Possibilities of Using Biologically Active Substances of Iceland Moss

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Abstract. Despite the emergence of new biotechnological methods of processing plant raw materials, modern medicine and pharmaceuticals are far from fully using phytotherapeutic drugs in treatment, due to the shortage of some types of medicinal plant raw materials. Mainly, a separate study of the constituent components is carried out, which makes it difficult to predict the pharmacological effect of the drug. Iceland moss is widespread in Russia and is a source of valuable substances. The article shows numerous studies of the beneficial properties of plant materials. The analysis of drugs and dietary supplements registered in the Russian Federation based on Iceland moss was carried out. The active substances in plant raw materials were determined, the conditions for the production of infusions and decoctions were proposed.

1. Introduction
Increasing life expectancy, maintaining human health is an important state problem, the solution of which is influenced by a healthy lifestyle, nutrition, the use of high-quality medicines and biologically active food additives, perfumery products that involve the use of biologically active substances. Plants are a constant source of biologically active substances.

Phytobiotechnology is successfully developing as the most important area of science and production and serves as the foundation for scientific and technological progress and economic growth of the agro-industrial complex and pharmaceuticals, with the obligatory effective interaction with traditional areas of science and economics and the necessary material and technical support from the state.

Biologically active substances have a complex chemical structure, synthesizing them is an expensive process, which leads to the need to extract substances from natural plant materials. In case of a shortage of plant raw materials, technologies for obtaining biologically active substances from the culture of plant cells and tissues are used. The production of useful and valuable secondary metabolites (a heterogeneous group of natural compounds – alkaloids, terpenes, phenols, and others) from cell cultures is an attractive alternative to traditional methods of plant growing [1]. In the case of the availability of raw materials and a wide area of distribution of plants, it is possible to use traditional methods for extracting active substances from a plant, while a scientifically grounded approach to the algorithm for obtaining the drug is required.

One of the medicinal plants that have a multifunctional effect on the human body is the Iceland moss (Cetraria islandica (L.)), a lichen from the Parmeliaceae family, which has a leafy-bushy thallus and fruit bodies (apothecia) that develop on the tops of the ribbon-like lobes in the form of dark brown saucer-shaped formations. Lichen is very widespread, so it can be classified as an almost cosmopolitan
species. In Russia, the reserves of Iceland moss are significant. However, it should be borne in mind that lichens regenerate rather slowly [2].

The chemical composition of the lichen thallus is varied: lichen acids make up 3–5% – these are cetraric, protocetrara, fumaroprotocetraric, paralyhesteric, usnic acids; polysaccharides 30–70%, the main part of which is lichenin (up to 64%), and isolichenin up to 10%. It also contains the bitter substance cetrarin, ascorbic and folic acids. Lichen is rich in mineral salts [2, 3]. Found mannitol, arabitol, trehalose, hemicellulose, umbilicin, thiamine, cyanocobalamin, ergosterol [4].

It is known that the polysaccharides of the Iceland moss are easily absorbed by the body, therefore it is used as a food product. For example, in Iceland, Iceland moss has long been added to bread, soup, and brewed instead of tea [5]. In addition, it is recommended as a nutritious decoction for patients with diabetes mellitus, tuberculosis and in recovery [2].

Iceland moss is used as a bitterness to stimulate appetite, an enveloping agent in inflammatory diseases of the gastrointestinal tract, and also, most often, in respiratory diseases, including in the complex treatment of tuberculosis [2]. It has been shown that lichen acids have antiviral, antitumor, antibiotic, and anti-inflammatory effects [4, 5].

The main antibiotic activity of lichens is due to the presence of usnic acid [2.6–diacetyl–7,9–dihydroxy–8.9–dimethyl–1.3(2H.9bH)–dibenzofurandione], which has become the most studied lichen metabolite of industrial importance. Usnic acid is found in many lichens, more often in the following genera: Alectoria, Cladonia, Usnea, Lecanora, Ramalina, Evernia. Various options for the extraction of usnic acid are used [6]. Usnic acid is added to creams, toothpastes, mouthwashes, deodorants and sunscreens, either as an active ingredient or as a preservative. In addition to antimicrobial activity against human and plant pathogens, usnic acid has antiviral, antiprotozoal activity. Insecticidal properties of usnic acid have also been identified [7]. Researchers are showing great interest in the ability of usnic acid to inhibit oxidative phosphorylation in bacterial cells and in antibacterial activity against Bacillus subtilis and Mycobacterium tuberculosis [8, 9].

In favor of the use of Iceland moss as a preservative in the food industry, research by domestic scientists who studied the ability of lichen broth to increase the shelf life of meat products says - the process of development of non-pathogenic microflora slows down during storage of prototypes of cooked sausages for five days, the number of bacteria was 8% less compared with control samples [10]. In addition, a high antioxidant activity of the broth was shown, which, moreover, has a high viscosity, which can be used as a thickener for food products [11]. The antioxidant activity of not only the decoction, but also water, hydroalcoholic and methanol extracts of Iceland moss has been proven [4].

Iceland moss is widely used as a herbal medicine in medical practice. Scopes of raw materials from lichens are biologically active additives (BAA), medicines, cosmetics and hygiene products. It has been shown that lichen acids have antiviral, antitumor, antibiotic, and anti-inflammatory effects [4]. When studying the effect of an aqueous extract of Iceland moss on antigen-induced arthritis in rats, it was found that experimental animals that were injected with an aqueous extract subcutaneously developed arthritis less often than the group that received saline, which confirms the anti-inflammatory effect of Iceland moss [12], extract from cetraria is part of the antimicrobial ointment [13]. The antioxidant activity of Iceland moss in diabetes is being actively studied [14, 15].

There are separate studies on the effects of Iceland moss on thyroid function. It was found that 30% tincture of Iceland moss thalass has thyrostatic activity [16].

In order to increase the antimicrobial activity against Staphylococcus epidermidis, a water-soluble complex of usnic acid and polyacrylamide has been proposed [17]. Usninat sodium is used externally in the treatment of trophic ulcers, infected wounds, and burns. Scientists are particularly interested in the use of Iceland moss in bronchopulmonary pathology. Lozenges for the symptomatic treatment of inflammation of the oral cavity or pharynx and associated dry cough and hoarseness are used in Germany and Slovenia; chewing gum is used in Germany. Syrups containing extracts from Iceland moss are allowed in Latvia and Slovenia. A concentrate of several herbs, including Iceland moss, for oral solution has been registered in Poland. In Austria, Lichen Islandicus is also a part of combined herbal teas, which are made directly in pharmacies and are sold only at the manufacturer’s pharmacy. Film-coated tablets,
capsules and herbal teas containing several herbal substances are available on the Belgian market; in Great Britain – a herbal medicine – capsules – containing 19 plant substances, including Iceland moss [18].

Cell cultivation has become the basis for obtaining many natural substances. Biotechnological cultivation has also been attempted with regard to Iceland moss. A method for in vitro cultivation of lichens was developed [19].

Today, research and development of multicomponent herbal preparations, which include Iceland moss, are relevant for the prevention and treatment of immunodeficiency states, given the fact that they act only on altered links of the immune system. Such formulations are capable of weakening the suppressive effect of some cytostatics, for example, azathioprine [20]. In addition, it has been proven that Iceland moss is an interferon inducer that helps to restore the protective barriers of the upper respiratory tract [21]. And the mucous substance contained in the moss thallus eliminates irritation, envelops the inflamed mucous membranes of the oral cavity, larynx, stomach, intestines. Therefore, it is promising to study it during the rehabilitation period after the transferred viral infections affecting the bronchopulmonary system. The use of infusions and extracts prepared from plant raw materials under these conditions preserves the entire complex of biologically active components.

The aim of the study was to study the content of usnic acid in the pharmaceutical raw materials of Iceland moss and the development of an algorithm for preparing a decoction in order to maximize the extraction of usnic acid for use in acute respiratory viral infections.

The following tasks were set:
1. To study the availability of raw materials and medicines of Iceland moss on the Russian pharmaceutical market. Analyze medicines and dietary supplements registered in the Russian Federation based on Iceland moss;
2. To study the content of usnic acid in the pharmaceutical raw materials of Iceland moss;
3. Develop and substantiate an algorithm for preparing a decoction.

2. Materials and methods

The assortment of biologically active additives (BAA) to food was studied according to the data of the search server according to the registers of Rospotrebnadzor and the Sanitary and Epidemiological Service of Russia.

Pharmacy raw materials are used for the preparation of infusions, decoctions. We studied the raw material – thallus of Iceland moss, contained in the dietary supplement of the manufacturer “Heritage of Nature” (Kamelia-LT LLC, Russia).

Organoleptic properties of raw materials: it is a dry lichen raw material, grayish in color, without visible impurities. Further, we studied the influence of technological factors (grinding of raw materials, time and temperature of infusion) on the quality of extracts, on the amount of release of usnic acid [22].

Thalls were crushed to a particle size of 7 mm, 3 mm and 1 mm and filled in at a ratio of 1:10 with purified water. Aqueous extracts were prepared by infusion at 90 °C and 100 °C. The quantitative determination of usnic acid was carried out after: 30 min, 1 hour and 2 hours, and 24 hours.

1 ml of the extract was taken and added to ethyl alcohol to a solution concentration of 75%. The amount of usnic acid was determined by measuring the optical density on a spectrophotometer at a wavelength of 290 nm in a cuvette with a layer thickness of 1 mm. 75% ethanol was used as a reference solution. The amount of usnic acid was found according to the calibration graph [23].

3. Results

On the Russian market, food products are widely represented, including BAA, which include extract or raw materials of Iceland moss.

According to the search engine in the registers of Rospotrebnadzor and the Sanitary and Epidemiological Service of Russia, certificates of state registration for raw materials of Iceland moss were obtained in 2011 by Ekos LLC (Pskov region), Apex LLC (St. Petersburg) and HORST Company LLC” (Altai territory); in 2014, Shalphey LLC (Irkutsk) and Denis Pharm Distribution LLC (St.
Petersburg); in 2017 Kamelia-LT LLC (Moscow region); in 2019 Lekra-SET LLC (Altai territory). The manufacturer and the recipient of the certificate are the same in all cases, except for the dietary supplement “Herbal tea Ilsaden”. These products are manufactured by the Lithuanian company JSC Svencioniu vaistazoles, and the certificate was obtained by Denis Pharm Distribution LLC.

These organizations have registered raw materials as BAA and produce it in crushed, powder, granular and briquetted form in packs or filter bags. Recommendations for the use of most manufacturers are reduced to the manufacture of an extract obtained by infusion of raw materials with hot water. The ratio of the extractant and raw materials is close to 1:50, the infusion time is from 15 to 30 minutes. It should be noted that there is a significant scatter of single and daily doses from different manufacturers: single doses from 100 to 200 ml of extract, daily doses from 200 to 800 ml. The duration of use is indicated by the manufacturers equal to 1 month. In accordance with the information on the product labels, contraindications are individual intolerance to the components, pregnancy, breastfeeding and children under 14 years of age.

The need for self-preparation of the extract at home is a negative factor for many consumers. Such buyers can purchase Icelandic moss in syrup, tablet, lozenge or capsule form. Iceland moss syrups are produced by Mirrolla LLC and RosProd LLC (St. Petersburg), pastilles – by Unic Pharmaceutical Laboratories LLC (Moscow), tablets and capsules – by Ekos LLC (Pskov region). These forms are also registered as dietary supplements. State registration certificates were issued in the period 2011–2019.

Among the combined herbal preparations containing Iceland moss, the most famous are the dietary supplements “Vzvar Tsetrazin” (LLC Zeleynaya Factory, Tomsk) and tablets Cetrazin (LLC Artlife, Tomsk). The drugs are marketed as antibacterial and antiviral agents. In them, Icelandic moss is used in conjunction with eucalyptus, St. John’s wort, propolis.

In the extracts we prepared, the content of usnic acid was determined by spectrophotometry [23], the results are shown in table 1.

| Degree of fineness | Temperature | Concentration of usnic acid, mg/ml |
|--------------------|-------------|-----------------------------------|
|                    | 0.5 h       | 1 h                               | 2 h              | 24 h             |
| 1 mm               | 90°C        | 29.57 ± 0.96                      | 38.84 ± 0.45     | 40.06 ± 0.62     | 17.15 ± 0.41     |
| 3 mm               | 90°C        | 27.01 ± 0.86                      | 28.16 ± 0.61     | 29.24 ± 0.68     | 20.31 ± 1.09     |
| 7 mm               | 90°C        | 22.27 ± 0.98                      | 23.87 ± 1.20     | 23.50 ± 0.94     | 15.08 ± 0.55     |
| 1 mm               | 100°C       | 39.42 ± 0.52                      | 46.39 ± 0.04     | 38.59 ± 0.77     | 15.83 ± 0.98     |
| 3 mm               | 100°C       | 29.77 ± 0.15                      | 37.57 ± 0.44     | 25.54 ± 0.96     | 12.51 ± 0.97     |
| 7 mm               | 100°C       | 26.16 ± 0.81                      | 26.94 ± 0.83     | 19.22 ± 0.56     | 10.00 ± 0.57     |

It was shown that infusion for more than 1 hour led to a decrease in the yield of the active substance from the thallus of Icelandic moss. An increase in the degree of fineness to 1 mm increases the yield of usnic acid at a temperature of 100 °C. With a grind of 3 and 7 mm and a temperature of 90 °C, the concentration of usnic acid increases insignificantly.

Thus, it was shown that the time and temperature of infusion significantly affect the transition of usnic acid into water, the grinding of raw materials has an effect at a temperature of 100 °C.

In the raw material, the amount of extractives and polysaccharides was determined. The amount of extractive substances was 28.74±0.012%. When determining polysaccharides, an analytical sample of raw materials was crushed to the size of particles passing through a sieve with holes of 1 mm, while the content of polysaccharides in thalli of Icelandic moss was 2.95±0.075%. We also noted that at 100 °C the yield of polysaccharides in extracts increased, but their amount was higher in larger raw materials.
4. Discussion
It was previously found that usnic acid is poorly soluble in water [24]. Therefore, for a more complete extraction of usnic acid in order to ensure its antimicrobial action, it is optimal to prepare a decoction from Icelandic moss, and not an infusion, as indicated on the packages. To increase the content of usnic acid, it is recommended to grind the raw material to 1 mm, followed by pouring water in a ratio of 1:10 and boiling for an hour. Our data do not contradict the results of other studies [11, 25]. It is also possible for the patient to purchase raw materials in filter bags with the subsequent implementation of the instructions of the instruction.

Boiling plant materials for a specified time does not destroy the polysaccharide components. To obtain the entire complex of biologically active substances from Iceland moss, it is necessary to carry out extraction at 100 °C using raw materials with an average particle size. This algorithm for preparing a decoction is relevant because the packaging of raw materials often lacks correct data on the method of its preparation.

The possibility of using other dosage forms of Icelandic moss has its limitations. For example, Russian manufacturers often introduce sucrose as a sweetener into syrups with phytoperparations, which limits their use by patients with diabetes mellitus and obesity [26].

5. Conclusion
Thus, it was shown that the time and temperature of infusion, the degree of grinding of raw materials significantly affect the transition of usnic acid into water. Therefore, the optimal time for preparing the broth is boiling for 1 hour, which will allow you to obtain the maximum concentration of usnic acid in the solution and preserve the effect of polysaccharides.

Iceland moss is widely used as a means of phytotherapy, research on the beneficial properties of the plant continues, a study of technological parameters has been carried out to increase the extraction of usnic acid and polysaccharides, and the conditions for making infusion and decoctions are shown.

The studied medicinal plant raw material – thallus of Iceland moss – is a promising raw material for the production of phytoperparations, as well as a source of biologically active substances for use in other sectors of the national economy.

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