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Chemical composition and microbiological evaluation of essential oil from *Hyssopus officinalis* L. with white and pink flowers

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Abstract: *Hyssopus officinalis* L. is a common plant that is most usually found in three color forms - f. *cyaneus* (blue), f. *ruber* Mill. (purple/pink) and f. *albus* Alef (white). In the present work, we evaluated the chemical composition and antimicrobial activity of essential oils obtained from Polish-sourced white- and pink-flowered *H. officinalis*. Gas chromatography-mass spectrometry analysis of the essential oil has shown that both forms of color have a different content of main components. The principle essential oil component of white-flowered *H. officinalis* L. was pinocamphone (51%), while pink-flowered *H. officinalis* L. contained almost equal amounts of pinocamphone (28.8%) and isopinocamphone (21.9%). Of note, the essential oil of the pink form was more active against Gram-positive bacteria, especially against *Bacillus subtilis*.

Keywords: Hyssop; white flowers; pink flowers; pinocamphnone; isopinocamphone; antimicrobial activity.

1 Introduction

Hyssop (*Hyssopus officinalis* L.), while grown in many countries around the world, is a plant native to southwestern Asia and southern Europe. Due to its camphor aroma and bitter taste, it is often used as a kitchen spice. In both the fresh and the dried state, it is also a supplement to salads, meats, vegetables, cottage cheese and pâté. Moreover, in the production of vermouth and bitter liqueurs (Chartreuse and Benedictine) hyssop supplies the unique spicy taste [1,2].

Hyssop blooms come mainly in three color forms: f. *cyaneus* Alef. – with blue crown petals (the most popular variety), f. *ruber* Mill – with purple (pink) crown petals, and f. *albus* Alef. – with white flowers [3]. The correlation between the chemical composition of the essential oil and the color of hyssop flowers is rarely described [3,4]. However, genotypic variability studies have shown variations between phenotypes [5,6]

Hyssop is an aromatic plant, although the composition of essential oil is not homogeneous. Literature data on the composition of the essential oil of *H. officinalis* exhibits major differences in the content thereof. As the main ingredient, the authors most often noted reference to isopinocamphone [7-20], pinocamphone [21-23], myrtenyl acetate [24], α-pinene [25], 1,8-cineole [26], pinocarvone [27] and linalool [28]. The ISO 9841: 2007 standard for hyssop oil specifies the highest content for: isopinocamphone (25-45%), pinocamphone (8.0-25%) and β-pinene (7.0-20.0%) [29].

The aim of this work was to evaluate the chemical composition and antimicrobial activity of essential oil obtained from Poland white and pink-flowered *H. officinalis* L. The present work is a continuation of previous research [7,30].

2 Experimental

2.1 Plant material

Flowering shoots (30 cm from the top) in full bloom of *H. officinalis* were collected from the Botanical Garden of the Department of Pharmacognosy, Medicinal Plant Unit, Medical University of Lublin (22°33'50.868"E, 51°15'22.8312"N, 187 m above sea level) during August, 2013

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A B

Photo 1: *Hyssopus officinalis* L. with white (A) and pink (B) flowers (Medicinal Plant Unit of Medical University, Lublin, Poland).

(Photo 1). The plant material was identified by Dr. Elwira Sieniawska, a Medicinal Plant Unit employee. All raw material was then dried at room temperature. Specimen HOW2013/HOP2013 is deposited above the Department of Pharmacognosy.

2.2 Extraction of essential oils

Essential oil hydro-distillation was carried out using a Deryng apparatus according to the previously described procedure [31]. Herein, 40 g of air-dried plant material plus 500 ml distilled water underwent 3 h distillation. Subsequently, anhydrous sodium sulfate was used to remove the water after extraction. The hyssop essential oils (HEO) were then collected into dark glass bottles and stored in a refrigerator at 4°C. The distillation of the essential oil was repeated 3 times.

2.3 Gas chromatography

Chromatographic analysis was carried out according to the previously described procedure [32]. In so doing, a GC-MS: ITMS Varian 4000 GC-MS/MS (Varian, USA) equipped with a CP-8410 auto-injector and a 30 m x 0.25 mm VF-5ms column (Varian, USA) was used. In this study, film thickness was 0.25 µm, carrier gas was He 0.5 ml/min, injector and detector temperature were, respectively, at 250 and 200°C; split ratio was 1:50; and inject volume was 5 µl. A temperature gradient was first applied (50°C) for 1 minute, then increased by 4°C/min to 250°C, with 250°C held for 10 minutes. The ionization energy was 70 eV; the range recorded was 35-1000 *m/z* and the scan rate was 0.80 s per scan. Data acquisition and processing, and instrumental control were performed by the Varian MS Workstation Version 6.42.

Subsequently, a GC/FID GC Varian 3800 (Varian, USA) equipped with a CP-8410 auto-injector and a 30 m x 0.25 mm DB-5 column (J&W Scientific, USA) was employed. The film thickness was 0.25 µm, the carrier gas was He 0.5 ml/min, injector and detector FID temperatures were 260°C; split ratio was 1:100; and injection volume was 5 µl. A temperature gradient was then applied (50°C for 1 minute, then increased by 4°C/min to 250°C, with 250°C held for 10 minutes).

Qualitative analysis was carried out via MS spectra, these were compared with the spectra library by means of the NIST MS Search Program (NIST 08, 2005), as well as with the data available in literature [33]. Identity of the compounds was confirmed by their retention indices, wherein the retention indices were determined in relation to a homologous series of *n*-alkanes (C_{10}–C_{40}) under the same operating conditions. Retention indices were also compared with literature data [34-37]. The percentages of main components of the essential oil were presented assuming that the sum of peak areas for all identified constituents is 100%.

2.4 Antimicrobial activity

Essential oils from the two color forms of *H. officinalis* were screened for antibacterial and antifungal activities by way of the micro-dilution broth method, using Mueller-Hinton broth either as Mueller-Hinton broth with 5% lysed sheep blood for growth of non-fastidious and fastidious bacteria, respectively, or as Mueller-Hinton broth with 2% glucose for growth of fungi. Minimal inhibitory concentration (MIC) of tested essential oils were evaluated for the panel of the reference microorganisms which belonged to the American Type Culture Collection (ATCC), including Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 9027, *Proteus mirabilis* ATCC 12453), Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 10240, *Streptococcus pyogenes* ATCC19615, *S. pneumoniae* ATCC49619, *S. mutans* ATCC25175) and fungi (*Candida albicans* ATCC 10231, *C. parapsilosis* ATCC 22019).

Stock cultures were maintained at -70°C in Tripticase Soy broth (containing 16% (v/v) glycerol) for bacteria, and Sabouraud dextrose broth (containing 16% (v/v) glycerol) for yeasts, until the study was performed. Before the

experiments, each bacterial and yeast strain was passaged onto fresh Mueller-Hinton agar or Mueller-Hinton agar with 5% lysed sheep blood at 35° for 24 h, and onto Sabouraud dextrose agar at 30° for 48 h, respectively.

The essential oils dissolved in dimethylosulfoxide (DMSO), were first diluted to the concentration (10 mg/ml) in the aforementioned and recommended broths for determining the antibacterial and antifungal activity. Subsequently, using the same media, serial two-fold dilutions were made in order to obtain final concentrations of plant extracts ranging from 0.156 to 10 mg/ml. The sterile 96-well polystyrene microtitrate plates (Nunc, Denmark) were prepared by dispensing 200 µl of appropriate dilution of plant material in culture broth per well. The inocula were then prepared with fresh microbial cultures in sterile 0.85% NaCl to match the turbidity of 0.5 McFarland standard, and 2 µl were added to the wells to obtain a final density of 1.5 x 10^6 CFU/ml and 5 x 10^4 CFU/ml for bacteria and yeasts, respectively. After incubation (35°C for 18-24 h), the MICs were assessed visually as the lowest oil concentration showing complete inhibition in the growth of the tested microorganisms. Appropriate DMSO (at a final concentration 10%), a positive control (containing inoculum without plant material) and negative control (containing plant material without inoculum) were included on each microplate. Reference compounds – vancomycin, ciprofloxacin for bacteria and fluconazole for yeasts were purchased from Sigma-Aldrich, (St. Louis, USA).

Minimal bactericidal concentration (MBC) or minimal fungicidal concentration (MFC) was obtained by subculturing 5 µl from each well that showed thorough growth inhibition, from the last positive one and from the growth control onto recommended agar plates. The plates were incubated at 35°C for 24 h and the MBC/MFC was defined as the lowest concentration of essential oil without growth of microorganisms. Each experiment was repeated in triplicate.

2.5 Statistical analysis

The average values of the component contents were calculated using Excel (Microsoft, USA).

Ethical approval: The conducted research is not related to either human or animals use.

3 Results and discussion

The HEO content obtained via hydrodistillation was (on average) 0.7% of total dry weight for white-flowered plants (HWF) and 0.5% of total dry weight for pink-flowered plants (HPF). In the oil of the HWF form, 44 components were identified, and in HPF – 49 components. The percentages of chemical constituents of essential oils are shown in Table 1.

Main HEO component-content may vary depending on the plant developmental stage, the age of the plant, the manner and place of cultivation and date of harvest, the plant part harvested and way of distillation, as well as the phenotype. During the development of the plant, the main constituents of the essential oil vary: pinocamphone dominates before flowering, and, during flowering, isopinocamphone [37]. Regarding the age of the plant, first year plants exhibit higher pinocamphone content, while isopinocamphone content dominates in three-year-old plants [22]. As to the manner of cultivation, Tavakoli and Aghajani [38] saw that the main ingredients of *H. officinalis* (pinocamphone or isopinocamphone) varied depending on the degree of irritation. Regarding altitude of cultivation, Fraternale et al. [39], in assessing the influence of climate on the content of the main components, noted that the oil obtained from the raw material growing below the 100 m above sea level contour contained a higher percentage of pinocamphone, while the oil obtained from the 1000 m above sea level contour contained a higher level of isopinocamphone. Moreover, the oil obtained from plants planted below the 100 m contour contained a much lower percentage of linalool and camphor compared to the oil obtained from plants grown at higher altitudes. Regarding the plant parts, the main components of the HEO were isopinocamphone (49.7-57.7%) in all parts of the plant, while β-pinene and pinocarvone concentrations were variable for each organ, and pinocamphone content never exceeded 1.5% [39]. As to place of occurrence, according to literature, the main HEO component content may vary depending on the place of occurrence, even in a small area. In the study of Bernotiene & Butkiene [27], isopinocamphone (33.6 and 16.8%) was the main constituent in two sites, and pinocarvone (21.1 and 28.1%) was the main component in another two sites. Wesolowska et al. [40], when comparing methods of obtaining HEO (hydrodistillation and steam distillation) did not find significant differences in the isopinocamphone content (the main ingredient). This varied, however, from 40.07% to 45.45% depending on the method. With regard to phenotype, the chemical composition of the three forms of *H. officinalis* growing in Yugoslavia was found to

Table 1: Percentage composition of essential oil obtained from white- (HWF) and pink- (HPW) flowered *Hyssopus officinalis* L.

No	Compound	RI exp.*	RI lit.**	HWF***	HPF***
1	α-Thujene	979	979 [a]	0.5	0.4
2	α-Pinene	981	981 [a]	1.0	0.7
3	Camphene	986	986 [a]	0.2	0.2
4	Sabinene	993	993 [a]	3.0	2.2
5	β-Pinene	995	994 [a]	12.4	9.8
6	3-Octanone	996	996 [b]	0.3	0.0
7	Myrcene	997	997 [b]	3.9	1.7
8	α-Terpinene	1019	1019 [c]	0.1	0.2
9	*p*-Cymene	1026	1028 [d]	0.1	0.0
10	Limonene	1031	1031 [c]	1.4	1.0
11	1,8-Cineole	1033	1035 [c]	7.6	1.9
12	β-Ocimene	1045	1050 [d]	0.4	1.0
13	γ-Terpinene	1058	1062 [d]	0.1	0.1
14	*cis*-Sabinene hydrate	1070	1070 [d]	0.2	0.1
15	Terpinolene	1085	1085 [c]	0.1	0.1
16	Linalool	1097	1099 [c]	0.1	0.8
17	*trans*-Sabinene hydrate	1101	1102 [c]	0.1	0.0
18	*cis*-Thujone	1107	1108 [d]	tr	0.1
19	*trans*-Thujone	1119	1120 [d]	0.5	0.4
20	*cis-p*-Menth-2-en-1-ol	1126	1129 [c]	0.1	0.0
21	*trans*-Pinocarveol	1145	1143 [a]	0.3	0.1
22	*trans-p*-Menth-2-en-1-ol	1159	1149 [c]	8.1	0.4
23	Pinocamphone	1167	1167 [d]	51.0	28.8
24	Borneol	1179	1176 [a]	0.1	0.0
25	Isopinocamphone	1184	1175 [d]	1.9	21.9
26	Myrtenol	1203	1196 [d]	0.6	4.7
27	Myrtenyl acetate	1332	1327 [d]	-	0.1
28	Δ-Elemene	1342	1338 [d]	0.1	0.1
29	α-Copaene	1384	1377 [d]	0.0	0.1
30	β-Bourbonene	1392	1388 [d]	0.2	0.8
31	β -Elemene	1396	1391 [d]	0.1	0.2
32	Methyl eugenol	1404	1402 [a]	-	0.4
33	α-Gurjunene	1418	1410 [d]	-	0.1
34	β-Caryophyllene	1433	1429 [d]	1.0	0.9
35	β-Copaene	1444	1432 [d]	0.0	0.1
36	α-Guaiene	1461	1440 [d]	0.0	0.1
37	α-Humulene	1474	1455 [d]	0.2	0.2
38	Alloaromadendrene	1479	1473 [d]	0.0	0.2
39	Germacrene D	1488	1495 [d]	0.0	0.1
40	γ-Amorphene	1502	1506 [c]	1.7	5.2
41	Bicyclogermacrene	1515	1507 [d]	2.0	3.3
42	γ-Cadinene	1530	1514 [d]	0.0	0.1
43	Elemol	1561	1560 [d]	-	5.4
44	Spathulenol	1589	1589 [d]	0.3	1.0
45	Viridiflorol	1598	1606 [a]	0.1	0.8
46	Ni.	1619	-	0.0	0.8
47	γ-Eudesmol	1648	1632 [d]	0.0	1.3
48	*epi-alpha*-Cadinol	1657	1640 [d]	0.1	0.6
49	A - Eudesmol	1675	1675 [c]	0.1	1.6
50	Bulnesol	1683	1672 [d]	-	0.2
	Total identified compounds (%)			99.6	99.0
	Grouped components (%)				
	Monoterpene hydrocarbons			23.2	17.4
	Oxygenated monoterpenes			70.6	59.3
	Sesquiterpene hydrocarbons			4.9	10.2
	Oxygenated sesquiterpenes			0.6	10.9
	Other compounds			0.3	1.2

* RI exp. – Retention indices determined relative to a series of n-alkanes (C10-C40) on the non
-on the non-polar capillary column VF5ms; ** RI lit. - RIs obtained from literature data: a [34],b [35],c [36],d [37]; ***Percentage values are means of three determinations, Ni. – unidentified compound.

be predominantly pinocamphone, isopinocamphone and pinocarvone [3]. Phenotypic variability in the composition of essential oils was also found in the hyssop growing in Moldova. Herein, the principal HEO components in the examined forms were isopinocamphone and pinocamphone [4]. In the present study, pinocamphone (51%) was the dominating compound in the essential oil of white flowers, while in pink flowers, the content of pinocamphone and isopinocamphone was comparatively 28.7% and 21.9%, respectively.

A comparison of the chemical composition of the essential oils from white- and pink-flowered *H. officinalis* with literature data suggests that the forms present in Poland are f. *albus* Alef. and f. *ruber* Mill. However, further taxonomic studies are needed.

Table 2: Antimicrobial activity of essential oil obtained from white- (HWF) and pink- (HPW) flowered *Hyssopus officinalis* L. and reference compounds – vancomycin, ciprofloxacin for bacteria and fluconazole for yeasts.

	HWF		HPF		Reference compounds	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (μg/ml)	MBC (μg/ml)
Gram-positive bacteria					Vancomycin	
Staphylococcus aureus ATCC25923	10	20	5	10	0.98	7.81
Staphylococcus epidermidis ATCC12228	5	10	2.5	5	0.98	0.98
Micrococcus luteus ATCC10240	2.5	5	2.5	5	0.12	0.12
Bacillus subtilis ATCC6633	5	5	0.625	2.5	0.24	0.49
Streptococcus pyogenes ATCC19615	0.625	1.25	0.312	0.625	0.24	0.49
Streptococcus pneumoniae ATCC49619	0.625	1.25	0.312	0.625	0.24	0.49
Streptococcus mutans ATCC25175	1.25	1.25	0.625	1.25	0.98	0.98
Gram-negative bacteria					Ciprofloxacin	
Escherichia coli ATCC25922	5	10	5	5	0.004	0.004
Proteus mirabilis ATCC12453	5	10	5	10	0.03	0.03
Klebsiella pneumoniae ATCC13883	5	10	5	10	0.12	0.12
Pseudomonas aeruginosa ATCC9027	5	10	5	10	0.49	0.98
Yeast					Fluconazole	
Candida albicans ATCC102231	0.625	2.5	0.625	2.5	0.98	1.95
Candida parapsilosis ATCC22019	1.25	5	0.625	1.25	1.95	1.95

The essential oils from the two cultivars of hyssop growing in Poland, showed moderate activity against *Streptococcus pyogenes, S. pneumoniae, S. mutans, Candida albicans* and *C. parapsilosis*, and poor activity against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, S. epidermidis, Bacillus subtilis* and *Micrococcus luteus* (Table 2).

The essential oils from pink-flowered *H. officinalis* was more active against bacteria Gram-positive: *Staphylococcus aureus, S. epidermidis*, *Streptococcus pyogenes, S. pneumoniae* and *S. mutans*. Moreover, it reveals exceptional activity against *Bacillus subtilis*. The activity of the essential oil against bacteria Gram-negative and yeast of the *Candida* spp. were similar. Tested essential oils showed lower activity compared to the reference components.

The antimicrobial properties of essential oils depend on a whole group of compounds having very different chemical structures, therefore, the essential oil antimicrobial effects can be explained through several diverse mechanisms. Probably the high lipophilicity of essential oils is very significant because it determines the cell and mitochondrial membrane penetrative qualities (considered as 'good'), the disruption of the structure and functions of the membranes, as well as the enhanced permeability [41]. Furthermore, in studies conducted by Hristova et al. [42], pinocamphnone, isopinocamphone, α– and β–pinene HEO show synergistic effects against *Candida* spp. The essential oil obtained from the hyssop showed a stronger activity than did pure ingredients.

Dehghanzadeh et al. [43] analyzed the composition and antimicrobial properties of the essential oil obtained from *H. officinalis* growing in Iran, and found it chemically different from the previously described hyssop oils. GC/MS analysis showed that the main components of this oil were thymol> β-bisabolol> carvacrol. This oil showed high antibacterial activity against *Klebsiella* sp. and *Erwinia amylovora* (responsible for the occurrence of fire blight on pear and apple crops) and no activity against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Xannthomonas axonopodis pv citri, Acidovorax sp. Streptomyces scabies* and *Pseudomonas florescence*. Antibacterial and antioxidant properties of the essential oil obtained from *H. officinalis* grown in Turkey were studied by Kizil et al. [10]. They showed that the chemotype is rich in isopinocamphone> β-pinene> terpinen-4-ol, and was active against *Streptococcus pyogenes*, *Staphylococcus aureus, Candida albicans* and *Escherichia coli*, while there was no activity against *Pseudomonas aeruginosa*.

As revealed, hyssop-derived essential oils have different antimicrobial activity. However, the use of pure

oil may be limited due to its neurotoxicity [44], and HEO oil is contraindicated in patients with epilepsy and during pregnancy.

4 Conclusions

An analysis of essential oils of the two flower forms of *Hyssopus officinalis* L. grown in Poland with white and pink flowers showed different chemotypes. A comparison of the obtained results and the literature data indicate that the essential oil from white-flowered plants contains mainly pinocamphone and β-pinene, whereas oil obtained from pink-flowered plants contained almost equal amounts of pinocamphone and isopinocamphone.

Conflict of interest: Authors state no conflict of interest.

References

[1] Hoope HA., Drogenkunde I., Valter de Gruyter Verl. Eds. Berlin-New-York, 1975.

[2] Charles D.J., Hyssop. In: Antioxidant Properties of Spices, Herbs and Other Sources. Springer, New York, NY, 2012.

[3] Chalchat J.C., Adamovic D., Gorunovic M.S., Composition of oils of three cultivated forms of *Hyssopus officinalis* endemic in Yugoslavia: f. *albus* Alef., f. *cyaneus* Alef. and f. *ruber* Mill., J. Essent. Oil Res., 2001, 13(6), 419-421.

[4] Goncearіuc M., *Hyssopus officinalis* L. genotypes with different content and composition of essential oil, Buletinul ASM. Stiintele vietii, 2013, 1(319), 86-96.

[5] Németh-Zámbori É., Rajhárt P., Inotai K., Effect of genotype and age on essential oil and total phenolics in hyssop (*Hyssopus officinalis* L.), J. Appl. Bot. Food Qual., 2017, 90, 25-30.

[6] Mutu A., Clapco S., Martea R., Port A., Duca, M., Intraspecific genetic variability of *Hyssopus officinalis* L., Analele Stiintifice ale Universitatii Al. I. Cuza Din Iasi-Serie Noua-Sectiunea II A Genetica si Biologie Moleculara, 2014, 15(3), 1-8.

[7] Baj T., Kowalski R., Swiatek L., Modzelewska M., Wolski T., Chemical composition and antioxidant activity of the essentials oil of hyssop (*Hyssopus officinalis* L. ssp. *officinalis*), Annales UMCS, 2010, 23, 55-62.

[8] Nikolić M., Marković T., Marković D., Glamoclija J., Ćirić A., Smiljković, M., Soković M., Antimicrobial activity of three Lamiaceae essential oils against common oral pathogens, Balk. J. Dent. Med., 2016, 20(3), 160-167.

[9] De Martino L., De Feo V., Nazzaro F., Chemical composition and in vitro antimicrobial and mutagenic activities of seven Lamiaceae essential oils, Molecules, 2009, 14(10), 4213-4230.

[10] Kizil S., Hasimi N., Tolan V., Karatas H., Chemical composition, antimicrobial and antioxidant activities of hyssop (*Hyssopus officinalis* L.) essential oil, Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 2010, 38(3), 99.

[11] Kizil S., Toncer O., Ipek A., Arslan N., Saglam S., Mahmood Khawar K., Blooming stages of Turkish hyssop (*Hyssopus officinalis* L.) affect essential oil composition. Acta Agr. Scand. Sect. B Soil Plant Sci., 2008, 58(3), 273-279.

[12] Myadelets M.A., Domrachev D. V., Cheremushkina V.A., A study of the chemical composition of essential oils of some species from the Lamiaceae family cultivated in the Western Siberian Region, Russ. J. Bioorg. Chem., 2013, 39(7), 733-738.

[13] Said-Al Ahl H.A., Abbas Z.K., Sabra A.S., Tkachenko K.G., Essential oil composition of *Hyssopus officinalis* L. cultivated in Egypt. Int. J. Plant Sci. Ecol., 2015, 1(2), 49-53.

[14] Mitic V., Dordevic S., Essential oil composition of *Hyssopus officinalis* L. cultivated in Serbia, Facta universitatis-series: Physics, Chemistry and Technology, 2000, 2(2), 105-108.

[15] De Almeida L.F.R., Frei F., Mancini E., De Martino L., De Feo V., Phytotoxic activities of Mediterranean essential oils, Molecules, 2010, 15(6), 4309-4323.

[16] Cvijovic M., Djukic D., Mandic L., Acamovic-Djokovic G., Pesakovic M., Composition and antimicrobial activity of essential oils of some medicinal and spice plants. Chem. Nat. Compd., 2010, 46(3), 481-483.

[17] Mazzanti G., Battinelli L., Salvatore G., Antimicrobial properties of the linalool-rich essential oil of *Hyssopus officinalis* L. var. *decumbens* (Lamiaceae). Flavour Fragr. J., 1998, 13(5), 289-294.

[18] Jianu C., Golet I., Misca C., Jianu A.M., Pop G., Gruia A.T., Antimicrobial properties and chemical composition of essential oils Isolated from six medicinal plants grown in Romania Against Foodborne Pathogens, Rev. Chim. (Bucharest), 2016, 67(6), 1056-1061.

[19] Mohan M., Seth R., Singh P., Lohani H., Gupta S., Composition of the volatiles of *Hyssopus officinalis* (L.) and *Thymus serpyllum* (L.) from Uttarakhand Himalaya, Natl. Acad. Sci. Lett., 2012, 35(5), 445-448.

[20] Kizil S., Güler V., Kirici S., Turk, M., Some agronomic characteristics and essential oil composition of hyssop (*Hyssopus officinalis* L.) under cultivation conditions. Acta Sci Polon-Hort Cultus, 2016, 15(6), 193-207.

[21] Garg S.N., Naqvi A.A., Singh A., Ram G., Kumar S., Composition of essential oil from an annual crop of *Hyssopus officinalis* grown in Indian plains. Flavour Fragr. J., 1999, 14(3), 170-172.

[22] Kotyuk L.A., Hyssop composition depending on age and plants development phases, Biotechnol. Acta, 2015, 8(5), 55-63.

[23] Khan R., Shawl A.S., Tantry M.A., Determination and seasonal variation of chemical constituents of essential oil of *Hyssopus officinalis* growing in Kashmir valley as incorporated species of Western Himalaya. Chem. Nat. Compd., 2012, 48(3), 502-505.

[24] Fathiazad F., Mazandarani M., Hamedeyazdan S., Phytochemical analysis and antioxidant activity of *Hyssopus officinalis* L. from Iran, Adv. Pharm. Bull., 2011, 1(2), 63-67.

[25] Ogunwande I.A., Flamini G., Alese O.O., Cioni P.L., Ogundajo A., Setzer W.N., A new chemical form of essential oil of *Hyssopus officinalis* L.(Lamiaceae) from Nigeria. Int. J. Biol. Chem. Sci., 2011, 5(1), 46-55.

[26] De Elguea-Culebras G.O., Sánchez-Vioque R., Berruga M.I., Herraiz-Peñalver D., González-Coloma A., Andrés M.F., Santana-Méridas O., Biocidal potential and chemical composition of industrial essential oils from *Hyssopus officinalis*, *Lavandula x intermedia* var. *super* and *Santolina chamaecyparissus*. Chem Biodiversity. 2017, Accepted Author Manuscript. doi:10.1002/cbdv.201700313

[27] Bernotiene G., Butkiene R., Essential oils of *Hyssopus officinalis* L. cultivated in East Lithuania. Chemija, 2010, 21(2-4), 135-138.
[28] Venditti A., Bianco A., Frezza C., Conti F., Bini L. M., Giuliani C. et al., Essential oil composition, polar compounds, glandular trichomes and biological activity of *Hyssopus officinalis* subsp. *aristatus* (Godr.) Nyman from central Italy. Ind. Crops Prod., 2015, 77, 353-363.
[29] Moro A., Zalacain A., Hurtado de Mendoza J., Carmona M., Effects of agronomic practices on volatile composition of *Hyssopus officinalis* L. essential oils, Molecules, 2011, 16(5), 4131-4139.
[30] Baj T., Sieniawska E., Kowalski R., Swiatek L., Modzelewska M., Wolski T., Chemical composition and antioxidant activity of the essentials oil of hyssop (*Hyssopus officinalis* L. ssp. *officinalis*). Part II. Free radical scavenging properties, Annales UMCS, 2011, 24, 103-109.
[31] Baj T., Ludwiczuk A., Sieniawska E., Skalicka-Woźniak K., Widelski J., Zieba K., Głowniak K., GC-MS analysis of essential oils from *Salvia officinalis* L.: comparison of extraction methods of the volatile components, Acta Polon. Pharm., 2013, 70(1), 35-40.
[32] Kowalski R., Baj T., Kowalska G., Pankiewicz U., Estimation of potential availability of essential oil in some brands of herbal teas and herbal dietary supplements, PloS One, 2015, 10(6), e0130714.
[33] Adams R.P., Identification of essential oil components by gas chromatography/mass spectrometry. 4th edn. Allured Publ. Corp, Carol Stream, 2007.
[34] Zawislak G., Nurzynska-Wierdlak R., Plant development and chemical composition of essential oil of yarrow (*Achillea millefolium* L.) cultivated under temperate climate. Annales Hort. 2017, 31-38.
[35] Nurzynska R., Zawislak G., Chemical composition and antioxidant activity of lavender (*Lavandula angustifolia* Mill.) aboveground parts. Acta Sci. Pol. Hortorum Cultus, 15(5) 2016, 225-241.
[36] Marzec M., Polakowski C., Chilczuk R., Kołodziej B., Evaluation of essential oil content, its chemical composition and price of thyme (*Thymus vulgaris* L.) raw material available in Poland. Herba Pol., 2010, 56(3), 37-52.
[37] Zawislak G., The chemical composition of essential hyssop oil depending on plant growth stage. Acta Sci. Pol. Hortum Cultus, 2013, 12(3), 161-170.
[38] Tavakoli M., Aghajani Z., The effects of drought stress on the components of the essential oil of *Hyssopus officinalis* L. and determining the antioxidative properties of its water extracts. J. Appl. Environ. Biol. Sci., 2016, 6(2), 31-36.
[39] Fraternale D., Ricci D., Epifano F., Curini M., Composition and antifungal activity of two essential oils of hyssop (*Hyssopus officinalis* L.). J. Essent. Oil Res., 2004, 16(6), 617-622.
[40] Wesołowska A., Jadczak D., Grzeszczuk M., Essential oil composition of hyssop (*Hyssopus officinalis* L.) cultivated in north-western Poland. Herba Pol., 2010, 56(1), 57-65.
[41] Burt S., Essential oils: their antibacterial properties and potential applications in foods – a review, Int. J. Food Microb., 2004, 94(3), 223-253.
[42] Hristova B.Y., Wanner J., Jirovetz L., Stappen I., Iliev I., Gochev V., Chemical composition and antifungal activity of essential oil of *Hyssopus officinalis* L. from Bulgaria against clinical isolates of *Candida* species, Biotechnol. Biotechnol. Equip. 2015, 29(3), 592-601.
[43] Dehghanzadeh N., Ketabchi S., Alizadeh A., Essential oil composition and antibacterial activity of *Hyssopus officinalis* L. grown in Iran, Asian J. Biol. Sci, 2012, 3(4), 767-771.
[44] Tisserand R., Essential oil safety II: Metabolism, neurotoxicity, reproductive toxicity., Int. J. Aromather., 1996, 7(4), 26-29.