lekarsky, Cyrano (Nemeth-Zambori et al., 2017). The crop can be productive for approximately 10 years and the plants are harvested preferably during the blooming season to produce essential oil by steam distillation (Hamida et al., 2020).

The objective of this study was to determine the chemical composition of essential oil of hyssop cv. "Domaći ljubičasti", grown in Serbia, was used to investigate its antimicrobial activity against 16 bacteria, mainly pathogens in the food industry.

Material and Methods

Plant material

The commercial hyssop variety "Domaći ljubičasti" (*H. officinalis* ssp. *officinalis* f. *cyaneus*) were grown at the Institute of Field and Vegetable Crops Novi Sad, during 2019. During the flowering stage (July), the aboveground parts were cut, dried and used for essential oil extraction.

Essential oil extraction

The dried aboveground parts of hyssop (*Hyssopi herba*) were subjected to hydro-distillation using an all-glass Clevenger-type apparatus to extract essential oils (*Hyssopi aetheroleum*). The samples were ground, homogenized and made into a fine powder. To extract the essential oils, 100 g of the powder was placed in 1 l conical flask and connected to the Clevenger apparatus. 500 ml of distilled water was added to the flask and heated to the boiling point. The steam combined with the essential oils was distilled into a graduated cylinder for 4 h and then separated from the aqueous layer. The essential oil amount was meagre, and therefore it was extracted with n-hexane, dried over anhydrous sodium sulfate and evaporated. The obtained oil was kept refrigerated at +4 °C until required for further analysis.

GC/FID and GC/MS analysis

The essential oil composition was determined by GC/FID and GC/MS. The GC analysis was performed on Agilent 6890N GC system equipped with 5975 MSD and FID, using HP-5 MS column (30 m × 0.25 mm, 0.25 μm film thickness). The injection volume was 2 μL and the injector temperature was 200 °C with a 10:1 split ratio. Helium was the carrier gas and its flow rate was 1.0 mL/min (constant flow mode). The column temperature was linearly programmed in the range 60–280 °C at a rate of 3°/min and held at 280 °C for 5 min. The transfer line was heated at 250 °C. The FID detector temperature was 300 °C. EI mass spectra (70 eV) were acquired in the m/z range 35–550. The retention indices were experimentally determined using n-alkanes (C8–C20 and C21–C40) injected under the same chromatographic conditions. The identification of the compounds was based on the comparison of their retention indices (RI), their retention times (tR) and mass spectra with those obtained from authentic samples and/or the NIST AMDIS (Automated Mass Spectral Deconvolution and Identification System) software, Wiley libraries, Adams database and literature.13 Relative percentages of the identified compounds were computed from the GC/FID peak area

Antimicrobial activity

The antimicrobial activity of the tested sample was evaluated by using 16 strains from American Type Culture Collection. There were seven tested Gram-negative bacteria in this investigation: *E. coli* (ATCC 8739 and ATCC 10536), *K. aerogenes* (ATCC 13048), *P. hauseri* (ATCC 13315), *P. aeruginosa* (ATCC 27853), *S.* Enteritidis (ATCC 13076) and *S.* Typhimurium (ATCC 14028). There were nine tested Gram-positive bacteria: *B. cereus* (ATCC 11778), *B. spizizenii* (ATCC 6633), *E. faecalis* (ATCC 29212), *L. innocua* (ATCC 33090), *L. ivanovii* (ATCC 19119), *L. monocytogenes* (ATCC 19111), *R. equi* (ATCC 6939), *S. aureus* (ATCC 25923) and *S. epidermidis* (ATCC 12228).

According to the National Committee for Clinical Laboratory Standards, the antimicrobial activity of hyssop essential oil was tested by the modified broth microdilution method (NCCLS 2002). The microbial strains suspensions were prepared from overnight broth cultures and were adjusted to 0.5 McFarland standard turbidity (corresponding to 1×10^8 CFU/mL), using a densitometer DEN-1 (Biosan, Riga, Latvia). Serial doubling dilutions of the tested essential oil was prepared in a 96/well microtiter plate over the range of 454.4-0.22 μL/mL in inoculated Mueller-Hinton broth (MHB, HiMedia). From the last well in row 100μL of the mixture was discharged. The test was performed in a total volume of 110 μL/mL with a final microbial concentration 10^6 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. Microbial growth was determined by adding 10μl of 0.01% resazurin (7-Hydroxy-3*H*-phenoxazin-3-one 10-oxide, HiMedia) aqueous solution. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the samples inhibiting visible growth (blue coloured pellet on the bottom of the wells after the addition of resazurin). To determine the minimal bactericidal concentration (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.

Results and discussion

Chemical composition

Hyssop essential oil is a light-yellow liquid, with a herbaceous, camphor-like odour with warm and spicy undernotes (Judžentiene, 2016). A total of 61 compounds were detected in the hyssop essential oil, among which bicyclic monoterpene ketones *cis*-pinocamphone (43.8%) and *trans*-pinocamphone (18.3%) were the most abundant, comprising 62.1%. Other significant compounds were: *β*-pinene (6.3%), pinocarvone (6.1%), limonene (3.5%), elemol (2.5%), myrtenol (2.0%), germacrene D (2.0%), *trans*-caryophyllene (1.1%) and one unidentified compound (1.1%). Other compounds were present in an amount less than 1.0% (Table 1). A GC-FID chromatogram of hyssop essential oil is shown in Figure 1.

Hyssop showed considerable variations in the relative content of its major components. It could be related to genotype, location, and climatic conditions (Ogunwande et al., 2011). Furthermore, high intraspecific diversity of hyssop is essential for adaptive evolution to diversify habitats (Galambosi et al., 1993, Mutu et al., 2014). According to this research, as well as research done in previous years, hyssop from Institute of Field and Vegetable Crops Novi Sad "Domaći ljubičasti" belongs to the chemotype with dominant *cis*-pinocamphone (=isopinocamphone) (Aćimović et al., 2019a, Aćimović et al., 2019b). Other than this chemotype, there are others such as: *trans*-pinocamphone, linalool, limonene, *trans*-pinocamphone + *cis*-pinocamphone and bicyclgermacrene chomotype (Ogunwande et al., 2011).

Antibacterial activity

Hyssop essential oil expressed the highest antibacterial activity against *S. aureus* (28.40 and 56.81 μL/mL MIC and MBC, respectively), as well as against *E. coli* (ATCC 8739) and *B. cereus* (56.81 μL/mL both, MIC and MBC). Against *E. coli* (ATCC 10536), *P. hauseri*, *L. monocytogenes*, *R. equi* and *L. ivanovii* the MIC was 56.81 μL/mL of essential oil, while MBC was 113.63 μL/mL. *S.* Enteritidis has the same value for MIC and MBC (113.63μL/mL). At the same time, for *E. faecalis*, *L. innocua* and *B. spizizenii* the MIC was 113.63 μL/mL of essential oil, while MBC was 227.25 μL/mL. Hyssop essential oil did not express antibacterial activity against *P. aeruginosa*, *Salmonella* Typhimurium, *K. aerogenes* and *S. epidermidis* (Table 2).

H. officinalis essential oil activity varied in terms of Gram-positive and Gram-negative (Venditti et al., 2015). Similar to our study, some previous studies have observed that essential oils have more activity against Gram-positive than Gram-negative isolates. This is thought to be due to the more complex, rigid outer membrane of Gram-negative bacteria with lipopolysaccharide that limits the diffusion of hydrophobic compounds. The complex outer membrane is not present in Gram-positive bacteria, and the peptidoglycan cell wall provides less resistance against the hydrophobic compounds (Chouhan et al., 2017). It is evident that antimicrobial potential depends on the chemical composition of essential oil (Saeedi and Morteza-Semnani, 2009). For example, in the same study, *H. officinalis* ssp. *officinalis* (with *cis*-pinocamphone as the main compound) was not effective against tested bacteria, while ssp. *decumbens* (with linalool as the dominant compound) inhibited almost all bacteria (Mazzanti et al., 1998).

Hyssop essential oil exhibited a varying degree of inhibitory effect against two tested *Salmonella* strains. In our study hyssop essential oil had the efficacy against *S.* Enteritidis but had not the efficacy against *S.* Typhimurium. Previous researches conducted by Busani et al. (2004) and Musgrove et al. (2006) showed that antimicrobial resistance in *Salmonella* spp. is serotypically dependent. They reported that among tested *Salmonella* isolates, *S.* Typhimurium was the most prevalent serotype and demonstrated the greatest multiple resistance against tested antimicrobial agents. The mechanism of the antimicrobial resistance in *Salmonella* spp. is complex, including its resistance at the cellular level and the adaptive resistance (Penesyan et al., 2015). The mechanism of resistance at the cellular level is related to the presence of certain genes, while adaptive resistance can be explained by the ability of producing a biofilm (Corona and Martinez, 2013).

Furthermore, it is shown that *H. officinalis* oil exhibited concentration-dependent antibacterial activity (Saeedi and Morteza-Semnani, 2009). Literature review of the main essential oil components and antibacterial properties of *H. officinalis* is shown in Table 3.

Table 1. Chemical composition of hyssop essential oil

No	R.T. min	Compound	RI	%
1	4.611	NI	880	tr
2	5.451	NI	918	tr
3	5.628	*a*-Thujene	924	0.1
4	5.820	*a*-Pinene	931	0.3
5	6.231	Camphene	946	0.1
6	6.916	Sabinene	970	0.9
7	**7.031**	***β*-Pinene**	**974**	**6.3**
8	7.404	Myrcene	988	1.0
9	7.523	NI	992	0.1
10	8.286	*a*-Terpinene	1014	0.1
11	8.562	*p*-Cymene	1022	0.2
12	**8.724**	**Limonene**	**1026**	**3.5**
13	8.794	*β*-Phellandrene	1028	0.3
14	9.004	*cis*-*β*-Ocimene	1034	0.1
15	9.381	*trans*-*β*-Ocimene	1044	0.5
16	9.798	*γ*-Terpinene	1055	0.3
17	10.108	*cis*-Sabinene hydrate (IPP vs OH)	1063	0.4
18	10.954	Terpinolene	1086	0.1
19	11.377	Linalool	1098	0.6
20	11.648	*cis*-Thujone	1104	0.2
21	12.094	*trans*-Thujone	1114	0.1
22	12.291	NI	1119	0.1
23	12.976	Nopinone	1135	0.1
24	13.022	*trans*-Pinocarveol	1136	0.5
25	13.846	NI	1155	1.1
26	**13.994**	***trans*-Pinocamphone**	**1159**	**18.3**
27	**14.068**	**Pinocarvone**	**1160**	**6.1**
28	**14.646**	***cis*-Pinocamphone**	**1174**	**43.8**
29	15.089	NI	1185	0.1
30	15.261	*a*-Terpineol	1188	0.2
31	**15.513**	**Myrtenol**	**1194**	**2.0**
32	15.617	Methyl chavicol	1197	0.3
33	17.810	NI	1246	0.1
34	20.009	Methyl myrtenate	1296	0.2
35	21.256	Myrtenyl acetate	1324	0.2
36	21.770	*δ*-Elemene	1335	0.6
37	23.858	*β*-Bourbonene	1383	0.5
38	24.176	*β*-Elemene	1390	0.1
39	24.747	Methyl eugenol	1403	0.2
40	24.933	*a*-Gurjunene	1407	0.2
41	**25.345**	***trans*-Caryophyllene**	**1417**	**1.1**
42	25.753	*β*-Copaene	1427	0.1
43	26.410	6,9-Guaiadiene	1443	0.1
44	26.790	*a*-Humulene	1452	0.2
45	26.930	*trans*-*β*-Farnesene	1456	0.1
46	27.101	9-*epi*-*trans*-Caryophyllene	1459	0.7
47	**27.957**	**Germacrene D**	**1480**	**2.0**
48	28.267	NI	1488	0.1
49	28.603	Bicyclogermacrene	1496	0.9
50	29.609	NI	1521	0.1
51	29.702	*δ*-Cadinene	1552	0.1
52	**30.760**	**Elemol**	**1548**	**2.5**
53	31.903	Spathulenol	1575	0.7
54	32.121	Caryophyllene oxide	1581	0.5
55	32.928	NI	1600	0.2
56	34.046	*γ*-Eudesmol	1629	0.1
57	34.303	NI	1636	0.1
58	34.400	NI	1639	0.1
59	34.768	NI	1648	0.1
60	34.887	NI	1651	0.2
61	41.912	NI	1845	0.1
		Total		**100%**
		NI		**2.5%**

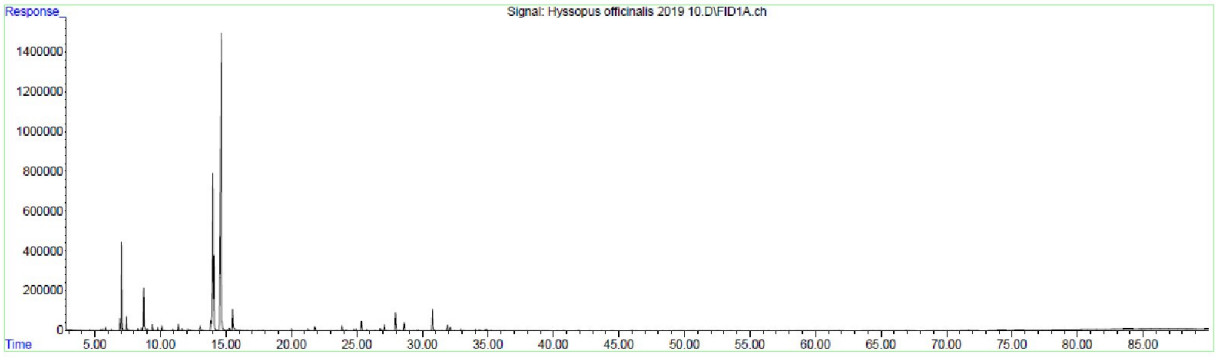

Figure 1. A GC-FID chromatogram of hyssop essential oil

Table 2. Antimicrobial properties of hyssop essential oil

Gram -	MIC (µL/mL)	MBC (µL/mL)	Gram +	MIC (µL/mL)	MBC (µL/mL)
Escherichia coli ATCC 8739	56.81	56.81	*Staphylococcus aureus* ATCC 25923	28.40	56.81
Escherichia coli ATCC 10536	56.81	113.63	*Bacillus cereus* ATCC 11778	56.81	56.81
Proteus hauseri ATCC 13315	56.81	113.63	*Listeria monocytogenes* ATCC 19111	56.81	113.63
Salmonella Enteritidis ATCC 13076	113.63	113.63	*Rhodococcus equi* ATCC 6939	56.81	113.25
Pseudomonas aeruginosa ATCC 27853	>454.50	>454.50	*Listeria ivanovii* ATCC 19119	56.81	113.63
Salmonella Typhimurium ATCC 14028	>454.50	>454.50	*Enterococcus faecalis* ATCC 29212	113.63	227.25
Klebsiella aerogenes ATCC 13048	>454.50	>454.50	*Listeria innocua* ATCC 33090	113.63	227.25
			Bacillus spizizenii ATCC 6633	113.63	227.25
			Staphylococcus epidermidis ATCC 12228	>454.50	>454.50

The *Z*-pinocamphone as the main compound (between 39.3 and 57.3%) was dominant in *H. officinalis* ssp. *officinalis* (Mazzanti et al., 1998, Kizil et al., 2010, Mahboubi et al., 2011, Aćimović et al., 2019b). The agar diffusion test did not show antimicrobial activity against *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Salmonella typhi*. In contrast, it showed more antimicrobial activity against *Klebsiella oxytoca*, *Escherichia coli*, *Enterococus faecalis* and *Staphylococcus aureus* was more active (Mazzanti et al., 1998). Furthermore, the disk diffusion test showed antimicrobial activity against *S. pyogenes*, *S. aureus*, *C. albicans* and *E. coli*, but not against *P. aeruginosa* (Kizil et al., 2010). Significant antibacterial activity was noted against *S. aureus*, *B. cereus* and *S. saprophyticus* while Gram-negative bacteria (*E. coli* and *P. aeruginosa*) were less sensitive (Mahboubi et al., 2011). Furthermore, the best antimicrobial activity was noted against *Bacillus cereus*, followed by *E. coli*, *S.* Enteritidis, *S. aureus* and *S. epidermidis* (Aćimović et al., 2019b). These results indicated the possibility that antimicrobial activity does not depend on the main compound (*Z*-pinocamphone), rather on the other compounds and their synergistic effect (Veras et al., 2012).

The dominant compound in *H. officinalis* ssp. *officinalis* was *E*-pinocamphone in f. *albus* (51.0%) and f. *ruber* (28.8%), where pink form (with almost equal amounts of both pinocamphone isomers) was more active against Gram-negative bacteria, especially against *B. subtilis* (Baj et al., 2018). Results also indicate synergistic and additive effects between essential oil compounds (Bassolé and Juliani, 2012, Boonyanugomol et al., 2017, Chouhan et al., 2017).

Generally, essential oils are comprised of two or three major components in relatively high concentrations (20-95%) while other components are present in traces. The major components of EOs determine their biological properties. However, some studies have demonstrated that EOs usually have higher antibacterial activity than the mixtures of their major components, suggesting that the minor

Table 3. Literature review of main components of essential oil and antibacterial properties of *H. officinalis*

Accession	*H. officinalis* ssp. *decumbens*	*H. officinalis* ssp. *officinalis*	*H. officinalis* ssp. *angustifolius*	*H. officinalis* ssp. *officinalis*	*H. officinalis*	*H. officinalis*	*H. officinalis* ssp. *aristatus*	*H. officinalis* f. *albus*	*H. officinalis* f. *ruber*	*H.officinalis* ssp. *officinalis*
Reference	Mazzanti et al. 1998	Mazzanti et al. 1998	Saeedi and Morteza-Semnani 2009	Kizil et al. 2010	Mahboubi et al. 2011	Dehghanzadeh et al. 2012	Venditti et al. 2015	Baj et al. 2018	Baj et al. 2018	Aćimović et al. 2019b
Main compounds in essential oil (%)	linalool 51.7% **1,8** cineole 12.3% limonene 5.1%	Z-pinocamphone 43.3% limonene 12.2% *β*-pinene 11.1%	dehydro-linalool 19.9% *β*-phellandrene 9.4% *β*-pinene 7.9%	Z-pinocamphone 57.3% *β*-pinene 7.2% terpinen-4-ol 7.1%	Z-pinocamphone 39.3% *E*-pinocamphone 22.1% 2-hydroxy-2,6,6-trimethylnorpinan-3-one 5.4%	thymol 19.0% *β*-bisabolol 10.62% carvacrol 7.7%	linalool 35.3% methyl eugenol 22.7% *Z*-*β*-ocimene 5.1%	*E*-pinocamphone 51.0% *β*-pinene 12.4% *E*-p-meth-2-en-1-ol 8.1%	*E*-pinocamphone 28.8% Z-pinocamphone 21.9% *β*-pinene 9.8%	Z-pinocamphone 41.1% *E*-pinocamphone 20.5% *β*-phellandrene 4.1%
	MIC/ MBC in %	MIC/ MBC in %	inhibition zone in mm/MIC in mg/ mL	inhibition zone in mm 5μL/10μ L	MIC/ MBC in mg/mL	MIC/ MBC inhibition zone in mm	inhibitio n zone in mm	MIC/ MBC in mg/mL	MIC/ MBC in mg/mL	MIC/MBC in μL/mL
B. subtilis			7.8/0.6					5.0/5.0	0.63/2.5	
B. cereus					1.0/1.0					14.2/28.4
E. faecalis	0.6/0.6	>1.2/>1.2					6.0			454.5/454.5
E. amylovora						7.8/7.8				
E. coli	0.3/0.3	>1.2/>1.2	-/1.3	20.3/23.3	4.0/4.0		8.9	5.0/10.0	5.0/5.0	227.3/227.3
Klebsiella sp.						6.0/6.0				
K. oxytoca	1.2/1.2	>1.2/>1.2								
K. pneumoniae								5.0/10.0	5.0/10.0	
M. luteus								2.5/5.0	2.5/5.0	
P. hauseri										227.3/454.5
P. mirabilis	1.2/1.2	>1.2/>1.2						5.0/10.0	5.0/10.0	
P. aeruginosa	0.3/0.3	>1.2/>1.2	-/2.5		4.0/4.0		6.0	5.0/10.0	5.0/10.0	454.5/454.5
S. enteritidis										227.3/227.3
S. typhi	0.6/0.6	>1.2/>1.2	7.2/0.6							
S. aureus	0.075/0.3	>1.2/>1.2	-/1.3	18.0/21.7	0.5/0.5		9.3	10.0/20.0	5.0/10.0	227.3/227.3
S. epideridis								5.0/10.0	2.5/5.0	227.3/227.3
S. saprophyticus					1.0/1.0					
S. pyogenes				19.0/23.6				0.63/1.25	0.31/0.63	
S. mutans								1.25/1.25	0.63/1.25	
S. pneumoniae								0.63/1.25	0.31/0.63	

components are critical to the synergistic activity (Bassolé and Juliani, 2012).

Linalool (35.3-51.7%) was the dominant compound in var. *decumbens* and ssp. *aristatus*, wild form of hyssop from Italy and France (Mazzanti et al., 1998, Venditti et al., 2015). Linalool may contribute to the greater antimicrobial activity of var. *decumbens* in comparison to *H. officinalis* (Mazzanti et al., 1998). In addition, dehydro-linalool (19.9%) was detected in *H. officinalis* ssp. *angustifolius* from Iran as the main compound (Saeedi and Morteza-Semnani, 2009). However, this compound is intermedier during synthesis of linalool from pinene (Kamatou and Viljoen, 2008).

Essential oil of *H. officinalis* from Iran contains thymol (19.0%), *β*-bisabolol (10.62%) and carvacrol (7.7%) as dominant compounds and shows high antimicrobial activities in vitro against *Klebsiella* sp. and *Erwinia amylovora*, two important plant pathogens (Dehghanzadeh et al., 2012). Furthermore, thymol is the main compound in *H. cuspidatus* from China, and this essential oil possesses contact toxicity against adult maize weevil (*Sitophilus zeamais*) (Li et al., 2013). Essential oil of *Hyssopus* sp. which contains thymol shows potential to be used as a natural pesticide.

Conclusions

The commercial variety of hyssop cv. "Domaći ljubičasti" has light-yellow essential oil, with a herbaceous, camphor-like odour with warm and spicy undernotes. GC-MS show the presence of 61 compounds, among which bicyclic monoterpene ketones *cis*-pinocamphone (43.8%) and *trans*-pinocamphone (18.3%) were the most abundant, comprising 62.1%. Other significant compounds were: *β*-pinene (6.3%) and pinocarvone (6.1%). Hyssop essential oil expressed antibacterial activity against 12 investigated bacteria (*S. aureus*, *E. coli*, *B. cereus*, *P. hauseri*, *L. monocytogenes*, *R. equi*, *L. ivanovii*, *S.* Enteritidis, *E. faecalis*, *L. innocua* and *B. spizizenii*). In contrast, it did not express activity against four investigated bacteria: *P. aeruginosa*, *Salmonella* Typhimurium, *K. aerogenes* and *S. epidermidis*. Further investigations will be focused on studying the antimicrobial activity of significant constituents of *H. officinalis* essential oil and potential synergistic or additive effects.

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Hemijski sastav etarskog ulja miloduha kultivara „Domaći ljubičasti" i ocena antimikrobne aktivnosti

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Sažetak: Miloduh (*Hyssopus officinalis* L., Lamiaceae) je višegodišnji žbun ili polužbun aromatičnog i oštrog mirisa zbog čega se koristi kao začinska biljka. Takođe je i ukrasna i medonosna biljka, ali i tradicionalni lek za bolesti organa za disanje i kod poremećaja varenja. Miloduh je cenjena eterično-aromatična biljka, a etarsko ulje se koristi u farmaceutskoj, parfimerijskoj i kozmetičkoj industriji kao i aromaterapiji. Cilj ovog istraživanja je bio da se determiniše hemijski sastav etarskog ulja miloduha kultivara „Domaći ljubičasti" gajenog u Srbiji, i da se ispita antimikrobna aktivnost na 16 bakterija, uglavnom patogena u prehrambenoj industriji. Ukupno 61 komponenta je detektovana u etarskom ulju, pri čemu su monoterpenski ketoni *cis*-pinokamfon (43.8%) i *trans*-pinokamfon (18.3%) bili najzastupljeniji, čineći 62.1%, a potom slede *β*-pinen i pinokarvon. Etarsko ulje miloduha je ispoljilo antibakterijsku aktivnost na: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Proteus hauseri*, *Listeria monocytogenes*, *Rhodococcus equi*, *Listeria ivanovii*, *Salmonella* Enteritidis, *Enterococcus faecalis*, *Listeria innocua* i *Bacillus spizizenii*, dok nije delovalo na *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, *Klebsiella aerogenes* i *Staphylococcus epidermidis*.

Ključne reči: antibakterijska aktivnost, etarsko ulje, *Hyssopus officinalis*, miloduh, pinokamfon