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**IDENTIFICATION AND COMPONENT ANALYSIS OF TRITERPENOIDS IN
MONARDA FISTULOSA L. AND *OCIMUM AMERICANUM* L. (*Lamiaceae*) AERIAL PARTS**

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Незважаючи на прогрес у створенні синтетичних препаратів, ліки рослинного походження не втрачають своєї актуальності до сьогодні. Тритерпеноїди є досить поширеною групою біологічно активних сполук рослин та виявляють широкий діапазон фармакологічної активності. Лікарські рослини родини Ясноткові (*Lamiaceae*) здатні накопичувати пентациклічні тритерпенові сполуки, які виявляють антиоксидантну, протизапальну, гепатопротекторну, протипухлинну та антимікробну дію. На сьогодні в Україні інтродуковано цілий ряд нових маловивчених видів родини, серед яких – монарда трубчаста (*Monarda fistulosa* L.) та васильки американські (*Ocimum americanum* L.).

Метою даного дослідження була ідентифікація, встановлення складу та компонентного вмісту тритерпенових сполук в надземній частині монарди трубчастої (*M. fistulosa*) і васильків американських (*O. americanum*) за умови їх культивування на території Західного Поділля.

Методи. Для встановлення наявності групи тритерпеноїдів в рослинній сировині використано пінну пробу, реакцію осадження та кольорову реакцію; для визначення компонентного вмісту тритерпенових сполук застосовано метод вискоєфективної рідинної хроматографії (ВЕРХ); визначення проводили на рідинному хроматографі Shimadzu LC 20 Prominence.

Результати. Наявність тритерпенових сполук було встановлено за допомогою пінної проби, реакцій ідентифікації та ВЕРХ. Ефективне екстрагування тритерпенових сполук з трави досліджуваних рослин було досягнуто з використанням 96 % та 70 % етанолу. Використання ВЕРХ-аналізу дозволило встановити істотні відмінності у вмісті урсолової, еускафової, торментинової та олеанолової кислот, бетуліну та лупеолу в надземній частині досліджуваних рослин. Домінуючими компонентами трави *M. fistulosa* та *O. americanum* були урсолова та еускафова кислоти.

Висновки. На основі проведених досліджень було ідентифіковано та встановлено компонентний вміст 6 тритерпеноїдів у надземній частині *M. fistulosa* та *O. americanum*. Отримані дані можна використати при плануванні фармакологічних досліджень та в хемотаксономії родини *Lamiaceae*

Ключові слова: *Lamiaceae*; *Monarda fistulosa*; *Ocimum americanum*; ВЕРХ; тритерпеноїди, надземна частина, етанольний витяг

1. Introduction

Despite the progress in the creation of synthetic drugs, herbal medicines do not lose their relevance nowadays. Phytopreparations are, as a rule, multicomponent composition, and, consequently, multipurpose, as in addition to the effect on target organs, the regulatory systems of the organism are also activated [1]. Medicinal plants with essential oils are an important source of medical and prophylactic treatments from ancient times to the present day, in connection with which scientists are interested in their phytochemical research [2, 3].

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

Medicinal plants of the *Lamiaceae* family containing essential oils have long been used in scientific and folk medicine as a source of therapeutic and prophylactic medicines [3, 4]. To date, a number of newly unknown species of the family [5] have been introduced in Ukraine, among them – Bee balm (*Monarda fistulosa* L.) and American basil (*Ocimum americanum* L.). The above-ground parts of these plants are used in folk medicine of different countries, but they are not used in official medicine [4]. It is important that, along with the

therapeutic and preventive action, both plants have good spice and taste qualities, which opens the possibility of their wide application in the manufacture of galenic preparations.

3. Analysis of recent studies and publications in which a solution of the problem are described and to which the author refers

A rather important group of compounds of secondary synthesis, which have a wide range of biological activity, are triterpenoids. According to various authors [6–8], representatives of the *Lamiaceae* family accumulate mainly ursane and oleanane derivatives that exhibit anti-inflammatory, antioxidant, hepatoprotective, anti-tumor, antiviral and antimicrobial properties. It is worth noting that some of these biological properties also show other compounds of secondary synthesis, previously found in various organs of plants of this family [9–11].

4. The field of research considering the general problem, which is described in the article

An important stage in the pharmacological research of poorly-studied, but promising species of medicinal plants is the analysis of compounds of secondary synthesis, which include triterpenoids. The peculiarities

of the accumulation of triterpene compounds in the herb of the non-fictional medicinal plants of the family *Lamiaceae*, in particular *M. fistulosa* and *O. americanum*, which have been introduced in Ukraine in recent years, remain not studied.

5. Formulation of goals (tasks) of article

The purpose of this work was to identify the composition and component content of triterpene compounds in the aboveground parts of *M. fistulosa* and *O. americanum* (under the condition of their cultivation on the territory of Western Podillya, Ukraine).

6. Presentation of the main research material (methods and objects) with the justification of the results

Extraction of triterpenic saponins from the herb of *M. fistulosa* and *O. americanum* was carried out using ethanol of various concentrations (50 %, 70 % and 96 %), which is considered an effective and environmentally justified extractive agent [5, 7]. The particles of 1 mm herb of investigated plants (5.0 g) were placed in a 250 ml circular flask, and 100 ml of ethanol of appropriate concentration was added. At first, ethanol-filled raw material was infused at room temperature for 3 hours, stirring periodically. Further, extraction was performed on a water heater at boiling point of the extractive agent for 1 hour. The cooled extract was filtered through a paper filter in a 100 ml volumetric flask and, if necessary, adjusted to the label with the appropriate extractant. An aliquot of each extract was evaporated to a dry residue and used for HPLC analysis. The remains were evaporated to 1/4–1/5 of the original volume and used to identify triterpene saponins.

Identification of triterpenoids (according to [12])

Foam test. Approximately 1 ml of extract for identification was diluted to 5 ml with purified water, closed with a stopper and vigorously shaken for 1 min. The formation of stable foam indicated the presence of triterpene saponins.

Sedimentation reaction. To 1 ml of the extract for identification, 3–4 drops of 10 % lead (II) acetate solution were added. The appearance of sediment indicated the presence of triterpene compounds.

Colour reaction. To 1 ml of the extract for identification, 1 drop of 10 % solution of copper (II) sulphate

and 1 ml of concentrated sulfuric acid solution were added, after which the mixture was carefully heated. The appearance of a blue-green colour indicated the presence of triterpene saponins.

HPLC analysis of triterpenoids (in accordance with [13, 14])

Investigation of the qualitative composition and quantitative content of triterpene compounds by the HPLC method was carried out on a liquid chromatograph Shimadzu LC 20 Prominence in a modular system equipped with a four-channel LC 20AD pump, a thermostat of columns 20A, an automatic sampler SIL 20A, SPDM 20A diode-matrix detector and ChemStation LC 20 A X-Bridge C18 column of 150 mm × 4.6 mm, a particle size of 5 μm (Waters, Ireland) was used; column temperature – 30 °C; detection wavelength – 205 nm; flow rate of the mobile phase – 1.0 ml / min; volume of the injected sample – 20 μl.

Moving phase: methanol and 0.2 % ammonium acetate solution (pH=6.75) in the ratio 80:20; elution mode isocratic. Identification of the components was carried out in accordance with the time of maintenance and compliance with the UV spectra of the substances-standards (oleanolic, ursolic, tormentic, and euscaphic acids, betulin and lupeol), which were determined in the range 190–800 nm.

Results and discussion. In the extracts of *M. fistulosa* and *O. americanum* raw materials obtained on the basis of high concentrations of ethanol (70 % and 96 %), a positive result was found regarding the presence of triterpene compounds in the use of the foaming test, as well as the colour and sedimentation reactions.

Based on the data of the analysis of UV spectra of standard models of triterpene compounds and investigated extracts, absorption peaks were found in the range of wavelengths of 200–210 nm, so their HPLC analysis was carried out at 205 nm. The results of the HPLC analysis of triterpenoids in the herb of the investigated plants are presented in Table 1 and on Fig. 1, 2; 6 compounds were identified.

As can be seen from the results obtained (Table 1, Fig. 1, 2), effective extractants for extracting triterpene compounds from the herbs of the studied plants were 96 % and 70 % ethanol; its 50 % concentration was less effective.

Table 1
Component content of triterpene compounds in the aboveground parts of *M. fistulosa* and *O. americanum*

Component	Content, %					
	<i>M. fistulosa</i>			<i>O. americanum</i>		
	96 % ethanol	70 % ethanol	50 % ethanol	96 % ethanol	70 % ethanol	50 % ethanol
Derivatives of ursane						
Ursolic acid	0.22	0.11	0.02	0.19	0.12	<0.01
Euscaphic acid	0.31	0.32	0.12	0.21	0.31	0.06
Tormentic acid	0.03	0.04	<0.01	0.03	0.06	0.01
Derivatives of oleanane						
Oleanolic acid	0.13	0.08	0.01	0.14	0.15	<0.01
Derivatives of lupane						
Betulin	0.09	0.11	0.05	0.06	0.1	0.03
Lupeol	–	–	–	0.05	–	–

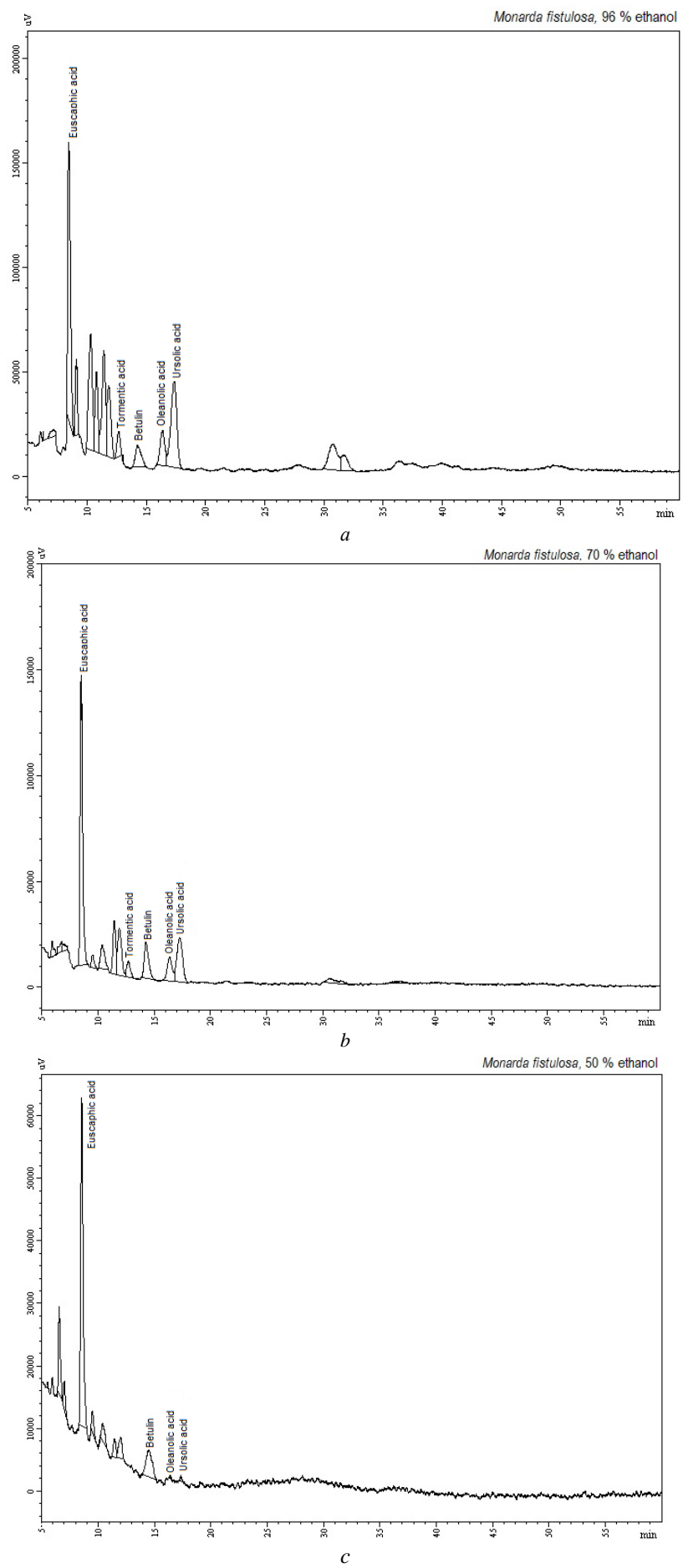


Fig. 1. Chromatogram of *M. fistulosa* herb extracts, obtained using ethanol of various concentrations: a – 96 %; b – 70 %; c – 50 % (at 205 nm)

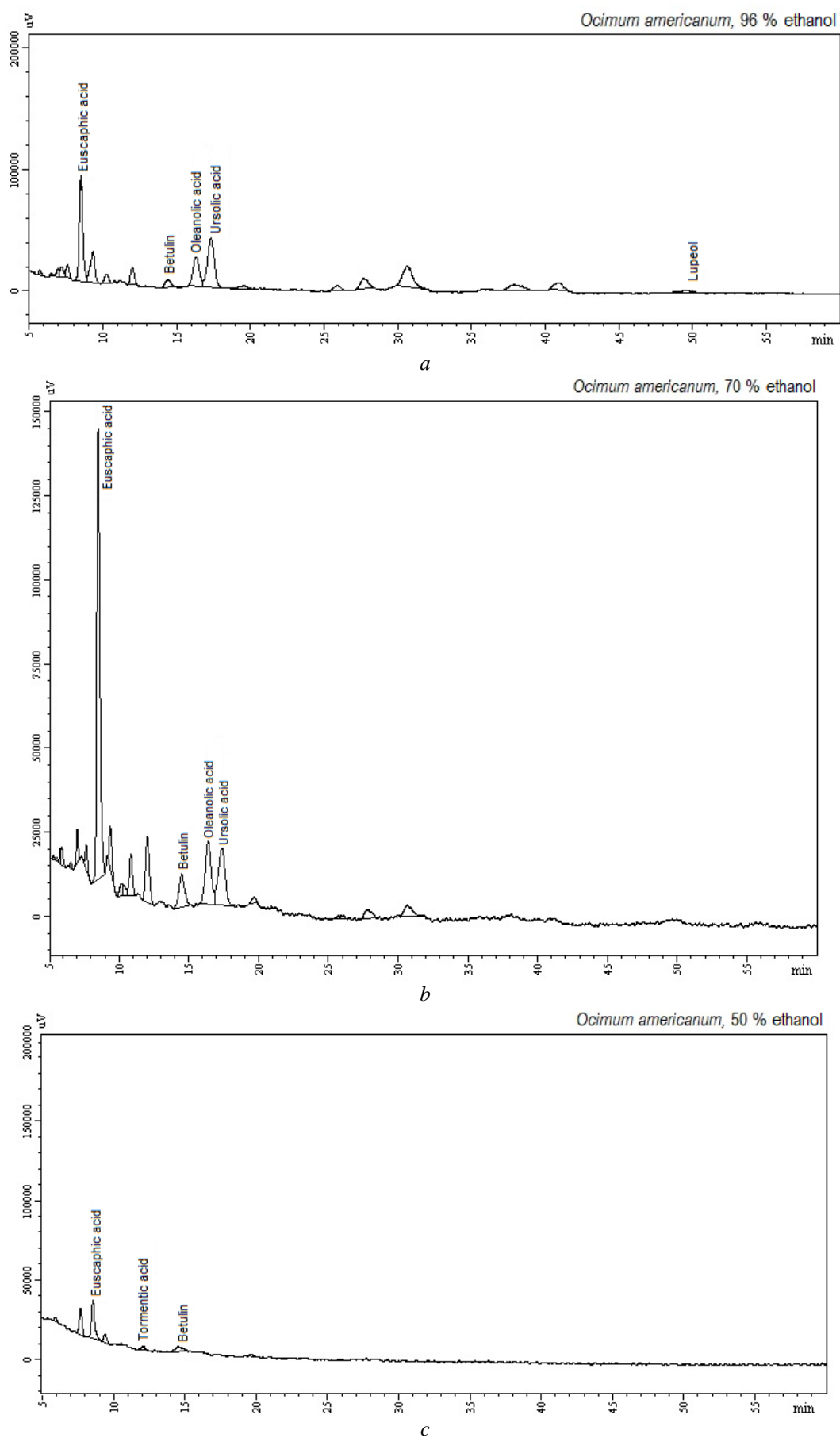


Fig. 2. Chromatogram of the *O. americanum* herb extracts, obtained using ethanol of various concentrations: *a* – 96 %; *b* – 70 %; *c* – 50 % (at 205 nm)

Ursolic and euscaphic acids (α -amine derivatives based on the ursane skeleton – Fig. 3) are the dominant components in 96 % and 70 % of ethanol extracts of both studied plants. As can be seen from Table 1, 96 % ethanol is the optimal extractant for extracting ursolic acid from the herbs of *M. fistulosa* and *O. americanum* (0.22 % and 0.19 %, respectively), while 70 % ethanol – euscaphic one (0.32 % and 0.31 %, respectively). The obtained results correlate with the literature data for other species of the *Lamiaceae* family with respect to the domination of the ursane derivatives. Thus, leaves of 8 representatives of the *Ocimum* genus, which grew on the territory of

Brazil [7], accumulated ursolic acid in the range of 0.29–2.02 %; the above-ground bodies of 4 species of the *Ocimum* genus, harvested in India, accumulated ursolic acid in the range of 0.008–0.296 % [15, 16]. The content of this compound in the aboveground part of 5 species of plants of the *Rosmarinus*, *Salvia* and *Satureja* genus ranges from 0.09 to 1.6 % [17]. Ursolic acid, which is a fairly common substance in the raw materials of the *Lamiaceae* family, has anti-inflammatory, antioxidant, hepatoprotective, antiviral, antimicrobial and cytostatic properties [6, 8]. The content of tormentic acid in the raw materials of the studied plants is low (Table 1, Fig. 1, 2).

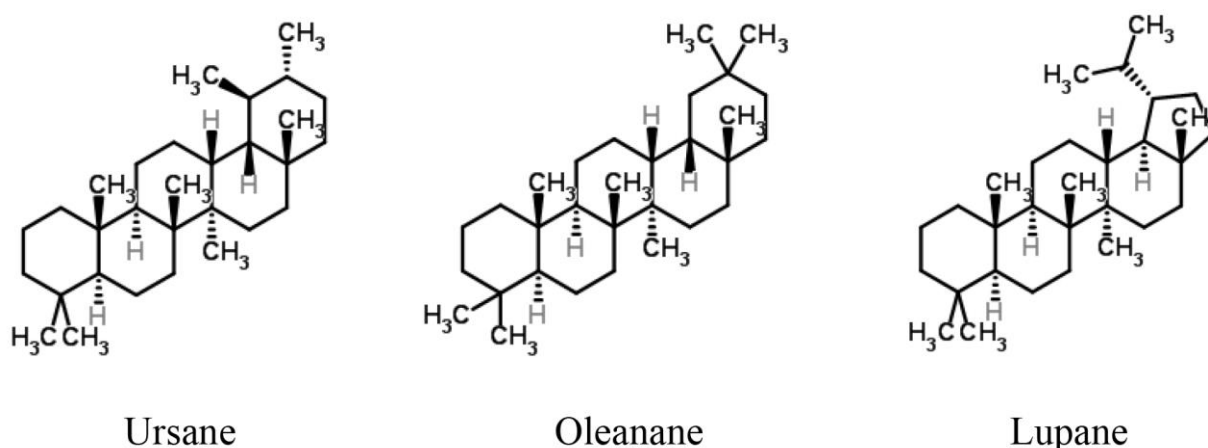


Fig. 3. The chemical structure of triterpene compounds, derivatives of which are found in herbs of *M. fistulosa* ra *O. americanum*

Oleanolic acid (a representative of the β -aminine group, which is based on the oleanane skeleton – Fig. 3), is usually identified in parallel with ursolic acid in the raw material of many plants of the *Lamiaceae* family [6, 14]. These triterpenoids are found both in the form of aglycones and in the form of glycosides, and have similar biological properties [6, 8]. The content of oleanolic acid is higher in the raw material of *O. americanum* in comparison with *M. fistulosa*, regardless of the ethanol concentration used (see Table 1). The obtained results correlate with the data of the literature, according to which [14] the above-ground part of *Ocimum gratissimum* accumulates 0.14 % of oleanolic acid; the content of this compound in the above-ground parts of the plants of the *Rosmarinus*, *Salvia* and *Satureja* genus ranges from 0.09 to 0.9 % [17].

The best solvent for extraction of betulin (a derivative of a lupane - see Figure 3) from the herb of both plants is 70 % ethanol (see Table 1). Another representative of the lupane group – lupeol – was found in a small amount in only 96 % ethanol extract of the *O. americanum* herb.

It is known that the content of different groups of biologically active substances in plants has a significant impact on the peculiarities of the climate and soils of the region of growth, the presence of chemotypes, peculiarities of care and preparation, selection

of extraction and analysis conditions. It should be noted that the use of HPLC analysis to determine the qualitative composition and quantitative content of triterpene saponins in plants is quite common [13, 15]; some scientists also use the method of gas chromatography after precolumn derivatization [17, 18]. Obviously, all these factors have a certain effect on the content of triterpene compounds in the raw material of *O. americanum* ra *M. fistulosa*.

7. Conclusions

1. It was identified the triterpenoids in ethanoic extracts obtained from herbs of *M. fistulosa* and *O. americanum*; the components of triterpenoids were determined by the HPLC method.

2. It has been determined that high concentrations of ethanol (96 % and 70 %) contribute to the more efficient extraction of triterpene compounds from the raw materials of the studied species than 50 % ethanol.

3. The peculiarities of the accumulation of 6 triterpene compounds (ursolic, oleanolic, euscaphic and tormentic acids, as well as betulin and lupeol) in the herb of *M. fistulosa* and *O. americanum* were established under cultivation of plants in the West Podillya (Ukraine). Ursolic and euscaphic acid are the dominant triterpene compounds in the herb of both species.

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