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The Study of Compounds Distilled with Water Vapor in *Cetraria islandica* (L.) Ach. Thalli Harvested in Ukraine

Abstract

The component composition and the quantitative content of compounds distilled with water vapor in the thalli of *Cetraria islandica* (L.) Ach. harvested in Ukraine were determined using the method of gas chromatography with mass spectrometric detection (GC/MS). 24 compounds distilled with water vapor were identified with the prevalence of fatty acids and their derivatives (57.86 ± 2.00 % of the total compounds), terpenoids and their derivatives (23.57 ± 0.97 % of the total compounds) and acyclic saturated hydrocarbons (10.99 ± 0.45 % of the total compounds). The highest percentage was observed for octadecadienoic (linoleic) acid (20.08 ± 0.67 % of the total compounds), hexadecanoic (palmitic) acid (19.21 ± 0.77 % of the total compounds) and 9,17-octadecadienal (18.57 ± 0.56 % of the total compounds). The presence of 4 monoterpenoids and 6 sesquiterpenoids in the raw material studied was determined for the first time.

Keywords: *Cetraria islandica*; thalli; compounds distilled with water vapor; GC/MS

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Дослідження сполук, дистильованих з водяною парою, що наявні у *Cetraria islandica* (L.) Ach. слані, заготовленої в Україні

Анотація

За допомогою методу газової хроматографії з мас-спектрометричним детектуванням (ГХ/МС) визначено компонентний склад та кількісний вміст дистильованих з водяною парою сполук, що наявні у слані *Cetraria islandica* (L.) Ach., заготовленої в Україні. Ідентифіковано 24 сполуки, дистильовані з водяною парою, серед яких переважали жирні кислоти та їхні похідні (57,86 ± 2,00 % від суми сполук), терпеноїди та їхні похідні (23,57 ± 0,97 % від суми сполук), ациклічні насичені вуглеводні (10,99 ± 0,45 % від суми сполук). Найвищий відсотковий вміст виявлено для октадекадієнової (лінолевої) кислоти (20,08 ± 0,67 % від суми сполук), гексадеканової (пальмітинової) кислоти (19,21 ± 0,77 % від суми сполук) та 9,17-октадекадієнового альдегіду (18,57 ± 0,56 % від суми сполук). Уперше для досліджуваної сировини виявлено наявність 4 монотерпеноїдів та 6 сесквітерпеноїдів.

Ключові слова: *Cetraria islandica*; слань; сполуки, дистильовані з водяною парою; ГХ/МС

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Introduction

It is known from the literature that lichen raw material has been an important component of traditional medicine, has long been used as a food product for humans and livestock, a source of spices, dyes and fragrances in the cosmetic and perfume industry in many cultures, mainly in Europe, South Asia and North America [1–3].

The widespread use of lichens for medical purposes and in different industries is due to the presence of various groups of biologically active substances (BAS) in their composition, such as polysaccharides, lichen acids, amino acids, proteins, fatty acids, vitamins, which have been the study subject of many scientific works [1, 4–6].

The group of compounds distilled with water vapor is not the main group of compounds that cause the pharmacological effect of the lichen raw material, but it is worth paying attention to it for deepening the knowledge about the qualitative composition and the quantitative content of the BAS groups of the medicinal raw material. Some representatives of this group of compounds, such as aliphatic acids and aromatic compounds, are synthesized in lichens in the same way as lichen substances – specific secondary metabolites, some of them are unique to lichens and rarely occur in higher plants or fungi [1]. According to some data, the number of secondary metabolites found in lichens exceeds 1000 and includes aliphatic and aromatic acids, depsides, depsidones, dibenzofurans [3, 7]. Their presence causes antibacterial, antifungal, antiviral, antiproliferative, antioxidant, and other types of pharmacological activity [2, 3, 7, 8].

It has been demonstrated that essential oils obtained from lichens of the genus *Evernia* (*Parmeliaceae*) and *Ramalina* (*Ramalinaceae*), which include, in particular, such substances as *β*-pinene, *α*-pinene, camphene, limonene, myrcene, *α*-phellandrene, *p*-cymene, have antimicrobial and antifungal effects [7, 9].

One of the most known representatives of the *Parmeliaceae* family, *Cetraria islandica* (L.) Ach. has been used in folk medicine in Central and Northern Europe for centuries [10]. In pharmacognostic texts and handbooks of folk medicine, there are examples of the use of this lichen as an ingredient for the dishes of traditional cuisine and food supplements in case of the gastrointestinal tract disorders and for the treatment of respiratory diseases [2, 11].

Nowadays thalli of *C. islandica* are the pharmacopoeial medicinal raw material in many countries; they are applied to manufacture drugs with the proven efficiency used in the treatment of the respiratory system inflammatory processes, contribute to the regulation of respiratory organs, have antibacterial, anti-inflammatory, antitussive, softening and antioxidant effects [8, 12, 13].

Using gas chromatography with mass spectrometric detection it was possible to detect 23 compounds in the raw material of *C. islandica*, 16 of them were identified. Their composition included fatty acids, aldehydes, phenols, and their ethers, olefins, and lichen acids [14].

Since we have found only fragmentary data on the component composition of compounds distilled with water vapor in the thalli of *C. islandica* in the literature available to us, the study of this group of BAS as a part of a comprehensive phytochemical study of the raw material of *C. islandica* harvested in Ukraine is expedient, relevant and has practical importance for the development of modern effective medicinal products.

The aim of this work was to study the component composition and determine the quantitative content of compounds distilled with water vapor in the raw material of *C. islandica* harvested in Ukraine to deepen knowledge about the qualitative composition and quantitative content of BAS in the domestic raw material and for further obtaining new substances and medicinal products based on it.

■ Materials and methods

Plant raw material

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

Equipment and conditions of chromatographic separation

The determination of the component composition of compounds distilled with water vapor in the raw material was carried out by the method of gas chromatography with mass spectrometric detection (GC/MS) [15, 16].

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 (length – 30 m, inner diameter – 0.25 mm, diameter of the sorbent grain – 0.25 µm) was used. The separation was carried out in the gradient mode. The initial temperature of 50 °C was maintained for 5 min, then increased to 220 °C with the rate of 4 °C per minute, then with the rate of 10 °C to 300 °C was maintained for 10 min. The flow rate of the carrier gas (helium) through the column was 1.0 mL min^{-1}. The temperature of the evaporator was 300 °C, the sample was injected in a split mode with the rate of 1:50, and the injection volume was 2 µL. The detection was carried out in the SCAN mode in the range of 38–400 m/z [17, 18].

Preparation of the raw material sample

The raw material previously crushed with a laboratory mill LGM-1 (Olis, Ukraine) was ground

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to a powdery state in a glass mortar. After that, 300 mL of water was added to 5.00 g (accurate weight) of the raw material and refluxed at a temperature of 100 °C for 3 h. The distilled water was extracted with heptane. The extract was evaporated to 100–200 µL in a stream of nitrogen [19].

Identification and quantification

The identification of compounds distilled with water vapor in the samples studied was performed using the NIST 02 mass spectrum library. The match percentage of the compounds detected with the compounds from the NIST 02 mass spectrum library was 80–99% [20, 21].

The quantitative content (%) of the total compounds was calculated by comparing the peak area of the components with the sum of the areas of all peaks on the chromatogram [15, 17].

■ Results and discussion

The GC/MS chromatogram obtained for compounds distilled with water vapor is shown in **Figure**. The results of the determination of the component composition and the quantitative content of compounds distilled with water vapor in the raw material of *C. islandica*, as well as their chromatographic parameters, are given in **Table**.

As a result of the study, 24 compounds distilled with water vapor were identified in the thalli of *C. islandica* harvested in Ukraine, including terpenoids and their derivatives, acyclic saturated hydrocarbons, dienes, fatty acids, esters.

Some of the compounds identified (2,2,4,4,6,6-hexamethyl-1,3,5,2,4,6-trioxatrisilinane, diisobutyl phthalate, 5-methyl-2-phenylindolizine) probably could have entered the samples studied from the outside during harvesting or transportation.

A significant part of the substances identified were terpenoids represented by 10 compounds and made up 23.57 ± 0.97% of the total quantitative content of compounds distilled with water vapor. Among them, 3 monocyclic monoterpenoids (*cis*-menthone, *trans*-menthone, menthol), bicyclic monoterpenoid *trans*-carane and 6 sesquiterpenoids (caryophyllene, *β*-cubebene, *γ*-muurolene, δ-amorphene, sesquiterpene ketone mayurone, hexahydrofarnesyl acetone) were found.

Among the compounds detected, the highest quantitative content was observed for polyunsaturated ω-6 fatty octadecadienoic (linoleic) acid – 20.08 ± 0.67% of the total compounds, saturated *n*-hexadecanoic (palmitic) acid – 19.21 ± 0.77% of the total compounds and 9,17-octadecadienal – 18.57 ± 0.56% of the total compounds.

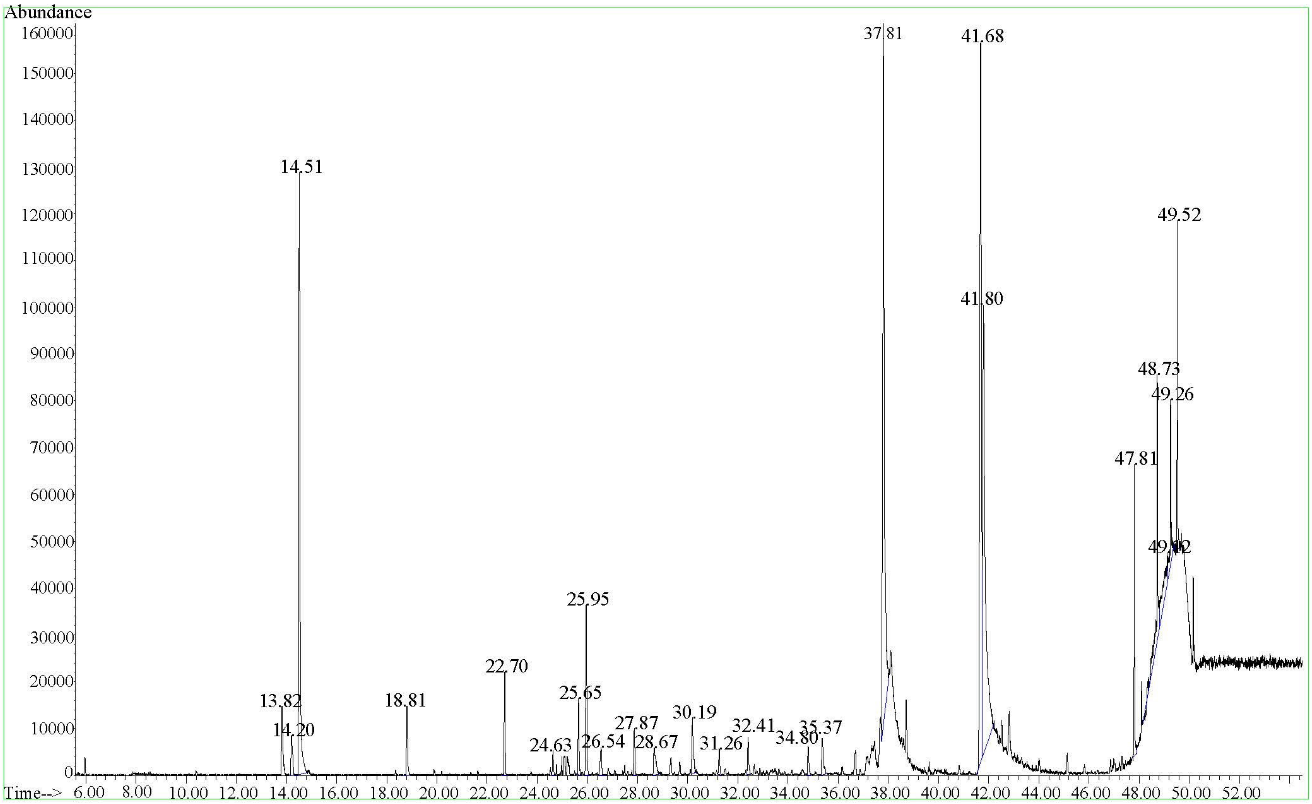


Figure. The GC/MS chromatogram of compounds distilled with water vapor in the thalli of *C. islandica* harvested in Ukraine

Table. The component composition and the quantitative content of compounds distilled with water vapor in the thalli of *C. islandica* (n = 5)

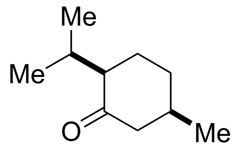
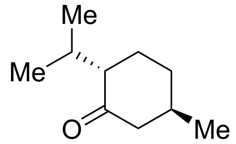
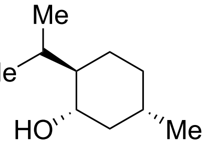
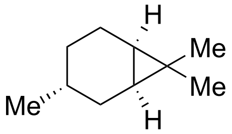
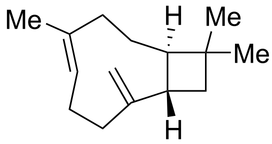
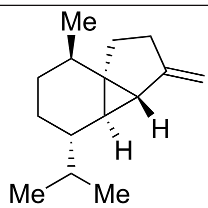
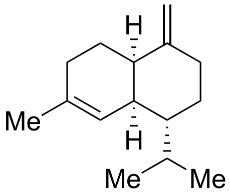
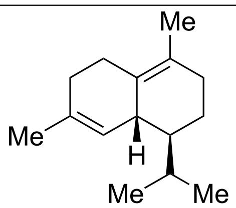
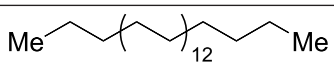
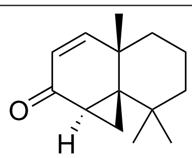
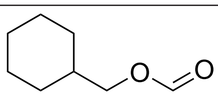
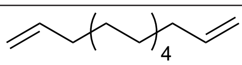
Retention time, min	The structure of the compound	The name of the compound	Content, %
1	2	3	4
13.83		*cis*-menthone	1.59 ± 0.04
14.20		*trans*-menthone	1.03 ± 0.05
14.51		menthol	11.95 ± 0.49
18.80		*trans*-carane	1.27 ± 0.06
22.70		caryophyllene	1.73 ± 0.08
24.63		*β*-cubebene	0.38 ± 0.02
25.65		*γ*-muurolene	1.27 ± 0.06
25.95		(+)-*δ*-amorphene	2.99 ± 0.12
26.55		hentriacontane	0.69 ± 0.03
27.87		mayurone	0.79 ± 0.02
28.67		cyclohexylmethyl formate	0.98 ± 0.04
30.19		1,13-tetradecadiene	1.24 ± 0.06

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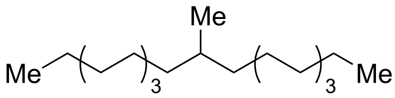
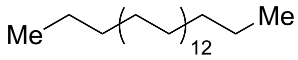
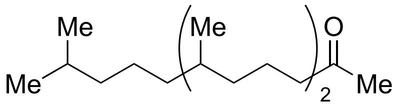
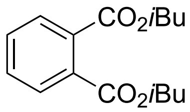
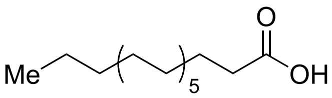
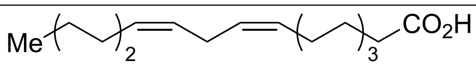
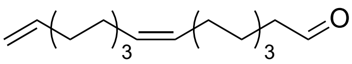
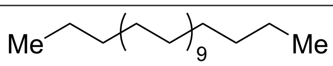
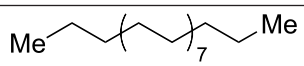
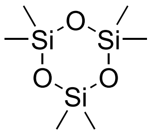
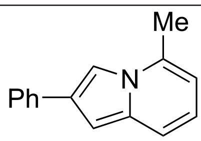
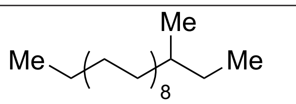
Table *(continued)*

1	2	3	4
31.26		10-methylnonadecane	0.47 ± 0.02
32.41		triacontane	0.68 ± 0.03
34.80		6,10,14-trimethylpentadecan-2-one	0.57 ± 0.03
35.37		diisobutyl phthalate	0.97 ± 0.04
37.81		palmitic acid	19.21 ± 0.77
41.68		linoleic acid	20.08 ± 0.67
41.80		9,17-octadecadienal	18.57 ± 0.56
47.81		pentacosane	2.38 ± 0.12
48.73		eicosane	3.99 ± 0.15
49.13		2,2,4,4,6,6-hexamethyl-1,3,5,2,4,6-trioxatrisilinane	2.28 ± 0.11
49.25		5-methyl-2-phenylindolizine	2.13 ± 0.8
49.52		3-methylheneicosane	2.78 ± 0.10

Six acyclic saturated hydrocarbons (10.99 ± 0.45% of the total compounds) were found in the raw material, namely hentriacontane, 10-methylnonadecane, triacontane, pentacosane, eicosane, 3-methyl-heneicosane, among them eicosane (3.99 ± 0.15% of the total compounds) dominated.

Such groups of BAS as fatty acid derivatives, aldehydes and dienes, which were detected as a result of our study, were also found in the results of the previous studies of the raw material of *C. islandica* conducted by the GC/MS method [14]. However, these researchers did not mention the presence of terpenoids and their derivatives in the raw material studied. Therefore, there is no data on the presence of *cis*-menthone, *trans*-menthone, menthol, *trans*-carane, caryophyllene, *β*-cubebene, *γ*-murolene, *δ*-amorphene, mayurone, hexahydrofarnesyl acetone; these data are provided for the first time.

According to literature data, phenols and their ethers, aldehydes, and lichen acids dominated among the compounds identified in the raw material, while according to the results of our study, fatty acids and their derivatives prevailed in percentage.

The presence of terpenoids and their derivatives, aldehydes in the raw material studied suggests that these compounds play a certain role in the anti-inflammatory, antiseptic, and expectorant pharmacological activity, which should be taken into account when developing new substances and medicinal products based on them.

Conclusions

The component composition of compounds distilled with water vapor in the raw material of *C. islandica* harvested in Ukraine has been studied by the GC/MS method. 24 compounds distilled with water vapor have been identified; among them fatty acids and their derivatives (57.86 ± 2.00% of the total compounds), terpenoids and their derivatives (23.57 ± 0.97% of the total compounds) and acyclic saturated hydrocarbons (10.99 ± 0.45% of the total compounds) predominate. For the first time, the presence of 4 monoterpenoids and 6 sesquiterpenoids has been determined in the raw material studied. The results obtained regarding the compounds distilled with water vapor in the raw material of *C. islandica* harvested in Ukraine indicate the need for further research, considering the batches of the raw material and places of harvesting, possibly in comparison with the raw material harvested outside the country.

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