STUDIES OF THE COMPOSITION OF THE CRYOGENIC GROUND CHAGA

E.Yu. Razumov, E.I. Baygildeeva A.V. Safina

The paper studies the properties and chemical composition of birch fungus - Chaga, which contains a unique complex of biologically active substances and is used as a raw material for the production of drugs and biologically active additives. The components listed prove their antioxidant, immunomodulating, antiviral, antidiabetic, and cytotoxic properties. The paper specifies the application of chaga in such areas as medicine, agriculture and food industry. It analyses the way the method of grinding of plant raw materials might influence the quality of the resulting drugs, including the sustaining the biological activity.

The results determined that the cryogenic grinding of chaga, which allows to preserve valuable substances of the fungus due to its grinding at low temperature in liquid nitrogen to be the most reasonable. The paper gives the analysis of the obtained dispersed phase by high performance liquid chromatography and atomic absorption spectroscopy. The results of the studies indicate an increase in the content of particular valuable components of the fungus in extracts of raw materials that have gone through the cryogenic grinding, compared with the extracts of raw materials obtained by the traditional grinding method. The data acquired can be of practical importance when chaga is used in medicine.

Key words: *birch mushroom, Chaga, cryogenic grinding, cryopowder.*

Introduction

Chaga is a perennial parasitic fungus of the Hymenochaetaceae family that lives on the bark of aged birch trees. It grows mainly in humid climates such as forests of Russia, Ukraine, Belarus, and Poland [1]. The fruit body consists of large brown solid growths inside, while closer to the wood the fruit body is softer and lighter. Fungus hypha destroys wood and causes tree decay [2]. Chaga contains a comprehensive set of substances that are essential for humans. In this context, the Chaga mushroom is widely used in the pharmaceutical industry.

Chaga containing drugs are generally used as anti-inflammatory and restorative. Chaga can also be used as part of the complex treatment of tumors of various localization, diseases of the gastrointestinal tract, it helps to mobilize the body's defenses by affecting metabolic processes [3-5]. Chaga-based drugs are biologically active. They are characterized by immunomodulatory, antiviral, antioxidant, antitoxic, radio protective, gene protective and adaptogene properties; these have the potential to regulate the activity of blood enzymes, as well as the activity of the cardiac, respiratory and nervous systems of the body [3-17].

Modern medicine uses chaga independently or as an ingredient of multicomponent drugs for internal and external use in the form of elixirs, decoctions, and ointments. Moreover, Chaga is used in animal husbandry; adding chaga to the diet of pigs stimulates the growth of piglets and weight gain. Extracted meal of Chaga can be good for crop production as a fertilizer to protect plants from late blight and stimulate growth. Food industry has applied Chaga extracts for preserving berry and vegetable juices [1].

Bulatov P.A. et al [18] studied the biology of fungus, the mechanisms for forming biologically active substances both in natural conditions and when grown in culture, determined methods for extracting and purifying medicinal substances in fungus for the purpose of further chemical and biochemical research. Shivrina A.A. et al [19] did a chemical analysis of fungus and fungus-contained concentrate, and studied the biosynthetic activity of the fungus.

X-ray fluorescence method, along with gravimetric analysis and atomic absorption spectroscopy identified the following elements that Chaga consists of: carbon (39%), potassium (9 - 10%), hydrogen (3.6%), nitrogen (0.4%), magnesium (0.64%), calcium (0.37%), chlorine (0.33%), phosphorus (0.23%), sodium (0.05%), rubidium (0.04%), sulfur (0.02%), manganese (0.02%), iron, copper, zinc, vanadium, chromium, traces of nickel, selenium, iodine, etc. Hydrolysis products contain 15 amino acids, among which are glycine, aspartic and glutamic acids (40% of all the acids), as well as tyrosine, serine, threonine, alanine, leucine, methionine, lysine, histidine, arginine, tryptophan, cystine and proline [1]. High biological activity of amino acids helps human body to adopt more effectively drugs from medicinal raw materials.

Chaga contains flavonoids, including flavones, flavonones, anthocyanins, catechins (presented by apigenin, mariningin, corin, and quercetin), triterin and sterol compounds (6 - 8%), acid-resistant lignin (25 - 30%), dietary fiber (2%), hemicellulose (12.5%), and other derivatives of pteroylglutamic acid. A study of the acid composition of chaga also showed the presence of such organic acids as acetic, butyric, oxalic, formic [1].

A study of the chemical composition showed that the chaga contains a unique polyphenolic chromogenic complex, including humic pigments such as melanins, called chagic acid, which are protectors from the effects of carcinogenic and mutagenic factors [20]. The chromogenic complex has a rich macro- and microelement composition (K, Mg, Na, Ca, Fe, Si, Zn, Al, etc.), includes derivatives of pyrocatechol and pyrogallol, pteric, steroid and terpene derivatives, lignin, polysaccharides, organic acids [21], and also has a wide range of biological and physiological effects: a high antitoxic effect, an effect on enzymatic processes.

The analysis of physical and chemical properties proved that the pigments obtained from chaga belong to melanins, i.e. group of high molecular weight black and brown pigments forming at the oxidative polymerization of phenols. Chaga melanins show high antioxidant and gene-protective activity. Specific structural features define a wide spectrum of the biological activity of melanins [22]. Sysoeva M.A. [23] researched the highly dispersed colloidal systems of water extracts of the chaga, systematized and developed a theoretical basis on the structural organization of chaga melanins.

Shivrina et al. [19] indicated that fraction of sterols and triterpenes extracted from chaga consist of lanosterol (a derivative of tetracyclic triterpen), inotodiol (triterpene alcohol), ergosterol, etc. These are insoluble in water; however, they are partially emulsified and extracted with hot water, and up to 80% remain in extracted meal while extracting. Inotodiol is known for an anti-blastoma activity.

Korsuna V.F. and Treskunova K.A. [24] reported that Chaga contains lectins. Lectins or agglutinins belong to the class of complex glycoproteins, which in some cases contain calcium, magnesium and other ions. They are capable of binding carbohydrates and participating in the processes of carbohydrates transporting and depositing, i.e. show the hypoglycemic effect, thus lowing the blood sugar level that people with diabetes suffer from. Furthermore, lectins can stimulate the growth and division of lymphocytes, participate in the regulation of immunological reactions, and block receptors of tumor cells, thus inhibiting their migration [1].

Kahlos K. [25] researched the composition of chaga terpenes, their biological activity, and antitumor effect in Finland. Taylor A. [26] from the United Stated studied the effects of aqueous chaga extracts in the treatment of adenocarcinoma. Scientists from Japan, namely, Nakajima Y. [27] research the extraction of chaga with the isolation of various components, define their structure and activity, and cultivate chaga. Chinese scientists, specifically Chung C.H. [28] examine the effect of substances isolated from chaga on cancer cells. Polish scientists [14, 15] study melanins secreted from chaga, as well as the effect mechanism of water extracts of birch fungus on the body in cancer treatment.

Chaga contains a complex of biologically active substances that are participating in the regulation of metabolism, as well as the correction and prevention of pathological disorders. In this context, it is crucially important to preserve these substances when receiving therapeutic agents. Technological problems related to the production of molecular ingredients that preserve the native structure and properties of the active complexes most important for the human body may arise while creating drugs based on biological raw materials [29].

Grinding is an essential technology in the pharmaceutical production. Conventional grinding has two significant drawbacks. The process involves the use of oxygen; hence, it causes intense oxidation and decomposition of substances of the processed raw materials. Moreover, heating of raw materials during grinding also results in the loss of valuable properties. Heating can be critical because of contact of the raw material with the surface of the working body of the grinder [30].

The technology for producing microdispersed materials will be considered effective only in case it ensures the reservation of the initial properties of the processed raw materials, in addition to fine grinding. The solubility of the plant material in biological fluids improves with the increase in the degree of grinding, thus boosting the therapeutic activity of the preparations obtained, and consequently resulting in the decrease of the amount of the plant material use.

Grinding materials to obtain pharmaceuticals is carried out in air at elevated temperatures associated with prolonged mechanical stress, which accelerates the destruction and oxidation of raw materials. In this context, it is reasonable to grind dry plant materials in a crisp, chilled state in the environment of chemically inert gases, for example, nitrogen, in order to avoid losses of thermolabile chemical compounds [31]. Cryogenic processing of plant raw materials allows you to fully preserve the native structure of not only the vitamins contained in it, but also molecular complexes containing the widest spectrum of trace elements essential for humans [32].

Such scientists as Kapitsa P.L., Landau L.D., Arkharov A.M., Gersh S.Ya., Belyakov V.P., Mikulin E.I., Marfenina E.V. etc made the greatest contribution to the development of cryology in the 20th century. Among other scientists, there are such names as Linde K., Claude J., Geylandt P., Simon F., Nernst V., and Carrier V. [33]. Cryogenic grinding of plant materials is used in molecular biology, microbiology and biochemistry to isolate substances and certain organelles of a plant cell. Raw materials cooled to low temperatures are subjected to cryogenic grinding, which helps avoid oxidation, caramelization and aggregation processes. At a low temperature, the bonds of biologically active substances with protein molecules become crisp, and further they are destroyed. This has a positive effect as protein molecules, having a large size and weight, interfere with the absorption of beneficial substances by the body. Cryogenic grinding leads to a decrease in microbial contamination of medicinal raw materials. The resulting cryopowder has a long shelf life and is a natural concentrate of biologically active substances, including vitamins and trace elements.

Cryogenic grinding has been widespread abroad. This method was initially used to extract and recover valuable materials from the wastes of the electrical and automotive industries in the United States, Japan, Germany, and France. Later the method became popular in the production of powders from vegetables, spices, heat-sensitive raw materials. Menry Balfaur LTD (USA) developed the technology of cryogenic grinding of chocolate; Cryopowider Servise (Great Britain) began to process polymer materials and pharmaceuticals in the similar way [30]. The French concern Arkopharma, which specializes in the production of parapharmaceuticals, uses the technology of cryogenic grinding of raw materials using liquid nitrogen to obtain ingredients for biologically active additives. In a liquid state, liquid nitrogen is colorless, non-toxic, therefore, when freezing food, it can be used in direct contact with a food product.

Liquid nitrogen has a low boiling point (-196^0C), it is chemically and biologically inert and is safe for working personnel. It makes a crushed mass fragility, protects it from overheating and deterioration. The presence of a dry inert atmosphere contributes to the preservation of the native properties of the source raw material.

Pavlyuk R.Yu. [30] indicated that as a result of cryogenic grinding of dried unconventional medicinal raw materials at a temperature of -10^0C and lower there is an increase in the concentration of biologically active substances, including beta-carotene, vitamin C, free amino acids, phenolic compounds with P-vitamin activity, compared to the source raw material. Such an effect can be explained by the degradation of bonds between biopolymers and low molecular weight compounds with the cleavage of the latter, and is also associated with a significant destruction of plant materials.

Pevneva O.P. and Schegolev A.A. point to the results of biochemical studies of cryogenically ground raw materials [31], during which it was found that 25–40% more essential oils are extracted from it than with traditional grinding and extraction methods. Studies prove that low-temperature grinding of medicinal plant materials leads to an increase in the yield of extractive substances by 5–45% depending on the source raw material, including carbohydrates by 2–25%, organic acids by 15–35%, and vitamin C by 15–45%.

The following advantages of cryogenic grinding of herbaceous medicinal raw materials in comparison with the traditional method of grinding were revealed as a result of research by Shchegoleva A.A. [34]. Cryogenic grinding preserves biologically active substances of ground products; it helps obtain a homogeneous particle size distribution of the crushed product; it obtains powders with particle sizes not achievable with traditional methods; finally, it prevents aggregation of particles resulting from the accumulation of static electricity in the case of traditional grinding.

The authors consider the use of cryogenic grinding when receiving drugs from the Chaga fungus reasonable taking into account the advantages of the product obtained from cryogenic crushed raw materials, including the preservation of its biological value, the increase in bioavailability of biologically active substances and the improvement of their adoption by human body. This will allow the preservation of its biologically active substances, that are widely used both in phyto- and fungotherapy.

Methods and materials

The authors chose as an object of study a birch mushroom – a chaga harvested in the winter in the forest zone of the Mari El Republic, far from industrial and housing infrastructure, and highways. Furthermore, the authors grounded two samples of room-dried chaga to compare the impact of the method of chaga grinding on the content of biologically active substances in it. Preliminary grinding of raw materials was carried out on a rotary knife mill RM-120.

A drum-type ball cryogenic mill (Fig. 1) with grinding bodies in the form of metal balls with a diameter of 20 mm was used for grinding. The experimental unit consists of a metal

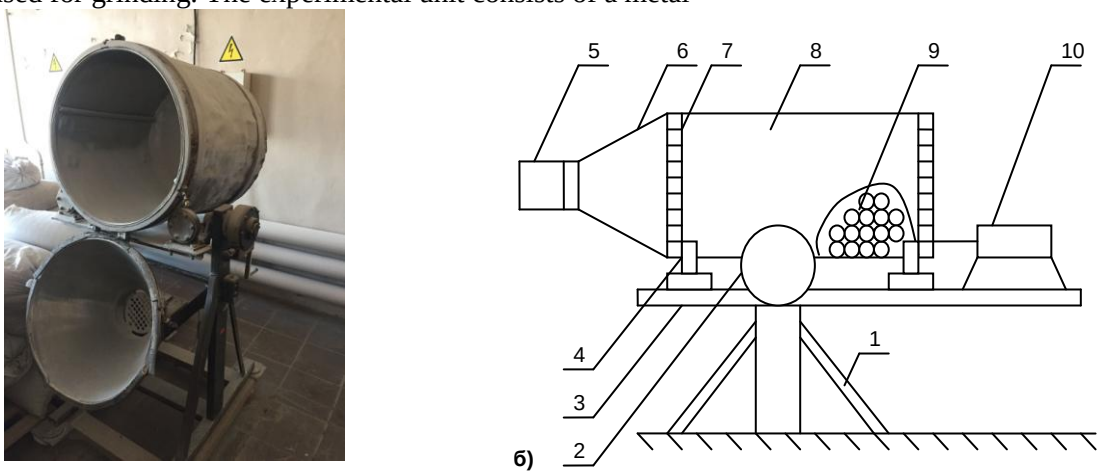

Figure 1. Experimental ball cryogenic mill: a - appearance; b - scheme: 1 - frame; 2 - worm reducer; 3 - plate; 4 - plain bearing; 5 – uploading container for the ground product; 6 - conical cover; 7 - belt fixing the axis of the cryogenic mill and electric motor; 8 - cryogenic mill body; 9 - grinding bodies; 10 - electric motor.

drum mounted on the frame and having a cylindrical and conical parts. The drum is tilted during operation and during unloading of ground raw materials. The conical part is supplemented by a cover for loading and unloading material and a grid to prevent the loss of grinding bodies during unloading. The rotation of the drum is carried out with the help of a drive mechanism and a system of ball bearings, which are mounted on a frame using bearings.

The grinding of the first sample was carried out for 40 minutes in a dry form with a drum rotation speed of 70 rpm. The grinding of the second sample was carried out after feeding liquid nitrogen to the mill, in the medium of which the raw material was aged for 5 minutes in order to completely freeze the chaga particles. The ratio of raw materials and liquid nitrogen was 1:2. The grinding process proceeded at atmospheric pressure and intensive evaporation of nitrogen. Particles with a size of not more than 60 microns were obtained as a result of grinding.

The extraction of crushed raw materials was carried out with water (for organic acids), as well as ethanol and methanol (for other components) according to traditional methods. To determine ceresins insoluble in water and alcohols, 5 ml per 50 ml of benzene was added to the extract gross volume.

Analysis of aqueous and alcoholic extracts of samples obtained by conventional grinding and cryogenic grinding was carried out by high performance liquid chromatography (HPLC) with a ratio of raw materials to extractant 1:50 and atomic absorption spectrometry (AAS) with a ratio of raw materials to extractant 1:25. HPLC analysis was carried out on a Shimadzu LC-20 chromatograph, Prominence series (Japan) with a diode array detector using a S-18 reverse-phase column from Diasfer (Russia) (250x4 mm), injection volume 20 mql. The solvent systems used are water-acetonitrile/methanol and water-acetonitrile/ethanol. Registration of the separated substances was carried out at two wavelengths: 270 nm and 325 nm. The components were identified spectrally in the wavelength range of 220 - 630 nm. AAS was carried out on a Shimadzu model AA-7000 spectrometer.

Results

The authors carried out a comparative analysis of the results of extraction of samples obtained by conventional grinding and cryogenic grinding in order to determine the effect of cryogenic grinding of chaga on the content of biologically active substances in it. The results are given in Table 1.

Table 1 - Results of High Performance Liquid Chromatography (HPLC) and Atomic Absorption Spectroscopy (ACC) of Chaga Extracts

Item number	Structural class	Concentration, mcg%	
		Ground sample	***Cryogenic sample, ground***
1	Amino acids:		
	Glycine	not detected	12.77
	Glutamine acid	1.44	14.71
	Asparagine acid	2.06	9.35
	Methionine	1.83	10.60
	Leucine	not detected	7.94
2	Indole-3acetic acid	17.27	24.54
3	Flavonoids	3.91	7.68
4	Hemicellulose	5.11	11.64
5	Lignin	27.33	9.90
6	Polysaccharose	8.57	2.24
7	Acetic acid	not detected	4.66
8	Oxalic acid	4.28	15.06
9	Butyric acid	2.02	8.75
10	Thiols	3.13	11.68
11	Melanin	13.42	24.22
12	Magnesium	0.61	0.64
13	Calcium	0.21	0.36
14	Pentole-1	not detected	7.55
15	Glycerin	not detected	3.99
16	Ash	29.48	7.58
17	Ceresin	not detected	1425

The analysis of amino acids in the samples indicates that biologically important compounds such as glycine and leucine were destructed in the process of conventional grinding. Glycine, which is one of the essential amino acids, is a metabolic regulator, normalizing and activating the processes of protective inhibition in the central nervous system, and increasing mental performance. Leucine is one of the most important amino acids, serves as a source of mental energy, stimulates growth hormone, and helps restore the body after injuries and operations. In the case of other amino acids, there is a significant increase in their content in the cryogenic ground sample, in particular, glutamine acid – by 10 times, asparagine acid – by 4.5 times. The amount of methionine, which is an essential amino acid, containing sulfur, which can produce other sulfur-containing molecules in the body and that is participating in the production of protein in the cells of the human body, increases by 6 times.

Indole-3acetic acid that is a substance of high physiological activity, and affects the processes of plant growth is found in both samples. However, the cryogenic ground samples contain 1.4 times more of that acid.

The amount of flavonoids and hemicelluloses increases in the cryogenic ground samples by two times. Flavonoids help to reduce pathologically increased permeability of capillaries, eliminate their fragility and ensure the preservation of ascorbic acid in the body, have a normalizing effect on the lymphatic flow and liver function, and have anti-inflammatory effects. Hemicellulose serves as a source of energy, affects lipid metabolism, plays the role of intestinal sorbent; it absorbs salts of heavy metals, and reduces cholesterol level.

The amount of lignin and polysaccharides in the cryogenic ground samples decreases almost three times. This effect can be explained by the destruction of the chaga lignin-carbohydrate complex in the process of freezing the raw materials.

The chaga components also include such organic acids as acetic, oxalic and butyric, which are crucial in normalizing the acid-base balance, and contribute to the elimination of toxins from the human body. No acetic acid was detected in the ground sample. All three organic acids are present in the cryogenic sample, while their content increased by 3.5 - 4.5 times.

The thiol content in the cryogenic sample increases almost 4 times. This component of chaga is an antioxidant. Thiol based drugs are used to increase the effectiveness of chemical and radiotherapy, and can stimulate the functioning of the human immune system.

The yield of the chromogenic complex is almost doubled, which can be explained by the stage of freezing the milled chaga. Moisture in raw materials under the influence of low temperatures turns into ice crystals, which destroy the cell walls of the fungus. Due to this, extractive substances, including melanins, become more accessible for extraction.

Among the selected components, magnesium and calcium are determined in both samples. However, the cryogenic ground samples show better results (by 5% and 71%, respectively). These elements are bound at the cellular level and, working together, contribute to optimal cellular metabolism.

Pentole is present only in the cryogenic ground sample. It serves as a carrier of a medicinal substance in therapeutic ointments, determines the speed and degree of absorption, affects the transport process through the skin, and contributes to the optimal therapeutic effect of the ointment.

Glycerin is determined only in the sample obtained by cryogenic grinding. It is an important component of the metabolic process in the body, that relates to nutritional supplements.

The ash content in the ground sample studied is more than 29%. As a result of cryogenic grinding, the ash content of raw materials is reduced by 4 times, which is a positive result. The high content of ash substances in chaga-based drugs can have a negative effect on the body, and therefore, reducing their content is a crucial objective.

Ceresin was found only in a cryogenic ground sample. With this component, metabolism and peripheral blood circulation are significantly increasing, which contributes to the absorbable, anti-inflammatory and antispasmodic effects of drugs containing it.

It was found that the sample obtained by cryogenic grinding of raw materials has a high content of valuable biologically active components as a result of a comparative analysis with high-performance liquid chromatography and atomic absorption spectroscopy of water and alcohol extracts of the fungus.

Conclusion

The complex of unique properties of chaga mushroom allows it to be widely adopted to obtain therapeutic and prophylactic preparations and food additives used to treat cancer and gastrointestinal diseases, diabetes, excretion of radionuclides, toxins, heavy metals, normalization of metabolism, etc. The following biologically active substances presented in chaga give it the therapeutic effect: a chromogenic polyphenol carbon complex (melanins), microelements, etc. The results of the study proved that the amount of therapeutic components of chaga-based products can be determined by the grinding method of the raw plant material. Thus, the problem of preserving the valuable components of the chaga can be solved by the use of cryogenic grinding technology.

The results of experimental studies showed that cryogenic grinding allows saving a larger number of biologically active substances of chaga compared to raw materials, ground by a conversional technology. Freezing raw materials in liquid nitrogen prevents the processes of oxidation, aggregation and caramelization. The bonds of biologically active substances with protein molecules are destroyed at low temperature, thus effecting a better absorption of the drug. Hence, chaga cryopowder is a product with a high content of valuable components. The data obtained can be of practical importance when using chaga as part of drugs and biologically active additives with targeted functional properties.

References

1. Shashkina M. Ja., P. N. Shashkin, and A. V. Sergeev. Chemical and medico biological properties of chaga (Inonotus obliquus) // Pharmaceutical Chemistry Journal . – 2006. – No10(40). – pp. 37-44. (In Russ.)

2. Karomatov I.D., Murodova M.M. Chaga, birch mushroom // Biology and Integrative Medicine. - 2017. - No. 2. - pp. 164-179. (In Russ.)

3. Korsun V.F., Krasnopolskaya L.M., Korsun E.V., Avkhukova M.A. Antitumor properties of fungi.– Moscow: Practical Medicine, 2012. – 210 p. (In Russ.)

4. Shashkina M.Ja., Shashkin P.N., Sergeev A.V. Chaga, Chagovit, Chagalux in medical and preventive practice. - Moscow: Edas. - 2009. - 64 p. (In Russ.)

5. Kuznetsova O.Yu. Physico-chemical characteristics and biological activity of aqueous extracts and the poly phenolic hydroxycarboxylic chaga complex: Dissertation for the Candidate of Chemical Sciences. - Kazan, 2004 .- 158 p. (In Russ.)

6. Lee S.H., Hwang H.S., Yun J.W. Antitumor activity of water extract of a mushroom, Inonotus obliquus, against HT-29 human colon cancer cells // Phytother Res. – 2009. – V.23. – P. 1784-1789.

7. Kim E. J., Lee Y.J., Shim H.K., Yoon J.H. A study on the mechanism by which the aqueous extract of Inonotus oblicuus induces apoptosis and inhibits proliferation in HT-29 human colon cancer cells // J. Korean Soc. Food Sci. Nutr. – 2006. – №5(35). – p. 516-523.

8. Ham S.S., Kim S.H., Moon S.Y., Chung M.J., Cui C.B., Han E.K., Chung C.K., Choe M. Antimutagenic effects of subfractions of Chaga mushroom (Inonotus obliquus) extract // Mutation Research. – 2009. – V. 672. – P. 55-59.

9. Chung M.J., Chung C.K., Jeong Y., Ham S.S. Anticancer activity of subfractions containing purecompounds of Chaga mushroom (Inonotus obliquus) extract in human cancer cellsand in Balbc/c mice bearing sarcoma-180 cells // Nutr. Res. Pract. – 2010. – V. 4. – p. 177-182.

10. Shin S.H., Kim Y. J., Park J. Effect of Cell Proliferation and Apoptosis by Inonotus obliquus in Human Glioblastoma U-87 MG Cells // J. Korean Soc. Food Sci. Nutr. – 2013. – №7(42). – p.1022-1028.

11. Babitskaya V.G., Shcherba V.V., Ikonnikova N.V. Melanin complex of the fungus Inonotus obliquus // Appl. Biochem. Microbiol. – 2000. – №4 (36). – p. 377-381.

12. Lee I.K., Kim Y.S., Jung Y.W., Yun B.S. New antioxidant polyphenols from the medicinal mushroom Inonotus obliquus // Bioorg. Med. Chem. Letters. – 2007. – V. 17. – P. 6678-6681.

13. Zhong X., Wang L., Sun D. Effects of Inotodiol Extracts from Inonotus Obliquus on Proliferation Cycle and Apoptotic Gene of Human Lung Adenocarcinoma Cell Line A549 // Chin. J. Integr. Med. – 2011. – V. 3. – p. 218-223.

14. Mazurkiewicz W., Rydel K., Podocki D., Lemieszek M.K., Langner E., Rzeski W.. Separation of an aqueous extract Inonotus obliquus (Chaga). A novel look at the efficiency of its influence on proliferation of A549 human lung carcinoma cells // Acta Poloniae Pharmaceuyica. – 2010. – №4 (67). – p. 397-406.

15. Mazurkiewicz W. Analysis of aqueous extract of Inonotus obliquus // Acta Poloniae Pharmaceuyica. – 2006. – №6 (63). – P. 497-501.

16. Sbitneva E.M. Chaga mushroom to fight a hundred diseases. - Moscow: RIPOL Classic, 2009. - 64 p. (In Russ.)

17. Kuznetsova O.Y. Review of modern advanced medicinal products containing the biologically active components of chaga mushroom birch. Drug development & registration. 2016; (1):128-141. (In Russ.)

18. Bulatov P.K., Berezina M.P., Yakimov P.A. Chaga, its properties and use in stage IV cancer. - Chaga and its therapeutic use. - Leningrad: Medgiz, 1959.- p. 7-22. (In Russ.)

19. Shivrina E.V. Lovyagina E.G. Platonov. Chaga and its therapeutic use in stage IV cancer. - Chaga and its therapeutic use. - Leningrad: Medgiz, 1959.- p. 55-71. (In Russ.)

20. Britton B. Biochemistry of natural pigments. - Moscow: Mir, 1986. - 422 p. (In Russ.)

21. Shashkina M.Ja., Shashkin P.N., Sergeev A.V. Chaga in oncology // Russian Journal of Biotherapy. - 2005. - No 4 (4). - p. 59-72. (In Russ.)

22. Kukulyanskaya T.A., Kurchenko N.V., Kurchenko V.P., Babitskaya V.G. Physicochemical properties of melanins produced by the sterile form of Inonotus obliquus ("Chaga") in natural and cultivated fungus. Applied Chemistry and Microbiology. 2002;1(38):68–72. (In Russ.)

23. Sysoeva M.A. Fine colloidal systems and chaga melanins. Monograph. - Kazan: KNITU, 2013 . 228 p. (In Russ.)

24. Korsun V.F., Treskunov K.A. Clinical herbal medicine in oncology. - Minsk: Belarus, 2003 . 366 p. (In Russ.)

25. Kahlos K., Kangas L., Hiltunen R. Antitumor activity of triterpenes in Inonotus obliquus // Planta Medica. – 52:554-556.

26. Taylor. A. Anticancer activity of plant extracts // Texas Reports on Biologea. Medicine. – 1956. – №14. – P. 543.

27. Nakajima Y. Antioxidant small phenolic ingredients in Inonotus obliquus (persoon) Pilat (Chaga) // Chem. Pharm. Bull (Tokyo). – 2007. – №2 (55). – P. 1222-1226.

28. Chung C.H. Anticancer activity and inhibition of multidrug resistance of cancer cells using substances isolated from Inonotus obliquus // Journal of Mycology and Plant Pathology. - 2007. 5 (41): 455-460. Anticancer activity of subfractions containing pure compounds of Chaga mushroom (*Inonotus obliquus*) extract in human cancer cells and in Balbc/c mice bearing Sarcoma-180 cells

29. Osetsky A.I., Graschenko V.I., Goltsev A.N> Kravchenko M.A., Stryuchkova E.V. Cryogenic Technologies in Production of Pharmaceutical, Cosmetic, Agrotechnical Formulations and Biologically Active Food Additives// Journal "Problems of Cryobiology and Cryomedicine (Problemy Kriobiologii i Kriomediciny. 2009. – 4(19):488-499. (In Russ.)

30. Pavlyuk R.Yu. Advanced technologies of dietary supplements from pollen and plant materials. Monograph. – Kiev. 2000. 133 p. (In Russ.)

31. Pevneva O.P., Schegolev A.A. Features of technology of microdispersion vegetable materials at negative temperatures and used in cosmeceuticals and industrial biotechnology. Forests of Russia and their economy. - 2015. 1(52):52-54. (In Russ.)

32. Georgievskiy V.P. Technology and standardization of drugs. – Kharkov: Riger, 2000. – Vol.2.- 781 p.

33. Arkharov A.M. The fundamentals of cryology. – Moscow: Bauman Moscow State University, 2014. 507 p. (In Russ.)

34. Schegolev A.A. Cryochemical technology for processing plant biomass to produce a complex of bioorganic compounds for medical purposes // New materials for medicine // Ed. M.G. Zuev, L.P. Larionov. Yekaterinburg: Ural Branch of the Russian Academy of Sciences, 2006. pp. 151 - 163. (In Russ.)