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IDENTIFICATION OF THE MAIN COMPOUNDS OF *AGASTACHE FOENICULUM* ESSENTIAL OIL BY TLC AND CHEMOTYPE EXPRESS-DETERMINATION

Topicality. *Agastache foeniculum essential oil has bactericidal, fungicidal and anti-inflammatory effects. Depending on the dominant component of the essential oil, five Agastache chemotypes have been distinguished. So, identification of the main compounds of Agastache foeniculum essential oil in other to find markers for the chemotype determination and standardization of raw materials is advisable.*

The purpose of the research *was to study the possibility of identifying the main terpenoids in the Agastache foeniculum raw materials by the method of "cold" thin layer chromatography (TLC) for the chemotype determination.*

Materials and methods. *The TLC was used for investigation with ethyl acetate – toluene (1:19) as mobile phase and special conditions: a temperature of elution +15°C and drying of chromatographic plates in a stream of cool air.*

Research results. *Menthol, linalool and pulegone by the method of "cold" TLC, in the Agastache foeniculum raw material grown in Kyiv were identified. The predominance of pulegone allows us to attribute the raw materials to pulegone chemotype. Estragole and limonene were identified in the Agastache foeniculum raw material grown in Kherson region with a predominance of estragole that allows us to attribute this raw material to the estragole chemotype.*

Conclusions. *The technique above of terpenoids identification ensures the specificity of the determination of the main components of Agastache foeniculum essential oil for the chemotype determination and its standardization.*

Key words: *agastache foenicilum, TLC, essential oil, qualitative analysis, pulegone, menthol, estragole, chemotype.*

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ІДЕНТИФІКАЦІЯ ОСНОВНИХ КОМПОНЕНТІВ ЕФІРНОЇ ОЛІЇ АГАСТАХЕ ФЕНХЕЛЬНОГО МЕТОДОМ ТШХ ТА ЕКСПРЕС-ВИЗНАЧЕННЯ ХЕМОТИПУ

Актуальність. *Ефірна олія Agastache foeniculum виявляє бактерицидну, фунгіцидну та протизапальну дію. Залежно від домінуючого компонента ефірної олії виділяють п'ять хемотипів Agastache. З метою пошуку маркерів для визначення хемотипу та стандартизації сировини Agastache foeniculum доцільно ідентифікувати основні компоненти його ефірної олії.*

Метою роботи *було вивчення можливості ідентифікації основних терпеноїдів у сировині Agastache foeniculum методом «холодової» тонкошарової хроматографії (ТШХ) для визначення хемотипу.*

Матеріали та методи дослідження. *Для дослідження використовували метод ТШХ, етилацетат – толуол (1:19) як рухому фазу та спеціальні умови: температура елюювання +15°С та висушування хроматографічних пластинок у потоці холодного повітря.*

Результати дослідження. *Методом «холодової» ТШХ у сировині Agastache foeniculum, вирощеної в Києві, було ідентифіковано ментол, ліналоол та пулегон. Переважний вміст пулегону дозволяє віднести таку сировину до пулегонового хемотипу. У сировині Agastache foeniculum, вирощеній у Херсонській області, виявлено естрагол та лімонен з переважанням естраголу, що дозволяє віднести цю сировину до естраголового хемотипу.*

Висновки. *Наведена методика ідентифікації терпеноїдів забезпечує специфічність визначення основних компонентів ефірної олії Agastache foeniculum для визначення хемотипу та його стандартизації.*

Ключові слова: *агастахе фенхельний, ТШХ, ефірна олія, якісний аналіз, ментол, естрагол, хемотип.*

Agastache foeniculum (Pursch) Kuntze, *Lamiaceae* family, is perennial aromatic medicinal plant. *Agastache foeniculum* herbs are quite widely used in Eastern folk medicine for colds and as a restorative agent. Its essential oil has bactericidal, fungicidal and anti-inflammatory effects (Najafi, 2022; Bălănescu, 2023). *Agastache foeniculum's* raw materials are abundant in phenylpropanoid and terpenoid metabolites. Depending on the dominant component of the essential oil, five *Agastache* chemotypes have been distinguished, based on the analysis of specimens from different geographical origins, cultivated in similar conditions: the 1st contains typical estragole (most common), 2nd – menthone, 3rd – menthone and pulegone, 4th – methyleugenol, and the 5th – methyleugenol and limonene-containing (Zielińska, 2014).

Given that the Pulegone chemotype aromatic profiles characterized by the predominant content of pulegone, which have to be monitored in the essential oil for their toxicity (Ribeiro-Silva, 2022), the other chemotypes are virtually free of pulegone and are safe for humans. To find out how raw materials can be of further use, it is important to identify their chemotype.

Therefore, identification of the main compounds of A*gastache foeniculum* essential oil in other to find markers for the chemotype determination and standardization of raw materials is advisable.

The aim of this work was to study the possibility of identifying the main terpenoids in the *Agastache foeniculum* raw materials by the method of "cold" thin layer chromatography (TLC) for the chemotype determination.

As objects of study, *Agastache foeniculum* herb was used. The raw plant materials were harvested during the mass flowering phase in 2020 at the different regions of Ukraine – at the experimental sites of A.V. Fomin Botanical Garden of Educational and Scientific Centre "Institute of Biology" of Taras Shevchenko National University of Kyiv, Kyiv (Ukraine), at the experimental sites of M.M. Gryshko National Botanic Garden of National Academy of Sciences of Ukraine (NASU), Kyiv (Ukraine) and at the State Enterprise Research Institute, "Novokahovske" of the Rice Institute of NAASU, Kherson oblast (Ukraine).

Materials and methods research. The chromatographic plates Merck 60 F_{254} 10*15 with a fixed layer of silicagel were used for chromatography. A mixture of solvents, which was used for further chromatographic separation was previously passed through the chromatographic plate and then dried in oven at 100°C. On the start line of the chromatographic plate, 5 μl of hexane extract of the raw material (sample 1 – *Agastache foeniculum* herb harvested at the experimental site of A.V. Fomin Botanical Garden of Educational and Scientific Centre "Institute of Biology" of Taras Shevchenko National University of Kyiv; sample 2 – at the experimental site of M.M. Gryshko National Botanical Garden of NASU in Kyiv; sample 3 – at State Enterprise Research Institute, "Novokahovske" of the Rice Institute of NAASU, Kherson oblast, Ukraine) and

standard samples of terpenoids such as linalool, limonene, isomenthone, menthol, pulegone, methyleugenol, estragole (Supelko, Merck KGaA), previously dissolved in hexane were applied on separate strips. The plates were eluted with ethyl acetate – toluene (1:19) (Imre, 2016; Ashida, 2019) as mobile phase at a temperature of +15°C and then dried in a stream of cool air. Then the plates were sprayed with anisaldehyde solution and kept in the oven at 100–105°C, for 5–10 minutes.

Results and discussion. There were revealed spots of standard substances on the chromatogram (the results of chromatographic separation are shown in Fig. 1): dark pink *Rf* = 0.04 (methyleugenol l), pink-purple *Rf* = 0.11 (estragole), slightly pink *Rf* = 0.23 (menthol), yellow-pink *Rf* = 0,31 (linalool), pink-orange *Rf* = 0,43 (pulegon), yellow *Rf* = 0.55 (isomentone) and blue *Rf* = 0,95 (limonene). The strip of the test extracts revealed spots at the level of three corresponding spots of terpenoids standard samples.

As a result of the research, by comparing the retention factors of standard solutions spots with the retention factors of investigated samples, it was observed that hexane extracts of the samples 1 and 2 contained menthol (*Rf*=0.23), linalool (*Rf*=0.31) and pulegone (*Rf*=0.41). More intensive color and size of pulegone spots on the chromatographic plates proved that the investigated samples contain pulegone in the highest amount. Hexane extract of the sample 3 contained estragole (*Rf*=0.11) and limonene (*Rf*=0.95). More intensive color and size of estragole spot on the chromatographic plates proved that the investigated sample contain estragole in the highest amount.

The analysis above allows us to attribute the raw material of *Agastache foeniculum* harvested in Kyiv, to the menthone and pulegone chemotype and the raw material of *Agastache foeniculum* harvested in Kherson region, to the estragole chemotype.

Conclusions. As a result of the research, menthol, linalool and pulegone were identified in the investigated raw material by the method of "cold" TLC. The "cold" TLC results confirmed the results determined by chromatography-mass spectrometry (GC-MS) (Konovalova, 2017) on the composition of volatile compounds of *Agastache foeniculum* herb obtained earlier. The above technique of identification of terpenoids ensures the specificity of the determination of the main components of *Agastache foeniculum* essential oil for the chemotype determination and its standardization. Considering that the dominant compounds for each *Agastache foeniculum* chemotype are fairly well separated by "cold" TLC in the system ethyl acetate – toluene (1:19), it is possible to recommend the specified method of chromatographic separation for express determination of the chemotype of *Agastache foeniculum* raw material. It should be noted that TLC qualitative analysis is cheaper and easier to use than GC-MS and can be used as a relatively fast methodology for quality control of essential oils.

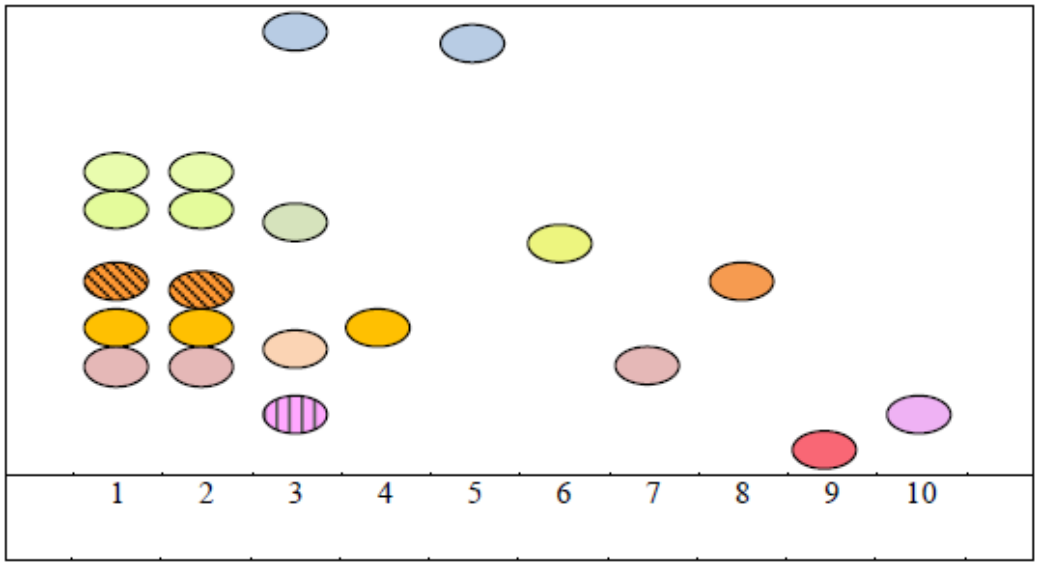

Fig. 1. Thin layer chromatogram of terpenoids from the Agastache foeniculum herb (lines 1–3 – hexane extracts of the samples 1–3 appropriately) with reference solutions containing the standards: line 4 – linalool; line 5 – limonene; line 6 – isomenthone; line 7 – menthol; line 8 – pulegone; line 9 – methyleugenol; line 10 – estragole; in the mobile phase ethyl acetate – toluene (1:19)

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