ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Hortorum Cultus 12(3) 2013, 161-170*

THE CHEMICAL COMPOSITION OF ESSENTIAL HYSSOP OIL DEPENDING ON PLANT GROWTH STAGE

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Abstract. Hyssop (*Hyssopus officinalis* L.) is a perennial plant of Mediterranean origin. The raw material is the herb (*Hyssopi herba*) that shows multidirectional medicinal activity. Essential oil is the main biologically active substance. The content of oil and its chemical composition determine the aroma of the raw material. The essential oil content changes during the period of plant growth. The aim of this study was to evaluate the qualitative and quantitative composition of hyssop oil depending on plant growth stage. Studies were conducted in the period 2007–2008 at the Experimental Farm of the Department of Vegetable Crops and Medicinal Plants, University of Life Sciences in Lublin. The hyssop herb was collected from plants at the following growth stages: vegetative stage (the middle of June), beginning of flowering (the middle of July), and full flowering (the middle of August). The herb was dried at a temperature of 30^{o}C and then ground through 4–5 mm mesh sieves. Essential oil was obtained from the ground herb by steam distillation. The main components of hyssop oil were as follows: cis-pinocamphone, trans-pinocamphone (monoterpene ketones), β-pinene (monoterpene hydrocarbon), germacrene D (sesquiterpene hydrocarbon), and elemol (sesquiterpene alcohol). Trans-pinocamphone was predominant in the oil extracted from hyssop collected at the vegetative stage, but its content decreased with plant growth, while the content of cis-pinocamphone increased. The content of β-pinene was the highest in the oil extracted from hyssop cut at the vegetative stage and more than twice lower in the oil obtained from plants harvested at the beginning of flowering and at full flowering.

Key words: *Hyssopus officinalis* L., cis-pinocamphone, trans-pinocamphone, β-pinene, germacrene D, elemol

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INTRODUCTION

Hyssop (*Hyssopus officinalis* L.) is a perennial plant of the family Lamiaceae. It is native to the Mediterranean Sea region. Hyssop reached northern Europe and Poland through the Benedictine and Cistercian orders [Strzelecka and Kowalski 2000]. This plant is well-acclimated in Poland. Hyssop raw material is obtained from field crops [Senderski 2004]. Hyssop is propagated from seed [Góra and Lis 2002], but good propagation results are also obtained under *in vitro* conditions [Nanova et al. 2007]. This plant was formerly used to treat many ailments, but currently it is on the fringe of phytotherapy. Therefore, detailed research is advisable to confirm the wide range of activity of hyssop [Jambor 2006].

The herbal raw material is the hyssop herb (*Hyssopi herba*) [Strzelecka and Kowalski 2000, Wyk and Wink 2009, Wesołowska et al. 2010]. Essential oil is one of the main groups of biologically active compounds [Baj et al. 2007]. Oil mainly accumulates in the flowers and leaves, whereas its amounts in the stems are insignificant [Wolski et al. 2006]. Hence, the stems are ballast that is not useful as herbal raw material. It is therefore advisable to rub raw material to obtain the herb without stems.

The hyssop herb also contains flavonoids, tannins [Kohlműnzer 2007, Wyk and Wink 2008, Zawiślak 2011], and marrubiin (a bitter) thanks to which the raw material shows multidirectional activity [Kohlműnzer 2007, Wyk and Wink 2008]. The hyssop herb stimulates the secretion of digestive juices and improves food digestion and absorption. Hyssop herbal remedies also have an expectorant effect [Jankovský and Landa 2002]. Diabetics have also been found to benefit from the use of hyssop [Strzemski 2008]. Woolf [1999] recommends the use of hyssop oil in nervous exhaustion. In veterinary medicine, hyssop remedies are given to animals to treat gastrointestinal disorders [Strzemski 2008]. Antibacterial and antifungal activity of hyssop oil has been demonstrated by many researchers [Mazzanti et al. 1998, Letessier et al. 2001, Tampieri et al. 2003, Fraternale et al. 2004, Wolski and Baj 2006].

The herbal industry requires high quality raw material with a uniform chemical composition [Zawiślak 2000]. The content of oil and its composition determine the quality of aromatic raw materials. According to Fraternale et al. [2004], the chemical composition of hyssop oil depends on the geographic origin of the plant and on cultivation technology. The study of Németh et al. [2000] showed that the oil content in hyssop substantially changed during the cultivation of this plant. According to Baj et al. [2007] and Zawiślak [2011], the oil content depended on plant growth stage. The aim of this study was to evaluate the qualitative and quantitative composition of hyssop oil depending on plant growth stage.

MATERIAL AND METHODS

The present study was conducted during the period 2007–2008. The experiment was established at the Experimental Farm of the Department of Vegetable Crops and Medicinal Plants, University of Life Sciences in Lublin (51°13’ N 22°34’ E). Hyssop seedlings were produced in a greenhouse. The plants were planted in the field in the middle

of May at 40 × 40 cm spacing. The plot was prepared in accordance with agronomic recommendations. Mineral fertilization was applied at an optimal level recommended for hyssop: 50 kg $N \cdot ha^{-1}$, 70 kg $P_2O \cdot ha^{-1}$, and 80 kg $K_2O \cdot ha^{-1}$. Phosphorus and potassium fertilizer was applied at the full rate during the preparation of the field before planting seedlings. Nitrogen fertilizer was applied in two doses: half of the rate was incorporated during field preparation, while the other half of fertilizer was applied to the plants in one dose after the seedlings became established. The study was carried out in a one-year plantation. Tending during the growth of the plants in the field involved hand weeding, which was done twice, and soil loosening. Average monthly air temperature from May to August in 2007 and 2008 were close to the long-term average temperature. Total precipitation May 2007 and 2008 was higher than the long-term average precipitation in June 2007. Rainfall was more than three times higher than in 2008. The herb was harvested at three times during the growing season, depending on the growth stage of the plant: in the middle of June (plants at the vegetative stage), in the middle of July (beginning of flowering), and in the middle of August (plants in full blooming). The plants were cut at a height of 8 cm above ground. The herb was dried in a drying oven at a temperature of 30°C. Drying time was 4 days. The whole dried herb was ground through 4–5 mm mesh sieves, thereby obtaining the ground herb. The ground herb was used to obtain hyssop oil by steam distillation [Polish Pharmacopoeia VII 2006]. Distillation time was 3 hours. 20 g of ground herb and 400 ml of distilled water were used for distillation.

The obtained results were statistically elaborated with the use of variance analysis or singular qualification at the significance level of $\alpha = 0.05$.

Analysis of essentials oil

GC-MS. The GC-MS instrument ITMS Varian 4000 GC-MS/MS (Varian, USA) was used, equipped with a CP-8410 auto-injector and a 30 m × 0.25 mm i.d. VF-5ms column (Varian, USA), film thickness 0.25 μm; carrier gas, helium at a rate of 0.5 ml/min; injector and detector temperature, 220°C and 200°C, respectively; split ratio, 1:20; injection volume, 1 μl. A temperature gradient was applied (60°C for 0.5 min, then incremented by 3°C/min to 246°C and held at this temperature for 10 min); ionization energy, 70 eV; mass range, 40–1000 Da; scan time, 0.80 s.

GC-FID. A Varian 3800 Series (Varian, USA) instrument with a DB-5 column (J&W, USA) was used, operated under the same conditions as GC-MS; FID, 256°C; split ratio 1:50.

The qualitative analysis was carried out on the basis of MS spectra, which were compared with the spectra of the NIST library [Mass Spectral Library] and with data available in the literature [Adams 2001]. The identity of the compounds was confirmed by their retention indices, taken from the literature [Adams 2001].

RESULTS AND DISCUSSION

The most oil was contained in the herb collection from plants in full blooming – 1.7%, and the lowest level of oil was found in the herb obtained from the plants that were in vegetative phase – 0.6% (Tab. 1). Rosłon et al. [2002] demonstrated that the content of oil in blooming period did not exceed 1%.

Table 1. Content of essential oil (%) in the herb of *Hyssopus officinalis* L.

Plant growth stage	2007	2008	2007–2008
In vegetative phase	0.8	0.5	0.6
Beginning of flowering	1.0	1.5	1.2
Full blooming	1.7	1.8	1.7
Mean	1.2	1.3	1.2
$LSD_{0.05}$	0.45	0.79	0.48

The chemical composition of essential oil in herbal plants depends on the place of origin of raw material [Özgüven and Tansi 1998, Fraternale et al. 2004, Góra and Lis 2004, Wolski and Baj 2006, Wesołowska et al. 2010] and on plant growth stage [Özgüven and Tansi 1998, Mirjalili et al. 2006, Sefidkon et al. 2007, Kizil et al. 2008, Nurzyńska-Wierdak and Dzida 2009, Kaškonienie et al. 2011]. A study conducted in south-eastern Poland showed the presence of 49 compounds in hyssop oil (Tab. 2). According to Garg et al. [1999], the number of identified compounds in hyssop oil was 21 and according Khizil et al. [2008] – 23. Baj et al. [2007, 2010] identified 63 compounds. The number of compounds identified by Wesołowska et al. [2010] depended on the oil distillation method. The authors identified 31 compounds in the oil obtained by steam distillation, 36 compounds in the oil extracted by simple distillation, and 27 compounds in the oil collected using a Dean – Stark distillation trap.

The main components of hyssop oil were as follows: cis-pinocamphone, trans-pinocamphone (monoterpene ketones), β-pinene (monoterpene hydrocarbon), germacrene D (sesquiterpene hydrocarbon), and elemol (sesquiterpene alcohol). The content of cis-pinocamphone in 2007 was 27.5–65.4% and it was higher than in 2008 (10.4–44.5%). In turn, the percentage of trans-pinocamphone in the oil obtained in 2007 was lower than in 2008 and it was, respectively, 13.0–34.0% and 18.0–53.0% (Tab. 2). Interesting are variations in the content of cis-pinocamphone and trans-pinocamphone depending on the growth stage of the plant (Fig. 1). The oil from plants at the vegetative stage contained almost three times less cis-pinocamphone (on average 18.9%) than the oil from plants in full bloom (on average 54.9%). The content of cis-pinocamphone increased with plant growth. On the other hand, the content of trans-pinocamphone was shown to decrease with plant growth. The average content of trans-pinocamphone in the oil from non-flowering plants (vegetative stage) was 43.5%, and it gradually decreased reaching a level of 15.5% at full flowering. The literature data show that cis-pinocamphone can predominate in hyssop oil, simultaneously with a low content of trans-pinocamphone [Mazzanti et al. 1998, Baj et al. 2010, Wesołowska et al. 2010].

Table 2. Chemical composition (%) of essential oil from the herb of *Hyssopus officinalis* L.

Compound	RI	2007			2008		
		vegetative phase	beginning of flowering	full blooming	vegetative phase	beginning of flowering	full blooming
α-thujene	931	tr.	tr.	tr.	tr.	tr.	tr.
α-pinene	939	tr.	tr.	tr.	0.5	0.3	0.5
camphene	955	tr.	tr.	tr.	tr.	tr.	tr.
sabinene	977	1.9	-	1.0	1.9	0.8	2.0
β-pinene	982	13.9	0.8	3.7	9.9	8.3	6.4
myrcene	993	1.7		0.9	2.4	1.4	3.1
p-cymene	1028	tr.	tr.	tr.	tr.	tr.	tr.
β-phellandrene	1030	3.4	1.5	1.5	7.5	2.4	1.3
sylvestrene	1032	tr.	tr.	tr.	tr.	tr.	tr.
(Z)- β-ocimene	1037	tr.	tr.	tr.	tr.	tr.	tr.
(E)- β-ocimene	1050	tr.	tr.	tr.	tr.	tr.	tr.
γ-terpinene	1060	tr.	tr.	tr.	tr.	tr.	tr.
cis-sabinene hydrate	1070	tr.	tr.	tr.	tr.	tr.	tr.
linalool	1089	-	1.0	-	0.6	0.8	1.1
cis-thujone	1108	tr.	tr.	tr.	tr.	tr.	tr.
trans-thujone	1120	tr.	tr.	tr.	tr.	tr.	tr.
trans-pinocarveol	1139	tr.	tr.	tr.	tr.	tr.	tr.
cis-pinene hydrate	1152	tr.	tr.	tr.	tr.	tr.	tr.
trans-pinocamphone	1167	34.0	23.8	13.0	53.0	27.9	18.0
pinocarvone	1165	1.4	-	1.8	-	1.1	-
borneol	1169	tr.	tr.	tr.	tr.	tr.	tr.
cis-pinocamphone	1175	27.5	49.4	65.4	10.4	37.7	44.5
myrtenol	1196	1.9	3.7	3.0	2.8	1.3	2.4
trans-2-hydroxy pinocamphone	1250	tr.	tr.	tr.	tr.	tr.	tr.
nopol	1280	tr.	tr.	tr.	tr.	tr.	tr.
thymol	1290	tr.	tr.	tr.	tr.	tr.	tr.
myrtenyl acetate	1327	tr.	tr.	tr.	tr.	tr.	tr.
δ-elemene	1338	tr.	tr.	tr.	tr.	tr.	tr.
α-copaene	1377	tr.	tr.	tr.	tr.	tr.	tr.
β-bourbonene	1388	0.7	1.2	tr.	0.3	1.2	0.4
β-elemene	1391	tr.	tr.	tr.	tr.	tr.	tr.
α-gurjunene	1410	tr.	tr.	tr.	tr.	tr.	tr.
E-caryophyllene	1429	2.5	2.4	1.2	0.6	1.9	1.3
β-copaene	1432	tr.	tr.	tr.	tr.	tr.	tr.
α-guaiene	1440	tr.	tr.	tr.	tr.	tr.	tr.
α-humulene	1455	tr.	tr.	tr.	tr.	tr.	tr.
allo-aromadendrene	1473	0.9	tr.	tr.	1.0	1.0	1.6
germacrene D	1495	3.7	4.5	1.4	6.2	5.6	5.0
bicyclogermacrene	1507	-	2.0	0.6	2.0	2.1	3.8
γ-cadinene	1514	tr.	tr.	tr.	tr.	tr.	tr.
δ-amorphene	1512	tr.	tr.	tr.	tr.	tr.	tr.
trans-calamenene	1529	tr.	tr.	tr.	tr.	tr.	tr.
elemol	1560	5.7	6.0	3.3	0.3	4.2	6.5
spathulenol	1589	-	1.0	0.2	-	0.6	1.0
caryophyllene oxide	1594	-	1.1	0.7	-	0.2	0.5
γ-eudesmol	1632	tr.	tr.	tr.	tr.	tr.	tr.
epi-α-cadinol	1640	tr.	tr.	tr.	tr.	tr.	tr.
α-eudesmol	1655	tr.	0.5	0.4	-	0.2	0.4
bulnesol	1672	tr.	tr.	tr.	tr.	tr.	tr.
Total		99.2	98.9	98.1	99.4	99.0	99.8
monoterpene hydrocarbons		20.9	2.3	7.1	22.2	13.2	13.3
monoterpene alcohols		1.9	4.7	3.0	3.4	2.1	3.5
monoterpene ketones		62.9	73.2	80.2	63.4	66.7	62.5
sesquiterpene hydrocarbons		7.8	10.1	3.2	10.1	11.8	12.1
sesquiterpene alcohols		5.7	7.5	3.9	0.3	5.0	7.9
oxygenated sesquiterpene		-	1.1	0.7	-	0.2	0.5

tr. – trace (< 0.05)

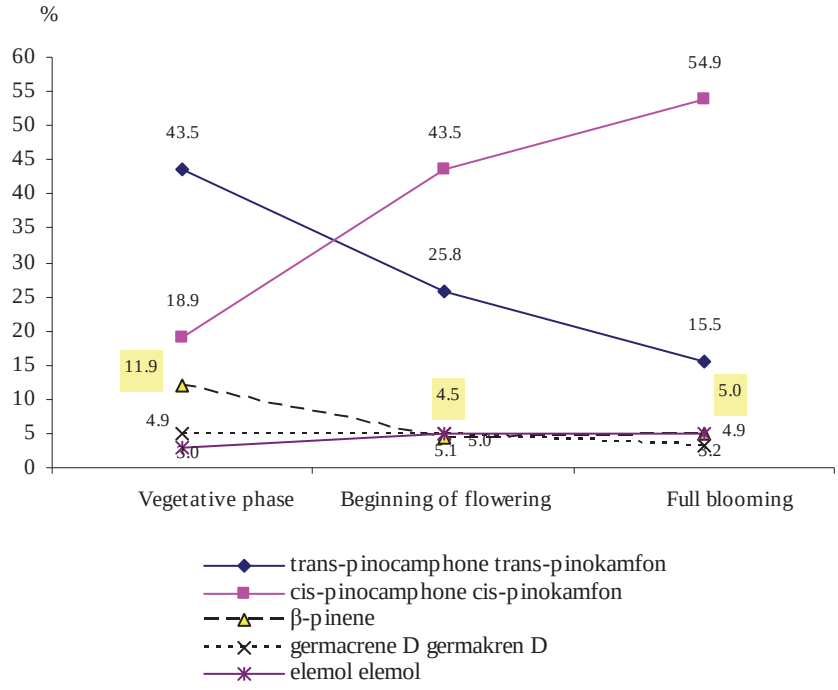

Fig. 1. The content of the main components in hyssop oil depending on plant growth stage (2007–2008)

According to Kizil et al. [2008, 2010], trans-pinocamphone was the dominant compound of hyssop oil (47.9–51.4% and 57.2%). Cis-pinocamphone and trans-pinocamphone were the dominant constituents in hyssop oil in the studies of Mitić and Dordević [2000], Fraternale et al. [2003], Rosłon et al. [2002], Rey et al. [2004], and Zheljazkov et al. [2012]. Mitić and Dordević [2000] showed the content of cis-pinocamphone to be at a level of 44.7%, whereas the content of trans–pinocamphone was lower (14.1%) than in the present study. According to Rey et al. [2004], the content of trans-pinocamphone in the hyssop oil of the cultivar 'Perlay' ranged from 40% to 60%. This oil contained a twice lower amount of cis-pinocamphone (20–30%). In the study of Garg et al. [1999], the hyssop oil contained more trans-pinocamphone (49.11%) than cis-pinocamphone (9.69%). The above-mentioned authors also showed that β-pinene was the dominant compound of hyssop oil (18.40%). In the present study, the content of β-pinene in the oil varied depending on the growth stage of the plants from which the oil was obtained. The highest amount of β-pinene was found to occur in the oil from hyssop plants at the vegetative stage (2007 – 13.9%; 2008 – 9.9%) (Tab. 2). In the study of Rosłon et al. [2002], the content of β-pinene in the oil isolated from the raw material collected both at the beginning of July and in the middle of August was higher and it was 14.88% and 15.07%, respectively. In the present study, the content of

β-pinene decreased with plant growth and it was more than twice lower in the oil obtained from hyssop harvested at the beginning of flowering and at full bloom (Fig. 1). The hyssop oil isolated from plants grown in the north-western part of Poland contained a very small amount of β-pinene (0.2%) [Wesołowska et al. 2010]. The oil from hyssop plants growing in the following countries was rich in β-pinene: Italy (11.15% and 10.5–10.8%) [Mazzanti et al. 1998, Fraternale et al. 2004], India (19.4%) [Garg et al. 1999], and Hungary (4–15%) [Rey et al. 2004].

The present experiment showed large variations in germacrene D content between years. The oil obtained in 2007 was characterized by a lower content of germacrene D (1.4–4.5%) than that isolated in 2008 (5.0–6.2%). The hyssop oil from full blooming plants was found to contain the lowest amount of this compound (Tab. 2). The literature data show that Indian oil [Garg et al. 1999] and Serbian oil [Mitić and Dordević 2000] contained small amounts of germacrene D, 0.65% and 1.6%, respectively.

The chemical analysis of the oil conducted in this study showed that the content of elemol varied between years. In 2007 it ranged from 3.3% to 6.0% and was the highest in the oil from plants harvested at the beginning of flowering. In 2008 the study showed higher variations in elemol content (0.3–6.5%) in hyssop oil – the oil from plants in full bloom contained the highest content of elemol (Tab. 2). Mitić and Dordević [2000] obtained oil in which the elemol content was 5.6%. Baj et al. [2010] found a slightly higher amount of elemol (7.4%). According to Wesołowska et al. [2010], the content of elemol was 17.21%. Mazzati et al. [1998] showed a very low content of this compound (1.7%). On the other hand, no presence of elemol in hyssop oil was found in the studies of Grag et al. [1999], Rosłon et al. [2002] and Fraternale et al. [2004].

Monoterpene ketones were shown to have the highest percentage in hyssop oils (Tab. 2). It was more than 62% in the oil obtained from plants at three different growth stages. Monoterpene hydrocarbons were the second largest group of compounds in the hyssop oils from plants at the vegetative stage (20.9–22.2%) and from plants in full bloom (7.1–13.3%). Sesquiterpene hydrocarbons (10.1–11.8%) were the second group of the dominant compounds in the oil from plants collected at the beginning of flowering. Monoterpenes have been the main group of compounds in hyssop oils in the studies of many researchers [Grag et al. 1999, Bernotienė and Butkienė 2010, Wesołowska et al. 2010].

CONCLUSIONS

1. The main components of hyssop oil were monoterpenes (cis-pinocamphone, trans-pinocamphone, β-pinene) and sesquiterpenes (germacrene D, elemol).

2. The study showed differences in the content of cis-pinocamphone and trans-pinocamphone in hyssop oil depending on the growth stage of plants harvested. Trans-pinocamphone was predominant in the oil extracted from hyssop collected at the vegetative stage, but its content decreased with plant growth, while the content of cis-pinocamphone increased.

3. The content of β-pinene was the highest in the oil extracted from hyssop harvested at the vegetative stage and more than twice lower in the oil obtained from plants harvested at the beginning of flowering and at full flowering.

4. Monoterpene ketones were found to have the highest percentage in the oil obtained from hyssop collected at all growth stages of the plant.

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SKŁAD CHEMICZNY OLEJKU ETERYCZNEGO HYZOPU LEKARSKIEGO W ZALEŻNOŚCI OD FAZY ROZWOJOWEJ ROŚLINY

Streszczenie. Hyzop lekarski (*Hyssopus oficinalis* L.) jest wieloletnią rośliną pochodzenia śródziemnomorskiego. Surowcem jest ziele (*Hyssopi herba*) o wielokierunkowym działaniu leczniczym. Główną substancją biologicznie aktywną jest olejek eteryczny. Zawartość olejku oraz jego skład chemiczny decyduje o aromacie surowca. W okresie wegetacji rośliny zmienia się zawartość olejku eterycznego. Celem badań była ocena składu

jakościowego i ilościowego olejku hyzopowego w zależności od fazy rozwojowej rośliny. Badania przeprowadzono w latach 2007–2008. Ziele hyzopu zebrano z roślin będących następujących fazach rozwoju: w fazie wegetatywnej (połowie czerwca), na początku kwitnienia (połowa lipca) oraz w pełni kwitnienia (połowa sierpnia). Ziele wysuszono w temperaturze 30°C, a następnie otarto na sitach o średnicy oczek 4–5 mm. Z ziela otartego otrzymano olejek eteryczny w procesie destylacji z parą wodną. Głównymi składnikami olejku hyzopowego były: cis-pinokamfon, trans-pinokamfon (ketony monoterpenowe), β-pinen (węglowodór monoterpenowy), germacrene D (węglowodór seskwiterpenowy) oraz elemol (alkohol seskwiterpenowy). W olejku z hyzopu zebranego w fazie wegetatywnej dominował trans-pinokamfon, jego zawartość malała w miarę rozwoju rośliny, natomiast zawartość cis-pinokamfonu wzrastała. Zawartość β-pinenu była największa w olejku z hyzopu ściętego w fazie wegetatywnej i ponad dwukrotnie mniejsza w olejku z roślin zebranych na początku i w pełni kwitnienia.

Słowa kluczowe: *Hyssopus officinalis* L., cis-pinokamfon, trans-pinokamfon, β-pinen, germakrene D, elemol

Accepted for print: 22.11.2012