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Articles

Antioxidant and antimicrobial activity of extracts of Cetraria islandica (L.) Ach. and Cladonia arbuscula (Wallr.) Flot.

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Lichens are symbiotic nature and produce unique secondary extracellular metabolites with high biological activity. In this paper, we compared the antioxidant and antimicrobial activity of two lichen species. Furthermore, we determined the quantitative content of antioxidants of low molecular weight and antimicrobial activity of 40% of the water ethyl extracts of *Cetraria islandica* and *Cladonia arbuscula* lichens depending on the layers' treatment methods and the ratio of dilution of the dry extract with water. Live parts of dried lichens collected in an ecologically clean area of Yakutia were used for extraction. The antioxidant activity of the water-ethanol extract of lichens was performed using a spectrophotometric method. Antimicrobial activity was established using the discodiffusion method in agar in various dilutions of dry lichen extract. The highest antioxidants of low molecular weight were found in *Cetraria islandica* in distilled water with solid to solvent ratio of 1:1 and in the *Cladonia arbuscula* extract – in a ratio of 1:5. At the same time, preliminary mechanochemical processing of lichen layer raw materials from lichen layers (particle size up to 1 mm) was shown to increase the yield of low-molecular antioxidants by up to 50% compared to coarse grinding (particle size 2 mm). The antibacterial activity of the studied lichens was tested in *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* by diluting the dry extract with water 1:1, 1:2, 1:3, 1:4, 1:5. The most significant antibacterial effect was found in the extract of the studied lichens in a 1:1 ratio. The diameter of the bacteriostatic zone in *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli* under the action of *Cetraria islandica* was 12±0.14 mm, 11±0.12 mm, and 12±0.13 mm, respectively. The suppression of the growth zone with *Cladonia arbuscula* against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* was 15±0.18 mm, 12±0.20 mm, and 13±0.20 mm in diameter, respectively. The results were in accordance with the action of oxacillin but were more effective than the action of penicillin, which served as a control.

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Keywords

Lichens, *Cetraria islandica*, *Cladonia arbuscula*, low molecular weight antioxidants

Introduction

Due to the growing resistance of pathogenic and opportunistic microorganisms to existing synthetic antibiotics, new drugs and the search for antibiotics of plant origin are currently required. Lichens are considered a promising source of raw materials for antibacterial properties (Srivastava et al. 2013; Tapalsky et al. 2017). Recently, the pharmacological properties of lichens, including their antiviral, antibiotic, and antitumor potential, have been actively studied (Fernandez-Moriano et al. 2016; Xu et al. 2016).

Lichens are an ecosystem consisting of mycobiota (fungi of the class ascomycetes and basidiomycetes) and phycobiont (green and yellow-green algae) (Xu et al. 2016). Due to a special symbiotic lifestyle, lichens have a pronounced biological activity, studied *in vitro* and *in vivo* (Boustie et al. 2011). The layers contain primary and secondary metabolites. Substances located right in the cell and intercellular space and involved in cellular metabolism are called primary metabolites (carbohydrates and enzymes). Secondary metabolites are the end products of metabolism. The high resistance of lichens to extreme physical influences is associated with the synthesis of numerous secondary metabolites in the layers of these plants.

Among lichen metabolites, lichen acids are considered the most studied. Among the phenolic metabolites of lichens, usnic acid is widely distributed. On the basis of their sodium salt, antibiotics have been developed that are used to treat wounds of various origins (Cocchietto et al. 2002). Tapalsky et al. (2017) claimed that minimum suppressive concentrations of lichen extracts can range from 100 to 1000 mcg ml^{-1}, which is superior to broad-spectrum antibiotics. Antimicrobial and antioxidant activity is also demonstrated by lichen, lichensteric, physodonic, and diffractaic acids (Kosanić & Ranković 2011; Fernández-Moriano et al. 2017).

The study of the antioxidant potential of lichen metabolites is a promising strategy to prevent or treating various diseases associated with oxidative stress. However, such mechanisms and signaling pathways associated with lichen antioxidant activity are still understudied (Gulluce et al. 2006; Xu et al. 2016; Fernández-Moriano et al. 2017).

The aim of this article is to explore the antioxidant and antimicrobial activity of the water-ethyl extracts of *Cetraria islandica* and *Cladonia arbuscula* lichens, which grow in Yakutia. To achieve the research goal, several tasks were set. First, it was necessary to determine the content of low molecular antioxidants in the studied lichens depending on the pretreatment methods of the raw materials. Second, we had to establish the content of low-molecular antioxidants depending on the concentration of dilution. Third, it was critical to evaluate the antimicrobial activity of extracts of two types of lichens (*Cetraria islandica* and *Cladonia arbuscula*) depending on the concentration of dilution.

Materials and Methods

The research object was the bushy lichen *Cetraria islandica* and *Cladonia arbuscula,* collected in the Republic of Sakha (Yakutia) in August 2017. The aboveground phytomass of plants was collected in 2017 in a 20 m^2 plot in a pine lichen forest and lingonberry near Yakutsk, Central Yakutia (61°55‘22.30” N, 129°32’5.79”E). The lichens were determined using standard procedures (Rykova 1996). The collected lichens were cleaned, washed, dried, and stored in paper envelopes. For the study, living parts of the plant layers were used.

Preparation of lichen extracts and isolation of components

To prepare the extracts, we took 5 g of the dried and crushed aboveground part of the raw materials and added 100 ml of 40% aqueous ethyl solution as extractant. The grinding of dried raw materials was carried out in two ways: coarse grinding (particle size 2 mm) and mechanochemical processing in a *TSEM-7-80* flow mill (particle size up to 1 mm). The extraction was carried out at room temperature for three days with periodic stirring. The extracts were filtered through paper and evaporated using a rotary evaporator Eyelaca-111 2 cl. Dry extracts of lichens weighing 1.0±0.01 mg were dissolved in distilled water. To obtain extract solutions with different concentrations – 1, 0.5, 0.33, 0.25, and 0.2 mg ml^{-1}, samples were prepared with a distilled water dilution of 1 mg per 5 ml (1:5), 1 mg per 4 ml (1:4), 1 mg per 3 ml(1:3), 1 mg per 2 ml (1:2) and 1 mg per 1 ml (1:1) distilled water, respectively.

Antioxidant activity

To evaluate the antioxidant properties of the extracts, we determined the content of low molecular weight antioxidants [LMWA] by their oxidation of ferric chloride (III) to ferric chloride (II). The amount of iron chloride (II) was determined by the intensity of the color of the resulting reaction product with ortho-phenanthroline on a PE-5400 UV spectrophotometer (λ=405nm) (Rogozhin 2006).

Antimicrobial activity

To evaluate the antimicrobial activity of the extracts obtained, we used the disco-diffusion method. Next, we prepared a suspension of test microorganisms at a concentration of 1.5 * 108 CFU / ml: Gram-positive bacteria *Staphylococcus aureus* Rosenbach, 1884*, Streptococcus pyogenes* Rosenbach, 1884,and Gram-negative *Escherichia coli* Migula, 1895,1 ml of suspension was added to a Petri dish with Muller-Hinton agar medium. Filter paper sterile discs were impregnated with 0.01 ml of the test extract. The dried discs with extracts at a distance of 2 cm from each other were spread out for sowing and incubated at 360 ° C for 18–24 hours. The results were recorded by measuring the bacterial growth suppression zones around the discs (including the diameter of the disc itself). With a growth suppression zone of up to 10 mm, bacterial resistance to the extract was observed, 11-15 mm - intermediate to the extract, 15-25 mm – as sensitive to the extract (“Federal Center for State Sanitary…”, 2004).

The results are presented as an arithmetic mean and a reliable confidence interval ($p<0.05$) according to the data from five experiments. We use Statistica v.11.0 software for Student’s t-test calculation (Glantz, 1998).

Results

For the effective extraction of biologically active substances from plants, the mechanical processing of plants plays an essential role. An increase in the extraction rate and the maximum yield of soluble substances can be achieved by increasing the contact area of the surface of substances, taking into account diffusion processes. Therefore, to increase the release of low-molecular substances from the lichen layers, we used the mechanochemical processing of raw materials. The content of LWMA in 1 g of dry extract obtained from the raw materials of the lichen layers after coarse grinding was 56 mcg eq g^{-1} in *Cetraria islandica* and 0.82 mcg eq g^{-1} in *Cladonia arbuscula*, compared to extracts after mechanochemical treatment (Fig. 1).

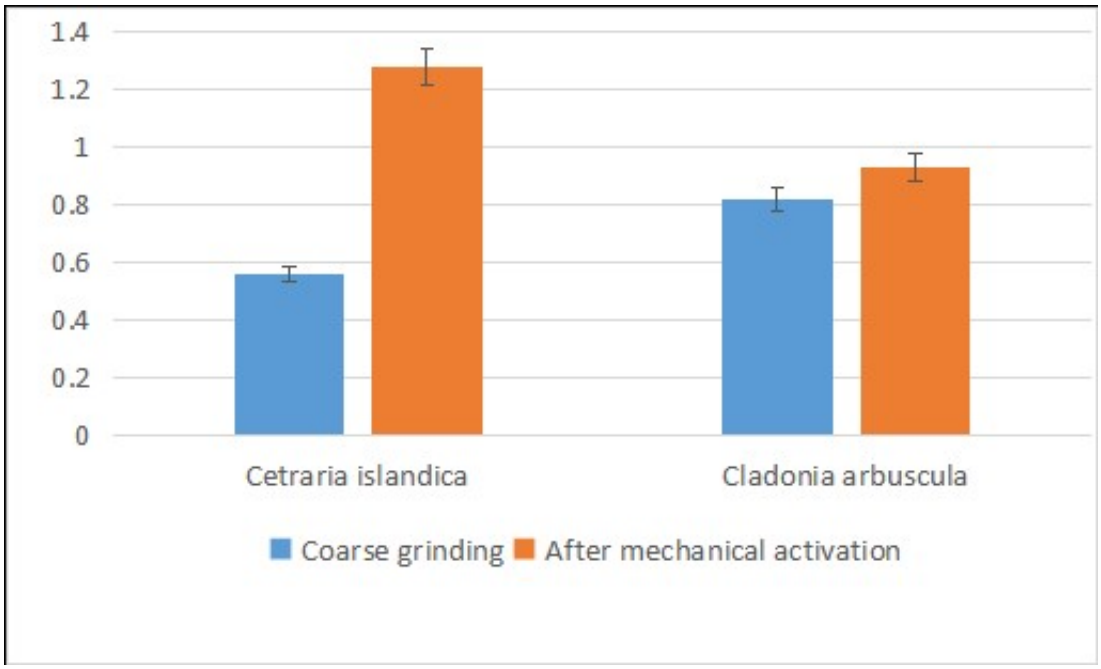

Figure 1. *The content of LWMA in Cetraria islandica and Cladonia arbuscula depends on the processing of raw materials during extraction, mcg eq g-1.*

Furthermore, the content of LWMA in solutions obtained by diluting the dry extract from 1 to 0.2 mg/ml in distilled water was determined. Samples were prepared with a dilution of 1: 5, 1: 4, 1: 3, 1:2, 1:1. In the studied samples of *Cetraria islandica* and *Cladonia arbuscula* lichens, a similar increase in the content of LWMA was observed, starting with a dilution of 1: 3 (Fig. 2).

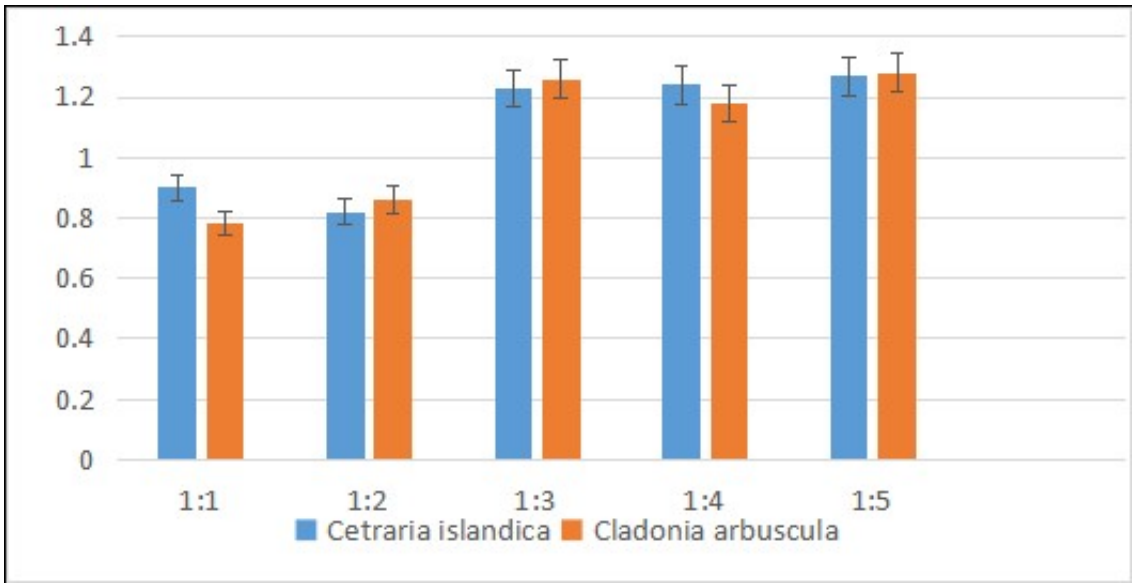

Figure 2. *The content of LWMA in Cetraria islandica and Cladonia arbuscula, depending on the dilution of the dry extract in water, mcg eq g-1.*

In our experiments, the effect of *Cetraria islandica* and *Cladonia arbuscula* extracts at a 1:1 dilution was maximally valid in inhibiting the growth of Gram positive bacteria (*S. aureus, S. pyogenes*) and Gram negative bacteria (*Escherichia coli).* It should be noted that in a 1:1 dilution, *Cladonia arbuscula* has more pronounced antibacterial properties than *Cetraria islandica*. The effect on Streptococcus pyogene growth of the studied extracts of *Cetraria islandica* extracts studied in dilutions 1:5, 1:4, 1:3 was determined to be not sensitive (6mm). *Staphylococcus aureus* in dilutions

1: 5 and 1: 4 was generally weaker. The most significant effect of *Escherichia coli* was shown by extracts at a 1:1 concentration of 1:1 *Cetraria islandica* and *Cladonia arbuscula*, the diameter of the bacteriostatic zone of which was 12±0.13 and 13± 0.20 mm, respectively, and when growth was suppressed by oxacillin, the diameter was 12± 0.12 mm, in case of penicillin it was 7± 0.09 mm (Table 1).

Dilution of dry extract	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Escherichia coli*
Cetraria islandica			
1 : 5	6 ± 0.10	6 ± 0.15	10 ± 0.13
1 : 4	6 ± 0.13	6 ± 0.12	10 ± 0.14
1 : 3	7 ± 0.11	6 ± 0.18	10 ± 0.12
1 : 2	8 ± 0.10	8 ± 0.12	8 ± 0.14
1 : 1	12 ± 0.14	11 ± 0.12	12 ± 0.13
Cladonia arbuscula			
1 : 5	7 ± 0.14	9 ± 0.15	10 ± 0.18
1 : 4	7 ± 0.14	9 ± 0.12	12 ± 0.16
1 : 3	7 ± 0.12	7 ± 0.15	7 ± 0.17
1 : 2	8 ± 0.16	11 ± 0.16	7 ± 0.13
1 : 1	15 ± 0.18	12 ± 0.20	13 ± 0.20

Table 1. *The diameter of the bacterial growth suppression zones (mm) with lichen extracts, depending on the breeding*

The antimicrobial effect of the extracts in dilutions of 1:5, 1:4 and 1:3 was less pronounced than that of oxacillin but more effective than that of penicillin. The antibacterial effect of *Cladonia arbuscula* extract on *Escherichia coli* was more significant than *Cetraria islandica* extract at a concentration due to differences in the content of biologically active substances (phenolic compounds).

Discussion

The results show the high biological potential of the studied lichens *Cetraria islandica* and *Cladonia arbuscula*, which grow in Yakutia. Currently, the study of the antioxidant capacity of lichens is an urgent topic. In most lichens, phenols capable of absorbing free radicals are important antioxidants (Ristić et al. 2016). It should be noted that extracts are a mixture of many biologically active substances in plant tissue; their antioxidant activity can result from individual components and their interactions.

In our investigation, the antioxidant activity of the *Cetraria islandica* and *Cladonia arbuscula extract* was probably the result of a synergistic effect of several compounds present in the extract, which in a related action contributes to a relatively high overall antioxidant effect. Other studies show that lichens are effective against sensitive bacterial strains and many other strains of multidrug resistant bacteria (Wimmerstedt & Kahlmeter 2008; Liu et al. 2017). Inhibition of the growth of multiresistant strains of *Streptococcus aureus* and mycobacteria is particularly important (Ferraz-Carvalho et al. 2016; Pompilio et al. 2016). Therefore, the research results also confirm the significance of lichens as a source of antimicrobial metabolites against both types of bacteria.

Conclusion

Thus, the results of our study demonstrate that mechanical activation of the lichen layers contributes to an increase in the LWMA yield of *Cetraria islandica* by 56% and *Cladonia arbuscula* by 10% compared to coarse ground layers. This situation indicates the possibility of obtaining active substances using an affordable and safe method. Comparatively high antioxidant properties of *Cetraria islandica* and *Cladonia arbuscula* extracts were manifested when prepared in a 40%

water-ethanol solution in the *Cetraria islandica* at a dilution of 1: 1 (1.28 mcg eq g^{-1}), and in the extract of the *Cladonia arbuscula* extract - 1: 5 (1.16 mcg eq g^{-1}). The antimicrobial properties were established by dilution of dry residue in a 1:1 ratio. The antibacterial effect of the extract in the 1:1 dilution of *Cetraria islandica* against *Staphylococcus aureus* was revealed to suppress the growth zone by 12± 0.14 mm, in *Streptococcus pyogenes* by 11±0.12 mm and *Escherichia coli* by 12±0.13 mm. The antibacterial effect of the extract in the 1:1 dilution of *Cladonia arbuscula* against *Staphylococcus aureus* was to suppress the growth zone by 15±0.18 mm, in *Streptococcus pyogenes* by 12±0.20 mm, and *Escherichia coli* by 13±0.20 mm. Furthermore, in the study of antimicrobial properties, *Cladonia arbuscula* was more effective than *Cetraria islandica*. The presented results can be used to further study these lichens as possible sources of substances with antimicrobial properties.

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