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PHARMACOGNOSTIC STUDY OF CETRARIA ISLANDICA (L.) ACH. THALLI MADE IN UKRAINE

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The aim *was to conduct a pharmacognostic study of C. islandica thalli harvested in Ukraine.*
Material and methods. *The study included seven series of C. islandica thalli harvested in Ukraine. Pharmacognostic research was conducted by using generally accepted methods. Quantitative determination of total polyphenols was performed by a spectrophotometric method in terms of pyrogallol and dry raw materials following the requirements of the SPhU 2.0 monograph. The component composition of flavonoid compounds and carboxylic acids was performed by HPLC.*
Results*. The pharmacognostic study of 7 series of the C. islandica raw materials was performed. Morphometric parameters were determined, including the fractional composition according to the size of thalli in each series. For the first time, fractions of the mineral and foreign organic matter were separated. The content of mineral foreign matter ranged from 0.22±0.01 % to 2.80±0.12 %; the content of organic foreign matter ranged from 0.15±0.01 % to 2.14±0.11 %. Due to the total foreign matter content, 6 series of the raw materials fulfilled the requirements of the SPhU 2.0 monograph. The accordance of morphological and anatomical description of the series to the requirements of the SPhU 2.0 monograph is shown. New distinctive diagnostic features of the morphological structure were found, namely: coalescence of blades with forming a membrane and branching of cilia along the edge of blades. For the first time for the series of raw materials harvested in Ukraine, the quantitative content of the sum of polyphenols in terms of pyrogallol and dry raw materials was determined, which ranged from 1.21±0.05 % to 1.73±0.04 %. For the first time for C. islandica thalli, the presence of flavonoid compounds: quercetin, luteolin, kaempferol and rutin, and carboxylic acids: sinapic, trans-cinnamic and quinic acids was determined.*
Conclusions*. The obtained results can be used as a basis for the relevant sections of the national part of the monograph "Cetraria Iceland" in SPhU 2.0*
Keywords*. Cetraria islandica, morphological structure, anatomical structure, total polyphenols, flavonoids, carboxylic acids*

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1. Introduction

Iceland moss, *Cetraria islandica* (L.) Ach., belongs to the lichens of the genus *Cetraria* of the family *Parmeliaceae*. The genus Cetraria, which takes one of the central positions in the family, was initially described by Acharius in 1803 and included 8 lichen species [1]. The morphological features of *C. islandica* thalli were commonly described in taxonomy. The first descriptions of the species and their belonging to the genus *Cetraria* were based on complex morphological features. Afterwards, the genus's taxonomic composition has repeatedly changed [2]. The number of species was gradually increasing, but modern molecular methods made it possible to determine the origin of the species and connections between the genera within the family more accurately [2–3].

According to the current databases, which are constantly updating information on the status of the species, today, the genus *Cetraria* includes 21 lichen species. [4–6]. Representatives of the genus *Cetraria* growing in Ukraine belong to 5 species: *C. islandica*, mainly spread in the western regions of Ukraine in the Carpathian forests; *C. sepincola* and *C. ericetorum*, primarily distributed in the forests of the central, rarer – in the west of areas, including the Carpathians [7, 8]; as well as *C. aculeata* and *C. steppae*, which are typical for the steppe regions of the East and South of Ukraine [9, 10]. *C. steppae* is considered vulnerable and belongs to the Red Book of Ukraine [11].

As of January 2022, the pharmaceutical market of Ukraine includes 5 medicinal products based on the raw materials of *C. islandica* such as Herbion Iceland moss syrup (KRKA, Slovenia), Isla-Moos and Isla-Mint pastilles (Engelhard Arzneimittel GmbH& Co. KG, Germany), Pectolvan phyto Iceland moss liquid extract (JSC Farmak, Ukraine) and Complex expectorant extract (Phytopharm Kleka SA, Poland), which are used for coughs and colds treatment and have antitussive, expectorant, anti-inflammatory, immunostimulatory and antibacterial effects [12]. In addition, *C. islandica* is a component of the dietary supplements that help to regulate respiratory function: Bronchalik lozenges (JSC Farmak, Ukraine), Herbal tea "Iceland moss" (LLC "Health Keys", Ukraine), Fiorda lozenges and Fiorda spray (PJSC "Liktravy", Ukraine),

Iceland moss and thyme syrup (LLC Research and Production Pharmaceutical Company "AIM"), as well as the dietary supplements which are used for the inflammatory processes of the respiratory system treatment: Islalor lozenges (Stella Nutrition, Poland), Bazooka Icelandic moss elixir (Delta Medical Promotions AG, Ukraine), Icelandic moss syrup (Ananta Medicare Ltd, India), Icelandic moss phytosyrup (LLC RPL Fitoproduct, Ukraine), Islafilipt tablets (LLC "Zdravopharm", Ukraine) [13].

Because using domestic origin plant raw materials in pharmacy is more rational and cost-effective, *C. islandica* has excellent potential for procurement in Ukraine and potential use as a domestic medicinal plant raw material. In this aspect, the study of the morphological and anatomical structure of domestic procurement of *C. islandica* is relevant because it allows choosing criteria for identifying raw materials based on the specific features of the external and internal structures and establishing differences between species within the genus [14].

Polymorphism is common for *C. islandica*, which means that morphological features of the thallus may vary depending on the influence of the habitat conditions. Icelandic scientists (Xu, 2018) described chemotypes in the *C. islandica* species complex from different regions of the country, which differed not only in chemical composition but also in morphological structure [15]. Significant intraspecific morphological differences in thalli size and blade width and the similarity in blade size and shape between *C. islandica* and *C. ericetorum* species were demonstrated.

Due to the symbiotic relationship between mycobiont and photobiont, lichens form specific secondary metabolites with biological activity. About 1000 secondary metabolites of lichens are known, a significant number of which are phenolic compounds [16]. In particular, depsides – atranorin and evernic acid, depsidones – physodic, norstictic and protocetraric acids, a dibenzofuran derivative – usnic acid, have antibacterial, anti-inflammatory, antioxidant, antiviral, antifungal, antiulcer and other types of pharmacological activity [16, 17]. In addition, there are also data on the thyreotropic properties of the *C. islandica* aqueous extract [18].

The presence of the compounds belonging to depsidones, xanthones, and anthraquinones in *C. islandica* thalli have been proved (Voicu, 2019) [19]. Among the phenolic compounds of *C. islandica*, lichen acids are the most thoroughly studied, as they are substances promising for use in pharmacy [20]. For instance, usnic acid sodium salt has been used as an antimicrobial agent for purulent wounds, burns and cracks for a considerable time [21]. High antioxidant and antimicrobial activity of acetone and ethanol extracts and pure usnic acid obtained from the *C. islandica* raw materials of the Romanian procurement has been demonstrated [22]. During the past decade, antiparasitic activity research has shown that protolichesterinic lichesterinic, protocetraric and fumaroprotocetraric acids isolated from *C. islandica* have an antiparasitic activity against *Trypanosoma brucei*, the causative agent of trypanosomiasis, also known as sleeping sickness [23].

Quantitative determination of the sum of polyphenolic compounds and flavonoids in *C. islandica* thalli was frequently combined with the study of antioxidant, antimicrobial, genotoxic and antitumor activity, particularly with these indicators in other lichen species [24–27]. The quantitative content of total polyphenols and total flavonoids was determined in aqueous, methanol, acetone and ethanol (Fito, 2020) extracts [24–27], but individual phenolic compounds were not identified in these studies.

In the study of the qualitative composition of water-soluble phenolic compounds carried out by the thin layer chromatography method, aromatic phenolic acids: 4-hydroxybenzoic and vanillic acids were found in *C. islandica* [28]. An attempt was made to determine the qualitative composition of some phenolic compounds in *C. islandica* aqueous and ethanolic extracts by the HPLC method. Unfortunately, it was not possible to identify all substances. Still, the authors point out the proximity of the spectrum of 4-hydroxybenzoic acid to the obtained results and suggest the presence of protocetraric acid [29].

Monographs on *C. islandica* are included in several pharmacopoeias of different countries [30–34]. There is also the USSR State Standard 13727-68 "Thalli of the Iceland moss lichen" [35]. Ukrainian scientists (Vladymyrova et al., 2013) conducted complex scientific research on the *C. islandica* thalli to develop the SPhU 2.0 monograph [36–37]. The monograph "Cetraria islandica" is a part of the State Pharmacopoeia of Ukraine 2.0, but it does not contain a national part [30]. The documents mentioned above regulate the quality of *C. islandica* raw materials for use as a medicinal product based on the external structure, foreign matter and lead content, loss on drying, total ash and swelling index [30–34].

In Table 1, the comparative characteristics of macro- and microscopic parameters of *C. islandica* thalli, which are included in the monographs of Pharmacopoeia of Ukraine [30], European Pharmacopoeia [31], British Pharmacopoeia [32], Pharmacopoeia of the Republic of Belarus [33] and Pharmacopoeia of the Republic of Kazakhstan [34], are given.

According to the data in Table 1, most descriptions of the features of the morphological and anatomical structure of *C. islandica* thalli are typical for the pharmacopoeial monographs of different countries. Monographs of the pharmacopoeias of Ukraine, the European Union, Great Britain and the Republic of Kazakhstan regulate raw materials on 13 macroscopic and 7 microscopic features. The monograph of the Pharmacopoeia of the Republic of Belarus includes 12 macroscopic and 3 microscopic features. The difference is that the monograph of the Pharmacopoeia of the Republic of Belarus describes the smell of the raw materials and the colour of the base of the thallus, which is absent in the monographs of the other pharmacopoeias and does not describe the appearance and location of the apothecia, algae cell size and spermogonia. Considering certain differences in the description of the raw materials in the pharmacopoeias, attention should be paid to the presence in the SPhU 2.0

monograph such diagnostic features as the colour of the base of the thallus, the size of algae cells and spermogonia, as well as separating fractions of the foreign matter into mineral and organic.

Table 1

Comparative characteristics of the quality indicators of *Cetraria islandica* (L.) Ach. thalli, listed in pharmacopoeias of different countries

Parameter	State Pharmacopoeia of Ukraine 2.0 [30]	European Pharmacopoeia 6.0 [31]	British Pharmacopoeia [32]	Pharmacopoeia of the Republic of Belarus [33]	Pharmacopoeia of the Republic of Kazakhstan [34]
Macroscopic parameters					
Raw material	Whole or cut, dried thalli of *Cetraria islandica* (L.) Acharius s.l	Whole or cut, dried thalli of *Cetraria islandica* (L.) Acharius s. l.	Whole or cut, dried thalli of *Cetraria islandica* (L.) Acharius s. l.	Whole or ground dried thalli of perennial foliose-fruticose lichen *Cetraria islandica* (L.) Acharius s. l.	Whole or cut, dried thalli of *Cetraria islandica* (L.) Acharius s. l.
Thallus size					
Length	About 15 cm	Up to 15 cm	Up to 15 cm	Up to 15 cm	Up to 15 cm
Blades width	0.3–1.5 cm	0.3–1.5 cm	0.3–1.5 cm	0.3–1.5 cm	0.3–1.5 cm
Blades thickness	0.5 mm	0.5 mm	0.5 mm	0.5 mm	0.5 mm
Branching	Irregularly dichotomous	Irregularly dichotomous	Irregularly dichotomous	Highly branched	Unevenly dichotomous
Thallus surface	Glabrous, groove-shaped or nearly flat, stiff, brittle	Smooth, groove-shaped or nearly flat, stiff, brittle	Smooth, groove-shaped or nearly flat, stiff, brittle	Glabrous	Smooth, groove-shaped
Thallus colour					
Upper surface	Greenish or greenish-brown	Greenish or greenish-brown	Greenish or greenish-brown	From greenish to greenish-brown	Greenish or greenish-brown
Lower surface	Greyish-white or light brownish	Greyish-white or light brownish	Greyish-white or light brownish	From greyish-white to pale brownish	Greyish-white or light brownish
The base of the thallus	–	–	–	Reddish-brown	–
Smell	–	–	–	Weak, peculiar	–
Cilia	On the edges of the blades	On the edges of the blades	On the edges of the blades	On the edges of the blades, short, dark brown	On the edges of the blades
Pseudocyphellae	Whitish, depressed spots	Whitish, depressed spots	Whitish, depressed spots	White, different sizes and shapes	Whitish, depressed spots
Apothecia					
Location	On the apices of the terminal lobes, very rarely	On the apices of the terminal lobes, very rarely	On the apices of the terminal lobes, very rarely	–	On the apices of branched lobes, very rarely
Colour	Brown	Brown	Brown	–	Brown
Shape	Discoid	Discoid	Discoid	–	Discoid
Microscopic parameters					
Fragments of the pseudoparenchyma					
Hyphae from the marginal layer	Narrow-lumened, thick-walled	Narrow-lumened, thick-walled	Narrow-lumened, thick-walled	Yellowish, tightly intertwined	Narrow-lumened, thick-walled
Hyphae from the adjacent layer	Wide-lumened, loosely entwined	Wide-lumened, loosely entwined	Wide-lumened, loosely entwined	–	Wide-lumened, loosely entwined
Algae cells					
Location	In the medullary zone	In the medullary zone	In the medullary zone	In the gonidial layer	In the medullary zone
Colour	Greenish or brownish	Greenish or brownish	Greenish or brownish	Green	Greenish or brownish
Size	Up to 15 μm in diameter	Up to 15 μm in diameter	Up to 15 μm in diameter	–	Up to 15 μm in diameter
Spermogonia					
Shape	Tube-like or cylindrical	Tube-like or cylindrical	Tube-like or cylindrical	–	Tube-like or cylindrical
Size	Up to about 160 μm wide and up to about 400 μm long.	Up to about 160 μm wide and up to about 400 μm long.	Up to about 160 μm wide and up to about 400 μm long.	–	Up to about 160 μm wide and up to about 400 μm long.

Note: "–" – no data given

The literature sources show the results of the study of the morphological and anatomical structure of *C. islandica*. Still, the place of the collection of the raw materials and the number of studied series are not indicated [38].

There is also data on the study of the chemical composition of *C. islandica* thalli harvested in the Ivano-Frankivsk region, in which ascorbic and usnic acids, polysaccharides and tannins were identified and quantified [39].

Therefore, in the sources available to us, no data on the morphological and anatomical structure of thalli and the quantitative content of the total polyphenols in the raw materials harvested in Ukraine. Also, there was no information on the component composition of flavonoid compounds and carboxylic acids in *C. islandica* thalli.

Given the above, from the perspective of the systematic research of *C. islandica* domestic raw materials, it is relevant and sensible to conduct a pharmacognostic study of *C. islandica* thalli, harvested in Ukraine, as well as to determine the component composition of flavonoid compounds and carboxylic acids in the raw materials.

The aim of the work was to conduct a pharmacognostic study of *C. islandica* thalli harvested in Ukraine.

2. Planning (methodology) of research

The stages of pharmacognostic study of the series of *C. islandica* thalli are shown in Fig. 1.

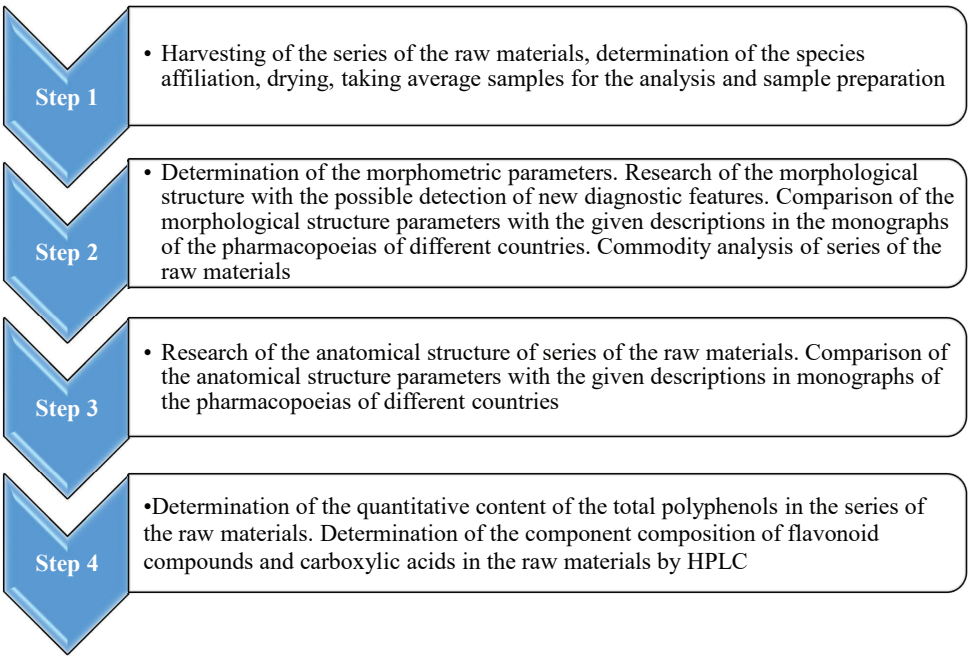


Fig. 1. Stages of the pharmacognostic study of *C. islandica* thalli harvested in Ukraine

3. Material and methods

3. 1. Plant material

The study included 7 series of *C. islandica* thalli. Raw materials were harvested during August - September 2019 in pine and mixed forests in the western regions of Ukraine: series 1 – Volyn region, Kamin–Kashyrskyi district; series 2 – Zakarpattia region, Rakhiv district; series 3 – Zakarpattia region, Uzhhorod district; series 4 – Zakarpattia region, Tyachiv district; series 5 – Ivano–Frankivsk region, Nadvirna district, series 6 – Chernivtsi region, Storozhynets district; series 7 – Rivne region, Sarny district.

Lichen thalli were collected from the soil substrate, dried to the air-dried condition in the open air undercover and stored in paper bags with labels indicating the place of collection, growing conditions and date of the collection. Species identification of the collected material was conducted by the head of the Department of Pharmacognosy of the National University of Pharmacy, PhD of Pharmacy, associate professor Mala O.S.

3. 2. Morphometric, morphological and anatomical research

A morphometric study was performed using generally accepted methods [31].

Macroscopic features were determined with the naked eye and a magnifying glass with a 2× and 10× magnification. Instruments such as a ruler and a calliper were used to measure the size of thalli.

Both air-dry and fixed in a mixture of glycerol-ethanol-water (1:1:1) raw materials were used to make sections from the surface and cross sections of lichen thalli [40]. Microscopic features were determined with a microscope Delta Optical BioLight 300 (Poland) with a 40×, 100× and 400× magnification.

The camcorder DLT-Cam Basic 2.0 mp and DLTCamViewer 2015 computer program with the following photo processing with Adobe Photoshop CS4 11.0.1 were used for recording the results.

3. 3. Determination of the quantitative content of total polyphenols

Quantitative determination of total polyphenols in the series of raw materials was conducted by the spectrophotometry method in terms of pyrogallol according to the requirements of SPhU 2.0 monograph 2.8.14 “Determination of tannins in medicinal raw materials” [41].

3. 4. Determination of the component composition of flavonoid compounds

Determination of the component composition of flavonoid compounds was performed by HPLC in series 2 of the raw materials. A portion of 0.3–0.6 g of the raw

material of each sample was extracted into 5 ml of 70 % ethyl alcohol solution in an ultrasonic bath at 80 °C for 5 hours in sealed glass vials with teflon lids. The obtained extract was centrifuged at 3000 rpm and filtered through disposable membrane filters with 0.22 μm pores.

Liquid chromatography was performed on an Agilent Technologies 1200 liquid chromatograph (USA). Acetonitrile (A) and 0.1 % formic acid solution in water (B) were used as the mobile phase. Elution was performed in a gradient mode: 0 min – A (30 %): B (70 %); 20 min – A (70 %): B (30 %); 22 min – A (100 %): B (0 %); 30 min – A (100 %): B (0 %). Separation was performed on a Zorbax SB-C18 chromatographic column (3.5 μm, 150×4.6 mm) (Agilent Technologies, USA), column flow rate 0.25 ml/min, thermostat temperature of 30 °C, volume injection of 4 μl. Detection was performed with a diode-matrix detector with signal registration at 280 and 365 nm and fixation of absorption spectra in the range of 210–700 nm [42].

Identification and quantitative analysis were performed with the standard samples solutions of flavonoid compounds (rutin, quercetin-3-b-glycoside, naringin, neohesperidin, quercetin, naringenin, kaempferol, luteolin). The quantitative content of flavonoid compounds (X) (μg/g) was determined with the formula:

$$X=c*V/m, \quad (1)$$

where c is the concentration of the compound, determined chromatographically, μg/ml; V is the volume of the extract, ml; m is the mass of raw materials from which the extraction was performed, g [42].

3. 5. Determination of the component composition of carboxylic acids

Determination of the component composition of carboxylic acids was performed by HPLC in series 2 of the raw materials. First, a portion of 0.4–06 g of the raw material of each sample was extracted in 5 ml of 60 % methanol solution in an ultrasonic bath at 80 °C for 4 hours in sealed glass vials with Teflon lids. The resulting extract was centrifuged at 3,000 rpm and filtered through disposable membrane filters with 0.22 μm pores.

Liquid chromatography was performed on an Agilent Technologies 1200 liquid chromatograph (USA). Methanol (A) and a 0.1 % formic acid solution in water (B) were used as the mobile phase. Elution was performed in a gradient mode: 0 min – A (25 %): B (75 %); 25 min – A (75 %): B (25 %); 27 min – A (100 %): B (0 %); 35 min – A (100 %): B (0 %). Separation was performed on a Zorbax SB-Aq chromatographic column (4.6 mm±150 mm, 3.5 μm) (Agilent Technologies, USA), column flow rate 0.5 ml/min, thermostat temperature 30 °C, volume injection capacity 4 μl. Detection was performed with a diode-matrix detector with signal registration at 250 and 275 nm and fixation of absorption spectra in the range 210–700 nm [43].

Identification and quantitative analysis were performed with the solutions of standard samples of calboxylic acids (gallic acid, hydroxyphenylacetic acid, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, *trans*-ferulic acid, sinapic acid, *trans*-cinnamic acid, quinic acid). The quantitative content of carboxylic acids (X) (μg/g) was determined by the formula:

$$X=c*V/m, \quad (2)$$

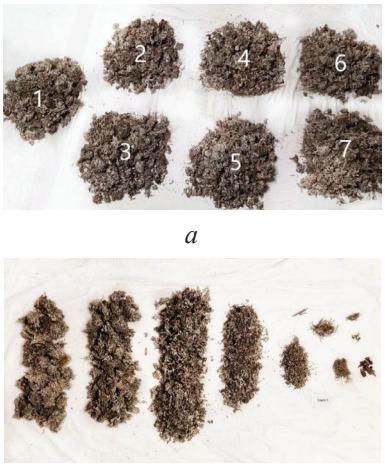
where c is the concentration of the compound, determined chromatographically, μg/ml;

V is the volume of extract, ml; m is the mass of raw material from which the extraction was performed, g.

4. Research results and discussion

4. 1. Determination of the morphometric parameters

The appearance of 7 series of the raw materials is shown in Fig. 2, *a*. The weight of the series ranged from 525 g (series 2) to 533 g (series 4). In the composition of each series, fractions of the raw materials according to the size of thalli were separated, as well as the content of mineral and organic foreign matter. For example, the fractional composition of a series of raw materials in the example of series 1 is shown in Fig. 2, *b*.



a

b

Fig. 2. The appearance of *C. islandica* thalli*: a* – the series of the raw material; *b* – fractional composition of series 1

The raw material was represented primarily by rectangular or square thalli of various sizes. The whole thalli 6–10 cm, 4–6 cm and 3–4 cm long and individual blades or their fragments 1–2 cm long and less than 1 cm long could be distinguished. The ratio of fractions in the series of raw materials is uneven (Fig. 3).

Thereby, the content of the 3–4 cm long thalli predominated in the series 1 (36.1 %); 3 (30.5 %); 5 (33.0 %); and 7 (27.8 %). The largest number of thalli 6–10 cm long was observed in series 2 (36.3 %) and 6 (30.5 %). Series 4 was dominated by thalli 4–6 cm long (28.5 %).

The SPhU 2.0 monograph regulates the foreign matter content in *C. islandica* at a maximum of 5 % [30]. For the first time, fractions of the mineral and foreign organic matter were separated. Mineral foreign matter included dust, sand, and small stones. Their content ranged from 0.22±0.01 % (series 2) to 2.80±0.12 % (series 5) (Fig. 4).

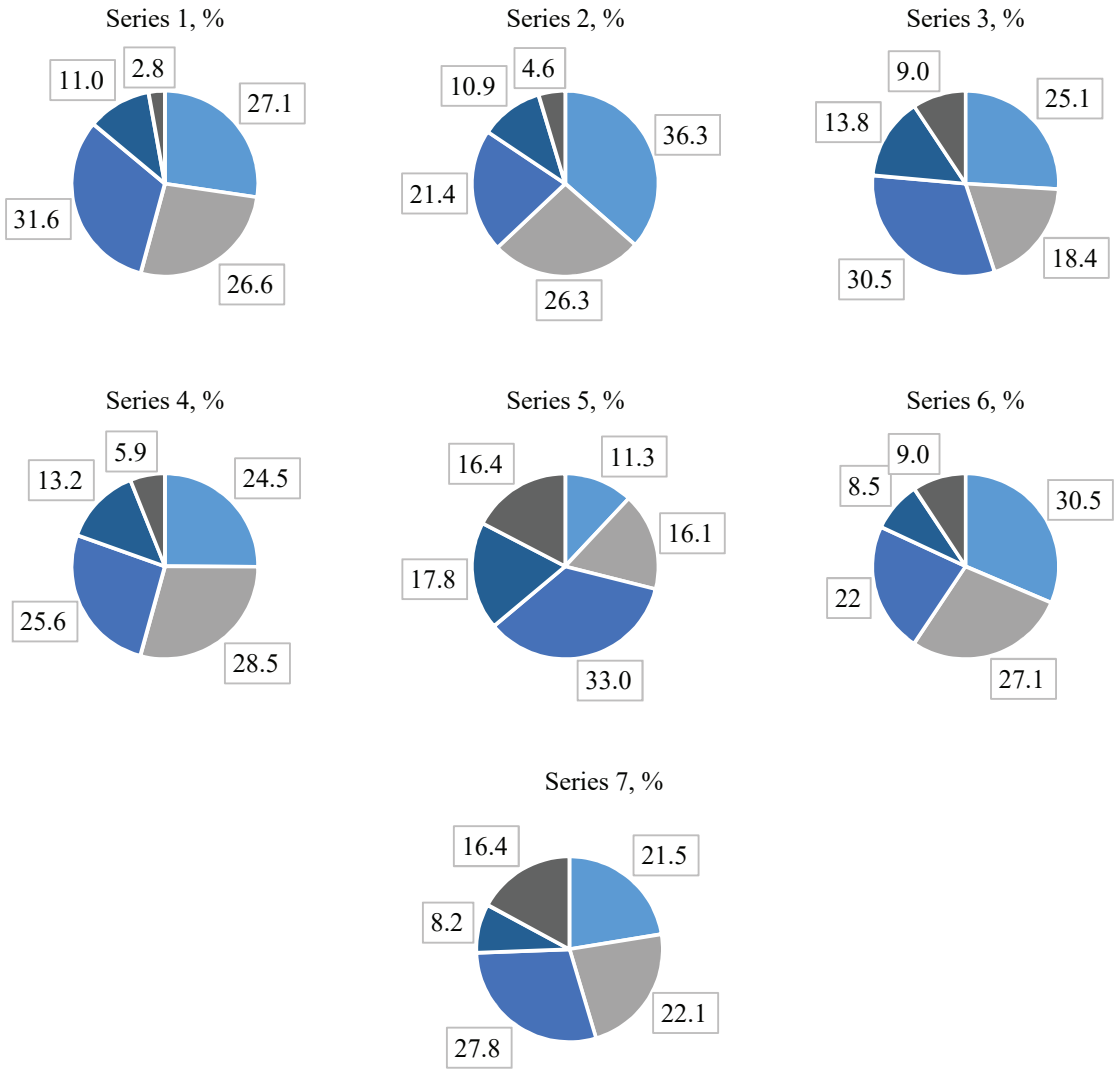


Fig. 3. Distribution of fractions in the series of the raw materials by thallus size: ■ – 6–10 cm; ■ – 4–6 cm; ■ – 3–4 cm; ■ – 1–2 cm; ■ – less than 1 cm

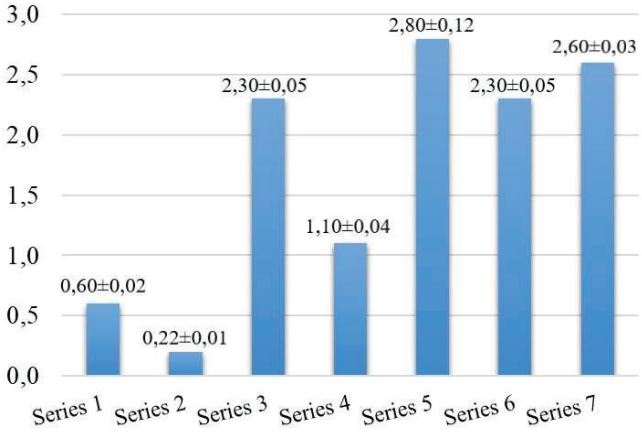


Fig. 4. The content of the foreign mineral matter in the series of the *C. islandica* raw materials (*m*=3)

The foreign organic matter was represented by parts of other plant species: sprigs and leaves of trees, conifer trees needles, fragments of mosses and lycopodium. The component composition and amount of foreign organic matter in each series are given in Table 2.

Table 2

The content of the foreign organic matter in the series of *C. islandica* raw materials (*m*=3)

Series No.	Quantitative content of the foreign organic matter, %				Total content of the foreign organic matter, %
	Leaves	Sprigs	Conifer tree needles	Other plant species	
1	0.03±0.01	0.01±0.00	0.20±0.02	0.01±0.00	0.25±0.02
2	0.01±0.00	0.20±0.02	0.02±0.00	0.05±0.01	0.27±0.02
3	0.11±0.01	0.04±0.00	0.30±0.02	0.06±0.01	0.52±0.02
4	0.02±0.00	–	0.81±0.04	0.01±0.00	0.83±0.04
5	0.80±0.04	0.20±0.01	1.12±0.06	0.02±0.00	2.14±0.11
6	0.02±0.00	0.01±0.00	0.10±0.01	0.02±0.00	0.15±0.01
7	–	0.08±0.01	1.40±0.03	–	1.48±0.04

Note: "–" – no component found

The foreign organic matter content in the *C. islandica* raw materials researched series ranged from 0.15±0.01 % (series 6) to 2.14±0.11 % (series 5). The total content of the foreign matter exceeded the regulated 5 % only in series 5. All other series of raw materials fulfilled this indicator's requirements of the SPhU 2.0 monograph (Table 2).

4. 2. Determination of the morphological features

The features of the morphological structure are shown in Fig. 5. The raw materials are foliose thalli with numerous blades, well-branched. Still, dichotomous branching is not clearly visible, which is more noticeable on flattened edges (Fig. 5, *a*). The blades are flat, thin, narrow at the base, extended at the top, strongly concave and folded at the edges, sometimes almost forming a tube intertwined. A distinctive feature not mentioned in the SPhU 2.0 monograph is that in some places, the blades coalesce, including the membranes, which provide a tight shape and vertical position of thallus in space (Fig. 5, *b*).

The length of the thalli typically did not exceed 10 (12) cm (Fig. 5, *c*), which does not coincide with the size of 15 cm described in the SPhU 2.0 monograph (Table 1). The width of the blades in the studied series was 0.2–4 cm (Fig. 5, *d*), in contrast to 0.3–1.5 cm, specified in the SPhU 2.0 monograph (Table 1). The thickness of the blades was approximately 0.5 mm, which corresponds to the data stated in the SPhU 2.0 monograph (Table 1). Dried thalli are brittle and have a rigid and cartilaginous structure. The surface of the blades is wrinkled and folded, with tiny grooves and recesses.

The upper surface of the thalli is darker, from greenish-grey to fulvous-brown. The lower surface is lighter, from greyish-olive to brown. Sometimes there are rhizines, with which thalli are attached to the ground. Eventually, the attachment organs die back, resulting in the base of the thalli becoming reddish (Fig. 5, *e*), and retaining on the substrate occurs through connections with other species of plants that form turf.

Pseudocyphellae are found along the entire lower surface of the thalli. There are structures not covered with a cortical layer which promote the exchange of water and air between the lichen and the environment. Pseudocyphellae are clearly delineated, slightly depressed, and whitish, with various shapes and sizes, mostly observed on the tops of the blades (Fig. 5, *f*). Sometimes fulfiseudocyphellae are covered with granular outgrowths – isidia (with the cortical layer) or soredia (without the cortical layer).

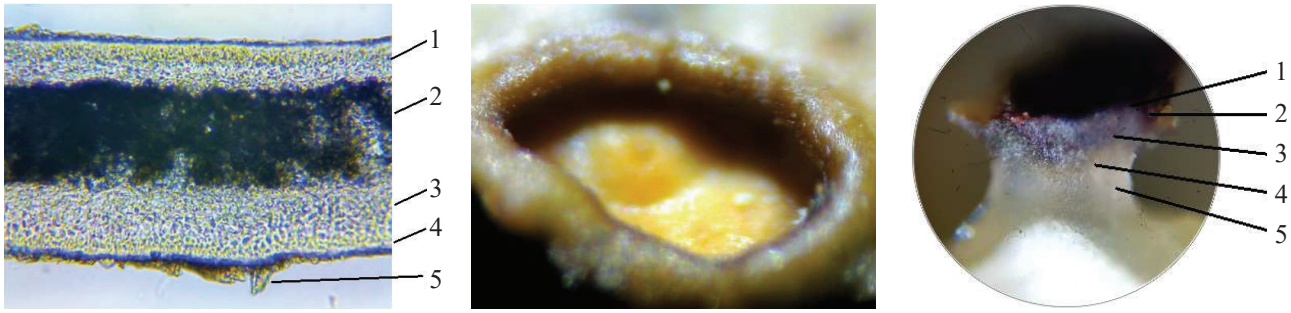
The blades' edges are rigid, straight, bristly cilia 0.1–1 mm long. Most cilia are simple, but there are also branched. (Fig. 5, *g*). Such a distinctive diagnostic feature as the branching of the cilia is mentioned for the first time and was not previously indicated in the descriptions of the raw materials. Furthermore, the colour of the cilia usually coincides with the colour of the blades: from grey-olive to dark brown.

On the upper surface of the blades, there are occasional dish-shaped apothecia on short stipe up to 10 mm in diameter (Fig. 5, *h*). Disks are dark brown, convex, and wrinkled, with a solid or intermittent edge.

Fig. 5. Features of the morphological structure of *C. islandica* thallus: *a* – the appearance of the blades of one thallus in a flattened form; *b* – a place of a coalescence of blades with forming a membrane; *c* – appearance and size of separate thalli; *d* – appearance and size of different blades; *e* – the base of the thallus; *f* – pseudocyphellae on the lower surface of the thallus; *g* – simple and branched cilia on the edge of the blades; *h* – apothecia on the upper surface of the blade

4. 3. Determination of the anatomical features

Features of the anatomical structure of *C. islandica* thallus are shown in Fig. 6. The thallus type is heteromeric; the gonidial layer, in which algae cells are concentrated, and the cortical layer can be distinguished. Differentiated structures located from top to bottom can be determined on the cross sections of the thallus (Fig. 6, *a*). The upper cortical layer is a prosoplectenchyma, an interweaving of the mycobiont's elongated (anisodiametric) hyphae (Fig. 6, *a*, 1). Under the cortical layer, there is the gonidial layer, divided into separate fragments of groups of algae cells surrounded by branches of fungal hyphae (Fig. 6, *a*, 2). The core layer below consists of the intertwined hyphae of the mycobiont and forms a loose plectenchyma (Fig. 6, *a*, 3). Finally, the lower cortical layer consists of tightly intertwined fungal hyphae (Fig. 6, *a*, 4). Sometimes, there may be rhizines – multicellular outgrowths of the lower cortical layer, which penetrate the substrate and perform the function of attaching the thallus (Fig. 6, *a*, 5).



a *b* *c*

Fig. 6. Features of the anatomical structure of the *C. islandica* thallus:
a – cross-section of the heteromeric thallus: *a*: 1 – upper cortical layer; 2 – gonidial layer; 3 – cortex; 4 – the lower cortical layer; 5 – rhizines; *b* – thallus edge of apothecia (top view); *c* – in-cross-section apothecia: 1 – epithecium; 2 – hymenium; 3 – hypothecium; 4 – algal layer; 5 – core layer

Apothecia is lecanorine and have a well-developed thallus edge (Fig. 6, *b*), in which a layer of algae cells and the core can be identified. The surface of the apothecia is covered with a thin layer – of epithecium, which performs a protective function (Fig. 6, *c*, 1). The hymenium (thecium) is located below. It is the generative part of the apothecia, which contains asci with spores (Fig. 6, *c*, 2). Below is the sterile part of the apothecia - hypothecium (Fig. 6, *c*, 3). Under the hypothecium and on the periphery of the disc of apothecia, is an intermittent layer of algae cells (Fig. 6, *c*, 4); below it, the core layer formed by hyphae of the mycobiont is located (Fig. 6, *c*, 5).

4. 4. Determination of the quantitative content of total polyphenols

The quantitative content of total polyphenols in the series of *C. islandica* raw materials is shown in a diagram form in Fig. 7. The highest content of total polyphenols – 1.73±0.04 % is observed in series 3 of raw materials, the lowest – 1.21±0.05 % – in series 4 (Fig. 7).

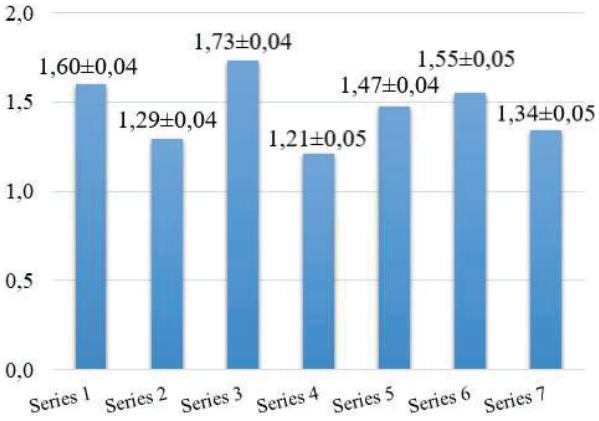


Fig. 7. Quantitative content of total polyphenols in the series of *C. islandica* raw materials in terms of pyrogallol and dry raw materials (*m*=5)

4. 5. Determination of the component composition of flavonoid compounds

The results of the determination of the component composition of flavonoid compounds by the HPLC method are given in Table 3 and Fig. 8.

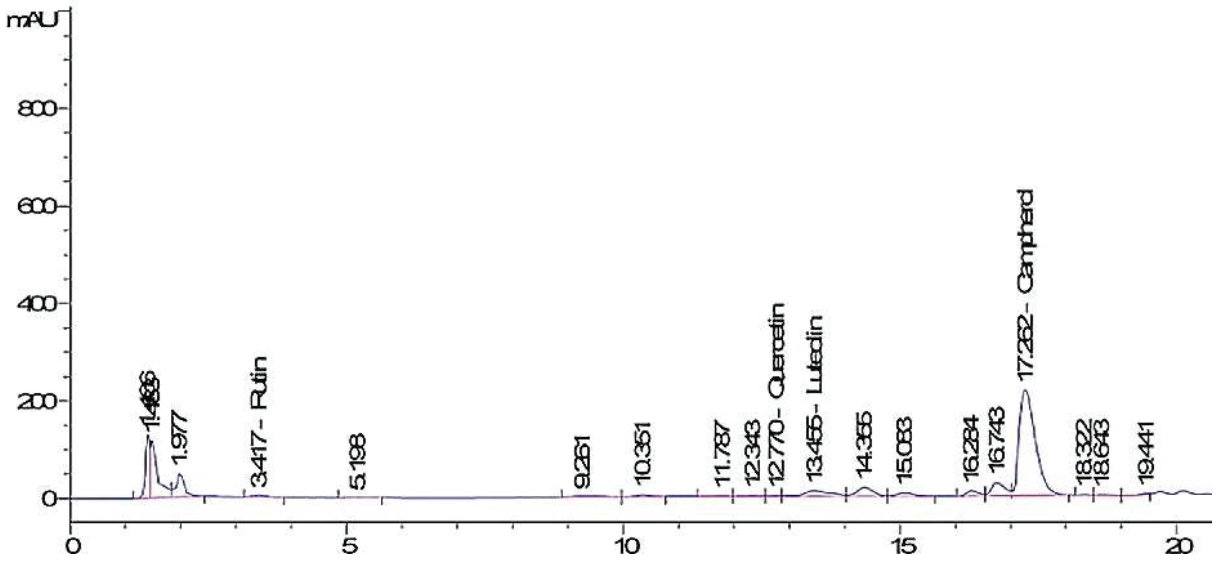


Fig. 8. HPLC chromatogram of flavonoid compounds in *C. islandica* raw materials

Table 3

Component composition of flavonoid compounds in *C. islandica* raw materials (*m*=3)

Substance	Content, μg/g of raw material
Rutin	62.19±2.13
Isoquercetin	–
Naringin	–
Neohesperidin	–
Quercetin	37.43±1.36
Luteolin	288.30±3.64
Naringenin	–
Kaempferol	943.81±9.51

Note: "–" – pol no component found

4 flavonoid compounds were found in the raw material. For the first time, flavonol aglycones quercetin, luteolin, kaempferol and quercetin derivative rutin was found in *C. islandica* thalli (Fig. 8).

4. 6. Determination of the component composition of carboxylic acids

The results of determining the component composition of carboxylic acids by the HPLC method are given in Table 4 and Fig. 9.

3 carboxylic acids were found in the raw materials. For the first time in the *C. islandica* raw materials, the presence of hydroxycinnamic sinapic acid, benzylidene acetic or *trans*-cinnamic acid and unsaturated polyhydroxycarboxylic quinic acid was found (Fig. 9).

Study limitations. The limitation may include the lack of other series of raw materials from other regions of Ukraine and different procurement periods due to the specific distribution of the studied species. It is also preferably to diversify the study of the raw materials by the HPLC method and expand the range of studied groups of natural compounds by using other sets of standard samples and chromatographic conditions.

It is pertinently to add systematicity to the study by determining the component composition of lichen acids and polysaccharides of the raw materials series.

Prospects for further research. The obtained results can be used as part of a systematic study of the series of *C. islandica* raw materials harvested in Ukraine, which can allow the developing of evidence-based approaches to integrated processing of the raw materials and can be used as a basis for relevant sections of the national part of the monograph "Cetraria islandica" in SPhU 2.0.

Table 4

Component composition of carboxylic acids in *C. islandica* raw materials (*m*=3)

Substance	Content, μg/g of raw material
Gallic acid	–
Hydroxyphenylacetic acid	–
Chlorogenic acid	–
Caffeic acid	–
Syringic acid	–
p-Coumaric acid	–
trans-Ferulic acid	–
Sinapic acid	3.37±0.08
trans-Cinnamic acid	2.71±0.06
Quinic acid	6.94±0.29

Note: «–» – no component found

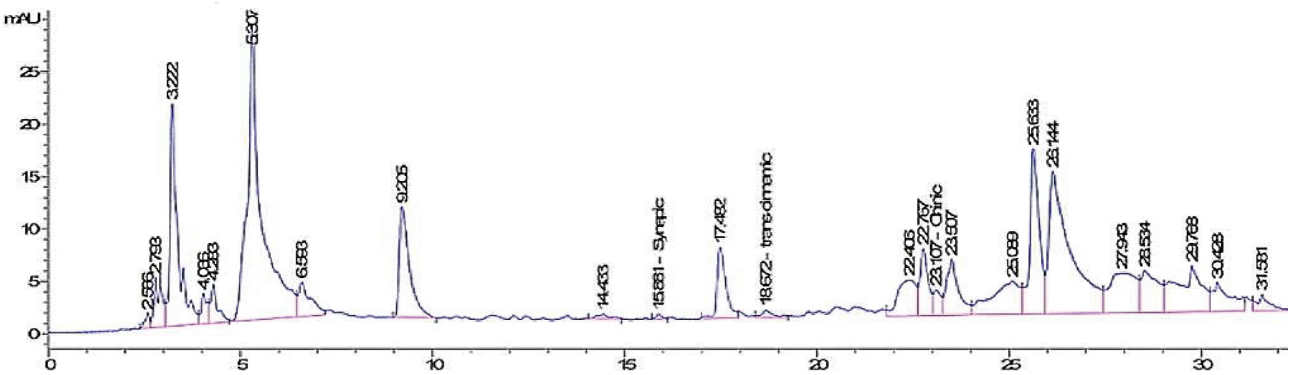


Fig. 9. HPLC chromatogram of carboxylic acids in *C. islandica* raw materials

5. Conclusions

1. The pharmacognostic study of 7 series of the *C. islandica* raw materials was performed for the first time. Morphometric parameters of 7 series of the raw materials harvested in Ukraine were determined, including the fractional composition according to the size of thalli in each series.

2. For the first time, fractions of the mineral and foreign organic matter were separated. The content of mineral foreign matter ranged from 0.22±0.01 % to 2.80±0.12 %; the content of organic foreign matter ranged from 0.15±0.01 % to 2.14±0.11 %. The total content of foreign matter in only series 5 exceeded 5 %, specified in the requirements of the SPhU 2.0 monograph. The other 6 series of raw materials fulfilled the requirements for this indicator.

3. The accordance of morphological and anatomical description of the series of *C. islandica* thalli harvested in Ukraine to the requirements of the sections "Identification A" and " Identification B" of the SPhU 2.0 monograph "Cetraria islandica" is shown. For the first time, new distinctive diagnostic features of the morphological structure were found: coalescence of blades with forming a membrane and branching of cilia along the edge of blades.

4. For the first time for the series of raw materials harvested in Ukraine, the quantitative content of the total polyphenols by the spectrophotometric method in terms of pyrogallol and dry raw materials following the requirements of the SPhU 2.0 monograph was determined. It ranged from 1.21±0.05 % to 1.73±0.04 %.

5. The component composition of flavonoid compounds and carboxylic acids was determined by HPLC method. As a result, for the first time for *C. islandica* thalli the presence of flavonoid compounds: quercetin, luteolin, kaempferol and rutin, and carboxylic acids: hydroxycinnamic sinapic acid, benzylidene acetic or *trans*-cinnamic acid and unsaturated polyhydroxycarboxylic quinic acid was determined.

6. The obtained results can be used as a basis for the relevant sections of the national part of the monograph "Cetraria islandica" in SPhU 2.0.

Conflict of interest

The authors declare that they have no conflict of interest in this research, whether financial, personal, authorship or otherwise, that could affect the study and its results presented in this paper.

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