THE STUDY OF FATTY ACID COMPOSITION OF THE RAW MATERIAL OF *CETRARIA ISLANDICA* (L.) ACH., HARVESTED IN UKRAINE

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Introduction

The direction of phytochemical research of plant raw materials is one of the up-to-date tasks of the pharmaceutical industry. Medicinal products of plant origin do not lose their importance and prominence even despite the availability of a wide range of synthetic drugs on the pharmaceutical market [1]. The elaboration of effective and safe plant medicinal products is possible only under the condition of providing quality of medicinal plant raw materials [2]. In this regard, it is relevant to carry out complex and comprehensive research of raw materials, which also includes the study of the groups of biologically active substances (BAS) that do not belong to the main active substances.

The procurement and use of local plant raw materials, including well-known traditional plants, which have found their application in global pharmaceutical practice, are particularly expedient and economically beneficial for the development of new domestic-origin drugs [2].

The study of the component composition of fatty acids in plant raw materials often attracts the attention of scientists, considering the fact that, this group of compounds plays an important role in adapting plants to environmental stress factors and defining their value as food crops and sources of raw materials for industrial use [3-4]. Modern scientific works are devoted not only to the search for new natural sources of fatty acids [5], but also to the study of the fatty acids profile and the new potential aspects of the pharmacological activity of plant raw materials, which are already used as medicinal [6-7].

In recent years, studies of the component composition of fatty acids have been actively conducted not only for higher plants, but also for fungi, algae and lichens [8-9], including studies related to their nutritional value and use as functional food products [10]. Fatty acid profiles also have significant importance for lichen studies, because they are used for the taxonomics and the differentiation of their genera [11].

Iceland moss (*Cetraria islandica* (L.) Ach.), a member of the *Parmeliaceae* family, belongs to lichens that have been traditionally used as food in the Northern European countries, China and India, as well as in folk medicine. [12-13]. Nowadays, *C. islandica* is a component of a number of drugs and dietary supplements which are used for inflammatory processes of the respiratory organs and help to regulate their functions [14].

A lot of recent scientific works are devoted to the study of the chemical composition of *C. islandica*, namely such groups of biologically active substances as carbohydrates, compounds of phenolic nature, in particular, lichen acids, and amino acids [13, 15-16].

Pharmacological studies of *C. islandica* have demonstrated a wide range of activity, including antibacterial, antiviral, anti-inflammatory, antioxidant, antifungal, anti-ulcer, and immunomodulatory [17-19].

In the literature sources there are only fragmentary and outdated data regarding the fatty acid composition of *C. islandica* [20]. Using the method of thin-layer chromatography, dutch scientists have identified in the raw material of *C. islandica* among others such fatty acids as: palmitic, stearic, oleic, linoleic and linolenic, which are also typical for higher plants. In addition, long-chain fatty acids such as behenic and eicosadienoic were also identified [20]. In the scientific sources available to us, we did not find data on the fatty acid composition of *C. islandica* raw material of Ukrainian origin.

Therefore, the study of the fatty acid composition of thalli of *C. islandica*, harvested in Ukraine, is a relevant issue as a part of the comprehensive study of the component composition of biologically active substances of such raw material.

The aim of the work was to study the component composition and to determine the quantitative content of fatty acids in thalli of *C. islandica*, harvested in Ukraine, as a part of the systematic pharmacognostic study of domestic raw material of *C. islandica* for the elaboration of new substances and medical products on its basis.

Materials and methods

Plant raw material. For the study, thalli of *C. islandica* harvested in autumn 2019 in the Rakhiv district of the Zakarpattia region were used. The raw material was dried to an air-dried condition in the open air under a cover and stored in paper bags in a dry place.

Conditions of chromatographic separation. The determination of fatty acids in the studied raw material was carried out by the method of gas chromatography with mass spectrometric detection (GC/MS). For the further analysis, methyl esters of fatty acids were obtained [21].

The chromatographic separation was performed on an Agilent 6890N gas chromatograph with a 5973 inert mass detector (Agilent technologies, USA). A HP-5ms capillary column (30 m×0.25 mm×0.25 μm, Agilent technologies, USA) was used. The temperature of the evaporator was 250^0C, the temperature of the interface was 280^0C. The separation was carried out in the following temperature programming mode: the initial temperature of 60^0C was held for 4 min, then was raised to 250^0C at the rate of 4^0C/min, and maintained for 6 min, then was raised to 300^0C at the rate of 20^0C and maintained for 5 min. 1 μl of the sample was injected in the split mode 1:20. Detection was carried out in the SCAN mode in the range of 38–400 m/z. The carrier gas (helium) flow rate through the column was 1.0 mL/min.

Sample preparation. The raw material was crushed by a laboratory mill LGM-1 (Olis, Ukraine) and ground to a powdery state in a glass mortar. Then 500 mg of the sample was placed in a glass vial, after which 3.3 mL of the reaction mixture of methanol:toluene:sulfuric acid (44:20:2 *v/v*) per sample and 1.7 mL of the solution of the internal standard (undecanoic acid in heptane solution) were added. The studied sample was kept at 80^0C for 2 hours, refrigerated and centrifuged for 10 minutes at 5000 rpm. 0.5 mL of the upper heptane phase, which contains methyl esters of fatty acids, was taken.

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Identification and quantification. The identification of fatty acid methyl esters was based on their retention times compared to the data of the mass spectral library NIST 02.

The quantitative analysis was carried out by the method of internal standards. The undecanoic acid solution was used as an internal standard.

The quantitative content of fatty acid (X, mg/kg) was calculated according to the formula:

$$X = \frac{S_x \times M_{inst} \times 1000}{S_{inst} \times m}$$

where:
S_x – the peak area of the studied fatty acid;
M_{inst} – the mass of the internal standard per the sample;
S_{inst} – peak area of the internal standard;
m – the mass of the sample of raw material, mg [21].

Results and discussion

The chromatogram of the methyl esters of fatty acids from thalli of *C. islandica* is shown in Figure 1. The results of the determination of the component composition and the quantitative content of fatty acids in the raw material of *C. islandica*, as well as their chromatographic parameters are shown in Table 1. According to the results of the study, six fatty acids were identified in the raw material of *C. islandica*; among them 3 belonged to saturated and 3 – to unsaturated. Arachidic (eicosanoic), pentadecylic (pentadecanoic) and stearic (octadecanoic) acids were found among the saturated ones. Among the unsaturated acids, linoleic (octadecadienoic), elaidic and oleic acids were identified. The amount of oleic acid, which belongs to ω-9 monounsaturated fatty acids and performs a number of important functions in the human body, including plastic and energetic, was 1.77±0.03 mg/g. Linoleic acid, an essential polyunsaturated ω-6 fatty acid that is part of cell membranes and is necessary for the synthesis of prostaglandins, was found in the amount of 1.95±0.03 mg/g.

The fatty acid composition of the raw material of *C. islandica* suggests the presence of reparative and anti-inflammatory action of the lipophilic fraction, which can be used for the further development of medicinal substances for various applications.

The quantitative content of identified fatty acids differed insignificantly and ranged from 1.57±0.02 mg/g (arachidic acid) to 1.95±0.03 mg/g (linoleic acid). The total quantitative content of fatty acids was 11.10±0.15 mg/g. The content of the sum of unsaturated fatty acids was 5.66±0.08 mg/g and slightly exceeded the content of the sum of saturated fatty acids (5.44±0.07 mg/g).

The literature sources provide data on the identification of elaidic, oleic, linoleic, arachidic, pentadecylic and stearic acids by the method of thin-layer chromatography in the raw material of *C. islandica*, as well as in some other species of terrestrial and tree-growing lichens [20], which has also been confirmed by the results of our research. However, in scientific sources available to us we did not find the data regarding the quantitative content of the above-mentioned fatty acids in the raw material of *C. islandica*.

Therefore, the data regarding the quantitative content of saturated arachidic, pentadecylic, stearic and unsaturated linoleic, elaidic and oleic fatty acids in the raw material of *C. islandica* were presumably given for the first time.

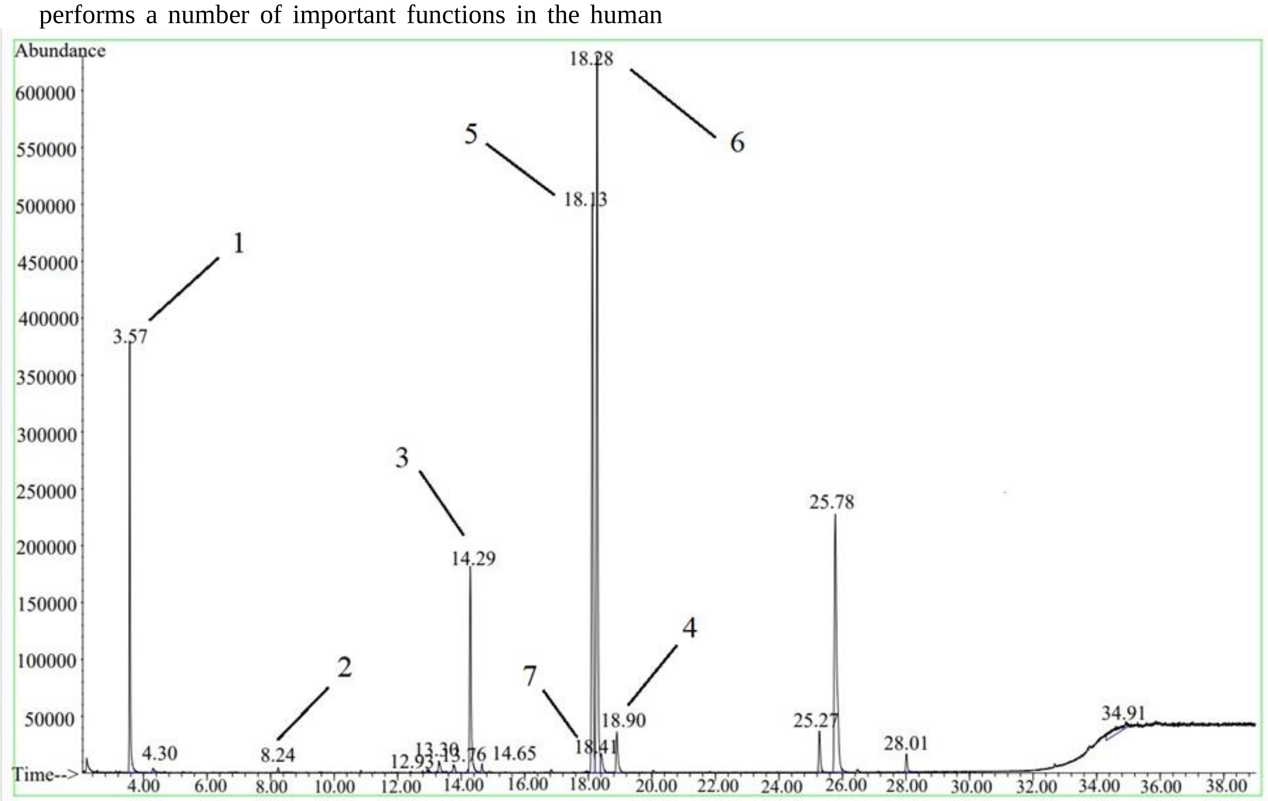

Figure. 1. GC/MS chromatogram of fatty acid methyl esters in thalli of *C. islandica*

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Table 1. The component composition and quantitative content of fatty acids in thalli of *C. islandica*

Peak number	Retention time	Peak area	Compound	Derivative	Content, mg/g
Saturated fatty acids					
1	3.5664	11.1716	Undecanoic acid	Undecanoic acid, methyl ester	Internal standard
2	8.2395	0.1626	Arachidic (eicosanoic) acid C 20:0	9-Eicosyne	1.57±0.02
3	14.2873	8.7417	Pentadecylic (pentadecanoic) acid C 15:0	Pentadecanoic acid, 14-methyl-, methyl ester	1.93±0.02
4	18.8978	2.1357	Stearic (octadecanoic) acid C 18:0	Octadecanoic acid, methyl ester	1.94±0.03
The sum of the saturated fatty acids					5.44±0.07
Unsaturated fatty acids					
5	18.1302	23.3001	Linoleic (octadecadienoic) acid C 18:2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	1.95±0.03
6	18.2775	29.0071	Elaidic acid C 18:1 (9-*trans*)	9-Octadecenoic acid, methyl ester, (E)-	1.94±0.02
7	18.4113	1.1934	Oleic acid C 18:1 (9-*cis*)	9-Octadecenoic acid (Z)-, methyl ester	1.77±0.03
The sum of the unsaturated fatty acids					5.66±0.08
Total					**11.10±0.15**

Conclusions

1. For the first time, the fatty acid composition of thalli of *C. islandica*, harvested in Ukraine, has been determined. In the raw material, six fatty acids were identified, among them 3 belonged to saturated.
2. The content of the sum of unsaturated fatty acids (5.66±0.08 mg/g) is comparable to the content of the sum of saturated fatty acids (5.44±0.07 mg/g). Among the saturated stearic acid (1.94±0.03 mg/g) prevailed. Linoleic acid (1.95±0.03 mg/g) predominated among the unsaturated ones.
3. The results obtained provide an opportunity to deepen knowledge about the component composition of biologically active substances in the raw material of *C. islandica*, harvested in Ukraine, and will be used for the elaboration of substances and medical products on its basis.

The study of fatty acid composition of the raw material of *Cetraria islandica* (l.) Ach., harvested in Ukraine

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Introduction. The study of the component composition of fatty acids in plant raw materials often attracts the attention of scientists, considering the fact that, this group of compounds plays an important role in adapting plants to environmental stress factors and defining their value as food crops and sources of raw materials for industrial use. Modern scientific works are devoted not only to the search for new natural sources of fatty acids, but also to the study of the fatty acids profile and the new potential aspects of the pharmacological activity of plant raw materials, which are already used as medicinal. In recent years, studies of the component composition of fatty acids have been actively conducted not only for higher plants, but also for fungi, algae and lichens, including studies related to their nutritional value and use as functional food products. Fatty acid profiles also have significant importance for lichen studies, because they are used for the taxonomics and the differentiation of their genera. A lot of recent scientific works are devoted to the study of the chemical composition of Iceland moss (*Cetraria islandica* (L.) Ach.), a member of the *Parmeliaceae* family, namely such groups of biologically active substances as carbohydrates, compounds of phenolic nature, in particular, lichen acids, and amino acids. Pharmacological studies of *C. islandica* have demonstrated a wide range of activity, including antibacterial, antiviral, anti-inflammatory, antioxidant, antifungal, anti-ulcer, and immunomodulatory. In the literature sources there are only fragmentary and outdated data regarding the fatty acid composition of *C. islandica*. Using the method of thin-layer chromatography, dutch scientists have identified in the raw material of *C. islandica* among others such fatty acids as: palmitic, stearic, oleic, linoleic and linolenic, which are also typical for higher plants. In addition, long-chain fatty acids such as behenic and eicosadienoic were also identified. Therefore, the study of the fatty acid composition of thalli of *C. islandica*, harvested in Ukraine, is a relevant issue as a part of the comprehensive study of the component composition of biologically active substances of such raw material. **The aim** of the work was to study the component composition and to determine the quantitative content of fatty acids in thalli of *C. islandica*, harvested in Ukraine, as a part of the systematic pharmacognostic

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study of domestic raw material of *C. islandica* for the elaboration of new substances and medical products on its basis. **Materials and methods.** For the study, thalli of *C. islandica* harvested in autumn 2019 in the Rakhiv district of the Zakarpattia region were used. The determination of the component composition of fatty acids in the studied raw material was carried out by the method of gas chromatography with mass spectrometric detection (GC/MS). The chromatographic separation was performed on an Agilent 6890N gas chromatograph with a 5973 inert mass detector (Agilent technologies, USA). A HP-5ms capillary column (30 m×0.25 mm×0.25 µm, Agilent technologies, USA) was used. For the further analysis, methyl esters of fatty acids were obtained. The identification of fatty acid methyl esters was based on their retention times compared to the data of the mass spectral library NIST 02. The quantitative analysis was carried out by the method of internal standards. The undecanoic acid solution was used as an internal standard. **Results and discussion.** In the raw material of *C. islandica*, harvested in Ukraine, six fatty acids were identified; among them 3 belonged to saturated. Among the saturated fatty acids arachidic (eicosanoic), pentadecylic (pentadecanoic) and stearic (octadecanoic) acids were found. Among the unsaturated fatty acids, linoleic (octadecadienoic), elaidic and oleic acids were identified. The quantitative content of identified fatty acids differed insignificantly and ranged from 1.57±0.02 mg/g (arachidic acid) to 1.95±0.03 mg/g (linoleic acid). The total content of the sum of fatty acids was 11.10±0.15 mg/g. The content of unsaturated fatty acids was 5.66±0.08 mg/g and slightly exceeded the content of the sum of saturated fatty acids (5.44±0.07 mg/g). The data regarding the quantitative content of saturated arachidic, pentadecylic, stearic and unsaturated linoleic, elaidic and oleic fatty acids in the raw material of *C. islandica* were presumably given for the first time. **Conclusions.** For the first time, the fatty acid composition of the raw material of *C. islandica*, harvested in Ukraine, has been determined. The content of the sum of unsaturated fatty acids (5.66±0.08 mg/g) is comparable to the content of the sum of saturated fatty acids (5.44±0.07 mg/g). Among the saturated stearic acid (1.94±0.03 mg/g) prevailed. Linoleic acid (1.95±0.03 mg/g) predominated among the unsaturated ones. The results obtained provide an opportunity to deepen knowledge about the component composition of biologically active substances in the raw material of *C. islandica*, harvested in Ukraine, and will be used for the elaboration of substances and medical products on its basis.

Keywords: *Cetraria islandica* (L.) Ach. thalli, component composition of fatty acids, GC/MS.

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