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Essential oil analysis of different hyssop genotypes from IFVCNS medicinal plant collection garden

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ABSTRACT
The aim of this study was to compare the chemical composition of different hyssop (*Hyssopus officinalis* L. subsp. *officinalis* L.) genotypes from medicinal plant collection garden of Institute of Field and Vegetable Crops Novi Sad. Essential oils were isolated from genotypes with white (f. *albus* Alef), pink (f. *ruber* Mill.) and violet-blue flowers (f. *cyaneus* Alef) by hydrodistillation in a Clevenger-type apparatus. The content of essential oil these three genotypes ranged between 0.47% (f. *albus* Alef) and 0.74% (f. *cyaneus* Alef). GC-MS analysis of essential oil identified 59 compounds, the major compounds being pinocamphone in cis and trans forms for all genotypes. The sum of these two compounds in all genotypes was between 61.1 and 74.4% depending on genotype.

KEY WORDS: *Hyssopus officinalis*, essential oil, *trans*-pinocamphone, *cis*-pinocamphone

INTRODUCTION

The genus *Hyssopus* belongs to the Lamiaceae family. It is native to Southern Europe, the Middle East, and the region surrounding the Caspian Sea (Ogunwande et al., 2011). Today, there is no consensus among the researchers on the taxonomy of the genus (Dumacheva et al., 2017). However, usually it is stated that genus *Hyssopus* comprises of about 10 to 12 species (Fathiazad and Hamedeyazdan, 2011). The genus name comes from a misinterpretation of the Hebrew word *adobe* or *ezob*, a biblical plant with purgative or cleansing properties (Andrews, 1961).

Hyssopus officinalis L. or hyssop is a perennial polymorphous plant species. The root is a strongly branching, multi-headed tap root. The plant is a typical xerophyte and is well adapted to drought and low input conditions. It forms many erect or decumbent woody stems, 30-60 cm high. Leaves are opposite each other, lanceolate or linear-lanceolate, 2-4 cm long and 0.5-1 cm wide, shiny dark-green. Flowers are hermaphrodite, clustered from 3 to 9 in nodes located in the axils of the leaves. The corolla is two-lipped, coloured in white, pink or blue-violet. The inflorescence is 20 to 25 cm long, false spike-like, composed of 4 to 10 flowered pseudoverticils in the terminal. It blooms from July through September and bears fruit from August through October (Nanova et al., 2007; Kizil et al., 2010; Fathiazad and Hamedeyazdan, 2011).

Hyssop herb (*Hyssopi herba*) is the herbal raw material. Essential oil of the *H. officinalis* (*Hyssopi aetheroleum*) is one of the main group of biologically active compounds. Essential oil is mainly accumulated in the flowers and leaves, whereas its amounts in the stems are insignificant (Zawiślak, 2013b). Despite having a slightly bitter taste and camphor aroma, *H. officinalis* is often used as a spice. Both fresh and dried, it is also a supplement to salads, meats, vegetables, cottage, cheese and pate. Moreover, in production of vermouth and bitter liquors (Chartreuse and Benedictine) it supplies the unique spicy taste (Baj et al., 2018).

Apart from this, *H. officinalis* is often used as condiment and spices in food industries due to its minty flavor (Fathiazad and Hamedeyazdan, 2011). It inhibits lipid oxidation and degradation of heme pigments caused by cooking and storage, and may be a useful additive for meat processing to prevent lipid oxidation and discoloration (Fernandez-Lopez et al., 2003). Furthermore, *H. officinalis* essential oil can inhibit the growth of some mycotoxicogenic fungal spoilers during cheese ripening (Moro et al., 2013). Essential oils of this plant is applied as flavoring agents in teas, soft drinks, candies, various snacks, chewing gum, baked goods, ice creams and frozen meals (Judžentienė, 2016).

In addition to their particular aroma, *H. officinalis* essential oil also exhibit antioxidative (Fathiazad et al., 2011; Vlase et al., 2014; Srivastava et al., 2018), antibacterial (Renzini et al., 2011) and

antifungal (Glamočlija et al., 2005; Hristova et al., 2015), as well as antiparasitic activities (Hikal and Said-Al Ahl, 2017). Apart from this, *H. officinalis* essential oil has anti-anxiety effects and can promote memory and learning under chronic immobilization stress (Salehi and Setorki, 2017). Furthermore, it has an anti-inflammatory effect through inhibiting the invasion of eosinophils and decreasing the levels of IgE, and also affects immune regulation (Ma et al., 2014). *H. officinalis* is widely used in cosmetics and in a variety of household products as a fragrance component (Kizil et al., 2010).

H. officinalis is a decorative shrub as well, with good resistance to shearing which allows it to be considered as a promising species in green infrastructure and gardening (Dumacheva et al., 2017). It is appreciated for its qualities to ameliorate eroded land and mobile sand genotypes (Goncearіuc and Balmu, 2013). The flowers are hermaphrodite and are pollinated by bees. The plant is commonly used by beekeepers to produce rich and aromatic honey (Moghtader, 2014). *H. officinalis* is a valuable melliferous plant, that attracts bees and other pollinating insects, giving 120-330 kg/ha of high-quality honey (Dumacheva et al., 2017). It is extensively cultivated in Russia, Spain, France and Italy (Kizil et al., 2010), as well as in the USA (Venditti et al., 2015). It is also cultivated in Serbia, however, on a small scale (Mitić and Đorđević, 2000; Aćimović et al., 2019).

As it mentioned above, *H. officinalis* is morphologically and genetically complex, with high variability between populations growing in different areas. So far, several subspecies have been recorded especially for Europe and northern Africa: subsp. *officinalis*, subsp. *aristatus* (Godr.) Nyman, subsp. *austro-oranensis* Maire, subsp. *canescens* (DC.) Nyman, and subsp. *montanus* (Jord. & Fourr.) Briq (Venditti et al., 2015). In Serbia, wild varieties are subsp. *pilifer* (Pant.) Murb. (Džamić et al., 2013), as well as subsp. *officinalis* in three forms: f. *cyaneus*, f. *ruber* and f. *albus* (Chalchat et al., 2001).

The aim of this study was to compare the chemical composition of different hyssop (*Hyssopus officinalis* L. subsp. *officinalis* L.) genotypes from medicinal plant collection garden of Institute of Field and Vegetable Crops Novi Sad.

Material and method

H. officinalis L. plants were collected during full flowering stage in August 2018 in collection garden of medicinal plants at Alternative Crops Department of Institute of Field and Vegetable Crops Novi Sad, Serbia. The taxonomic identification of the plant material was confirmed by a senior plant taxonomist, Milica Rat, in the Department of Biology, University of Novi Sad, Serbia. The voucher specimen was deposited at the Herbarium of the Department of Biology, and Ecology (BUNS), as 2-1453 (f. *albus*), 2-1454 (f. *ruber*) and 2-1455 (f. *cyaneus*).

The plant material was dried in shade and ground in a grinder with a 2 mm diameter mesh. In order to extract the essential oils, 100 g of the ground and homogenized plant material 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 in combination with the essential oils was distilled into a graduated cylinder for 3 h and then separated from aqueous layer. The essential oil obtained was dried over anhydrous sodium sulphate and stored at +4°C until tested and analyzed.

Gas chromatographic-mass spectrometric analysis was performed using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 Network mass selective detector (MSD) (both Agilent, Santa Clara, USA), in positive ion electron impact (EI) mode. The separation was achieved using Agilent 19091S-433 HP-5MS 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 index and by comparison with reference spectra (Wiley and NIST databases).

Results and discussion

The hydrodistillation of *H. officinalis* subsp. *officinalis* gave a pale yellow oil. The yield for all three genotypes ranged between 0.47% (f. *albus* Alef), 0.55% (f. *ruber*) and 0.74% (f. *cyaneus* Alef).

Different content of essential oil in white-flowered plants (0.7%) and pink-flowered plants (0.5%) was established in Poland (Baj et al., 2018). The essential oil content varied between 0.79-1.30% for plants with white flowers, 0.83-1.00% for plants with pink flowers as well as 0.83-1.20% for plants with blue flowers depending on weather conditions during the year (Wesolowska and Jadczak, 2018). The

content of essential oil is different for different genotypes and in Moldova conditions very high and f. *albus* - 1.43%, f. *ruber* - 2.53%, f. *cyaneus* - 1.88% (Goncearіuc and Balmus, 2013).

Investigations of the three cultivated forms of *H. officinalis* endemic in former Yugoslavia showed that f. *cyaneus,* which is characterized by blue flowers, yielded between 4.9 and 5.8 t of fresh plant material per hectare, and essential oil in yields ranging from 0.65-0.75%. Furthermore, f. *ruber*, characterized by pink-flowers, could achieve 3.9-5.1 t/ha of fresh plant material as well as 0.7-1.1% of essential oil, while f. *albus*, characterized by white flowers, achieved a yield is from 4.5 to 6.5 t/ha of fresh plant material, while the essential oil content ranged from 0.6-1.0% (Chalchat et al., 2001).

GC-MS analysis of essential oils identified 59 compounds from all *H. officinalis* genotypes. The percentage of the composition of the essential oils of *H. officinalis* is given in Table 1. Constituents were listed in order of elution from HP-5 capillary column. However, cis-pinocamphone and trans-pinocamphone were the most abundant.

The content of trans-pinocamphone is lowest in f. *albus* (16.4%), followed by f. *cyaneus* (22.3%), and highest in f. *ruber* (58.3%). However, content of cis-pinocamphone is the lowest in f. *ruber* (16.1%), while in f. *cyaneus* and f. *albus* it is higher (38.8% and 45.1%, respectively). As it can be seen, the sum of these two compounds in all tested genotypes is above 60% (61.1-74.4% depending on the genotype). Other significant components were: sabinene (1.1% at all genotypes), β-pinene (6.4-8.4%), myrcene (0.8-1.6%), β-phellandrene (0-5.9%), one NI compound (0.7-2.4%), pinocarvone (0.5-2.7%), myrtenol (1.5-2.5%), trans-caryophyllene (0.8-1.6%), allo-aromadendrene (0.6-1.4%), germacrene D (1.6-3.0%), bicyclogermacrene (1.0-2.5%) and elemol (0-1.9%) (Tab. 1).

Table 1.
Essential oil composition of three different *H. officinalis* L. subsp. *officinalis* L. genotypes
Tabela 1.
Hemijski sastav etarskih ulja različitih genotipova izopa (*H. officinalis* L. subsp. *officinalis* L.)

	Compound name	**f. *albus* Alef**	**f. *ruber* Mill.**	**f. *cyaneus* Alef**
1	α-thujene	0.1	0.1	0.1
2	α-pinene	0.3	0.4	0.3
3	camphene	0.1	0.1	0.1
4	sabinene	1.1	1.1	1.1
5	β-pinene	6.4	8.4	6.6
6	myrcene	1.5	0.8	1.6
7	α-phellandrene	tr	0.9	-
8	α-terpinene	0.1	-	0.2
9	p-cymene	tr	-	0.1
10	β-phellandrene	5.6	-	5.9
11	1,8-Cineole	-	0.3	-
12	cis-β-ocimene	-	0.2	0.2
13	trans-β-ocimene	tr	0.6	0.6
14	γ-terpinene	0.1	-	0.4
15	cis-sabinene hydrate	0.1	-	0.4
16	terpinolene	0.1	-	0.1
17	linalool	0.9	0.3	0.5
18	cis-thujone	0.2	-	0.2
19	trans-thujone	0.1	0.3	0.2
20	NI	tr	-	0.1
21	nopinone	-	0.1	tr
22	trans-pinocarveol	0.3	0.3	0.3

23	NI	2.4	0.7	1.0
24	trans-pinocamphone	16.4	58.3	22.3
25	pinocarvone	2.7	0.5	0.7
26	cis-pinocamphone	45.1	16.1	38.8
27	terpinen-4-ol	0.3	0.2	0.9
28	cryptone	0.1	-	tr
29	α-terpineol	0.2	0.2	0.2
30	myrtenol	1.5	2.5	2.0
31	methyl myrtenate	0.1	tr	0.1
32	δ-elemene	0.1	tr	0.1
33	β-bourbonene	0.7	1.1	0.9
34	methyl eugenol	0.1	tr	0.1
35	α-gurjunene	0.3	0.2	0.4
36	trans-caryophyllene	1.6	0.8	1.4
37	β-copaene	0.1	0.1	0.1
38	NI	tr	0.1	0.1
39	α-humulene	0.3	0.1	0.2
40	allo-aromadendrene	1.1	0.6	1.4
41	NI	tr	0.1	tr
42	germacrene D	2.8	1.6	3.0
43	β-selinene	0.1	0.1	0.1
44	bicyclogermacrene	2.2	1.0	2.5
45	γ-Cadinene	0.1	0.1	0.1
46	δ-Cadinene	0.1	tr	0.1
47	elemol	1.9	-	1.7
48	hedycaryol	-	0.6	-
49	spathulenol	0.5	0.4	0.6
50	caryophyllene oxide	0.5	0.3	0.4
51	NI	0.2	0.1	tr
52	NI	tr	-	0.2
53	γ-eudesmol	0.3	tr	0.3
54	NI	0.1	-	0.1
55	epi-α-Cadinol	0.2	0.2	0.1
56	β-eudesmol	0.2	-	0.2
57	α-eudesmol	0.3	0.1	0.3
58	NI	0.1	0.1	0.1
59	NI	0.1	-	0.1
	Essential oil content	**0.47**	**0.55**	**0.74**

NI – Compound not determined, tr – Compound present in traces

Essential oil composition of *H. officinalis* showed considerable variations in the relative concentration of its major components. The essential oil biosynthesis is mainly attributed to genetic factors, but other factors have an important effect on essential oil production such as climate and soil condition, development stage and plant age, harvest time, as well as postharvest processing (Ogunwande et al., 2011; Zheljazkov et al., 2012; Yousefzadeh and Naghdi Badi, 2017). A polymorphism between populations from different geographical areas was found. Intraspecific diversity is an important component of adaptive evolution, determining the ability of plants to survive in more diverse habitats, to colonize habitats of wide ecological amplitude and to respond to different environmental changes (Mutu et al., 2014).

However, in the essential oil obtained from the white-flowered *H. officinalis* from Poland, the most abundant compounds were pinocamphone (51.0%), followed by *β*-pinene (12.4%), *trans-p*-menth-2-en-1-ol (8.1%) and 1,8-cineole (7.6%), while in the pink-flowered form the major components were cis-pinocamphone (28.8%), trans-pinocamphone (21.9%), *β*-pinene (9.8%), elemol (5.4%) and *γ*-amorphene (5.2%) (Baj et al., 2018). Furthermore, GC-MS analysis of *H. officinalis* essential oil from Moldova identified 30-38 compounds for different genotypes. The essential oil separated from the f. *ruber* contains 66.94% of pinocamphone: trans-pinocamphone (33.31%) and cis-pinocamphone (33.63%), and f. *albus*, the main component is trans-pinocamphone (61.1%), while the concentration of cis-pinocamphone was only 2.15% (Goncearіuc and Balmu, 2013).

Essential oil composition of *H. officinalis* from Crimea included 60 compounds, mainly synthesized pinocamphone, trans-pinocamphone, α- and β-pinene, sabinene, myrcene, β-phellandrene, linalool, myrtenol, methyleugenol, elemol and etc. Comparison study of plant distribution by biosynthesis of pinocamphone and trans-pinocamphone in essential oil of different forms presented the following: maximum level of pinocamphone mass fraction in essential oil was marked for f. *ruber* plants (60.48%), f. *cyaneus* (3637%), f. *albus* (35,24%). Rather different results were obtained in biosynthesis of trans-pinocamphone. Maximum mass fraction of trans-pinocamphone occurred in essential oil of f. *albus* (61.12%), a bit less for f. *cyaneus* (57.93%) and the minimum concentration was registered for f. *ruber* (38.14%) (Rabotyagov and Shibko, 2014).

All other research is focused on *H. officinalis* with violet-blue flowers from different countries. The cluster analysis of the principal components of *H. officinalis* can classify all varieties in several groups: pinocamphone-rich, linalool-rich, limonene-rich, pinocamphone + trans-pinocamphone, trans-pinocamphone-rich and bicyclogermacrene-rich chemotypes. However, the dominant constituents of the oil of *H. officinalis* from Nigeria are α-pinene (70.9%) and β-pinene (10.9%) (Ogunwande et al., 2011).

Generally, *H. officinalis* subsp. *officinalis* is mainly characterized by bicyclic monoterpene ketones such as cis-pinocamphone and trans-pinocamphone, and by smaller amounts of β-pinene (i.e. the biogeneticprecursor of trans-pinocamphone), pinocarvone, limonene, 1,8-cineole, linalool and camphor (Fathiazad and Hamedeyazdan, 2011). It is validated by a number of studies conducted in Italy (Fraternale et al., 2004), Switzerland (Rey et al., 2004), Hungary (Rey et al., 2004; Németh-Zámbori et al., 2017), Turkey (Kizil et al., 2010), Finland (Galambosi et al., 2010), Poland (Zawiślak, 2013a; Németh-Zámbori et al., 2017), Germany (Németh-Zámbori et al., 2017), Iran (Moghtader, 2014), India (Pandey et al., 2014), Ukraine (Kotyuk, 2015), Bulgaria (Hristova et al., 2015) as well as Mississippi (Zheljazkov et al., 2012).

However, *H. officinalis* plants collected in six localities in East Lithuania show the presence of pinocarvone (21.1-28.1%) as a major component in four oils, and trans-pinocamphone (16.8–33.6%) in two oils. Apart from pinocarvone, notable amounts of trans-pinocamphone (11.5–15.9%), β-pinene (7.0-11.4%), germacrene D (3.7-5.5%) and hedycaryol (4.1-4.8%) were found in four oils. The other main constituents in two other oils were pinocarvone (9.0-13.6%), β-pinene (7.2-8.6%) and hedycaryol (4.0-9.1%). Compounds with the pinane carbon skeleton made up 43.6-56.9% of the essential oils. Oxygenated monoterpenes were the most characteristic components of the oils (36.0-50.7%) (Bernotienė and Butkienė, 2010).

H. officinalis subsp. *pilifer* (Pant.) Murb. is a wild growing taxon in Eastern Serbia, as well as in Bulgaria and N. Greece, north-western Croatia. Among the 30 compounds identified in the oil, the main were 1,8-cineole (36.43%), β-pinene (19.55%), trans-pinocamphone (15.32%) and trans-pinocamphone (6.39%) (Džamić et al., 2013). Furthermore, the essential oil from *H. officinalis* grown in Spain was also characterized by a high content of 1,8-cineole (52.89%) and β-pinene (16.82%) as the main components (Garcia Vallejo et al., 1995).

H. officinalis subsp. *aristatus* present in Italy contains linalool (5.3-51.2%) and methyl eugenol (7.3-22.7%) as the main constituents, while minor quantities of cis-β-ocimene(5.1-5.8%), trans-β-ocimene (2.1–5.3%), limonene (3.7-4.4%) and germacrene D (1.9-4.1%) were recorded (Venditti et al., 2015).

While, *H. officinalis* subsp. *decumbens* (Jordan & Fourr.) Briq. from France, also contains linalool (51.7%), 1,8-cineole (12.3%) and limonene (5.1%) as the main compounds (Mazzanti et al., 1998).

Wild *H. officinalis* L., which was collected from Montenegro (around Petnjica) contains methyl eugenol (38.3%), limonene (37.4%) and β-pinene (9.6%) as the main components (Gorunovic et al., 1995).

Conclusion

GC-MS analysis of *H. officinalis* L. subsp. *officinalis* essential oil from Institute of Field and Vegetable Crops Novi Sad medicinal plant collection garden showed that all three genotypes (f. *albus* Alef, f. *ruber* Mill. and f. *cyaneus* Alef) belong to pinocamphone-rich chemotype.

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Analiza hemijskog sastava etarskih ulja različitih genotipova izopa iz IFVCNS kolekcije lekovitog bilja

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SAŽETAK
Cilj ovog istraživanja bio je da se uporedi hemijski sastav etarskih ulja različitih genotipova izopa (*Hyssopus officinalis* L. subsp. *officinalis* L.) iz kolekcije lekovitog bilja Instituta za ratarstvo i povrtarstvo Novi Sad. Etarsko ulje dobijeno je od genotipova sa belim (f. *albus* Alef), roze (f. *ruber* Mill.) i plavo-ljubičastim cvetovima (f. *cyaneus* Alef) postupkom hidrodestilacije na aparaturi po Klevendžeru. Sadržaj etarskog ulja u sva tri genotipa se kretao između 0.47% (f. *albus* Alef) i 0.74% (f. *cyaneus* Alef). GC-MS analizom etarskih ulja utvrđeno je 59 komponenti, među kojima su najzastupljeniji bili cis-pinokamfon i trans-pinokamfon za sve genotipove. Suma ova dva izomera se kretala od 61.1 do 74.4% u zavisnosti od genotipa.

KLJUČNE REČI: *Hyssopus officinalis*, etarsko ulje, *trans*-pinokamfon, *cis*-pinokamfon

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