Comparative chromato-mass spectrometric study of tinctures based on Echinacea purpurea (L.) Moench

Yu. I. Korniievskyi1,A,F, D. Yu. Skoryna1,B,C,D, V. H. Korniievska1,B,C, N. V. Kandybei2,C

1Zaporizhzhia State Medical University, Ukraine, 2PJSC VIOLA Pharmaceutical Factory, Zaporizhzhia, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

An urgent problem today is the spread of secondary immunodeficiencies associated with increased stress, urbanization, and negative changes in the environment that lead to impaired immune function. Herbal remedies are milder, less likely to become addictive and have side effects, and are long-lasting. A promising plant with immunomodulatory action is Echinacea purpurea (L.) Moench, a member of the family Asteraceae. The main active ingredients of echinacea are phenolic compounds, carbohydrates, and alkalimides. The chemical composition of echinacea is also represented by flavonoids, tannins, saponins, higher fatty acids, amino acids, betaine, essential oil, macro- and microelements (Se, Co, Ag, Mo, Zn, Mg, K, Na, Fe, etc.). Drugs have immunostimulatory, antioxidant, membrane-stabilizing effect, promote healing of wounds, burns, ulcers, are used in infectious and viral diseases, especially HF. They are traditionally used for furunculosis, septicemia (infection of the blood), pyorrhea, tonsillitis, especially for the treatment of boils, carbuncles, and abscesses. It is also important that in modern pharmaceutical science the leading place is occupied by research related to the introduction into medical practice of herbal medicines, the study of their chemical composition, standardization, development of optimal technologies for the manufacture of phytopreparations. That is why the objects of our research were tinctures based on Echinacea purpurea.

The aim of the work is to study and compare the component composition of tinctures based on Echinacea purpurea (L.) Moench using gas chromatography (GC).

Materials and methods. Tinctures of rhizomes with roots of Echinacea purpurea were used for the study: experimental – made according to industrial technological recipe (1:5, extractant – ethanol 70 %) from raw materials harvested in July 2019 at the research site of Zaporizhzhia State Medical University and control – finished products LLC “Zhytomyr Pharmaceutical Factory”, series 20319. Tinctures were investigated on a gas chromatograph Agilent 7890B with mass spectrometric detector 5977B. The NIST14 mass spectrum library was used to identify the components.

Results. 31 (experimental) and 23 (control) components were identified by GC in rhizomes with rhizomes of Echinacea purpurea. The results of the study indicate that the qualitative and quantitative chemical composition of rhizomes with roots of Echinacea purpurea was characterized by complexity and variability. This fact complicates the process of standardization of phytopreparations and can affect their effectiveness and safety. Therefore, members of the genus Echinacea should be the subject of further in-depth chemical study.

Conclusions. A comparative analysis of the component composition of tinctures based on Echinacea purpurea (L.) Moench was studied and performed with the help of GC. The GC method is suitable for the determination of natural BAS in the composition of phytopreparations from echinacea and can be used in the development of methods for their standardization. The results of the study were of value for further development and improvement of analytical regulations for raw materials and phytopreparations from Echinacea purpurea. They can also be the basis for the creation of new substances with immunomodulatory activity based on the underground organs of echinacea.

Key words: gas chromatography, Echinacea, component composition, immunomodulatory action.

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Порівняльне хромато-мас-спектрометричне дослідження настойок на основі Echinacea purpurea (L.) Moench

Ю. І. Корнієвський, Д. Ю. Ско́ріна, В. Г. Корнієвська, Н. В. Кандибей

Актуальною проблемою є поширення вторинних імунодефіцітів, пов’язаних із підвищенням стресовими навантаженнями, урбанизацією та негативними змінами в екології, що призводять до порушення функціонування імунної системи. Препарати, які виробляються на основі лікарських рослин, характеризуються більш якісною дією, меншою ієрархією розвитку звикання та побічних ефектів, а також можливістю доволі тривалого застосування. Ехінацея пурпурова (Echinacea purpurea (L.) Moench), представник родини Asteraceae, – перспективна рослина з імуномодулювальною дією, сприяє загоєнню ран, опіків, виразок; їх застосовують при інфекційних і вірусних захворюваннях, особливо HF. Вони традиційно використовуються при фурункулозі, сепсисі (загострення кровообігу), pyorrhea, тонзилліти, особливо для лікування язв, карбункулів, абсцесів. Отже, важливо, що в сучасній фармацевтичній науці основою є дослідження, пов’язані з впровадженням на медицинську практику природних медикаментів, дослідженнях їх хімічного складу, стандартизації, розробці оптимальних технологій для виготовлення фітопрепаратів. Це є причиною того, що об’єктами нашого дослідження стали настойки на основі Echinacea purpurea.

Це дослідження посвячено порівняльному хромато-мас-спектрометричному дослідженню настойок на основі Echinacea purpurea (L.) Moench, виконаного за допомогою газової хроматографії (GC).

Матеріали та методи. Настойки коренів з коріннями Echinacea purpurea були використані для дослідження: експериментальних – виготовлені за індустріально-технологічним рецептом (1:5, екстрактант – етанол 70 %) з сироватки, зібраної в лікарському полі Запорізького державного медичного університету в липні 2019 року, з контрольними – готовими виробами ПІВО “Зітomyrський фармацевтичний завод”, серія 20319. Настойки вивчені на газовій хроматографії Agilent 7890B з мас-спектрометричним дістECTом 5977B. Базу NIST14 було використано для ідентифікації компонентів.

Результати. 31 (експериментальних) та 23 (контрольних) компонентів було визначено за допомогою GC в коренях з коріннями Echinacea purpurea. Результати дослідження підтверджують, що хімічний склад коренів з коріннями Echinacea purpurea характеризується комплексністю та варіабельністю. Це складається з об’єктом нашого дослідження, що повинен набувати більш інтенсивного вивчення у глибоких хімічних дослідженнях.

КонCLUSIOnS. Порівняльний аналіз компонентного складу настойок на основі Echinacea purpurea (L.) Moench був проведений та здійснений середством GC. Інструмент хроматографії GC справляється з детермінації ноців біологічного значення у складі фітопрепаратів від Echinacea purpurea і може бути використаний для розроблення методик їх стандартизації. Результати дослідження були цінними для подальшого розвитку та усунення аналітичних нормативів для сировинних і фітопрепаратів з Echinacea purpurea. Вони також можуть бути основою для створення нових засобів з імуностимулюючою дією на основі нижніх органів Echinacea.

Ключові слова: газова хроматографія, Echinacea, компонентний склад, імуномодулююча дія.

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Справдиве хромато-мас-спектрометричне ісследування настоек на основі Echinacea purpurea (L.) Moench Ю. І. Корниєвський, Д. Ю. Скорина, В. Г. Корниєвская, Н. В. Кандебей

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Today is the actual problem there is a spread of secondary immunodeficiencies, related to high-stress loads, urbanization, and negative changes in ecology, that lead to violations functioning immune system. These states inevitably need it to carry out immune correction. Use for this purpose synthetic drugs complicated a significant risk of developing unwanted reactions and usually low economic availability of such medicines for the population. In return, drugs, which are produced based on medicinal plants, are characteristic with softer action, less likely to get used to it and side effects, as well as the opportunity long enough application [1,2]. Therefore, in the practice of rehabilitation of persons with secondary immunodeficiency states, including as support for chemotherapy in cancer, it is advisable to use medicinal plants that have immunotropic effects [1,3,4].

The promising plant with immunomodulatory action is purple Echinacea (Echinacea purpurea (L.) Moench), representative of the family Asteraceae. It is known that phytopreparations based on this plant exhibit immunostimulatory, antioxidant, membrane stabilizing, antiseptic action, and are capable of stimulating the central nervous system [4–10].

It is also important that in modern pharmaceutical science the leading place is occupied with the researches connected with the introduction into medical practice of herbal medicines, studying of their chemical composition, standardization, development of optimum technologies of production of herbal preparations. That is why tinctures based on purple Echinacea have become the object of our research [11].

Aim
The purpose of the work is to study and comparative analysis of the component composition of tinctures based on purple Echinacea (Echinacea purpurea (L.) Moench) using gas chromatography (GC).

Materials and methods
Tinctures of rhizomes with purple Echinacea roots were used for the study:

1) experimental – manufactured according to an industrial, technological recipe (1:5, extractant – ethanol 70 %) from raw materials, which was harvested in July 2019 at the experimental site of the Zaporizhzhia State Medical University. The receipt of this tincture was carried out according to validated technology by the approved technological regulations. The main stages of production are reflected in the flow chart (Fig. 1);

2) control – finished products of LLC “Zhytomyr Pharmaceutical Factory”, series 20319.

The tinctures were examined on an Agilent 7890B gas chromatograph with a 5977B mass spectrometry detector. Chromatography conditions: DB-5ms column, 30 m long, with an internal diameter of 250 μm and a phase thickness of 0.25 μm. The carrier gas (helium) velocity is 1.3 ml/min. Injection volume – 0.5 μl. Flow split – 1:5. The temperature of the sample input block is 265 °C. Thermostat temperature: programmable – 70 °C (holding 1 min), up to 150 °C at a speed of 20 °C/min (holding 1 min), up to 270 °C at a speed of 20 °C/min (holding 4 min). A NIST14 mass spectra library was used to identify the components.

Results
In the analysis of the obtained chromatograms (Fig. 2, 3, Table 1) and the processing of the results of chromatographic mass spectrometry determination of tincture components on the basis of purple Echinacea Echinacea purpurea (L.) Moench, identified biologically active substances (BAS) belong to derivatives of: carboxylic acids (2, 15, 21, 22, 23); esters (4, 17, 26, 27, 34, 35); lactones (6); aldehydes (10); ketones (1, 3, 5, 9); glycosides (11); carbocyclic compounds (31, 33, 36); nitrogen-containing heterocycles (13, 14, 16, 19, 24); oxygen-containing heterocycles (7, 8, 28, 30, 32, 37); organosilicon compounds (12).

Discussion
31 (experimental) and 23 (control) components were identified using GLC in tinctures of rhizomes with purple Echinacea roots. There were 9 components in the experimental tincture:

1) 17.291 Rt pyridine, 4-(3-mercapto-4-methyl-5(4H)-1,2,4 triazolyl)-, 12.14 %;
2) 10.503 Rt benzaldehyde, 2-hydroxy-6-methyl-, 7.11 %;
3) 12.948 Rt ethyl a-D-glucopyranoside, 7.01 %;
4) 19.556 Rt N-(2-methylbutyl)undecac(2E,4Z)-diene-8,10-diyamidine, 4.60 %;
5) 3.51 Rt dihydroxacetone, 4.15 %;
6) 6.53 Rt 4H-pyran-4-one, 2,3-dihydroxy-3,5-dihydroxy-6-methyl-, 1.59 %;
7) 20.459 Rt 1H-indene, 2-butyl-3-hexyl-, 1.48 %;
8) 8.084 Rt 4-hydroxy-2-methylacetophenone, 1.15 %;
9) 22.805 Rt 9,12-octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl-, 0.79 %.

The control tincture is dominated by 7 components:

1) 22.584 Rt γ-sitosterol, 17.62 %;
2) 17.999 Rt cis-vaccenic acid, 8.37 %;
3) 13.064 Rt 1,3,5-cycloheptatriene, 7,7-dimethyl-3-(trimethylsilyl)-, 8.17 %;
4) 10.524 Rt benzaldehyde, 2-hydroxy-6-methyl-, 7.8 %;
5) 3.423 Rt dihydroxyacetone, 2.77 %;
6) 7.521 Rt benzo furan, 2,3-dihydro-1.38 %;
7) 19.547 Rt 2H-pyran-2-one, 6-[2E-(4-tolyl)ethenyl]-4-methoxy-, 1.27 %.

In the tinctures studied, there were 15 components in common, with only 2 components in quantitative terms: benzaldehyde, 2-hydroxy-6-methyl- (7.11 % and 7.80 %) and 2H-pyran-2-one, 6-[2E-(4-tolyl)ethenyl]-4-methoxy- (1.59 % and 1.27 %).

The results of the study show that the qualitative and quantitative chemical composition of rhizomes with roots of purple Echinacea was characterized by complexity and variability. This fact complicates the standardization of herbal preparations and may affect their effectiveness and safety.
Raw materials, intermediate products and materials

- Detergents and disinfectants from the warehouse, water purified from the water treatment site

Production preparation

- Sanitary preparation of industrial premises, technological equipment and inventory, personnel; technical preparation of premises and equipment

Control in the production process

- Microbiological purity of air

Preparation of raw materials

- Input quality control of raw materials

- Quality indicators according to specifications

- Temperature and time of washing, drying, storage; absence of mechanical inclusions and microbiological purity

- Raw material weighing

- Mass of raw materials

Production of tincture

- Preparation of the extractant

- The weight of the components, the mixing time, the mass and concentration of the extraction

- Component mass, temperature, vacuum depth, extraction time

- Extractant regeneration

- Mass and concentration of alcohol-distillation

- Weight of components, temperature and settling time, parameters of filters and centrifugation, control of intermediate products, mass of intermediate obtained and completeness of unloading

- Defending, clarifying or filtering and unloading the tincture

- Weight of components, temperature and settling time, parameters of filters and centrifugation, control of intermediate products, mass of intermediate obtained and completeness of unloading

- Packing, marking and packaging of the tincture

- Transfer of the intermediate to the drug packing shop

- Dosage and closure

- Accuracy of dosing sealing, mechanical inclusions

- Marking and packaging

- Correct marking, completeness, quantity of packing

- Quarantine storage of finished goods

- Finished goods control (according to the MCA), issuance of a marketing authorization

- Warehouse of finished products

Fig. 1. Technological scheme of production of experimental tincture of rhizomes with purple Echinacea roots.
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Fig. 2. Chromatogram of experimental tincture of rhizomes with purple Echinacea roots.

Fig. 3. Chromatogram of control tincture of rhizomes with purple Echinacea roots.

Table 1. Comparative chromatographic mass-spectrometric characteristics of tincture components based on purple Echinacea (Echinacea purpurea (L.) Moench)

| No. | The name of the component | Gross formula | Experimental | Control |
|-----|---------------------------|---------------|--------------|---------|
|     |                           |               | Rt           | Contents, % | Rt           | Contents, % |
| 1.  | Acetone                   | C₃H₆O         | 1.906        | 1.65       |              |             |
| 2.  | Acetic acid               | C₂H₄O₂        | 2.022        | 5.73       | 1.89        | 5.31        |
| 3.  | 2-Propanone, 1-hydroxy    | C₃H₆O         | 2.199        | 4.30       | 2.082       | 3.72        |
| 4.  | Propanoic acid, 2-oxo-, methyl ester | C₄H₆O₃ | 2.584 | 2.42 | – | – |
| 5.  | Dihydroxyacetone          | C₂H₄O₂        | 3.51         | 4.15       | 3.423       | 2.77        |
| 6.  | 2-Hydroxy-γ-butyrolactone | C₃H₆O         | 4.524        | 2.24       | 4.467       | 1.74        |
| 7.  | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | C₄H₆O₃ | 6.53 | 1.59 | – | – |
| 8.  | Benzofuran, 2,3-dihydro    | C₃H₆O         | 7.523        | 0.94       | 7.521       | 1.38        |
| 9.  | 4-Hydroxy-2methyl acetophenone | C₄H₈O       | 8.804        | 1.15       | 8.804       | 1.43        |
| 10. | Benzaldehyde, 2-hydroxy-6-methyl- | C₄H₆O₂ | 10.503      | 7.11       | 10.524      | 7.80        |
| 11. | Ethyl α-D-glucopyranoside  | C₈H₁₂O₃       | 12.948       | 0.01       | 12.869      | 2.98        |
| 12. | 1,3,5-Cycloheptatriene, 7,7-dimethyl-3-(trimethylsilyl)- | C₈H₁₄Si | – | – | 13.064 | 8.17 |
| 13. | 4(1H)-Quinazolinone, 2,3-dihydro-1,3-dimethyl-2-thioxoo- | C₈H₁₄N₂S | – | – | 15.787 | 1.54 |
| 14. | (1f)-Quinolin-4-ol-2-one, 8-nitro- | C₉H₁₄N₂O₂ | 15.787 | 1.23 | – | – |
| 15. | n-Hexadecanoic acid       | C₁₈H₃₄O₂ | 16.277 | 1.52 | 16.284 | 2.75 |
| 16. | 1,2,4-Triazole, 3-mercatto-4-phenyl-5-methyl- | C₇H₂₅N₃S | 16.71 | 5.27 | 16.695 | 1.85 |
| 17. | Hexadecanoic acid, ethyl ester | C₁₈H₃₄O₂ | – | – | 16.598 | 2.7 |
| 18. | N-Isobutylundeca-(2E,4E)-diene-8,10-diynamide | C₁₅H₂₀NO | 17.218 | 1.93 | – | – |
Therefore, representatives of the genus Echinacea should be the object of further advanced chemical study.

Conclusions

1. A comparative analysis of the component composition of tinctures based on purple Echinacea was studied and performed using GC (Echinacea purpurea (L.) Moench). The GC method was suitable for the determination of natural BAS in the composition of Echinacea herbal preparations and can be used in the development of methods for their standardization.

2. In the experimental tincture of rhizomes with roots of purple Echinacea identified 31 components, and in the control – 23. The composition of the tinctures was characterized by complexity and variability. In the tinctures studied, 15 components were common, with only 2 components in quantitative terms.

3. The results of the study were of value for the further development and improvement of analytical regulatory documentation for raw materials and herbal preparations of purple Echinacea. They may also be the basis for the creation of new immunomodulatory substances based on the underground organs of Echinacea.

Prospects for further research. Tinctures based on Echinacea purpurea (L.) Moench can be considered as a basis for the creation of new substances with immunomodulatory activity based on the underground organs of echinacea.

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Information about authors:

Korniievskyi Yu. I., PhD, Associate Professor of the Department of Pharmacognosy, Pharmacology and Botany, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0001-7863-6736

Skoryna D. Yu., PhD, Senior Lecturer of the Department of Pharmaceutical Chemistry, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0002-8851-8757

Korniievska V. H., PhD, Associate Professor of the Department of Pharmacognosy, Pharmacology and Botany, Zaporizhzhia State Medical University, Ukraine.
ORCID ID: 0000-0001-8307-1282
Comparative chromato-mass spectrometric study of tinctures based on Echinacea purpurea (L.) Moench

Kandybei N. V., PhD, Teaching Assistant of the Department of Clinical Pharmacy, Pharmacotherapy, Management and Organization of Pharmacy of FPE, Zaporizhzhia State Medical University, Ukraine.

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