The study of fatty acids in Anchusa officinalis L. herb of the Ukrainian flora

The presence of different groups of biologically active substances in the medicinal plant raw material determines its wide use in medical practice. The study of the chemical composition and possible pharmacological effects of medicinal plants are priorities in the process of their research.

Aim. To study the content and the composition of fatty acids in Anchusa officinalis L. herb of the Ukrainian flora using the gas chromatography/mass spectrometry method (GC/MS).

Materials and methods. The samples of Anchusa officinalis L. herb were harvested in June 2017 in the Ivano-Frankivsk region. The study was performed using the GC/MS method. To identify the components, the spectra obtained were considered on the basis of general laws of fragmentation for molecules of organic compounds under the action of the electron impact, as well as by comparing the results with data from NIST05 and WILEY 2007 mass spectrum libraries. The data analysis was performed using Statistic 7.0, Excel 7.0 statistical program package for Microsoft Office for Windows.

Results and discussion. This study presents the results the fatty acids investigation in Anchusa officinalis L. herb of the Ukrainian flora. For the first time, 8 fatty acids were identified. They were myristic acid (163.36 mg/kg), palmitic acid (2324.39 mg/kg), linoleic acid (1498.33 mg/kg), linolenic acid (2696.36 mg/kg), stearic acid (410.16 mg/kg), arachidic acid (240.43 mg/kg), behenic acid (409.23 mg/kg), lignoceric acid (498.38 mg/kg). The total mass of unsaturated fatty acids (linoleic and linolenic acid) was 4045.95 mg/kg, while the total mass of saturated fatty acids (myristic, palmitic, stearic, arachidic, behenic and lignoceric acid) was 4194.69 mg/kg. It indicates an insignificant predominance of unsaturated acids in the plant raw material. The total content of fatty acids in Anchusa officinalis L. herb was 8240.64 mg/kg (0.8 % by weight of the plant raw material).

Conclusions. The study of fatty acids in Anchusa officinalis L. herb of the Ukrainian flora has been conducted for the first time. In the raw material, the following 8 fatty acids have been identified: unsaturated fatty acids (linoleic and linolenic acid) and saturated fatty acids (myristic, palmitic, stearic, arachidic, behenic and lignoceric acid). The total content of fatty acids in Anchusa officinalis L. herb is 0.8 % by weight of a dry raw material. The dominant compound among fatty acids is linolenic acid (2696.36 mg/kg of a dry raw material). Taking into account the above the further search for the method of obtaining the fatty acid complex from Anchusa officinalis L. herb is promising in order to develop the effective and safe herbal medicines.

Key words: Anchusa officinalis L.; herb; GC/MS; biologically active substances; fatty acids

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Висновки. Дослідження жирних кислот у траві воловика лікарського (Anchusa officinalis L.) флори України проведено вперше. У сировині виявлено 8 жирних кислот: ненасичені жирні кислоти (лінолеву та ліноленову) та насичені жирні кислоти (міристинову, пальмітинову, стеаринову, арахідову, бегенову та лігноцеринову). За- гальний вміст жирних кислот у траві Anchusa officinalis L. становить 0,8 % від маси сухої сировини. Домінантною сполукою серед жирних кислот є ліноленова кислота (2696,36 мг/кг сухої сировини). З оптику на визначеннє перспективним постає шлях до отримання комплексу жирних кислот із трави воловика лікарського з метою розроблення ефективних та безпечних фітопрепаратів.

Ключові слова: Anchusa officinalis L.; трава; ГХ/МС; біологічно активні речовини; жирні кислоти

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Изучение жирных кислот в траве воловика лекарственного (Anchusa officinalis L.) флоры Украины

Наличие различных групп биологически активных веществ в лекарственном растительном сырье предопределяет его широкое использование в медицинской практике. Изучение химического состава и возможных фарма- кологических эффектов лекарственных растений является приоритетом в процессе их исследования.

Цель. Изучить содержание и состав жирных кислот в траве воловика лекарственного (Anchusa officinalis L.) флоры Украины с использованием метода газовой хроматографии / масс-спектрометрии (ГХ/МС).

Материалы и методы. Образцы травы воловика лекарственного (Anchusa officinalis L.) были заготовлены в Ивано-Франковской области в июне 2017 года. Исследование проведено методом ГХ/МС. Для идентификации компонентов полученные спектры рассмотрены в отношении общих законов фрагментации молекул органических соединений под влиянием электрона, а также путем сравнения результатов с данными библиотек масс-спектра NIST05 и WILEY 2007. Анализ данных выполнен с использованием статистического программного пакета Statistic 7.0, Excel 7.0 для Microsoft для Windows.

Результаты и их обсуждение. В данной статье представлены результаты изучения жирных кислот в траве воловика лекарственного (Anchusa officinalis L.) флоры Украины. Впервые было выявлено 8 жирных кислот, в частности: миристиновую (163,36 мг/кг), пальмитиновую (2324,39 мг/кг), линолевую (1498,33 мг/кг), линоленовую (2696,36 мг/кг), стеариновую (240,43 мг/кг), арахідову (409,23 мг/кг), бегеновую (409,23 мг/кг), лігноцериновую (498,38 мг/кг) кислоты. Общая масса ненасыщенных жирных кислот (линолевой и линоленовой) составляет 4045,95 мг/кг; общая масса насыщенных жирных кислот (миристиновой, пальмитиновой, стеариновой, арахідовой, бегеновой и лігноцериновой) – 4194,69 мг/кг. Это свидетельствует о незначительном преобладании ненасыщенных кислот в данном сырье. Общее содержание жирных кислот в траве Anchusa officinalis L. составляет 8240,64 мг/кг, что составляет 0,8 % от массы растительного сырья.

Выводы. Исследование жирных кислот в траве воловика лекарственного (Anchusa officinalis L.) флоры Украины проведено впервые. В сырье выявлены 8 жирных кислот: ненасыщенные жирные кислоты (линолевая и линоленовая) и насыщенные жирные кислоты (миристиновая, пальмитиновая, стеариновая, арахідовая, бегеновая и лігноцериновая). Общее содержание жирных кислот в траве Anchusa officinalis L. составляет 0,8 % от массы растительного сырья. С учетом вышеизложенного перспективным является дальнейший поиск способа получения комплекса жирных кислот из травы воловика лекарственного с целью разработки эффективных и безопасных фитопрепаратов.

Ключевые слова: Anchusa officinalis L.; трава; ГХ/МС; биологически активные вещества; жирные кислоты

Introduction. Despite significant advances in the study of medicinal plants, all the possibilities associated with their use in medicine have not yet been exhausted. Currently, the selection and study of individual biologically active substances from plants are the main areas of research when developing new drugs. Often, when using medicinal plants, not just any substance, but a complex of substances is active. At the same time, substances that do not have a self-expressed pharmacological effect can contribute to the therapeutic effect. The presence of different groups of biologically active substances in the medicinal plant raw material determines its wide use in medical practice. Biologically active substances of plants are inorganic and organic compounds; their common characteristic is high activity in relatively small quantities. The study of the chemical composition and possible pharmacological effects of medicinal plants are priorities in the process of their research.

Fatty acids are not synthesized by the human body. Different types and concentrations of fatty acids are presented in many plant and animal sources. Unsaturated fatty acids, in particular linoleic and linolenic, regulate the lipid metabolism, provide the growth and development of organisms and increase their resistance to harmful environmental factors [1, 2]. Anchusa L. is one of the largest genera concentrated in the Mediterranean. They grow mainly in Europe, West Asia and Tropical Africa [3-5]. Anchusa L. species are herbaceous, annual and perennial plants, mostly densely covered with rough hairs. 11 species of Anchusa L. grow in Ukraine. They are divided into 4 sections by morphological characteristics: Euanchusa Griseb., Buglossum (Gaertn.) Reichenb., Buglosellum Gusul. (pro subgen.) and Cynoglottis Gusul. (pro subgen.) [6-9]. Anchusa L. species are a valuable source of natural compounds with potential therapeutic effects. Due to the variety of biologically active substances, Anchusa genus species show diverse pharmacological actions [9]. Anchusa officinalis L. is the most common type and it is widespread in muddy and sandy places and in fields almost all over Ukraine [9].

There is limited number of reports in the world on studying fatty acids in Anchusa genus species. Al-Salihi F. et al. (2007) investigated fatty acids in Anchusa strigosa L.
dry flowers. The highest content was recorded for n-hexadecanoic and octadecanoic acids – 3.6404 µgm and 4.6040 µgm /100 g of a dry raw material, respectively [2]. Kukukboyaci N. et al. (2013) determined the content of fatty acids in the seed oils of Anchusa species of the Turkish flora – Anchusa azurea Miller var. azurea, Anchusa leptophylla Roem. & Schultes subsp. leptophylla, Anchusa arvensis (L.) Bieb. subsp. orientalis (L.) Nordh. and Anchusa undulata L. subsp. hybrida (Ten.) Coutinho. The composition of fatty acids for all species was the same, and it was represented by oleic, palmitic, linolenic, γ-linolenic and α-linolenic acids [1]. Ozcan T. (2008) studied the fatty acid composition in seeds of two informal groups of Anchusa officinalis L. of the Turkish flora. It was found that predominant acids were linoleic, oleic, and α- and γ-linolenic [10]. Thus, the study of fatty acids of Anchusa officinalis L. herb of the Ukrainian flora is relevant and is conducted for the first time.

The aim of this work was to study the content and the composition of fatty acids in Anchusa officinalis L. herb of the Ukrainian flora using the gas chromatography/mass spectrometry method (GC/MS).

Materials and methods. The samples of Anchusa officinalis L. herb were harvested in June 2017 in the Ivano-Frankivsk region. The plant was identified at the Department of Pharmacy, Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine, and the authenticated voucher specimens were deposited there. All reagents were of the highest purity (Sigma-Aldrich, St. Louise, USA). The study was performed by the GC/MS method using an Agilent Technologies 6890 chromatograph with a 5973 mass spectrometric detector and a HP-5ms capillary column (diameter – 0.25 mm, length – 30 m). The speed of the carrier gas (helium) was 1.0 ml/min. The temperature of the sample heater was 250 °C. The temperature of the thermostat was programmed from 60 °C to 320 °C with a speed of 7 °/min.

To obtain fatty acid methyl esters, 0.05 g of the crushed herb was placed in 2 ml vials, adding n-tridecane as an internal standard and 2 ml of 2 % solution of acetyl chloride in methanol. The vials were incubated for 2 h at 37 °C. Fatty acid methyl esters were extracted with 500 µl of hexane.

To identify the components, the spectra obtained were considered on the basis of general laws of fragmentation...
for molecules of organic compounds under the action of the electron impact, as well as by comparing the results with data from NIST05 and WILEY 2007 mass spectrum libraries with a total of more than 470000. The quantitative content of components (X, mg/kg) was determined relative to the internal standard by the formula:

$$X = \frac{P_1 \times 50}{P_2 \times m},$$

where: $P_1$ – is the peak area of the substance studied; 50 – is the mass of the internal standard introduced into the sample, µg; $P_2$ – is the peak area of the standard; m – is the weight of the raw material, g.

The data analysis was performed using Statistic 7.0, Excel 7.0 statistical program package for Microsoft Office for Windows.

**Results and discussion.** This study presents the results the fatty acids investigation in *Anchusa officinalis* L. herb of the Ukrainian flora. For the first time, 8 fatty acids were identified: 2 polyunsaturated – linoleic, γ-linolenic and 6 saturated – palmitic, stearic, myristic, arachidonic, behenic, lignocerinic acids. However, 6 compounds were not identified, but it was determined that 4 of them belonged to alkanes. The data obtained are presented in Fig.

The results of the analysis of fatty acids in *Anchusa officinalis* L. herb are given in Table.

### Table

| Peak number | R.T., min | Substance | Chemical nomenclature | Content, mg/kg |
|-------------|-----------|-----------|----------------------|----------------|
| 1.          | 7.636     | Tetradecanoic acid (Myristic acid), methyl ester | C 14:0 | 163.36 |
| 2.          | 12.095    | Hexadecanoic acid (Palmitic acid), methyl ester | C 16:0 | 2324.39 |
| 3.          | 15.844    | 9,12-Octadecadienoic acid (Linoleic acid), methyl ester, (Z,Z)- | C 18:2n6 | 1498.33 |
| 4.          | 15.987    | 9,12,15-Octadecatrienoic acid (Linolenic acid), methyl ester, (Z,Z,Z)- | C 18:3n6 | 2696.36 |
| 5.          | 16.589    | Octadeconoic acid (Stearic acid), methyl ester | C 18:0 | 410.16 |
| 6.          | 18.750    | Nonadecanoic acid | internal standard |
| 7.          | 20.838    | Eicosanoic acid (Arachidic acid), methyl ester | C 20:0 | 240.43 |
| 8.          | 24.793    | Docosanoic acid (Behenic acid), methyl ester | C 22:0 | 409.23 |
| 9.          | 27.886    | Unidentified component (alkane) | – | 238.69 |
| 10.         | 28.466    | Tetracosanoic acid (Lignoceric acid), methyl ester | C 24:0 | 498.38 |
| 11.         | 31.528    | Unidentified component (alkane) | – | 1639.11 |
| 12.         | 34.773    | Unidentified component (alkane) | – | 3398.70 |
| 13.         | 35.291    | Unidentified component | – | 295.85 |
| 14.         | 37.545    | Unidentified component (alkane) | – | 982.02 |
| 15.         | 38.049    | Unidentified component | – | 748.84 |
| **Total**   |           |           |                      | 15543.85 |
| **The amount of saturated fatty acids** | | | | 4045.95 |
| **The amount of unsaturated fatty acids** | | | | 4194.69 |

The results of the analysis of fatty acids in *Anchusa officinalis* L. herb are given in Table.

### Results of the analysis

**Table**

| Peak number | R.T., min | Substance | Chemical nomenclature | Content, mg/kg |
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| **The amount of saturated fatty acids** | | | | 4045.95 |
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**Conclusions and prospects of further research**

The study of fatty acids in *Anchusa officinalis* L. herb of the Ukrainian flora has been conducted for the first time.

In the raw material, the following 8 fatty acids have been identified: unsaturated fatty acids (linoleic and linolenic acid) and saturated fatty acids (myristic, palmitic, stearic, arachidic, behenic and lignoceric acid).

It has been determined that unsaturated acids are predominant in the plant raw material. The dominant compound among fatty acids is linolenic acid (2696.36 mg/kg) and linoleic acid (1498.33 mg/kg), stearic acid (410.16 mg/kg), arachidic acid (240.43 mg/kg), behenic acid (409.23 mg/kg), and lignoceric acid (498.38 mg/kg). The total mass of saturated fatty acids (linoleic and linolenic acid) was 4045.95 mg/kg, while the total mass of saturated fatty acids (myristic, palmitic, stearic, arachidic, behenic and lignoceric acid) was 4194.69 mg/kg. It indicates an insignificant predominance of unsaturated acids in the plant raw material. The total content of fatty acids in *Anchusa officinalis* L. herb was 8240.64 mg/kg (0.8 % by weight of the plant raw material).

**Conflict of interest:** the author has no conflict of interests to declare.
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