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GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC CHARACTERISTICS OF ESSENTIAL OILS OF PLANTS OF THE GENUS *PYCNANTHEMUM* (LAMIACEAE) AND PECULIARITIES OF THEIR APPLICATION IN PRACTICE

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Background. Not all plants are capable of producing essential oil (EO). There are 3.000 different plant EOs described in the world, but only 10 % of them are widely used by humans. In particular, perfumery and the food industry use the largest amount of natural EOs – 30 % and 40 % of the world production, respectively.

A thorough study of EOs, knowledge of their components in both chemical and pharmacological sense will facilitate the improvement of the quality of human life.

The purpose of our experimental study was to determine the EO potential of four species of the genus *Pycnanthemum* Michx. (Lamiaceae): *P. montanum* Michx., *P. muticum* (Michx.) Pers., *P. tenuifolium* Schrad., *P. verticillatum* var. *pilosum* (Nutt.) Cooperr. introduced in the forest-steppe zone of Ukraine. The above plants are representatives of the flora of North America.

Methods. The EOs were obtained in laboratory conditions by hydrodistillation using a Clevenger apparatus. The raw material of the plants – a blended mass of leaves, inflorescences and the herbaceous part of the stem was used in an air-dry state. Harvesting of raw materials was carried out during the flowering phase of plants. The EOs content is given in terms of an absolute dry weight. The identification of compounds and their amount in each EO was determined by the method of gas chromatography-mass spectrometry.

Results. In the conditions of the forest-steppe of Ukraine, the introduced species of plants of the genus *Pycnanthemum* show a high level of EOs biosynthesis – 0.53–3.34 %. The dominant compounds common to the obtained essential oils are Pulegone, Isomenthone, and Isopinocamphone. The indicators of the content of pulegone exceed those of other compounds and reach 48.14–73.93 %. Pulegone is a controversial organic compound, since it exhibits a high biological activity and possesses pharmacological properties suitable for use in medical practice, but when cleaved, it forms toxic metabolites, in particular mentofuran.

Conclusions. The obtained results indicate that plants of the genus *Pycnanthemum*, which are little known in Ukraine, have a high EO potential under the proposed growth conditions. Due to this, they are potential objects for use in domestic cultural phytocenoses. Considering the component composition of EOs with the dominant Pulegone, their consumption requires correct dosage.

Keywords: *Pycnanthemum* sp., essential oils, GC-MS, Pulegone

INTRODUCTION

The role of the plant world in human life cannot be overestimated. Among the infinite ”gifts” of plants are their secondary metabolites – essential oils (EOs). However, not all plants are capable of biosynthesizing EOs. Therefore, those that possess such a property are referred to a separate group of EOs plants. At present, when extraordinary attention is paid to “naturalness” in all areas of human life, plant EOs are in high demand and there is a tendency to increase their production and use (Global Essential Oil…, 2021; Cosmetic Oil…, 2022).

It is known that EOs are found among plants from 60 families distributed throughout the world. Of the 3000 different EOs described so far, only about 300 are available on the world market (Raut *et al.*, 2014).

EOs are highly concentrated liquids of a complex mixture of unique volatile chemical compounds such as terpenoids and their oxygen derivatives, including aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones, and compounds containing nitrogen and sulfur, homologues of phenylpropanoids. Typically, EOs consist of terpenoids, alcohols, ethers, ethers, ketones, and aldehydes in various concentrations (Božović *et al.*, 2017; Nazir *et al.*, 2022; Barbouchi *et al.*, 2021).

The chemical composition of EOs determines their aroma and pharmacological effects. Due to their high biological activity, they are used in pharmacy (drugs, herbal remedies, nutritional supplements) and aromatherapy. It is known, for example, that EOs containing mainly aldehydes and phenols are characterized by the greatest antibacterial activity. Components such as monoterpenes, sesquiterpenes and triterpenes have a strong antiviral effect, for example, against rhinoviruses and herpes viruses (Nazir *et al.*, 2022; García-Nieto, 2000). Also, EOs are in demand as natural flavors and preservatives in cosmetology, perfumery, food industry, in household chemicals, etc. (Moré, 2009). Currently, perfumery utilizes 30 % of the world production of EOs plants, while food industry – 40 % (García-Nieto, 2000).

The study of plant EOs, their component composition and, correspondingly, the areas of their practical application is timely. In our work, we aimed to establish the EO potential (the quantitative content and qualitative composition) of several species of the genus *Pycnanthemum* Michx. (Lamiaceae): *P. montanum* Michx., *P. muticum* (Michx.) Pers., *P. tenuifolium* Schrad., *P. verticillatum* var. *pilosum* (Nutt.) Cooperr. These are herbaceous perennial plants with a pleasant minty aroma, which represent the flora of North America (Weakley, 2008). Currently, the genus *Pycnanthemum* is little known in Ukraine. Individual representatives are studied within the collection of Non-Traditional EO Plants of the Department of Cultural Flora of the M. M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (forest-steppe zone).

MATERIAL AND METHODS

Preparation of plant raw materials. The fresh aerial part of the plants was chopped into 1–1.5 cm fragments and left to wither for 24 hours in room conditions. The raw materials were then dried to an air-dry state using an Eridri ULTRA FD1000 dryer.

Isolation of the essential oils. The EOs were obtained by hydro distillation using an apparatus with a Clevenger nozzle. The samples were weighed on a VLKT-500 g-M scale. The weight of one sample was 35 g. The volume of water in the flask was 500 mL. The experiments were performed in triplicate. The exposure time – 2 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) capillary column (0.25 mm × 25.0 m). Conditions: gas velocity-carrier – 1.0 mL/min, flow split ratio – 1:20, evaporator temperature – 250 ºC, detector temperature (DEP) – 280 ºC, column temperature regime – gradual heating from 60 ºC to 185 ºC.

The component composition of the EOs was determined on a gas chromatograph with a mass spectrometric detector HP 6890. Mass spectrometric detector 1.6 – 800 a.o.m., EI ionization, Scan Mode & SIM Mode, "Hewlett Packard", USA. Chromatographic column – capillary HP–5ms (0.25 mm × 30.0 m). Carrier gas – helium. Carrier gas velocity 1.2 mL/min. Sample injection heater temperature – 180 ºC. Oven temperature programmable from 62 to 165 ºC at a rate of 5 deg/min. Sample injection (1 μL) without flow split.

For the identification of the EO components, the NIST mass spectrum library was used in combination with programs for identification by time of content AMDIS.

RESULTS AND DISCUSSION

The EO potential of plants of the genus *Pycnanthemum*: *P. montanum, P. muticum, P. tenuifolium, P. verticillatum* var. *pilosum*, introduced into the soil and climatic conditions of Ukraine, was studied for the first time. The quantitative content of EOs in raw materials of the experimental species in terms of absolute dry weight, as well as its chromatographic profile, has been determined.

P. montanum. The content of EO in the raw materials of the introduced plants is 2.37±0.1 %. The EO is transparent, colorless and has a pleasant mint aroma. 16 constituent compounds were detected, of which 15 were identified (**Table 1**, **Fig. 1**).

Table 1. **Chemical composition of *Pycnanthemum montanum* Michx. essential oil**

No	Compounds	Recording time, min	Content index, %
1	1-Octen-3ol	12.578	0.31
2	Myrcene	13.695	0.73
3	1.8-Cineol	17.146	5.14
4	Limonene	17.238	0.08
5	β-Ocimene	17.441	0.02
6	*cis*-Sabinene hydrate	18.243	0.63
7	Menthone	18.990	0.01
8	Isomenthone	25.545	14.04
9	*trans*-Isopulegone	25.712	0.45
10	Isopinocamphone	26.168	1.52
11	Pulegone	26.615	73.93
12	Neryl acetate	30.525	0.06
13	*Unidentified*	32.255	1.76
14	Germacrene D	34.409	0.66
15	Bicyclogermacrene	35.472	0.01
16	Caryophyllene oxide	36.420	0.29
Total			100

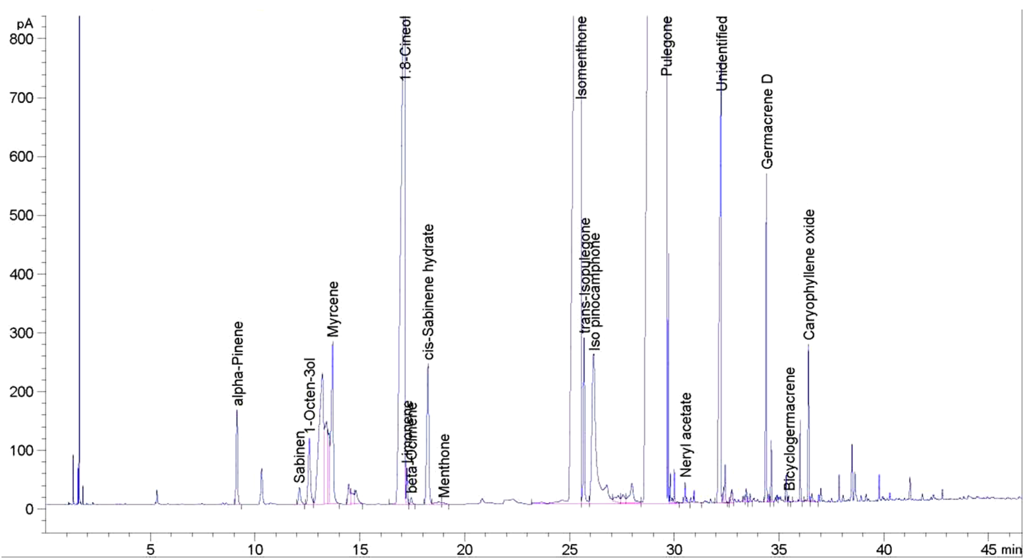


Fig. 1. GS-MS Chromatogram of essential oil compounds of *Pycnanthemum montanum* Michx.

P. muticum. The content of EO in the raw material is 2.58±0.2%. The oil is transparent, colorless. The aroma of the EO is herbal-mint, moderately sharp. 22 components were detected, 21 were identified (**Table 2**, **Fig. 2**).

Table 2. **Chemical composition of *Pycnanthemum muticum* (Michx.) Pers. essential oil**

No	Compounds	Recording time, min	Content index, %
1	1-Octen-3ol	12.601	0.31
2	Myrcene	13.733	0.54
3	*p*-Cymene	16.511	0.03
4	1.8-Cineol	17.071	2.34
5	Limonene	17.195	0.09
6	β-Ocimene	17.436	0.11
7	*cis*-Sabinene hydrate	18.283	0.89
8	*cis*-Linalool oxide	19.008	0.03
9	Isomenthone	25.829	20.39
10	*trans*-Isopulegone	26.106	6.61
11	Isopinocamphone	26.411	2.79
12	Pulegone	29.744	63.47
13	Thymol	30.534	0.14
14	Carvacrol	31.851	0.02
15	Caryophyllene	33.641	0.02
16	*Unidentified*	32.255	1.76
17	Germacrene D	34.423	0.54
18	β-Farnesene	35.098	0.03
19	Bicyclogermacrene	35.360	0.06
20	Spathulenol	36.072	0.64
21	Caryophyllene oxide	36.435	0.43
22	Isospathulenol	37.527	0.01
Total			100

P. tenuifolium. The content of EO in the raw material is 0.53±0.03%. The EO is transparent, slightly lemon-colored. The herbal aroma is fresh and pleasant. 17 components were detected and identified (**Table 3**, **Fig. 3**).

P. verticillatum* var. *pilosum. The EO content is 3.34±0.01%. The EO is transparent and colorless. The aroma is herbal-mint with pronounced grassiness. 22 components were detected, 21 were identified (**Table 4**, **Fig. 4**).

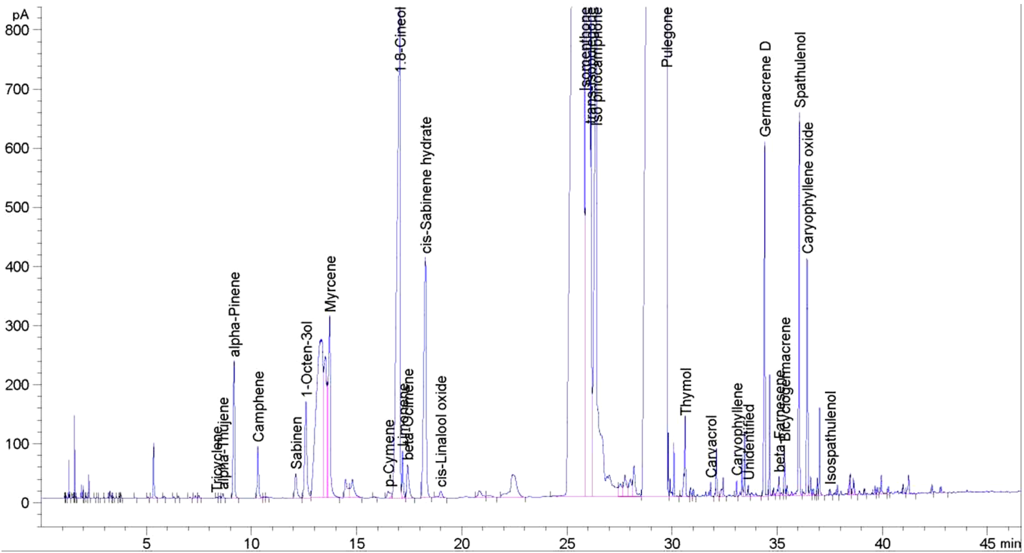


Fig. 2. GS-MS Chromatogram of essential oil compounds of *Pycnanthemum muticum* (Michx.) Pers.

Table 3. **Chemical composition of *Pycnanthemum tenuifolium* Schrad. essential oil**

No	Compounds	Recording time, min	Content index, %
1	1-Octen-3ol	12.332	0.66
2	β-Pinene	13.064	5.29
3	Myrcene	13.476	1.09
4	1.8-Cineol	16.909	8.58
5	Limonene	17.176	0.58
6	*cis*-Sabinene hydrate	18.217	0.62
7	Camphor	23.263	1.78
8	*cis*-Linalool oxide	19.008	0.03
9	Isomenthone	25.051	9.39
10	*trans*-Isopulegone	25.490	3.22
11	Isopinocamphone	25.999	1.98
12	Myrtenol	27.224	0.25
13	Pulegone	28.812	48.14
14	Linalyl acetate	29.445	2.15
15	Germacrene D	34.340	4.37
16	Bicyclogermacrene	35.458	2.21
17	Caryophyllene oxide	36.404	9.71
Total			100

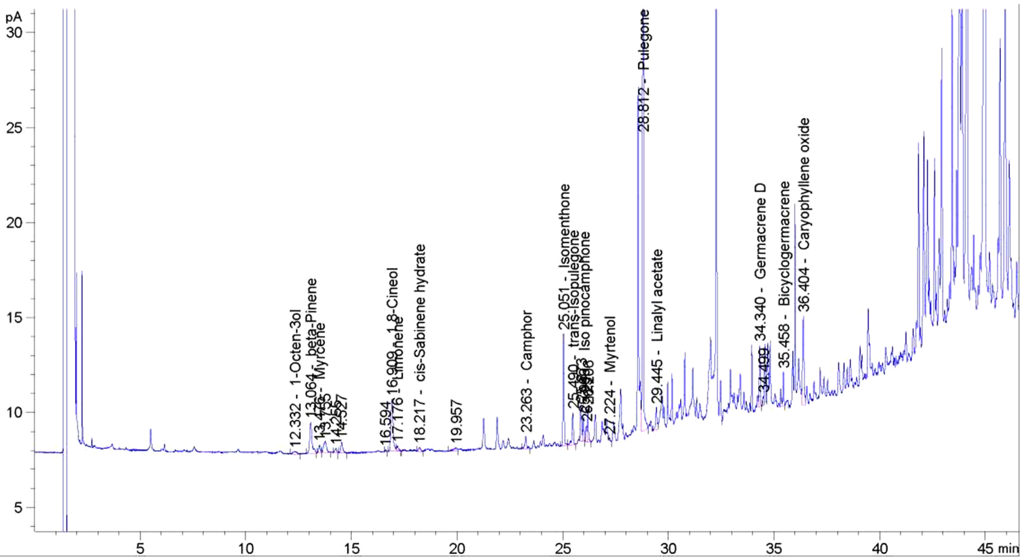


Fig. 3. GS-MS Chromatogram of essential oil compounds of *Pycnanthemum tenuifolium* Schrad.

Table 4. **Chemical composition of *Pycnanthemum verticillatum* var. *pilosum* (Nutt.) Cooperr. essential oil**

No	Compounds	Recording time, min	Content index, %
1	2	3	4
1	α-Pinene	9.158	0.25
2	Camphene	10.301	0.02
3	Sabinen	12.111	0.07
4	1-Octen-3ol	12.595	0.29
5	Myrcene	13.587	0.17
6	1.8-Cineol	16.887	0.59
7	Limonene	17.110	0.11
8	*cis*-Sabinene hydrate	18.137	0.03
9	Isomenthone	25.751	16.99
10	*trans*-Isopulegone	26.008	4.31
11	Isopinocamphone	26.329	1.74
12	Hexyl butyrate	27.012	0.04
13	Myrtenol	27.342	0.02
14	Pulegone	29.799	73.66
15	Thymol	30.595	0.10
16	Carvacrol	31.856	0.07

End of Table 4

1	2	3	4
17	Caryophyllene	33.641	0.02
18	*Unidentified*	32.857	0.55
19	Germacrene D	34.394	0.22
20	Bicyclogermacrene	35.355	0.06
21	Spathulenol	36.066	0.55
22	Caryophyllene oxide	36.414	0.17
Total			100

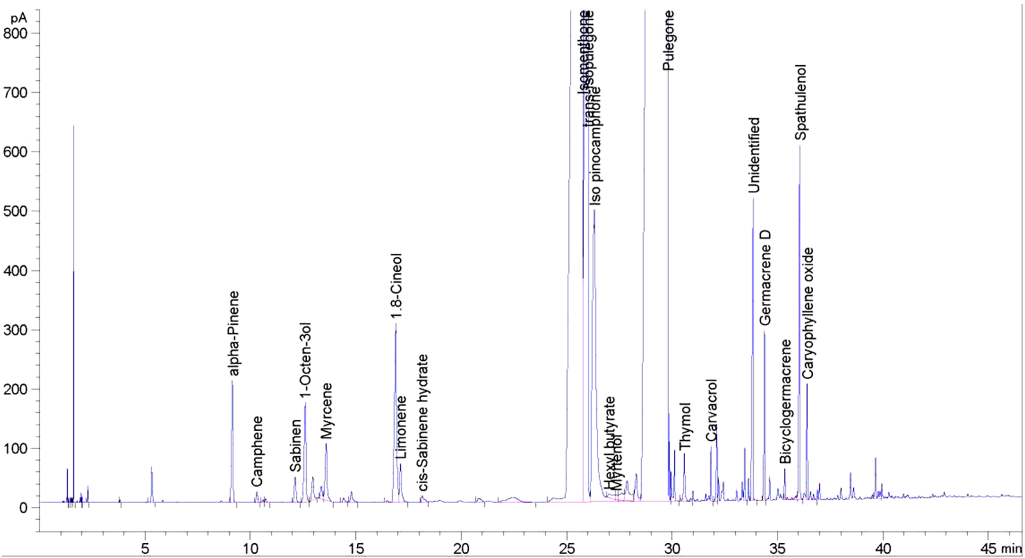


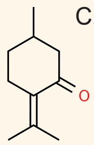
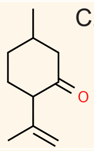
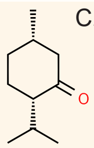
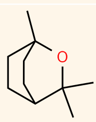
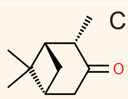
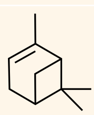
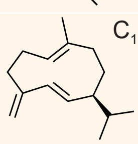
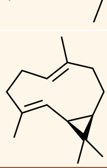
Fig. 4. GS-MS Chromatogram of essential oil compounds of *Pycnanthemum verticillatum* var. *pilosum* (Nutt.) Cooperr.

The results of the experiments show that the studied plant material is characterized by a high content of EOs. Among the 4 screened species, *P. verticillatum* var *pilosum* stands out for its powerful EO biosynthesis – 3.34±0.01 %, in contrast, this indicator for *P. tenuifolium* was the smallest – 0.53±0.03 %. Although, when considering *P. tenuifolium* separately, the content of EO is high. In general, the obtained results testify to the extraordinary EO potential of the introduced plant species in the conditions of Ukraine.

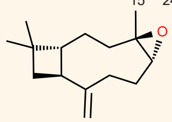
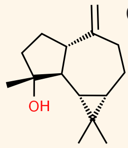
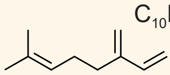
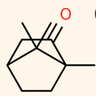
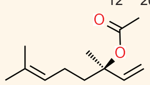
A critical analysis of the component composition of EOs indicates that the common dominant compounds are Pulegone, Isomenthon, Isopinocamphone. Other important components are different and depend on the EO of a specific plant species. The greatest differences in the component composition were found in the EO of *P. tenuifolium*. Among the dominant compounds of this oil are Germacrene D, Bicyclogermacrene, Caryophyllene oxide, Myrcene, which are found in other EOs, but in amounts less than 1 (<1) (**Table 5**). At the same time, the EO of *P. tenuifolium* contains such compounds as β-Pinene, Camphor, Linalyl acetate, which are absent from other EOs. Mirtenol was found only in two EOs – that of *P. tenuifolium* (0.25 %) and *P. verticillatum* var. *pilosum* (0.02 %).

The shared components for the EOs of *P. muticum* and *P. verticillatum* var. *pilosum* are Thymol, Carvacrol, and Caryophyllene at 0.14 and 0.10 %: 0.02 and 0.07 %; 0.02 and 0–02 %, respectively.

Table 5. **Comparative indications of dominant compounds found in essential oils of *Pycnanthemum* sp.**

Dominant compounds	Formula, *structure	Chemical class	Species of genus *Pycnanthemum*			
			P. montanum	*P. muticum*	*P. tenuifolium*	*P. verticillatum* var. *pilosum*
1	2	3	4	5	6	7
Pulegone	$C_{10}H_{16}O$ 	Monoterpene ketone	+	+	+	+
trans-Isopulegone	$C_{10}H_{16}O$ 	Menthane monoterpenoid	< 1	+	+	+
Isomenthone	$C_{10}H_{18}O$ 	Menthane monoterpenoid	+	+	+	+
1.8-Cineol (Eucalyptol)	$C_{10}H_{18}O$ 	Bicyclic monoterpene	+	+	+	< 1
Isopinocamphone	$C_{10}H_{16}O$ 	Bicyclic monoterpene	+	+	+	+
β-Pinene	$C_{10}H_{16}$ 	Monoterpene	–	–	+	–
Germacrene D	$C_{15}H_{24}$ 	Sesquiterpenes	< 1	< 1	+	< 1
Bicyclogermacrene	$C_{15}H_{24}$ 	Sesquiterpenoid	< 1	< 1	+	< 1

End of Table 5

1	2	3	4	5	6	7
Caryophyllene oxide	$C_{15}H_{24}O$ 	Sesquiterpenoid	< 1	< 1	+	< 1
Spathulenol	$C_{15}H_{24}$ 	Tricyclic sesquiterpene	–	< 1	–	< 1
Myrcene	$C_{10}H_{16}$ 	Acyclic monoterpenoid	< 1	< 1	+	< 1
Camphor	$C_{10}H_{16}O$ 	Terpenoid and a cyclic ketone	–	–	+	–
Linalyl acetate / Licareol acetate	$C_{12}H_{20}O_2$ 	Acyclic monoterpenoid	–	–	+	–

Note * Graphic images of the structure of compounds were obtained from the web resource: ChemSpider Search and Share Chemistry. Chemical-Structure. http://www.chemspider.com

Among the dominant compounds in EO samples, Pulegone apparently plays a decisive role. The content of this compound in the EO of *P. montanum*, *P. muticum*, *P. tenuifolium*, *P. verticillatum* var. *pilosum* and *P. tenuifolium* is high – 73.93; 63.47; 48.14; 73.66 %, respectively. Thus, it is Pulegone that determines the pharmacological properties of EOs of the experimental *Pycnanthemum* species.

Pulegone is a colorless oily liquid (at room temperature), highly lipophilic, non-volatile, has a pleasant, refreshing smell that resembles something between peppermint and camphor. Pulegone is practically insoluble in water, but miscible with ethanol, diethyl ether, and chloroform. Derived from Terpinolene via Piperithenone, it is also a precursor of Menthone, Isomenthone and Isopulegone (Cosmetic Oil…, 2022; Ainane *et al.*, 2023).

According to literature, EOs with the predominance of Pulegone are used for flavoring pharmaceutical preparations, alcoholic beverages, pastries, candies, ice cream, etc. It is also used as a natural fragrance in cosmetics and personal hygiene products, in detergents and as one of the most powerful insecticides. It is believed that Pulegone is easily absorbed by the human body (International Agency…, 2016; Ainane *et al.*, 2023; Pulegone…, n.d.). It has been proven that Pulegone has a potential therapeutic effect and can be used for the prevention of inflammatory diseases, as a mucolytic and a gastrointestinal distresser (Roy *et al.*, 2018; Pulegone…, n.d.). It is also considered as a psychoactive compound having analgesic profile (Sousa *et al.*, 2011).

According to the results of tests on animals, Pulegone both alone and in combination with another component of EOs – eugenol can have either a positive or negative effect. The effects of exposure depend on the dosage (Ribeiro-Silva *et al.*, 2022). It also shows a synergistic effect with Thymol and 1.8-Cineol, increasing the qualitative indicators of action almost twice, but with linalool it can act as an antagonist (Cosmetic oil…, 2022).

It is known that an excessive dose of Pulegone causes gastrointestinal disorders in humans manifested by pain, nausea and vomiting. Eventually, it can lead to liver and kidney failure, bleeding, seizures, multiple organ failure, and even death (Pennyroyal oil … (n.d.); Da Rocha *et al.*, 2012). This occurs as a result of Pulegone breakdown in the body with the formation of toxic metabolites – Mentofuran, Piperithenone, Piperitone and Menthone and their effects (Ribeiro-Silva *et al.*, 2022).

Since 1965, the United States Food and Drug Administration has granted Pulegone generally recognized as safe (GRAS) status for use in foodstuffs (21 CFR 172.515) and was included by the Council of Europe in 1974 in the list of artificial flavoring substances that can be temporarily added to foodstuffs without danger to health) (Božović *et al.*, 2017).

However, in 2002, the Scientific Committee on Food (SCF) issued an opinion on Pulegone and Menthofuran. Mentofuran is the main metabolite of Pulegone, so they were evaluated together. Restrictions and permissible norms of use were established. Maximum levels of Pulegone addition: 25 mg/kg in food products, 100 mg/kg in beverages, except for 250 mg/kg in mint beverages and 350 mg/kg in mint confectionery (Opinion of …, 2008).

Today, there is a way to reduce the level of Pulegone and Menthofuran in the EO. It consists in adding a Lewis acid to an EO with a high content of Pulegone. A reaction takes place followed by neutralization and distillation (Johnson, 1993).

Summing up the above material, we can confidently state that in the conditions of Ukraine, species of the genus *Pycnanthemum* of the Lamiaceae family: *P. montanum*, *P. muticum*, *P. tenuifolium*, *P. verticillatum* var. *pilosum* have a high rate of EO production – 0.53–3.34 %. Based on this, they can be considered as potential objects in the list of domestic EO plants suitable for replenishing cultural phytocenoses. It was established that the dominant compounds of the obtained EOs are Pulegone, *trans*-Isopulegone, Isomenthone. However, the determining role in terms of content in all four EOs is played by Pulegone. This is decisive in the formation of the spectrum of pharmacological action of EOs *P. montanum*, *P. muticum*, *P. tenuifolium*, *P. verticillatum* var. *pilosum*. Its content is 48.14–73.93 %. This calls for the correct use of EOs of these types of plants, so that the positive effect does not acquire negative aspects.

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

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ГАЗОВА ХРОМАТО-МАССПЕКТРОМЕТРИЧНА ХАРАКТЕРИСТИКА ЕФІРНИХ ОЛІЙ РОСЛИН РОДУ *PYCNANTHEMUM* (LAMIACEAE) ТА ОСОБЛИВОСТІ ЇХНЬОГО ПРАКТИЧНОГО ВИКОРИСТАННЯ

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Вступ. Далеко не всі рослини здатні продукувати ефірну олію. У світі описано 3000 різних рослинних ефірних олій, але тільки 10 % з них людина застосовує масштабно. Зокрема, парфумерія використовує натуральних ефірних олій 30 % світової продукції, а харчова промисловість – 40 %.

Досконале вивчення ефірних олій, знання їхніх складових як у хімічному, так і у фармакологічному аспектах дають змогу покращувати якість життя людини, пропонуючи натуральний продукт.

Мета нашого експериментального дослідження – встановити ефіроолійний потенціал 4-х видів роду *Pycnanthemum* Michx. (Lamiaceae): *P. montanum* Michx., *P. muticum* (Michx.) Pers., *P. tenuifolium* Schrad., *P. verticillatum* var. *pilosum* (Nutt.) Cooperr., інтродукованих у лісостеповій зоні України. Вказані види рослин є представниками флори Північної Америки.

Матеріали та методи. Ефірну олію отримано в лабораторних умовах методом гідродистиляції з використанням апарату Клевенджера. Сировину рослин (купажована маса з листків, суцвіть і трав'яної частини стебла) використовували у повітряно-сухому стані. Заготівлю сировини проведено у фазу квітування рослин. Вміст ефірної олії подано в перерахунку на абсолютно суху масу. Ідентифікацію сполук і їхню кількість у складі кожної ефірної олії визначено методом газової хромато-масспектрометрії.

Результати. В умовах лісостепу України інтродуковані види рослин роду *Pycnanthemum* виявляють високий рівень біосинтезу ефірної олії – 0,53–3,34 %. Домінантними сполуками, спільними для отриманих ефірних олій, є пулегон, ізоментон, ізопінокамфон. Показники вмісту пулегону перевершують решту і сягають 48,14–73,93 %. Пулегон є неоднозначною органічною сполукою, оскільки виявляє високу біологічну активність і фармакологічні властивості, придатні для застосування в лікувальній практиці, але водночас під час розщеплення утворює токсичні метаболіти, зокрема, ментофуран.

Висновки. Отримані результати свідчать, що маловідомі в Україні рослини роду *Pycnanthemum* в запропонованих умовах зростання мають високий ефіроолійний потенціал. Завдяки цьому вони є потенційними об'єктами для використання у вітчизняних культурфітоценозах. Зважаючи на компонентний склад ефірних олій і домінуючий пулегон, їхнє вживання потребує коректності у дозуванні.

Ключові слова: *Pycnanthemum* sp., ефірні олії, ГХ-МС, пулегон

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