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## BIOCHEMICAL COMPOSITION OF ESSENTIAL OIL OF *HYSSOPUS SERAVSCHANICUS* (DUBJ.) PAZIJ. (LAMIACEAE) INTRODUCED INTO THE CONDITIONS OF UKRAINE (FOREST-STEPPE ZONE)

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**Background.** In today's world, there is an increased demand for natural essential oils as one of the sources of improvement of human life. This is due to the wide range of biologically active effects of essential oils, which determines their wide use in medicine, food industry, aromatherapy, cosmetology, technical cleaning products, etc. Expanding the range of cultivated essential plants contributes to the preservation of natural floral resources and enriches the range of natural essential oils. The purpose of our study is to evaluate the quantitative and qualitative composition of the essential oil of *Hyssopus seravschanicus* (Dubj.) Pazij. (Lamiaceae) as a new plant species introduced in the conditions of the Right Bank Forest-Steppe Zone of Ukraine.

**Methods.** To obtain the essential oil of *H. seravschanicus*, the method of hydrodistillation using a Clevenger apparatus was applied. Air-dry raw material of plants – the above-ground herbaceous part collected in the phase of mass flowering of plants was studied. The determination of compounds and their relative participation in the composition of the essential oil was determined by the method of gas chromatography-mass spectrometry.

**Results.** The seed population of *H. seravschanicus* introduced in the conditions of the Right Bank Forest Steppe of Ukraine is characterized by a high biosynthesis of essential oil with a yield rate of  $0.72 \pm 0.04$  %. 27 essential oil compounds have been identified, which by their chemical nature belong to terpenes, terpenoids and sesquiterpenes.



The dominant compound is Isopinocampone (61.58 %). An equally important role in the aromatic composition of the oil is played by  $\beta$ -Pinene (14.66 %), Terpinen-4-ol (8.53 %), Sabinen (2.24 %), 1,8-Cineol (1.77 %) and Myrcene (1.77 %), Spathulenol (1.5 %).

**Conclusions.** Thus, the high essential oil productivity of the introduced species *H. seravschanicus* was experimentally established in new growth conditions – the Right-Bank part of the Forest-Steppe Zone of Ukraine. This allows us to consider this type of plant as a source of natural essential oils of the isopinocampone chemotype – *H. seravschanicus* st. Isopinocampone.

**Keywords:** *Hyssopus seravschanicus*, essential oil, compounds, Forest Steppe of Ukraine

## INTRODUCTION

While improving the quality of human life, it is impossible to do without pure natural substances such as essential oils. The popularity of essential oils has been revived due to the growing awareness of properties, which is driving the demand for their extraction and use. The coronavirus pandemic that swept the world has also increased the demand for essential oils in the aromatherapy and personal care industries (Essential Oils..., 2022; Natural Flavors..., 2022).

The world market is developing mainly due to the sustainable trend of green consumerism, and essential oils occupy a prominent place in this process. Growth of the world population along with GDP are the factors which are expected to drive further growth in the use of essential oils in the food and beverage industry, aromatherapy, perfumery, as well as cosmetics and personal care, healthcare and spa, which are today's key end-users of essential oils.

Today, Europe accounts for the largest share of the global essential oil market, with the Asia-Pacific region and North America sharing the second place (Pertuzzi, 2022; Cosmetic Oil Market..., 2022).

However, with the growing scale of urbanization and industrialization, natural populations of ether-bearing plants are depleted or disappear altogether. Essential oils are rather an expensive product. Research work on the introduction of essential oil plants contributes to the preservation of plant diversity. At the same time, it ensures the search for new types of plants, the quantitative content and qualitative composition of essential oil of which has the potential for wide application.

In this regard, representatives of the genus *Hyssopus* L. (Lamiaceae), in particular *H. seravschanicus* (Dubj.) Pazij, are interesting research objects. The natural habitat of *H. seravschanicus* is concentrated in Afghanistan, Pakistan, Kyrgyzstan, Tajikistan and Uzbekistan (Safarov, 2013). In Ukraine, *H. seravschanicus* occurs to a limited extent in the collections of botanical institutions or private nurseries. Nevertheless, as our own research has shown, plants grow well, develop and go through a full cycle of development in the conditions of the Right Bank Forest-Steppe. That is, there are no barriers to growing this type of plant, at least in the Forest-Steppe of Ukraine.

Within the framework of complex introduction studies, experimental work was carried out on the study of the quantitative content of essential oil in raw materials of introduced plants *H. seravschanicus* and its component composition. The purpose of this study is to establish the essential oil potential of introduced plants and compare them with the published data related to the study of *H. seravschanicus* plants from natural populations.

## MATERIALS AND METHODS

**Plant source.** The flowering above-ground herbaceous part of *H. seravschanicus* plants was used for experimental studies. The plants grow in the collection of „Non-traditional essential oil plants” of the Cultural Flora Department of the M. M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (NBG) (Right Bank Forest-Steppe). They were introduced in 2018 seed material. The seeds were received by the „Index Seminum” system from the botanical institution of Vakraot (Hungary).

**Isolation of the essential oils.** To obtain oil, the hydrodistillation method was used by apparatus with a Clevenger-type nozzle. We hold the opinion that the distillation process is considered more gentle for extracting essential oil from raw materials, since it is highly sensitive to temperatures and can lose its aroma if more powerful processes are used (Essential Oils..., 2022).

The raw material of *H. seravschanicus*, which was previously dried to an air-dry state using an Eridri ULTRA FD1000 dryer was subjected to distillation.

Sample weight 50 g. Multiplicity of experiment 4-fold. Exposure time – 1.45 hours (from the moment the water boils).

**Gas chromatography – Mass spectrometry analysis (GC-MS).** The chromatographic profile was obtained on an Agilent Technologies 7890 gas chromatograph using a vf-5ms (5%-phenyl)-methylpolysiloxane) 25 m long capillary column, with the internal diameter of 0.25 mm and the stationary phase thickness of 0.33  $\mu\text{m}$  under the following conditions: gas velocity-carrier – 1.0 mL/min, flow split ratio – 1:20, evaporator temperature – 250  $^{\circ}\text{C}$ , detector temperature (DEP) – 280  $^{\circ}\text{C}$ , column temperature regime – gradual heating from 60  $^{\circ}\text{C}$  to 185  $^{\circ}\text{C}$ .

The component composition of the essential oil was determined on a gas chromatograph with a mass spectrometric detector HP 6890 with a mass spectrometric detector 5973. Mass spectrometric detector 1.6 – 800 a.o.m., EI ionization, SIM & Scan mode), „Hewlett Packard”, USA. Chromatographic column – capillary HP–5ms (5%-phenyl)-methylpolysiloxane), outer diameter 0.25 mm, length 30 m. Carrier gas – helium. Carrier gas velocity – 1.2 mL/min. Sample injection heater temperature – 180  $^{\circ}\text{C}$ . Oven temperature programmable from 62 to 165 deg at a rate of 5 deg/min. Sample injection (1  $\mu\text{L}$ ) without flow split. For the identification of essential oil components, the NIST mass spectrum library was used in combination with programs for identification by time of content AMDIS.

## RESULTS AND DISCUSSION

The genus *Hyssopus* includes 10 species (according to The Plant List) that are common in Central Asia, Eastern Mediterranean and Mongolia (Genus *Hyssopus*. The Plant List... n.d.; Royal Botanic Gardens Kew... n.d.).

Within the genus, the most researched to date is *H. officinalis* L. (Sharif-Rad *et al.*, 2022). Not many studies have been devoted to another species, *H. seravschanicus* (Dubj.) Pazij., which has a number of synonymous names – *H. ferganensis* Boriss., *H. officinalis* var. *seravschanicus* Dubj., *H. tianschanicus* Boriss., under which publications about this plant can be found.

*H. seravschanicus* is a type of plant that belongs to the Irano-Turanian phryganoids – the group of mesoxerophytic shrubs and semi-shrubs and some perennial grasses (Safarov, 2013). It is known to be used for medical, food purposes, in perfumery,

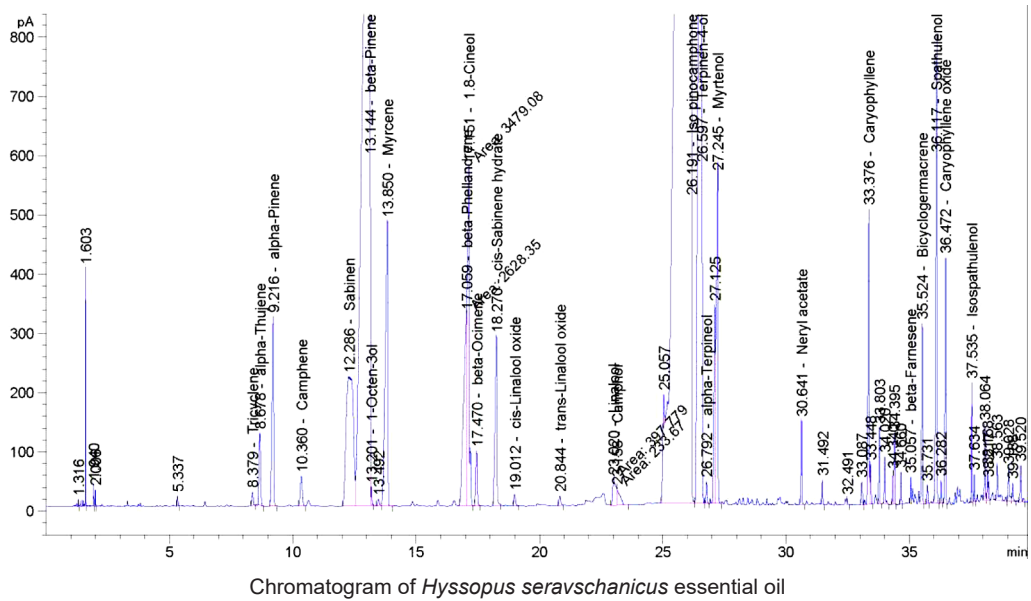
and as a powerful honey carrier. The content of essential oil in the aerial part of plants of natural populations varies depending on the region of raw material collection and is within 0.34–1.0 % (Dzhumaev *et al.*, 1990; Sharopov *et al.*, 2012; Azimova *et al.*, 2012).

*H. seravschanicus*, introduced under the conditions of NBG, produces essential oil with a yield of  $0.72 \pm 0.04$  %, which is undoubtedly a high indicator. The oil is transparent, has a yellow color and a pleasant strong aroma.

The obtained essential oil samples were subjected to GC-MS analysis. 27 components have been identified that make up 100 % of the total oil. The dominant compound is the bicyclic monoterpene Iso pinocamphone (61.58 %), other important compounds are  $\beta$ -Pinene (14.66 %), Terpinen-4-ol (8.53 %), Sabinen (2.24 %), 1.8-Cineol (1.77 %) and Myrcene (1.77 %), Spathulenol (1.5 %). In general, from a biochemical point of view, all the volatile compounds found in the essential oil of *H. seravschanicus* belong to the class of terpenes (monoterpenes that form the light volatile fractions of the essential oil and sesquiterpenes that make up the heavy volatile fractions) and their terpenoid and sesquiterpenoid derivatives. The components of the monoterpene group predominate quantitatively (Table, Figure).

Components of *Hyssopus seravschanicus* essential oil

No	Compounds	Recording time, min	Content index, %
1.	Tricyclene	8.379	0.05
2.	$\alpha$ -Thujene	8.678	0.28
3.	$\alpha$ -Pinene	9.216	0.80
4.	Camphene	10.360	0.15
5.	Sabinen	12.286	2.24
6.	$\beta$ -Pinene	13.144	14.66
7.	1-Octen-3ol	13.201	0.04
8.	Myrcene	13.850	1.77
9.	$\beta$ -Phellandrene	17.059	1.34
10.	1.8-Cineol	17.151	1.77
11.	$\beta$ -Ocimene	17.470	0.21
12.	<i>cis</i> -Sabinene hydrate	18.270	0.80
13.	<i>cis</i> -Linalool oxide	19.012	0.05
14.	<i>trans</i> -Linalool oxide	20.844	0.04
15.	Linalool	23.020	0.20
16.	Camphor	23.158	0.12
17.	Iso pinocamphone	26.191	61.58
18.	Terpinen-4-ol	26.597	8.53
19.	$\alpha$ -Terpineol	26.792	0.06
20.	Myrtenol	27.245	1.45
21.	Neryl acetate	30.641	0.21
22.	Caryophyllene	33.376	0.84
23.	$\beta$ -Farnesene	35.057	0.06
24.	Bicyclogermacrene	35.524	0.46
25.	Spathulenol	36.117	1.50
26.	Caryophyllene oxide	36.472	0.62
27.	Isospathulenol	37.535	0.19
Totals			100



It is known from literary sources that *H. seravschanicus* from natural populations, for example, in Uzbekistan, contains Pinocamphone (71.0 %), β-Pinene (8.6 %), 1.8-Cineole (6.4 %), Carvacrol (1.6 %), cis-Ocimene (1.4%), p-Cymene (1.3 %) and Sabinene (Mamadaliyeva *et al.*, 2017). From Tajikistan – cis-Pinocamphone (57.0–88.9 %), β-Pinene (0.4–6.0 %), 1.8-Cineol (1.8–3.6 %), Camphor (0.5–4.0 %), Spathulenol (0.1–5.0 %) (Egamberdieva *et al.*, 2018). From Kyrgyzstan – the components Camphene, β-Pinene, Pinocamphone, 1.8-Cineol, Linalool, α-Terpinyl-acetate, Bornyl acetate, Myrcene, Limonene (Eisenman *et al.*, 2012).

It is obvious that the essential oils of plants from different regions of growth differ in terms of dominant components and their quantitative content. However, the main dominant component is Isopinocamphone, cis-pinocamphone or pinocamphone. That is, there are different chemotypes of *H. seravschanicus* with regard to the main component.

The high biosynthesis of Isopinocamphone (bicyclic monoterpene ketone) by of *H. seravschanicus* plants under NBG conditions suggests that this type of plant can be considered as a source of natural essential oils of the iso-pinocamphonic chemotype – *H. seravschanicus* st. Isopinocamphone and prompts to focus on the selection of productive lines in the subsequent breeding process, since this compound is considered the main component of commercial oils.

It should be noted that the presence of Isopinocamphone, cis-pinocamphone or pinocamphone in the essential oil requires its moderate use for medicinal purposes while respecting the safety profile. This is due to the fact that these compounds have a neurotoxic effect and can cause convulsions in humans in excess doses.

Thus, the plants of the seed population of *H. seravschanicus*, introduced in the conditions of the NBG (Right Bank part of the Forest-Steppe of Ukraine) have a high content of essential oil in the above-ground herbaceous part in the phase of mass flowering. The indicator is 0.72±0.04 % based on absolutely dry mass. 27 compounds

were identified, among which Isopinocampone (61.58 %) was dominant. The obtained results allow us to consider the introduced plant species as a new potential essential oil plant in the territory of Ukraine and as a source of one of the isomers of Pinocampone.

## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## AUTHOR CONTRIBUTIONS

Conceptualization, [K-V.S.M.]; methodology, [K-V.S.M.; L.I.V.; G.O.V.]; validation, [K-V.S.M.; L.I.V.]; formal analysis [K-V.S.M.]; investigation, [K-V.S.M.; L.I.V.; G.O.V.]; resources, [K-V.S.M.; L.I.V.; R.D.B.]; data curation, [K-V.S.M.; L.I.V.]; writing – original draft preparation, [K-V.S.M.; L.I.V.]; writing – review and editing, [K-V.S.M.]; visualization, [K-V.S.M.; L.I.V.; G.O.V.]; supervision, [K-V.S.M.]; project administration, [K-V.S.M.; L.I.V.; R.D.B.]; funding acquisition, [K-V.S.M.; R.D.B.].

All authors have read and agreed to the published version of the manuscript.

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## БІОХІМІЧНИЙ СКЛАД ЕФІРНОЇ ОЛІЇ *HYSSOPUS SERAVSCHANICUS* (DUBJ.) PAZIJ. (LAMIACEAE), ІНТРОДУКОВАНОГО В УМОВАХ УКРАЇНИ (ЛІСОСТЕПОВА ЗОНА)

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**Вступ.** Сьогодення демонструє підвищення у світі попиту на натуральні ефірні олії як одне із джерел покращення життя людини. Це пов'язано з широким спектром біологічно активної дії ефірних олій, внаслідок чого актуальним є їхнє використання в лікарській, харчовій галузі, ароматерапії, косметології, технічних засобах очищення та інше. Розширення асортименту культивованих рослин-ефіроносів сприяє збереження природних флористичних ресурсів і збагачує асортимент натуральних ефірних олій.

Мета нашого дослідження – оцінити кількісний і якісний склад ефірної олії *Hyssopus seravschanicus* (Dubj.) Pazij. (Lamiaceae) як нового виду рослин, інтродукованого в умовах Правобережного Лісостепу України.

**Матеріали та методи.** Для отримання ефірної олії *H. seravschanicus* застосовано метод гідродистиляції із використанням апарату Клевенджера. Досліджували повітряно-суху сировину рослин – надземну трав'яну частину, зібрану у фазі масового квітання рослин. Встановлення сполук і їхньої дольової участі у складі ефірної олії визначено методом газової хромато-мас-спектрометрії.

**Результати.** Для насінної популяції *H. seravschanicus*, інтродукованої в умовах Правобережного Лісостепу України, характерний високий біосинтез ефірної

олії із показником виходу  $0,72 \pm 0,04$  %. Ідентифіковано 27 компонентів ефірної олії, які за своєю хімічною природою належать до терпенів, терпеноїдів і сесквітерпенів. Домінуючою сполукою є Isopinocampone (61,58 %). Не менш важливу роль в ароматичній композиції олії відіграють  $\beta$ -Pinene (14,66 %), Terpinen-4-ol (8,53 %), Sabinen (2,24 %), 1.8-Cineol (1,77 %) та Myrcene (1,77 %), Spathulenol (1,5 %).

**Висновки.** Отже, експериментальним способом встановлена висока ефіро-олійна продуктивність інтродукованого виду *H. seravschanicus* в нових умовах зростання – Правобережній частині лісостепової зони України. Це дає змогу розглядати цей вид рослин як джерело натуральних ефірних олій ізопінокамфонного хемотипу – *H. seravschanicus* st. Isopinocampone.

**Ключові слова:** *Hyssopus seravschanicus*, ефірна олія, сполуки, Лісостеп України