Water vacuum-oscillating extraction of chaga

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Abstract. Studies on the birch fungus (chaga) are quite broad are versatile, because of its vital properties for man. In this regard, research into increase of efficiency of the extraction process in order to increase the yield of nutrients and acceleration of the process are of current interest. Thereby, the paper presents a method of water vacuum-oscillating extraction of Chaga, aimed at increased yield of valuable substance – melanin. The extraction method, that has been developed, consists of periodic cycles of water heating of raw materials and boiling in vacuum. Comparative analysis of this method of extraction of chaga with the classical one, which includes two stages, showed that the proposed technology can increase the quantitative yield of melanin by more than 1.5 times when carrying out a one-step process and reducing a total duration of the process by 1.4 times. Thus, it was found that further research in this direction, aimed at rationalization of regime parameters is appropriate.

Keywords: chaga, melanin, water extraction, vacuum-oscillating process

Introduction

Inonotus Obliquus is widely used in medicine as a prophylactic and therapeutic agent; while it was discovered that the chemical composition of Chaga sharply differs from other bracket fungi. Thus, Chaga contains water-soluble pigments, some organic acids, polysaccharides, phenylchromone, steroid substances, a rich set of mineral compounds, microelements, etc. The natural complex of these substances stipulates the medicinal properties of Chaga [1]. There were medical scholars in the XIX century who claimed Chaga could be effective to fight cancer. In 1858 E. Froben described the case of the cure with Chaga extract of a seriously ill patient with an inoperable form of parotid gland cancer; in 1862 A. Furkht described the cure for cancer of the lip and submandibular gland without surgery with Chaga brew, although the blastema was 3/4 of the lower lip size. The thick brew of the fungus was taken inside and in the form of applications 3 times a day. The treatment lasted several months and ended with the complete disappearance of the cancer blastema. However, all attempts to check the clinically therapeutic effect of Chaga failed and did not prove positive effects. The possible cause of failure can be the faulty use of other species of fungi or, rather, fruit bodies of fungi instead of Chaga or the use of low-quality Chaga, collected from dead trees that do not possess the necessary set of biologically active substances, and consequently, have low therapeutic activity [1].

Recently there has been a great interest among foreign researchers in Chaga for the treatment of blastema diseases. Therefore, a microbiologist from China tested the water extract of Inonotus Obliquus on bone marrow cells in mice with chemical immunodeficiency. He injected Chaga extract daily for 24 days to mice treated with cyclophosphamide (400 mg/kg of body weight), an immunosuppressive alkylating agent, which revealed that the level of TNF-α (blastema necrosis factor) can be effectively reduced by the extract of Chaga [2].
The medicinal properties of Chaga were thoroughly studied by Chinese scholars, namely, Liuping Fan [3] used sepharose (a medication for the purification method and protein separation based on their selective interaction with the ligand). They proved that the fungus contains a water-soluble polysaccharide (ISP2a), which not only showed antitumor activity, but also could significantly enhance the immune response of tumor mice. In addition, ISP2a significantly improved the proliferation of lymphocytes and increased the production of TNF-α. The results of these studies showed that ISP2a has potential applications as a natural antitumor agent with immunomodulating activity.

Chinese researchers played a significant role in the study of Chaga. They proved the hepatoprotective activity of the water extract against the oxidative liver damage induced peptide hydroperoxide in the primary cultured rat hepatocyte [4]. Michelle Co et al. [5] found that birch bark contains betulin, a compound used to fight cancer. However, betulin cannot be safely used by people in its current form. While Chaga absorbs betulin from the birch and synthesizes it in such a way that it becomes safer for humans’ consumption. Today, betulin and betulinic acid are studied not only to fight cancer, but also as agents in the fight against HIV.

Serbian scientists [6] reported on the treatment with Chaga of a dog, that had mammary adenocarcinoma and there were many skin metastases. 3 months later the general condition of the animal improved, it had appetite and it recovered some body weight, the solid tumor node softened, and multiple skin metastases decreased. Histological changes in tumor tissue were in liquefactive necrosis and intensive tissue proliferation. The authors later confirmed these data in experiments on two dogs with spontaneous breast cancer. Doctors of Biological Sciences from China [7] in a number of studies have investigated the antitumor properties of various extracts of Chaga and bespoke the effectiveness of the natural product in fighting against blastemas. They sought to investigate the effect of continuous intake of Chaga water extract on suppressing the tumor.

Scholars from the Chinese laboratory of biotechnology of medicinal plants Zheng W. et al. [8] tried to obtain phenolic compounds from I. Obliquus by fermentation. The process was carried out in a continuously stirred reactor to find out how it accumulates phenolic compounds in different culture media and whether these compounds have antioxidant activity. The phenolic compounds produced by I. Obliquus in the control medium consisted of melanin, flavonoids, polyphenols and other smaller phenols. It became evident that their accumulation was influenced by the addition of H2O2, to the medium, which resulted in an increase in the content of total intracellular phenols and melanin, a change in the content of extracellular phenols was less intense. The simultaneous exposure of H2O2 and arbutin led to a further increase in the production of intracellular phenols and a decrease in the accumulation of extracellular phenols. Hence, it was agreed that the production of phenolic compounds based on I. Obliquus is enhanced by the introduction of an oxidizing agent, which allows it to be used subsequently as a reliable source of pharmaceutically important phenolic compounds.

Bao-Zhong Diao et al. [9] investigated the therapeutic effect of polysaccharides from Inonotus Obliquus on streptozotocin, induced diabetic symptoms and their potential mechanisms. They studied the influence of polysaccharides Inonotus Obliquus on body weight, blood glucose level, damaged pancreatic cells, oxidative stresses, pro-inflammatory cytokines and enzymes that metabolize glucose in the liver. The results showed that the introduction of the polysaccharide can restore anomalous oxidative indices near normal levels. Damaged streptozotocin pancreatic rat β cells, were partially restored after mice were injected with this component in 6 weeks time.

Jung-Han Lee and Chang-Kee Hyun found that a water-soluble melanin complex extracted from I. Obliquus improves insulin sensitivity and reduces obesity in high-fat mice (HF). When the melanin complex was treated with adipocytes, insulin-stimulated glucose uptake significantly increased, and its effect was proved in the treatment with Wortmannin. Moreover, they observed a pre-dependent increase in the activity of actoforelation and transporter of glucose transcription into the plasma membrane in melanin complexes treated with melanin. The fat tissue of melanin-treated HF-mice had a higher expression of oxidative fatty acid genes without a significant change in the expression of
lipogenetic genes. These results indicate that the water-soluble complex of I. Obliquus melanin has an adventurous lipid-metabolic effect, which makes it a good candidate for an antidiabetic agent [10].

Thus, the studies devoted to birch fungus - Chaga are ample and versatile, in view of its vital properties for humans. In this regard, we continue studies on how to boost the efficiency of the extraction process in order to increase the output of nutrients and accelerate the qualitative and quantitative processes.

Methods and materials

The material used for the experiment was Chaga, which was collected in the autumn in one of the regions of the central part of the Russian Federation. Autumn and spring are regarded as the most favorable periods for collecting Chaga, since the greatest amount of antioxidant substances are accumulated in Chaga, which subsequently influence the properties of the melanin obtained [11]. Chaga was collected by cutting it off the birch with a knife or an ax. Later the Chaga was ground into fractions by the use of a drum crushe and placed in a cardboard container at room temperature. Before the experiment, the crushed Chaga was sorted into fractions with a shaker. 3-5 mm fractions were used in the experiment.

Classical method of extracting

The classical method of Chaga water extraction serve prototype; the methods suggests that the ground Chaga is filled within stillled water in a ratio of 1:6 and place in the chamber for 5 hours at a constant temperature of 70°C. Further, the extract obtained is segregated from the meal, which is then poured with water in a ratio of 1:4 and extracted additionally for 5 hours at the same constant temperature. This is called an extract of the second stage, which is necessary for more complete extract of important substances. The first stage extract is filtered and stored for further mixing with the extract of the second stage and melanin preparation.

The classical method required a great amount of time, and the extract is prepared in two stages, which can be regarded as a disadvantage. The main task of the development of an alternative method was to shorten the time for the process and achieve the output of the extract in one step, that is why we decided to use a vacuum medium [3].

Extracting in a vacuum

When the Chaga was extracted with vacuum, the ground Chaga was pre-vacuum ed for 10 minutes in order to release the holes from air and moisture vapor and conduct efficient impregnation of the extractant. Further, the authors added distilled water in the ratio 1:10 and placed in a vacuum heating chamber. The extraction temperature was 70°C. During the extraction process, the pressure dropped hourly to 0.3 atm., which contributed to the boiling of the extractant in a vacuum medium. This procedure lasted 5-7 minutes, depending on the boiling point of the extract. After that, the extract was removed from the chamber, filtered and stored for further production of melanin. Totally, the process took no more than 7 hours.

Quantitative characteristics of melanin output

Melanin is the main active component of Chaga, which his responsible for it’s the rapeutic activity. When water is extracted from the Chaga mushroom, a colloidal system is formed with melanin in a dispersed phase. When acids such as sulfuric, hydrochloric are added to the Chaga water extract melanin precipitates. The method of extracting and the nature of the precipitating agent affect the structural organization and biological activity of the melanin obtained, in particular the content of ash substances involved in the melanin particles formation [11, 13].

To obtain the desired melanin precipitate, we added 25% hydrochloric acid solution to the Chaga water extract, to a pH of 1.0-2.0, the resulting mixture was stirred and left for 30 minutes. The precipitated dark brown melanin precipitate was separated and dried. The process of melanin drying is carried out at 70°C also in two ways: under atmospheric pressure and in vacuum. We used electron
microscopy method to study the microstructures of both the Chaga mushroom and the melanin obtained by various methods.

**Results**

An electron microscopy analysis of the microscopic structure of the Chaga mushroom (Fig. 1) allows us to conclude that the outer layers of the fungus, characterized by a high melanin content in comparison with the inner layers, can also be described by a denser structure, which complicates the extraction process, so the intensification of this process by various methods is urgent for researchers.

We elicited that melanin output by using the vacuum extraction technology proposed by the authors has increased more than 1.5 times, which is supported by the data in Tables 1 and 2. In addition, the use of vacuum reduced the extraction process to one stage and shortened the total duration from 10 to 7 hours compared with the classical method.

Thus, we can conclude that the preliminary vacuuming of the raw material and periodic boiling in a vacuum intensify the extraction process, and make further studies in this filed be feasible [12, 14]. Moreover, melanin obtained as a result of vacuum drying has a more gill-bearing microstructure (Figure 2), which can contribute to more effective digestion in the human body when used as a biologically active additive.

![Figure 1 – Microscopic structure of Chaga: (a) the outer layer of the fungus; (b) the inner layer of the fungus.](image)

| No | Chaga weight | Steps | Water extraction Vol., ml | Melanin output g | % |
|----|--------------|-------|--------------------------|------------------|---|
| 1  | 10           | 1     | 28                       | 0.4568           | 4.57 |
|    |              | 2     | 36                       | 0.2192           | 2.19 |
|    |              | Total | 64                       | 0.6761           | 6.76 |
| 2  | 10           | 1     | 36                       | 0.4117           | 4.12 |
|    |              | 2     | 36                       | 0.2171           | 2.17 |
|    |              | Total | 72                       | 0.6287           | 6.29 |
| 3  | 10           | 1     | 36                       | 0.4936           | 4.94 |
|    |              | 2     | 33                       | 0.1820           | 1.82 |
|    |              | Total | 69                       | 0.6756           | 6.76 |
| 4  | 10           | 1     | 39                       | 0.4499           | 4.50 |
Table 2 – Melanin output by vacuum extraction

| No | Chaga weight | Water extraction Vol., ml | Melanin output | Melanin output |
|----|--------------|---------------------------|----------------|----------------|
| 1  | 10           | 96                        | 1.0486         | 10.49%         |
| 2  | 10           | 96                        | 0.9837         | 9.84%          |
| 3  | 10           | 94                        | 1.0163         | 10.16%         |
| 4  | 10           | 96                        | 1.0373         | 10.37%         |

Figure 2 – Melanin, obtained during the drying process: (a) by atmospheric pressure (b) in a vacuum

Conclusion

The technology of vacuum water extraction of the Chaga fungus was developed, which is as follows. The crushed Chaga was previously evacuated for 10 minutes to release air and moisture from the pores and ensure effective impregnation of the extractant. Further, distilled water was added in a ratio of 1:10 and placed in a vacuum heating chamber. The extraction temperature was 70 °C. During the extraction process, the pressure in the chamber was reduced every hour to 0.3 atm, which promoted the boiling of the extractant in a vacuum medium. This procedure lasted 5-7 minutes. Then the pressure was again raised to atmospheric value. Six cycles were performed. In general, the process took no more than 7 hours. After this, the extract was removed from the chamber, filtered and stored for further production of melanin. A comparative analysis of the developed vacuum method of Chaga water extraction with a classical method, including two steps proved that the proposed technology allows to increase the quantitative output of melanin by more than 1.5 times while decreasing the total duration of the procedure by 1.4 times. Thus, further studies, which deal with the development of reasonable regime parameters for the preliminary evacuation of raw materials and periodic boiling in a vacuum are worthwhile.

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