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# Chemical Components of Essential Oils from Aerial Parts of *Pycnanthemum virginianum* and *P. californicum* (Lamiaceae) Plants

## Abstract

**Aim.** The research is aimed at determining the qualitative and quantitative content of essential oils in the aerial part of two species of the genus *Pycnanthemum* Michx. (Lamiaceae) – *P. virginianum* (L.) T. Durand & B.D. Jacks. ex B.L. Rob & Fernald and *P. californicum* Norr. exDurand. The plants were introduced in the M. M. Gryshko National Botanical Garden of National Academy of Sciences of Ukraine (Forest-Steppe zone). These are representatives of the flora of North America, and they are little known in Ukraine. Plants have useful medicinal and nutritional properties, but the biochemical composition of their essential oils has not been sufficiently studied in the world.

**Materials and methods.** In the experiment, the aerial herbal part of plants collected during the flowering phase was used. The quantitative content of the essential oil was determined by the hydrodistillation method, and its qualitative characteristics were found by the GC-MS analysis. The chromatographic profile was obtained on an Agilent Technologies 7890. The component composition of the essential oil was determined on a gas chromatograph with a HP 6890 mass spectrometric detector with a mass spectrometric detector 5973. We used a mass spectrometric detector 1.6 – 800 a.o.m., EI ionization, SIM & Scan mode, "Hewlett Packard", USA. Identification of essential oil components was performed using the NIST mass spectrum library in combination with AMDIS content-time identification programs.

**Results and discussion.** *P. virginianum* was found to produce 1.96 ± 0.17 % of essential oil, in which 12 compounds out of 13 were identified; *P. californicum* had 2.66 ± 0.13 % of essential oil, 13 compounds out of 15 were identified. The essential oil samples obtained have pulegone as the dominant component: *P. virginianum* – 44.65 %, *P. californicum* – 86.07 %. In addition to it, they also contain thymol, myrcene, 1.8-cineole, menthone, limonene and other compounds.

**Conclusions.** For the first time, the qualitative and quantitative composition of the essential oils of plants of *P. virginianum* and *P. californicum* species introduced in Ukraine has been determined. The results obtained indicate that when introduced plants have a high biosynthesizing ability to produce essential oil. Pulegone has been found to be the dominant component; therefore, the essential oil can be classified as a pulegone-type essential oil. We believe that the raw material of *P. virginianum* and *P. californicum* are potentially suitable for use in perfumery, cosmetics, aromatherapy, personal care products, dentistry, and in the pharmaceutical and food industries.

***Keywords:*** introduced plants; *Pycnanthemum;* components of essential oils; GC-MS

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## Хімічні компоненти ефірних олій із надземних частин рослин *Pycnanthemum virginianum* і *P. californicum* (Lamiaceae)

## Анотація

**Мета.** Визначити якісний склад та кількісний вміст ефірних олій у надземній частині двох видів роду *Pycnanthemum* Michx. (Lamiaceae) – *P. virginianum* (L.) T. Durand & B.D. Jacks. ex B.L. Rob & Fernald та *P. californicum* Norr. ex Durand. Рослини інтродуковано в Національному ботанічному саду імені М. М. Гришка НАН України (лісостепова зона). Це представники флори Північної Америки, а в Україні вони маловідомі. Рослини мають корисні лікувальні та харчові властивості, але біохімічний склад їхніх ефірних олій у світі вивчено недостатньо.

**Матеріали та методи.** В експерименті використано надземну трав'яну частину рослин, зібрану у фазу квітування. Кількісний вміст ефірної олії визначали методом гідродистиляції, а її якісні характеристики – методом ГХ-МС. Хроматографічний профіль отримували на хроматографі Agilent Technologies 7890. Компонентний склад ефірної олії визначали на газовому хроматографі HP 6890 з мас-спектрометричним детектором 5973. Мас-спектрометричний детектор 1,6 – 800 а.о.м., EI іонізація, SIM & Scan mode, «Hewlett Packard», США. Ідентифікацію компонентів ефірної олії виконували за допомогою бібліотеки мас-спектрів NIST у поєднанні з програмами ідентифікації вмісту AMDIS.

**Результати та їх обговорення.** Виявлено, що *P. virginianum* продукує 1,96 ± 0.17 % ефірної олії, у якій ідентифіковано 12 сполук із 13 виявлених; *P. californicum* – 2,66 ± 0,13 %, ідентифіковано 13 сполук із 15. Отримані зразки ефірної олії за домінантний компонент мають пулегон: *P. virginianum* – 44,65 %, *P. californicum* – 86,07 %. Окрім нього, зразки також містять тимол, мірцен, 1,8-цинеол, ментон, лімонен та інші сполуки.

**Висновки.** Уперше визначено якісний та кількісний склад ефірних олій рослин видів *P. virginianum* та *P. californicum*, інтродукованих в умови України. Отримані результати свідчать, що за інтродукції рослини мають високу біосинтезувальну здатність продукувати ефірну олію. Виявлено, що домінантним компонентом є пулегон, тому ефірну олію можна кваліфікувати як ефірну олію пулегонного типу. Вважаємо, що сировина *P. virginianum* та *P. californicum* потенційно придатна для використання в парфумерії, косметиці, ароматерапії, засобах особистої гігієни, стоматології, у фармацевтичній та харчовій промисловості.

***Ключові слова:*** інтродуковані рослини; *Pycnanthemum;* компоненти ефірної олії; ГХ-МС

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## ■ Introduction

There was a need for a wider study of the chemical composition of the essential oils of plants of the genus *Pycnanthemum* (Lamiaceae), as evidenced by the analysis of literary sources. In general, a comprehensive assessment of the essential oils of plants of the genus *Pycnanthemum* has not yet been carried out. However, to fully understand the application and benefits of *Pycnanthemum* sp. a deeper understanding of the chemical composition of plants is needed [1].

To date, it is known that the quantitative content of essential oil and especially its qualitative composition in different types of *Pycnanthemum* is different, but the main components are pulegone (medicinal mint), menthone (fresh mint), isomenthone (cereal mint), limonene (citrus), piperitone (mint and camphor). The essential oil of *Pycnanthemum floridanum* E. Grant & Epling has been most fully evaluated and it contains 40 volatile components [2–5].

The consequence of the different composition of the essential oil is the difference in aroma. Differences in aromas are observed not only at the species level. There are reports of different plant aromas among populations of *P. virginianum* – some have a distinct citrus scent similar to *Melissa officinalis* L. or *Monarda citriodora* Cerv ex Lag. Similar information regarding variability in essential oil composition and aroma is available for *Pycnanthemum loomisii* Nutt. Thus, different chemotypes may exist within one species [1, 6].

Considering a wide spectrum of the biological activity of related genera due to the essential oil content, it can be assumed that *Pycnanthemum* sp. has similar properties, in particular, antioxidant, antitumor, antiviral, antifungal and antibacterial properties. Due to their high content of terpenes, *Pycnanthemum* plants have a wide potential for application in pharmaceuticals, cosmetics, culinary and food flavoring [1, 7].

According to the latest taxonomy, the genus *Pycnanthemum* includes 22 taxa, 20 species and 2 varieties [8]. The natural range covers certain areas of North America, administratively subordinate to the United States (mainly the states of the eastern part of the country) and Canada (2 southern provinces). The mountains of North Carolina (USA) are considered to be the center of species diversity [9].

Plants of the genus *Pycnanthemum* are herbaceous perennials up to 80 cm high. The aerial part of the plants contains secondary metabolites – essential oils. On the American continent there is a long tradition of using *Pycnanthemum* sp. by the aboriginal population for medicinal purposes



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(in indigestion, colitis, dyspepsia, colds, headaches), in cooking and in ritual ceremonies [10, 11]. An interesting historical reference about the use of *Pycnanthemum* is a book of the 19th century. The 1898 edition of the "Royal American Dispensary" mentions the genus *Pycnanthemum* and its medicinal properties: "*Pycnanthemum* is diaphoretic, stimulant, antispasmodic, carminative and tonic. A warm infusion is very useful for postpartum, remitting and other forms of fever, cough, cold, catarrh, etc., and it is very useful for spasmodic diseases, especially colic, stomach spasms, spasms in babies." [12].

*Pycnanthemum* sp. are also used as good honey plants and ornamental plants [13].

In Ukraine, the *Pycnanthemum* genus is practically not found either in scientific or private collections and is little known. However, taking into account the useful properties of plants of this genus and the possibility of their complex use, they are promising for research. In particular, insufficient study of the biochemical composition prompted us to conduct a series of experimental studies. The results of one of them are presented in this work.

## Materials and methods

**Plant source**

The studies used plants of two species, *P. virginianum* and *P. californicum*. These plants were introduced into the Department of Cultural Flora of M. M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (NBG) in 2014 and 2017, respectively.

The plants were grown from seed material obtained from scientific botanical institutions in the Czech Republic within the framework of the "Index Seminum" exchange system. The growth conditions were as follows: open soil, Forest-Steppe zone of Ukraine. Generatively adult plants are shown in Figure 1 (a, b).

In 2021, the raw material was harvested and analyzed. Harvesting of the raw material was carried out during the phase of mass flowering of plants. Under the raw material of these species, we mean the above-ground herbal leafy part – a mixture of leaves, inflorescences and stems.

**Isolation of the essential oils**

The fresh above-ground part of plants was collected on a sunny day, during lunch hours. Then they were crushed into fragments of 1–1.5 cm and left to wither for 24 hours. Next, the raw material was dried to an air-dry state using an Eridri ULTRA FD1000 dryer. Weighing samples of the raw material was carried out on the VLKT–500 g–M scales. The essential oil was obtained by hydrodistillation using an apparatus with a Clevenger-type nozzle. The sample weight was 35 g. The multiplicity of the experiment was 3-fold. The exposure time was 1.5 hours (from the moment the water boils).

**The 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, 25 m long, with an internal diameter of 0.25 mm and a stationary phase thickness of 0.33 μm under the following conditions: gas velocity – carrier – 1.0 mL $min^{-1}$; flow split ratio – 1:20; evaporator temperature – 250 °C; detector temperature (DEP) – 280 °C; column temperature regime – gradual heating from 60 °C to 185 °C.

***a*** ***b***

**Figure 1**. *Pycnanthemum virginianum* (a) and *P. californicum* (b) introduced in the M. M. Gryshko National Botanical Garden of the NAS of Ukraine (Kyiv, Ukraine)

The component composition of the essential oil was determined on a gas chromatograph with a HP 6890 mass spectrometric detector with a mass spectrometric detector 5973. We used a mass spectrometric detector 1.6 – 800 a.o.m., EI ionization, SIM & Scan mode, "Hewlett Packard", USA. The chromatographic conditions were as follows: 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 $min^{-1}$; sample injection heater temperature – 180 °C.

The oven temperature was programmable from 62 to 165 °C at a rate of 5 deg $min^{-1}$. The sample injection (1 μL) was without flow split. For the identification of essential oil components, the NIST mass spectrum library was used in combination with AMDIS content-time identification programs.

## ■ Results and discussions

Under the conditions of the NBG, the introduced species *P. virginianum* and *P. californicum* have a high content of essential oil in the raw material. It has been experimentally determined that in the aerial part of *P. virginianum* the content of essential oil is 1.96 ± 0.17%, in *P. californicum* – 2.66 ± 0.13%. They differ in color – in *P. virginianum*, the essential oil is transparent with a barely noticeable yellowish tinge, and it is colorless transparent in *P. californicum*. There were 13 components found in the essential oil of *P. virginianum*, 12 of them were identified. As can be seen from Table 1, the main odorants include pulegone (44.65%), thymol (20.16%), menthone (6.61%), isomenthone (5.75%), limonene (2.73%), caryophyllene (2.72%), *p*-cymene (2.36%), myrcene (1.91%). The chromatogram of the *P. virginianum* essential oil is shown in Figure 2.

The presence of the chemical characteristics of *P. virginianum* essential oil in the literature made it possible to compare it with our sample shown in Table 2.

The publication covering the results of studying the essential oil of 4 varieties of *P. virginianum* (Alabama A&M University Research Station, North Alabama, USA) was used [7]. In the samples from North Alabama, the chemical profiles of 4 samples were taken into account, which harvesting was in October 2020 on the 155$^{th}$ day after planting the plants from the greenhouse into the open ground. We analyzed the entire flowering aerial herbal part, and colleagues presented the results of screening the essential oil extracted from leaves of the plants. Despite the differences in the experimental material and the lack of other information, we nevertheless used this material and made a comparison.

Obviously, according to the concentration of pulegone, thymol, and caryophylene in the essential

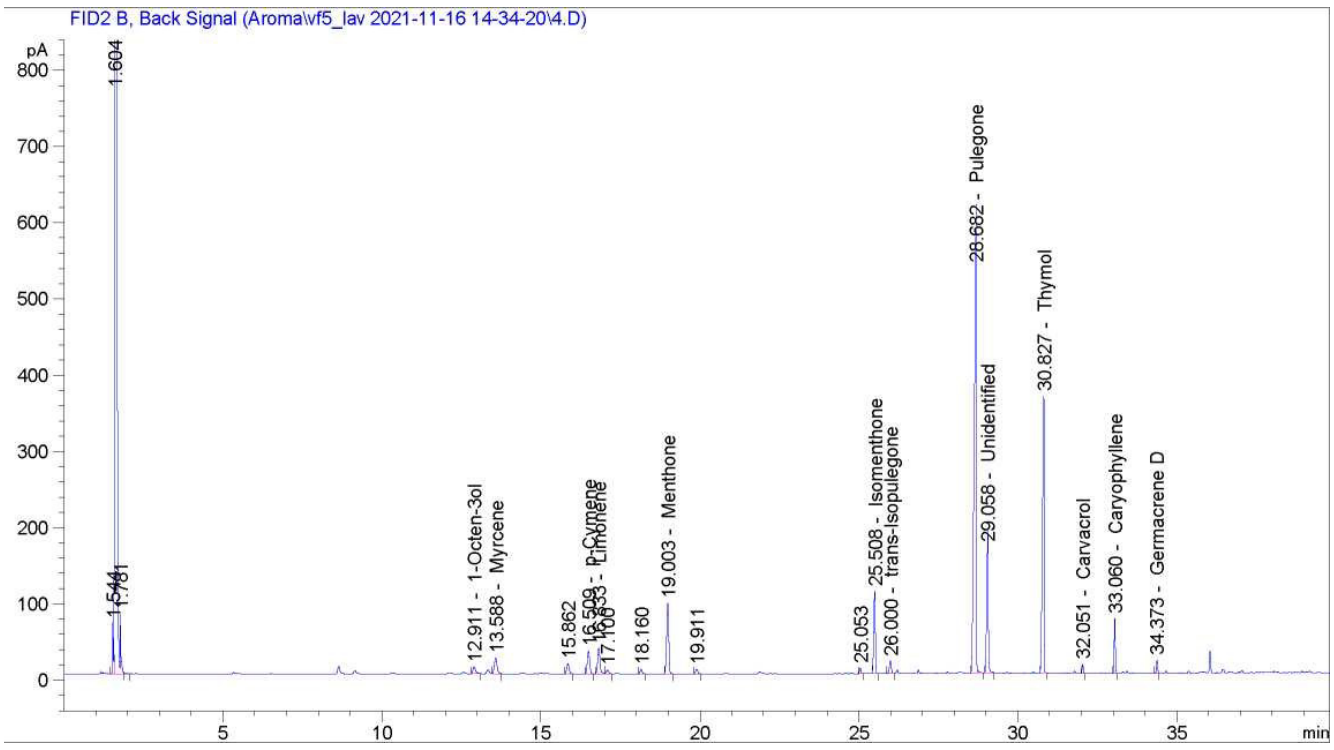


**Figure 2.** Chromatogram of the *Pycnanthemum virginianum* essential oil


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**Table 1**. Chemical constituents of the essential oil of *Pycnanthemum virginianum*

| No | Compounds | RetTime, *min* | Content index, % |
|---|---|---|---|
| 1 | 1-Octen-3-ol | 12.911 | 0.88 |
| 2 | Myrcene | 13.588 | 1.91 |
| 3 | *p*-Cymene | 16.509 | 2.36 |
| 4 | Limonene | 16.833 | 2.73 |
| 5 | Menthone | 19.003 | 6.61 |
| 6 | Isomenthone | 25.508 | 5.75 |
| 7 | *trans*-Isopulegone | 26.000 | 1.09 |
| 8 | Pulegone | 28.682 | 44.65 |
| 9 | Unidentified | 29.058 | 9.93 |
| 10 | Thymol | 30.827 | 20.16 |
| 11 | Carvacrol | 32.051 | 0.48 |
| 12 | Caryophyllene | 33.060 | 2.72 |
| 13 | Germacrene D | 34.373 | 0.71 |
| | | Total | 99.98 |

**Table 2**. The content of major components in the essential oil of *Pycnanthemum virginianum*, in the samples of NBG (our own analysis) and North Alabama (*Setzer et al.*, 2021)

| Compounds | Content of dominant compounds, % | |
|---|---|---|
| | NBG | North Alabama |
| 1-Octen-3-ol | 0.88 | 1.8–2.0 |
| Myrcene | 1.91 | 0.4–2.6 |
| *p*-Cymene | 2.36 | 7.1–8.7 |
| Limonene | 2.73 | 1.3–5.5 |
| Menthone | 6.61 | 3.8–9.9 |
| Isomenthone | 5.75 | 0.4–54.7 |
| *trans*-Isopulegone | 1.09 | 1.1–4.1 |
| Pulegone | 44.65 | 10.8–29.8 |
| Thymol | 20.16 | 0.2–22.1 |
| Carvacrol | 0.48 | 1.4 |
| Caryophyllene | 2.72 | 0.2–1.0 |
| Germacrene D | 0.71 | 0.7–1.5 |

oil, the *P. virginianum* sample from NBG occupies a leading position. This is despite the fact that the whole herbaceous aboveground part of the plants was subject to analysis, i.e., the sample included not only leaves and inflorescences as organs of the highest encountering frequency of essential oil glands, but also stems, which had a low indicator them.

15 components were found in the essential oil of *P. californicum*, 13 of them were identified. The key odorants were pulegone (86.07%), isomenthone (8.77%), trans-isopulegone (1.85%), 1,8-cineole (1.14%), 1-octen-3-ol (0.33%), myrcene (0.2%), neryl acetate (0.21%) and limonene (0.11%) as evidenced by the data in Table 3. The chromatogram of *P. californicum* essential oil is shown in Figure 3.

In both species, the dominant component of the essential oil is pulegone (*P. californicum* – 86%, *P. virginianum* – 45%) although the qualitative set of components that form the aroma and pharmacological properties of the essential oil by the quantitative content differ in these two introduced species.

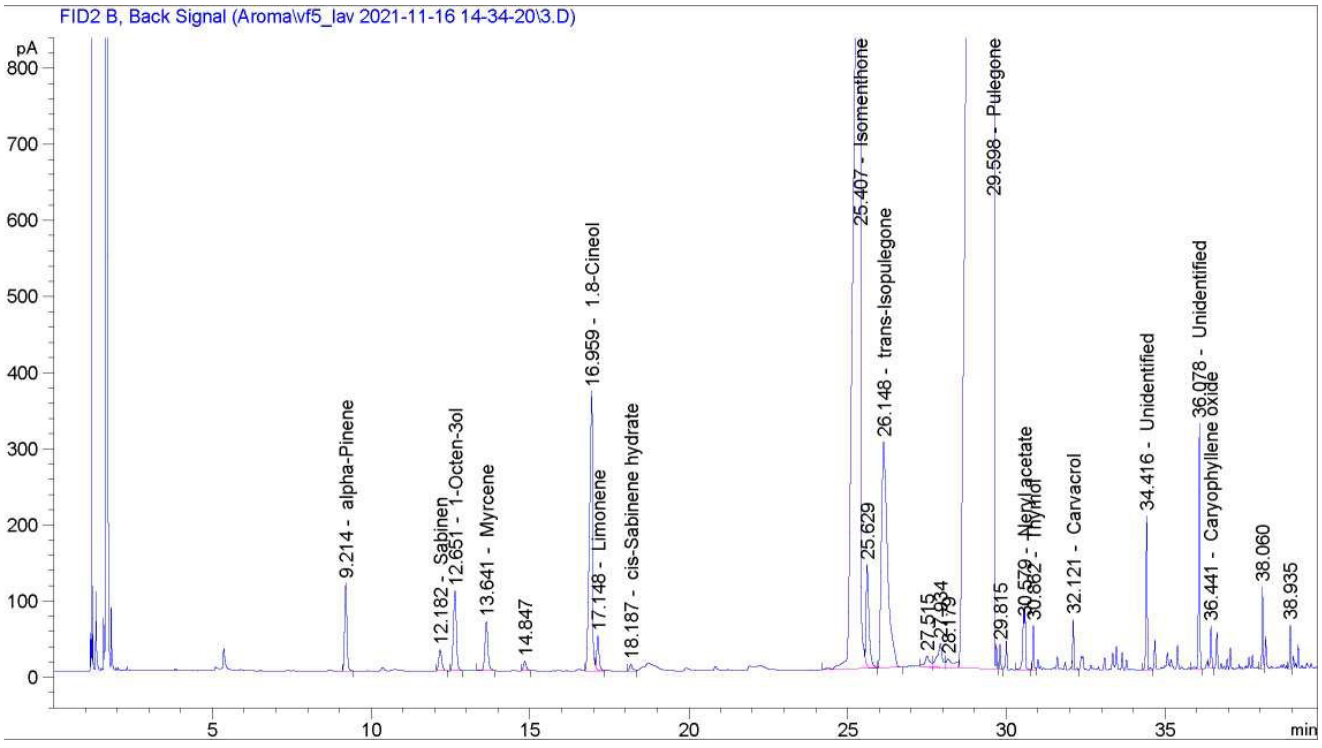


**Figure 3.** Chromatogram of the *Pycnanthemum californicum* essential oil

**Table 3**. Chemical constituents of the essential oil of *Pycnanthemum californicum*

| No. | Compounds | RetTime, *min* | Content index, *%* |
|---|---|---|---|
| 1 | Sabinen | 12.182 | 0.09 |
| 2 | 1-Octen-3-ol | 12.651 | 0.33 |
| 3 | Myrcene | 13.641 | 0.20 |
| 4 | 1.8-Cineol | 16.959 | 1.14 |
| 5 | Limonene | 17.148 | 0.11 |
| 6 | *cis*-Sabinene hydrate | 18.187 | 0.03 |
| 7 | Isomenthone | 25.407 | 8.77 |
| 8 | *trans*-Isopulegone | 26.148 | 1.85 |
| 9 | Pulegone | 29.598 | 86.07 |
| 10 | Neryl acetate | 30.579 | 0.21 |
| 11 | Thymol | 30.862 | 0.07 |
| 12 | Carvacrol | 32.121 | 0.09 |
| 13 | Unidentified | 34.416 | 0.27 |
| 14 | Unidentified | 36.078 | 0.44 |
| 15 | Caryophyllene oxide | 36.441 | 0.08 |
| | | Total | 99.74 |

Pulegone belongs to the group of monoterpene ketones, has a sweetish pleasant aftertaste and a refreshing aroma reminiscent of mint. In this, it is similar to other "minty" terpenes – camphor and isopulegon, which also have a cool minty aroma.

Six genera of *Lamiaceae* (*Acinos, Calamintha, Cyclotrichium, Mentha, Micromeria* and *Ziziphora)* have been found to contain pulegone as the main component of their oils. Pulegone was first obtained from *Mentha pulegium* L., hence the name. Taking into account the dominant role of pulegone in the composition of *P. virginianum* and *P. californicum* essential oils, the raw material of these species is promising for practical use, for example, as a substitute for *M. pulegium*, in particular the well-known spice "ombalo" – a mixture of dry leaves and inflorescences from *M. pulegium* [14]. According to published data, the content of pulegone in the essential oil of *M. pulegium* varies within 40–45% depending on the origin of the raw material – 43.5% (Egypt) [15], 40.98% (Morocco) [16], 48.7% (Iran) [17], 65.0–83.1% (India) [18], 28.9% (Turkey) [19]. At the same time, it is known that the commercial essential oil of *M. pulegium* contains about 84% pulegone (Center for Aromatic Plant Research, Lehi, Utah, USA) [7].

Pulegone is used in perfumery, cosmetics, aromatherapy, personal care products, dentistry, and as a source of menthol. In medicine, it is applied as a remedy for colds and coughs, and in the food industry – as a flavoring of foods and alcoholic beverages. Taking into account all the positive characteristics, you should still adhere to certain rules for using pulegone. To date, there are no toxicokinetic studies in humans regarding the effects of pulegone, but there are some studies done in other mammals. When pulegone is ingested, it is broken down in the liver and reacts to form numerous toxic metabolites that can cause harm to the body, in particular a necrotic effect on the liver. Some metabolites identified are mentofuran, piperitenon, piperitone and menthone. If there are no restrictions on the use of pulegone in perfumery and cosmetics, they do exist in the food industry. In particular, for food products, the rate of use of pure pulegone is 25 mg $kg^{-1}$, soft drinks, sweets – 100–350 mg $kg^{-1}$ [20]. Due to the high concentration of pulegone in the essential oil of *P. californicum* and *P. virginianum* introduced, their raw material can also be offered as an alternative to synthetic pesticides and as a natural repellent.

## Conclusions

In the M. M. Gryshko National Botanical Garden of the NAS of Ukraine (Forest-Steppe zone), plant species of the North American flora – *P. virginianum* and *P. californicum* family *Lamiaceae,* which are new to Ukraine, have been introduced. According to the results of laboratory studies, it has been found that plants of these species during the flowering period have a high content of essential oil in the aerial part: in *P. virginianum*, the content of essential oil is 1.96 ± 0.17%, *P. californicum* it is 2.66 ± 0.13%. The qualitative and quantitative content of the essential oil in these plant species show that they contain the essential oil of a pulegone type since this substance is the prevailing component in their oil. This fact has an impact on the practical use of essential oils, as well as the aerial herbal part of plants of these species. We believe that the raw material of *P. virginianum* and *P. californicum* are potentially suitable for use in perfumery, cosmetics, aromatherapy, personal care products, dentistry, and in the pharmaceutical and food industries.


ISSN 2308-8303 (Print) / 2518-1548 (Online)

## References


1. Dein, M. Key Odorants of *Pycnanthemum incanum* and Stereochemistry of Chiral Compounds. Master's Thesis, University of Tennessee, **2019**.
2. https://trace.tennessee.edu/utk_gradthes/5523.
3. Ebadollahi, A; Ziaee, M; Palla, F. Essential oils extracted from different species of the Lamiaceae plant family as prospective bioagents against several detrimental pests. *Molecules* **2020**, *25* (7), 1556. https://doi.org/10.3390/molecules25071556.
4. Karpinski, T. M. Essential oils of Lamiaceae family plants as antifungals. *Biomolecules* **2020**, *10* (1), 103. https://doi.org/10.3390/biom10010103.
5. Shu, C.-K.; Lawrence, B.M.; Miller, K.L. Chemical Composition of the Essential Oil of *Pycnanthemum floridanum* E. Grant and Epling. *J. Essent. Oil Res.* **1994**, *8* (5), 529–531. https://doi.org/10.1080/10412905.1994.9698442.
6. Dein, M.; Munafo, J.P. Characterization of key odorants in hoary mountain mint, *Pycnanthemum incanum. J. Agric. Food Chem.* **2019**, *67* (9), 2589–2597. https://doi.org/10.1021/acs.jafc.8b06803.
7. Dein, M.; Munafo, J. P. Characterization of Odorants in Loomis' Mountain Mint, *Pycnanthemum loomisii. J. Agric. Food Chem.* **2022**, *70* (45), 14448–14456. https://doi.org/10.1021/acs.jafc.2c05492.
8. Setzer, W. N.; Duong, L.; Pham, T.; Poudel, A.; Nguyen, C.; Mentreddy, S. R. Essential Oils of Four Virginia Mountain Mint (*Pycnanthemum virginianum*) Varieties Grown in North Alabama. *Plants* **2021**, *10*, 1397. https://doi.org/10.3390/plants10071397.
9. The Plant List: *Pycnanthemum* Michaux. http://www.theplantlist.org/tpl1.1/search?q=Pycnanthemum (accessed Oct 20, 2022).
10. Flora of the Carolinas, Virginia, Georgia, northern Florida, and surrounding areas *Pycnanthemum* Michaux (Mountain-mint, Wild-basil). Working Draft of 7 April 2008. https://ncbg.unc.edu/wp-content/uploads/sites/963/2020/06/WeakleyFlora_2008-Apr.pdf (accessed Feb 28, 2022).
11. Cozzo, D. N. Ethnobotanical classification system and medical ethnobotany of the eastern band of the Cherokee Indians. Athens, Georgia, USA, 2004. https://getd.libs.uga.edu/pdfs/cozzo_david_n_200405_phd.pdf (accessed Sep 09, 2022).
12. Small, E. Mountain Mint. *North American Cornucopia: Top 100 Indigenous Food Plants*, 1st Ed. CRC Press: 2013, 463–466. https://doi.org/10.1201/b15818.
13. Felter, H. W.; Lloyd, J. U. King's American Dispensatory. Cincinnatti, USA: Ohio Valley Co, 1898. Scan version by H. Kress https://www.henriettes-herb.com/eclectic/kings/pycnanthemum.html (accessed 2023-02-02).
14. Jenderek, M. M.; Holman, G. E.; Ellis, D. D.; Reed, B. M. Long-term preservation of *Pycnanthemum* genetic resources. In *Book of Abstracts*, *World Congress on In Vitro Biology Meeting*, Bellevue, WA, United States, June 3–7, 2012.
15. Ombalo (m'yata bolotna, bloshyna, bloshnitsa): shcho tse take, chym zaminyty, osoblyvosti vyroshchuvannya, zastosuvannya [Ombalo (swamp mint, flea): what it is, what to replace it with, features of cultivation, application, *in Ukrainian*]. https://release.com.ua/?p=37313 (accessed Aug 17, 2022).
16. El-Ghorab, A. H. The Chemical Composition of the Mentha pulegium L. Essential Oil from Egypt and its Antioxidant Activity. *J. Essent. Oil-Bear. Plants* **2006,** *9* (2), 183–195. https://doi.org/10.1080/0972060X.2006.10643491.
17. Bouyahya, A.; Et-Touys, A.; Bakri, Y.; Talbaui, A.; Fellah, H.; Abrini, J.; Dakka, N. Chemical composition of Mentha pulegium and Rosmarinus officinalis essential oils and their antileishmanial, antibacterial and antioxidant activities. *Microb. Pathog.* **2017**, *111*, 41–49. https://doi.org/10.1016/j.micpath.2017.08.015.
18. Nickavar, B.; Jabbareh, F. Analysis of the Essential Oil from *Mentha pulegium* and Identification of its Antioxidant Constituents. *J. Essent. Oil-Bear. Plants* **2018**, *21* (1), 223–229. https://doi.org/10.1080/0972060X.2018.1433073.
19. Agnihotri, V. K.; Agarwal, S. G.; Dhar, P. L.; Thappa, R. K.; Baleshwar; Kapahi, B. K.; Saxena, R. K.; Qazi, G. N. Essential oil composition of *Mentha pulegium* L. growing wild in the North-Western Himalayas India. *Flavour Fragr. J.* 2005, *20* (6), 607–610. https://doi.org/10.1002/ffj.1497.
20. Yasa, H.; Onar, H. C.; Yusufoglu, A. S. Chemical Composition of the Essential Oil of *Mentha pulegium* L. from Bodrum, Turkey. *J. Essen. Oil-Bear. Plants* **2015**, *15* (6), 1040–1043. https://doi.org/10.1080/0972060X.2012.10662609.
21. Da Rocha, M. S.; Dodmane, P. R.; Arnold, L. L.; Pennington, K. L.; Anwar, M. M.; Adams, B. R.; Taylor, S. V.; Wermes, C.; Adams, T. B.; Cohen, S. M. Mode of Action of Pulegone on the Urinary Bladder of $F_{344}$ Rats. *Toxicol. Sci.* **2012,** *128* (1), 1–8. https://doi.org/10.1093/toxsci/kfs135.



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