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HYSSOP COMPOSITION DEPENDING ON AGE AND PLANTS DEVELOPMENT PHASES

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The aim of this paper was to investigate biochemical composition of *Hyssopus officinalis* L. (*Lamiaceae*) in relation to plant age and phenological growth stage under conditions of Ukrainian Polissia, in order to determine the optimal harvest dates of the herbal material and its application spheres.

The raw material samples under analysis were cut at various growth stages: the vegetative, budding, blooming, and ripening stages. To study the hyssop oil composition, areal parts of H. officinalis were used. The composition analysis was aimed at determining absolute dry matter (by drying samples at $105\,^{\circ}\text{C}$ up to the constant mass), "crude" cellulose, amounts of protein, fats, calcium, potassium, phosphorus, ascorbic acid, carotene, discernible sugars and tannins and essential oil.

The present study has proved that in the plant ontogenesis the amount of essential oil, obtained from H. officinalis areal parts, does not markedly decrease: volatile oil yield in plants of the first, second and third years of life amounted to 1.007%, 0.75% and 0.71% respectively. The composition of volatile oil in the plants of the first year of life reveals 46 components, of which pinocampone (53.73%), isopinocampone (4.66%), myrtenol (9.35%) and camphor (3.86%) prevailed. In H. officinalis volatile oil of the third year 30 components were identified, the prevailing of which were isopinocampone (44.43%), pinocampone (35.49%), myrtenol (5.26%), germacrene D (3.15%), pulegone (2.93%) and bicyclogermacrene (1.35%).

It was observed the change in the quantitative and qualitative composition of H. officinalis volatile oil throughout the entire vegetation period. Thus, in the phase of vegetative growth it was identified 25 compounds, the most predominant being elemol (33.25%), germacren D (21.59%) and bicyclogermacrene (15.78%). In the phase of blossoming 30 components can be identified, a high amount of isopinocampone and pinocampone (44.43% and 35.49%) and lover amount of myrtenol (5.26%), pulegone (2.93%) and bicyclogermacrene (1.35%) can be noted. During the fruit-bearing period 21 compounds with the prevalence of elemol (44.46%), bicyclogermacrene (10.30%), germacren D (5.86%), spatulenol (4.36%), β -eudesmol (4.34%), α -eudesmol (4.04%) and γ -eudesmol (3.92%) can be identified.

H. officinalis raw material from one-year-old plants is to be used in food industry, whereas plants collected at the blooming stage are preferable for cosmetics and perfumery.

Key words: Hyssopus officinalis L., biological active substances, essential oil, pinocampone, isopinocampone.

Hyssop (*Hyssopus officinalis* L.) is one of valuable aromatic, essential, spicy, medicinal, honey-bearing plants, containing numerous biologically active substances, due to which it is used in pharmacy as well as food, cosmetic and perfumery industries [1–3]. *H. officinalis* raw material obtained from one-year-old plants is rich in proteins, total sugars, ascorbic acid, carotene, calcium, phosphorus. Contents of dry matter, fats and tannins increase with plant maturity.

Areal parts of H. officinalis in the blooming stage are known to contain from 0.36 up to 1.3% essential oil on a dry matter basis, as well as flavonoids (diosmin, hesperidin), triterpene acids (ursolic, oleonolic), tannins, bitter substances, tars, resins, vitamins and others [4, 5].

The hyssop oil major components, distilled from H. officinalis cultivated in Iran, are: myrtenol acetate (74.08%), camphor (6.76%), germacrene D (3.36%), spathulenol (2.14%), caryophyllene oxide (2.13%), β -caryophyllene (2.10%)[6].

Essential oil, extracted from plants cultivated in Serbia, contains: cis-pinocamphone (42.9%), trans-pinocamphone (14.1%), germacrene-D-11-ol (5.7%) and elemol (5.6%) [7], in Spain — up to 19.75% pinocamphone, 24.58% isopinocamphone, 20.54% β -pinene, 4.76% germacrene [8], in the Western Himalayas — cis-pinocamphone (49.7-57.7%), pinokarvon (5.5-24.9%), β -pinene (5.7-9.3%), 1,8-cineol (2.9-8.0%),

β-phellandrene (1.8–3.2%), myrtenol methyl ester (2.7–3.0%), sabinene (0.8–1.9%), myrtenol (1.4–1.7%), myrcene (0.5–1.3%) and trans-pinocamphone (<0.05–1.3%)[9].

In essential oil, obtained from herbs grown in Lublin (Poland) and cut in full bloom, the dominant components detected were: cis-pinocamphone ranging from 44.5 (2008) to 65.4% (2007), trans-pinocamphone — from 13 (2007) to 18% (2008), germacrene D — from 1.4 (2007) to 5.0% (2008) [10]. Further research identified cis-pinocamphone (48.6%), elemol (7.4%), β -pinene (6.1%), 1.8-cineol (5.8%) and caryophyllene oxide (5.5%) [11].

In essential oil from herbs cultivated in Moldova, depending on genotypical properties, 30-38 components were identified, among which dominated *cis*- and *trans*-pinocamphone. Essential oil from *H. officinalis* L. f. cyaneus contained 51.77% cis-pinocamphone and 6.70% trans-pinocamphone; from H. officinalis L. f. ruber 33.31% and 33.31%, respectively; and from H. officinalis L. f. albus -2.15% and 61.1%, respectively. Similarly, there was detected a considerable amount of β-pinene (f. cyaneus — 61.1%, f. albus — 7.38%, f. ruber — 4.15%), β -phellandrene (f. cyaneus — 4.83%, f. albus — 6.79%, f. ruber -3.64%) and carvacrol (f. cyaneus -0.34%, f. albus -0.34%, f. ruber -3.31%)[12].

The chemical composition of essential oil, obtained from different cultivars of *H. officinalis* grown in Ukrainian Polissia, has shown that the total amount of pinocamphone and isopinocamphone in the cultivar Atlant constituted 90.432%; in the cultivar Markiz — 89.228% and in Vodohrai — 89.096% [13].

Ukrainian findings showed that the mass portion and chemical composition of essential oil from *H. officinalis*, grown in the Pre-Mountainous Crimea, varied during a 24-hour period [13, 14].

Composition and amounts of essential oil components are viewed as characteristic chemotaxonomic properties of plants, which determine their biological activity and change when introduced in non-native environment [15-17].

The percentage composition of hyssop oil demonstrates that its biologically active compounds vary depending on various plant development conditions and stages of growth. Thus, to determine the optimal harvesting dates and spheres of the herb raw material application, the present research was done to analyze hyssop oil composition in relation to the phenological growth stage and plant age in conditions of Ukrainian Polissia.

Materials and Methods

The present research was conducted on experimental plots of Botanical Gardens of Zhytomyr National Agroecological University in 2008–2013, oil composition was analyzed in 2010–2013. Hyssop seedlings were obtained from botanical collection of New Cultures Department of Hryshko National Botanical Gardens of the National Academy of Sciences of Ukraine.

The study was based on *Hyssopus* officinalis L. cv. Markiz with dark blue and violet corolla (Fig. 1).



Fig. 1. The blooming stage of H. officinalis, grown in Botanical Gardens of Zhytomyr National Agroecological University

Plant material was cut at different stages of growth: the vegetative, budding, blooming and ripening stages. To analyze the chemical composition of hyssop oil, areal parts of 15 plants were cut, chopped and stirred so as mean samples could be obtained. Analysis of each sample was repeated three times. The absolute dry matter was determined by drying the samples at 105 °C until achieving the constant mass; fats content was found by obtaining solids-not-fat; "crude" cellulose according to Henneberg and Stockmann; calcium — by the trylonometric method [17]; proteins — by the Kjeldahl method; phosphorus — by the volumetric method with ammonium-molybdate solution [18]; ash — by burning in a muffle furnace (300–700 °C); wet ashing — by the Kurkayev method; ascorbic acid — according to Murrey [19]; carotene was determined with spectrophotometer UNICO 28 with the use of Kalosh gasoline solvent [20]; total discernible sugars and tannins were found according to Kryshchenko [21]; potassium — in flame photometer CL 378 (ELICO Limited, India) [19]. Essential oil content was determined according to the Clevenger method [22].

To obtain essential oil from raw material and to determine its qualitative and quantitative composition, a load of material (0,5 g) was put into a 20 ml capacity vessel, to which 10 ml water was added. The sample was hydrodistilled for 2 h in an air-cooled reflux condenser. In the process of hydrodistillation, volatile substances were adsorbed on the inner wall of the reflux condenser. After the system cooled, the adsorbed substances were slowly washed out by adding 3 ml of especially pure pentane into a dry 10 ml capacity vessel. The washout was concentrated by blowing (100 ml/min) especially pure nitrogen till the extract residual volume became 10 µl, which was then injected into a chromatographic injector. Further concentration of the sample was performed in the injector itself till the volume became 2 µl. The sample was injected into the chromatographic column in a splitless mode, i.e. without splitting the flow, which enabled to avoid the sample distribution loss and increase 10–20 times the chromatography sensitivity. The injecting rate was 1.2 ml/min for 0.2 min.

The gas chromatography analysis was carried out with gas chromatograph Agilent Technologies 6890, equipped with mass spectrometric detector 5973. The analysis conditions: a 30 m×0.25 mm i. d. DB-5 capillary column; carrier gas — helium, at a flow rate of 2 ml/min; the heater temperature at introduction — 250 $^{\circ}$ C. The thermostat was programmed at 50-320 °C, at a rate of 4 °C/ min. Identification of the constituents was based on mass spectra and retention times, which were compared with those given in NIST05 Mass Spectral Library, containing more than 470,000 spectra, besides, identification programmes AMDIS and NIST were used [23].

The obtained results were statistically processed with Microsoft Excel -10. Each test was repeated three times. Mean values and standard deviations $(M \pm m)$ were calculated. The difference was evaluated for significance level of P < 0.05 in accordance with the Student's test.

Results and Discussion

The obtained results demonstrate that the highest amount of dry matter is found in 3-year-old plants, which is 1.5 times higher than that of 1-year-old herbs (Table 1). The amounts of protein, ash and total sugar in 3-year-old plants decrease in comparison with 1-year-olds 1.3; 1.8;

3 times, respectively. The content of cellulose in raw material from 1-year-old herbs constitutes $37.81\pm0.90\%$, 2-year-olds — $41.2\pm0.76\%$, 3-year-olds — $37.81\pm0.90\%$ of absolutely dry mass, the content of fats is 2.7 ± 0.03 ; 3.8 ± 0.09 ; $3.81\pm0.26\%$, respectively. The amount of ascorbic acid in 1-year-old hyssop raw material is 2 times higher than that of 2-year-olds, and 5.6 times higher than that of 3-year-olds.

Nevertheless, tanning have not revealed the above tendency, the highest amount occurring in 3-year-old plants $(9.01\pm0.61\%)$ and the lowest — in 2-year-olds $(2.73\pm0.24\%)$. 1-year-old herbs contained $6.59\pm0.11\%$ of tannins. The carotene content in 1-year-old plants has proved to be 1.8 times higher than that of 2-year-olds, and 3.3 times higher if compared with 3-year-olds. Phosphorus is the lowest in 2-year-old plants $(0.15\pm0.006\%)$, and the highest in 1-year-old herbs (0.51± 0.001%). The calcium content hasn't displayed considerable variation. The highest potassium amounts have been detected in 2-year-old plants ($2607.4 \pm 94.64 \text{ mg}\%$), the lowest — in 3-year-olds (1126.7 \pm 121.37 mg%), in 1-yearolds this amount being $1486.81\pm94.00 \text{ mg}\%$ (Table 1).

The present study has proved that the yield of essential oil extracted from the areal parts of plants, cultivated in Ukrainian Polissia, does not significantly decrease with age. Thus, determined on a dry matter basis, the essential oil yield from 1-year-old plants constitutes 1.007%, from 2-year-old ones — 0.78% and from 3-year-olds — 0.71% (Fig. 2).

The present study of hyssop oil, obtained from areal parts of 1-year-old plants has identified 46 compounds, among which only

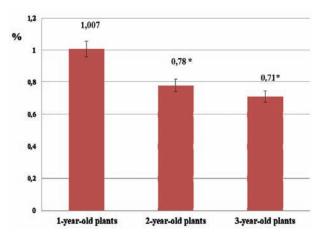


Fig. 2. H. officinalis essential oil yield obtained from plants in the blooming stage, cultivated in Ukrainian Polissia (% on a dry matter basis)

| | Ţ | The component amount,% | | | |
|--|------------------|------------------------|-------------------|--|--|
| Component | | Year of growth (age) | | | |
| | 1 | 2 | 3 | | |
| Dry matter | 20.16 ± 0.82 | 28.53 ± 2.30 | 29.76±0.23* | | |
| Protein | 20.91±0.91 | 19.18±0.92 | 16,37±0.83* | | |
| Ash | 8.30±0.72 | 7.20 ± 0.46 | 4.42±0.89* | | |
| Total sugar | 9.86±0.11 | 6.09±0.25* | 3.34±0.68* | | |
| Cellulose | 37.81±0.90 | 41.20±0.76* | 37.18±0.57 | | |
| Fats | 2.73±0.03 | 3.81±0.09* | 3.81±0.26* | | |
| Ascorbic acid, mg/100 g | 211.31±4.1 | $105.15 {\pm} 0.7 {*}$ | 37.88±1.87* | | |
| Tannins | 6.59±0.11 | 2.73±0.24* | 9.01±0.61* | | |
| Carotene, $\mathrm{mg}/100~\mathrm{g}$ | 2.51 ± 0.07 | $1.37 {\pm} 0.04$ | 0.77±0.01* | | |
| Phosphorus | 0.51±0.001 | 0.15±0.006* | $0.48 {\pm} 0.06$ | | |
| Calcium | 6.59±0.11 | 2.73±0.24* | 2.7±0.15* | | |
| Potassium, mg/100 g | 1486.8±94 | 2607.4±94.6* | 1126.8±121.4 | | |

Table 1. Composition of H. officinalis areal raw material in relation to plant age

Note: here and thereafter * — P < 0.05 in comparison with 1-year-old plants.

13 exceed 1%. The main components are: pinocamphone — 53.73%, isopinocamphone — 4.66%, myrtenol — 9.35%, camphor -3.86%.

Hyssop oil also displays homo myrtenol, α -thujene, β -bourbonene, neral, geranial, eugenol, terpinen-4-ol and other substances, the amount of which constituted from 0.01 to 0.9% (Table 2).

In hyssop oil obtained from 3-year-old plants 30 components have been identified, among which dominated 6 components: isopinocamphone (44.43%), pinocamphone (35.49%), myrtenol (5.26%), germacrene D (3.15%), pulegone (2.93%), biciclogermacrene (1.35%). There were also detected (from 0.01 to 1%): β -pinene, myrcene, limonene, β -phellandrene, cis-ocimene, myrtenal, bicicloelemen, β -bourbonene, β -caryophyllene and other substances (Table 2).

Study of the hyssop optimal harvest dates in conditions of Ukrainian Polissia allows to determine the terms, when the maximum of essential oil can be obtained. Its highest yield has been achieved at the blooming stage (0.72%), and the lowest — at the ripening stage (0.29%) (Fig. 3).

The present analysis of hyssop oil extracted from plants at the vegetative stage has detected 25 components, among which dominated monocyclic sesquiterpenes — elemol (33.25%), germacrene D (21.59%), biciclogermacrene (15.78%).

The given analysis has also identified bicyclic sesquiterpenes — β -caryophyllene (8.83%), humulene (1.68%), tricyclic sesquiterpenes — aromadendrene (3.68%),

isomer of tertiary sesquiterpene alcohol—eudesmol (1.43%), etc. These compounds are used in perfumery as aromatic and aroma fixing substances, in medicine as antihelminthics.

In hyssop oil, obtained from plants at the budding stage, 32 components have been identified with a high amount of pinocamphone — 70.42%. There is also isopinocamphone — 4.31%, germacrene D — 3.96%, elemol — 3.51%, myrtenol — 3.31%, pinokarvon — 2.87%, biciclogermacrene — 2.50% and other compounds (Table 3).

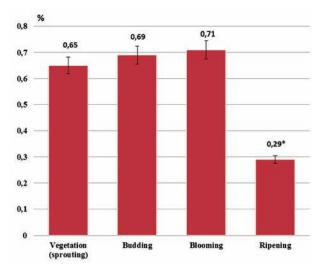


Fig. 3. Yield of hyssop oil from plants at the vegetative stage cultivated in Ukrainian Polissia (% on a dry matter basis; * — P < 0.05 in comparison with the vegetation (sprouting) stage)

 $Table\ 2.$ Composition of H. officinalis essential oil at the blooming stage in relation to plant age

| 2.0 | | The component amount,% | | |
|-----|------------------------|-------------------------------------|-----------------------|--|
| № | Component | 1-year-old plants 3-year-old plants | | |
| 1 | Pinocamphone | 53.73 ± 3.00 | $35.49 \pm 2.49 *$ | |
| 2 | Isopinocamphone | 4.66±0.44 | 44.43±3.38* | |
| 3 | Myrtenol | 9.35±1.11 | $5.26 {\pm} 0.73 {*}$ | |
| 4 | Pulegone | 0 | $2.93{\pm}0.25$ | |
| 5 | Biciclogermacren | 0.63±0.08 | 1.35±0.07* | |
| 6 | Phenylacetaldehyde | 3.07±0.19 | 0 | |
| 7 | β-Thujene | 1.48±0.07 | 0.15±0.03* | |
| 8 | Camphor | $3.86 {\pm} 0.44$ | 0 | |
| 9 | Methyleugenol | 1.16±0.09 | 0.14±0.03* | |
| 10 | Spathulenol | 1.95±0.28 | 0.19±0.03* | |
| 11 | Terpinen-4-ol | 1.30±0.30 | 0 | |
| 12 | α-Terpineol | 1.66±0.21 | 0 | |
| 13 | Epoxy linalyl acetate | 1.73±0.22 | 0 | |
| 14 | Viridiflorol | 2.25±0.11 | 0.06±0.007* | |
| 15 | Sabinene | 0.05 ± 0.006 | 0.22 ± 0.07 | |
| 16 | β-Pinene | 0.05±0.008 | 0.97±0.06* | |
| 17 | Myrcene | 0.15±0.03 | 0.50±0.024* | |
| 18 | Limonene | 0.09 ± 0.005 | 0.25±0.03* | |
| 19 | β-Phellandrene | 0.17±0.02 | $0.64 {\pm} 0.05 {*}$ | |
| 20 | Cis-ocimene | 0.17±0.03 | 0.22 ± 0.03 | |
| 21 | Trans-sabinene hydrate | 0.01±0.004 | 0.07±0.009* | |
| 22 | Linalool | 0.16 ± 0.03 | 0.66±0.08* | |
| 23 | Myrtenal | 0.12±0.03 | 0.19 ± 0.02 | |
| 24 | Neral | 0.08±0.008 | 0.04 ± 0.004 * | |
| 25 | Mertenil acetate | 0 | $0.06 {\pm} 0.005$ | |
| 26 | Bicicloelemen | 0 | 0.38±0.05 | |
| 27 | β-Bourbonene | 0.39 ± 0.05 | $0.48 {\pm} 0.07$ | |
| 28 | β-Elemene | 0 | $0.10 {\pm} 0.007$ | |
| 29 | α-Gurjunene | 0 | 0.13±0.03 | |
| 30 | β-Caryophyllene | $0.22 {\pm} 0.05$ | $0.54 {\pm} 0.05 {*}$ | |
| 31 | Allo-aromandendrene | 0 | 0.28 ± 0.04 | |
| 32 | Elemol | 0.43±0.08 | 0.12±0.03* | |
| 33 | Ledol | $0.16 {\pm} 0.02$ | $0.09 \pm 0.005 *$ | |
| 34 | α-Kadinol | 0 | $0.04{\pm}0.003$ | |
| 35 | Terpinolene | 0.01±0.004 | 0 | |
| 36 | α-Thujone | $0.27 {\pm} 0.04$ | 0 | |
| 37 | Homo myrtenol | $0.34{\pm}0.05$ | 0 | |
| 38 | 1(7).4.8-o-Mentatriene | 0.35±0.06 | 0 | |
| 39 | Borneol | 0.08±0.004 | 0 | |
| 40 | Aromadendrene | $0.34{\pm}0.04$ | 0 | |
| 41 | Germacrene D | 1.36±0.14 | 3.15±0.14* | |
| 42 | Globulol | $0.99 {\pm} 0.05$ | 0 | |
| 43 | β-Eudesmol | $0.17{\pm}0.02$ | 0 | |
| 44 | 1-Octene-3-ol | 0.07±0.007 | 0 | |
| 45 | Methylchavicol | 0.03±0.006 | 0 | |
| 46 | Geranial | 0.07 ± 0.007 | 0 | |
| 47 | Heranil acetate | 0.02 ± 0.005 | 0 | |
| 48 | Eugenol | 0.58±0.07 | 0 | |
| 49 | Cis-jasmone | 0.02±0.003 | 0 | |
| 50 | Caryophyllene oxide | 0.28±0.06 | 0 | |
| 51 | Humulene | 0.01±0.001 | 0 | |
| 52 | Trans-linalool oxide | 0.25 ± 0.03 | 0 | |
| 53 | Epoxy linalyl acetate | 1.73±0.22 | 0 | |

Table 3. Composition of H. officinalis essential oil with reference to the vegetation stage

| Component amount depending on the vegetation stage,% | | | | | |
|--|------------------------|-----------------|-----------------|-----------------|--|
| Component | vegetation (sprouting) | budding | blooming | ripening | |
| Linalool | 0.64±0.08 | 0.68±0.08 | 0.66 ± 0.07 | 0 | |
| Terpinen-4-ol | 0.30±0.02 | 0.00=0.00 | 0.00=0.01 | 0 | |
| α-Terpineol | 0.90±0.06 | 0.32±0.06* | 0 | 0.12±0.01* | |
| Myrtenol | 0.50±0.02 | 3.31±0.54* | 5.26±0.11* | 0.47 ± 0.05 | |
| Geraniol | 0.71±0.04 | 0 | 0 | 0 | |
| Bicicloelemen | 1.51±0.1 | 0.21±0.05* | 0.38±0.04* | 1.01±0.05* | |
| β-Bourbonene | 1.69±0.1 | 0.65±0.06* | 0.48±0.03* | 0.52±0.03* | |
| β-Elemene | 0.65±0.07 | 0 | 0.10±0.03* | 0 | |
| α-Gurjunene | 0.82±0.08 | 0 | 0.13±0.03* | 0 | |
| β-Caryophyllene | 8.83±0.9 | 1.50±0.34* | 0.54±0.05* | 0 | |
| β-Kubeben | 0.43±0.05 | 0 | 0 | 0.21±0.03* | |
| Humulene | 1.68±0.10 | 0.40±0.06* | 0 | 0 | |
| Aromadendrene | 3.68±0.43 | 0.37±0.06* | 0 | 1.19±0.23* | |
| Germacrene D | 21.59±1.63 | 3.96±0.54* | 0.15±0.03* | 5.86±0.31* | |
| Biciclogermacren | 15.78±1.05 | 2.50±0.41 | 1.35±0.07 | 10.30±1.04 | |
| α-Amorphen | 0.38±0.04 | 0 | 0 | 0 | |
| Elemol | 33.25±1.82 | 3.51±0.10* | 0.12±0.03* | 44.46±1.59* | |
| Globulol | 0.71±0.07 | 0.16±0.03* | 0 | 0 | |
| Viridiflorol | 0.72±0.06 | 0 | 0.06±0.005* | 0.46±0.03* | |
| Spathulenol | 0.52±0.02 | 0.21±0.03* | 0.19±0.04* | 4.36±0.81* | |
| Ledol | 0.94±0.10 | 0.16±0.02* | 0.09±0.003* | 0 | |
| γ-Eudesmol | 1.07±0.06 | 0 | 0 | 3.92±0.19* | |
| α-Kadinol | 0.46 ± 0.05 | 0.19±0.03* | 0.04±0.02* | 0 | |
| α-Eudesmol | 0.82±0.08 | 0 | 0 | 4.04±0.69* | |
| β-Eudesmol | 1.43±0.1 | 0.23±0.05* | 0 | 4.34±0.64* | |
| Cis-3-Hexen-1-ol | 0 | 0.13±0.03 | 0 | 0 | |
| β-Pinene | 0 | 0.19±0.03 | 0.97±0.05** | 0 | |
| Myrcene | 0 | 0.15±0.02 | 0.50±0.04** | 0 | |
| Limonene | 0 | 0.11±0.03 | 0.25±0.03** | 0 | |
| β-Phellandrene | 0 | 0.13±0.03 | 0.64±0.05** | 0 | |
| Trans-ocimene | 0 | 0.08±0.005 | 0 | 0 | |
| Cis-ocimene | 0 | 0.47±0.04 | 0.22±0.03** | 0 | |
| Trans-sabinene hydrate | 0 | 0.12±0.05 | 0.07±0.004 | 0 | |
| β-Thujone | 0 | 0.48±0.05 | 0.15±0.02** | 0 | |
| Phenylacetaldehyde | 0 | 0.24 ± 0.05 | 0 | 0 | |
| Pinokarveol | 0 | 0.17±0.03 | 0 | 0 | |
| Pinocamphone | 0 | 70.42±3.54 | 35.49±2.36** | 0.09±0.003** | |
| Isopinocamphone | 0 | 4.31±0.23 | 44.43±2.42** | 0.06±0.005** | |
| Pinokarvon | 0 | 2.87±0.34 | 0 | 0 | |
| Methylchavicol | 0 | 0.24 ± 0.05 | 0 | 0 | |
| 1.6-Germacradien-5-ol | 0 | 0.13±0.04 | 0 | 0 | |
| Krypton | 0 | 0 | 0 | 0.07±0.003 | |
| Anisic acid methyl ester | 0 | 0 | 0 | 0.35 ± 0.05 | |
| β-Caryophyllene | 0 | 0 | 0 | 0.81±0.08 | |
| Isoelemol | 0 | 0 | 0 | 1.22 ± 0.14 | |
| Caryophyllene oxide | 0 | 0 | 0 | 0.93 ± 0.05 | |
| Pulegone | 0 | 0 | 2.93±0.42 | 0 | |
| Methyleugenol | 0 | 0 | 0.14±0.02 | 0 | |
| Sabinene | 0 | 0 | 0.22±0.04 | 0 | |
| Myrtenal | 0 | 0 | 0.19±0.03 | 0 | |
| Neral | 0 | 0 | 0.04±0.004 | 0 | |
| Mertenil acetate | 0 | 0 | 0.06±0.002 | 0 | |
| Alloaromadendren | 0 | 0 | 0.28±0.03 | 0 | |

Note: * — P < 0.05 compared with the sprouting stage, ** — with the budding stage.

In the blooming stage there have been identified 30 components with high amounts of isopinocamphone and pinocamphone 44.43% and 35.49%), somewhat lower is the content of myrtenol (5.26%), pulegone (2.93%), biciclogermacrene (1.35%).

The dominant constituents of hyssop oil at the ripening stage are: elemol (44.46%), biciclogermacrene (10.30%), germacrene D (5.86%), spathulenol (4.36%), β -eudesmol (4.34%), α -eudesmol (4.04%), γ -eudesmol (3.92%). The total number of identified compounds is 21 (Table 3).

The quality of hyssop oil is known to depend on the ratio of the main components — pinocamphone and isopinocamphone, the total amount of which should exceed 55%. The abovementioned compounds are monoterpene ketones, which are characterized by a considerable biological activity and have anticeptic, anti-inflammatory, sudorific, antihelminthic, tonic, wound healing, and in case of overdosing — toxic properties [24].

In different growing conditions, the total content of pinocamphone and isopinocamphone, detected in hyssop oil, is: in Spain — 44.33 [8], Serbia — 57.0, Western Himalayas — 49.7–57.7 [9], Moldova — 58.47 [12], Poland — 83.4% of the total oil content [11].

At the budding and blooming stages, the total content of pinocamphone and isopinocamphone in the plants, introduced in Ukrainian Polissia, constitutes 70.42% and 74.73% of the total oil content, which testifies to favorable growing conditions. It should be noted that the total content of the above components in 1-year-old plants was 58.39%, and in 3-year-olds — 79.92%.

Plants at the vegetative stage have displayed no pinocamphone and isopinocamphone, and the ripening stage is characterized only by traces of these compounds (0.09 and 0.06%). Thus, these stages are favorable for collecting raw material to be used in potential preparations for internal usage, because they exert no toxic effect.

Hyssop phyto material cultivated in Ukrainian Polissia is a source of vitamins,

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macro elements and fats. One-year-old plants have the highest content of proteins, total sugars, ascorbic acid, carotene, calcium, phosphorus. The amount of dry matter, fats and tannins in the raw material increased with plant age.

It has been found that the total amount of essential oil, obtained from the plants areal parts, does not decrease considerably: 1-year-old plants yielded 1.007%, 2-year-old — 0.78% and 3-year-old — 0.71%. One-year-old plants displayed 46 components, among which dominated: pinocamphone — 53.73%, isopinocamphone—4.66%, myrtenol—9.35%. Essential oil from 3-year-old *H. officinalis* exhibited 30 components, among which dominated: isopinocamphone (44.43%), pinocamphone (35.49%), myrtenol (5.26%).

In the vegetation (sprouting) stage 25 components have been detected, among which predominated elemol (33.25%), germacrene D (21.58%), biciclogermacrene (15.78%). At the blooming stage 30 components have been identified, with a high content of isopinocamphone and pinocamphone (44.43% and 35.49%). In the ripening stage 21 compounds have been revealed, with predominance of elemol (44.46%), biciclogermacrene (10.30%) and germacrene D (5.86%).

Areal parts of 1-year-old plants have proved to be rich in ascorbic acid (211.31 mg%) and carotene (2.51 mg%), which possess immune modulating and antioxidant properties. Therefore, it seems useful to add hyssop green sprouts or dry raw material to daily rations in order to replenish the content of vitamins in a human body.

The obtained data shows that the herb material from 1-year-old plants can be used in food and pharmaceutical industries, while plants collected at the blooming stage are preferable for perfumery. Cultivation of H. officinalis in Ukrainian Polissia has a great potential and can be employed in biotechnology with the aim to expand the assortment of biologically active food additives and antimicrobial bio preparations.

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СКЛАД ГІСОПУ ЛІКАРСЬКОГО ЗАЛЕЖНО ВІД ВІКУ ТА ФАЗ РОЗВИТКУ РОСЛИНИ

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Метою роботи було дослідження біохімічного складу *Hyssopus officinalis* L. (*Lamiaceae*) залежно від віку та фенологічних фаз розвитку в умовах Полісся України для встановлення оптимальних термінів збирання та напрямів використання фітосировини.

СОСТАВ ИССОПА ЛЕКАРСТВЕННОГО В ЗАВИСИМОСТИ ОТ ВОЗРАСТА И ФАЗ РАЗВИТИЯ РАСТЕНИЯ

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Целью работы было исследование биохимического состава *Hyssopus officinalis* L. (*Lamiaceae*) в зависимости от возраста и фенологических фаз развития в условиях Полесья Украины для установления оптимальных сроков сбора и направлений использования фитосырья. Сировину збирали в різні фази розвитку рослин: вегетативного росту, бутонізації, цвітіння та плодоношення. Для аналізу складу використовували надземну частину рослин. Визначали абсолютно суху речовину шляхом висушування зразків за 105 °С до постійної маси, «сиру» клітковину, вміст протеїну, жирів, кальцію, калію, фосфору, аскорбінової кислоти, каротину, водорозчинних цукрів і дубильних речовин, ефірної олії.

Встановлено, що в онтогенезі рослин масова частка ефірної олії, виділена з надземної частини H. officinalis, знижується неістотно: вихід ефірної олії у рослин першого року життя становив 1,007%, другого — 0,78%, третього – 0,71%. У рослин першого року життя у складі ефірної олії виявлено 46 компонентів, серед яких домінували: пінокамфон — 53,73%, ізопінокамфон — 4,66%, міртенол — 9,35%, камфора — 3,86%. В ефірній олії H. officinalis третього року життя ідентифіковано 30 компонентів, серед яких переважали: ізопінокамфон — 44,43%, пінокамфон — 35,49%, міртенол — 5,26%, гермакрен D — 3,15%, пулегон — 2,93%, біциклогермакрен — 1,35%.

Відзначено зміну кількісного та якісного складу ефірної олії *H. officinalis* упродовж вегетаційного періоду. У фазі вегетативного росту ідентифіковано 25 сполук, серед яких домінували елемол — 33,25%, гермакрен D – 21,59%, біциклогермакрен — 15,78%. У фазі цвітіння ідентифіковано 30 компонентів, зафіксовано високий вміст ізопінокамфону та пінокамфону — 44,43% і 35,49%), дещо нижчий — міртенолу — 5,26%, пулегону — 2,93%, біциклогермакрену — 1,35% . Під час плодоношення ідентифіковано 21 сполуку з домінуванням елемолу — 44,46%, біциклогермакрену — 10,30%, гермакрену D -5,86%, спатуленолу — 4,36%, β -евдесмолу — 4,34%, α -евдесмолу — 4,04%, γ -евдесмолу — 3,92%.

Зроблено висновок про доцільність використання рослинної сировини гісопу лікарського першого року життя у харчовій промисловості, а у фазі цвітіння — у косметології та парфумерії.

Ключові слова: Hyssopus officinalis, біологічно активні речовини, ефірна олія, пінокамфон, ізопінокамфон.

Сырье собирали в разные фазы развития растений: вегетативного роста, бутонизации, цветения и плодоношения. Для анализа состава использовали надземную часть растений. Определяли абсолютно сухое вещество путем высушивания образцов при 105 °С до постоянной массы, «сырую» клетчатку, содержание протеина, жиров, кальция, калия, фосфора, аскорбиновой кислоты, каротина, водорастворимых сахаров и дубильных веществ, эфирного масла.

Установлено, что в онтогенезе растений массовая доля эфирного масла, выделенного из надземной части H. officinalis, снижается незначительно: выход эфирного масла у растений первого года жизни составлял 1,007%, второго — 0.78%, третьего — 0.71%. У растений первого года жизни в составе эфирного масла обнаружено 46 компонентов, среди которых доминировали: пинокамфон -53,73%, изопинокамфон — 4,66%, миртенол — 9,35%, камфора — 3.86%. В эфирном масле H. officinalis третьего года жизни идентифицировано 30 компонентов, среди которых преобладали: изопинокамфон — 44,43%, пинокамфон — 35,49% , миртенол — 5,26% , гермакрен D — 3,15%, пулегон — 2,93%, бициклогермакрен — 1,35%.

Отмечено изменение количественного и качественного состава эфирного масла H. officinalis в течение вегетационного периода. Во время фазы вегетативного роста идентифицировано 25 соединений, среди которых доминировали элемол — 33,25%, гермакрен D — 21,59%, бициклогермакрен 15,78%. В течение фазы цветения идентифицировано 30 компонентов, отмечено высокое содержание изопинокамфона и пинокамфона — 44,43% и 35,49%, несколько ниже — миртенола — 5,26%, пулегона (2,93%) и бициклогермакрена — 1,35%. Во время плодоношения идентифицировано 21 соединение с преобладанием элемола — 44,46%, бициклогермакрена — 10,30%, гермакрена D — 5.86%, спатуленола — 4.36%, lpha-эвдесмола — 4,04%, eta-эвдесмола — 4,34% и γ -эвдесмола — 3,92%.

Сделан вывод о целесообразности использования растительного сырья иссопа лекарственного первого года жизни в пищевой промышленности, а в фазе цветения — в косметологии и парфюмерии.

Ключевые слова: Hyssopus officinalis, биологически активные вещества, эфирное масло, пинокамфон, изопинокамфон.