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Influence of development conditions on qualitative indicators of Fungus Chaga

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Abstract: The paper gives description of the growth conditions on the output of valuable components from the fungus chaga. The samples of chaga represent two different regions of Eurasia – Western Siberia and the Czech Republic. The results of the research proved that the yield of melanin in water extracts from the chaga grown in Western Siberia was 1.7% higher than that of the Czech analogue. Consequently, the authors concluded that the harsher conditions of growth, which cause a slow growth of the fungus, lead to a greater accumulation of useful substances.

Kew words: extraction, melanin, infrared spectrum analysis, preventive measures, cancer medicine

Introduction

One of the types of natural raw materials that has long been used in folk medicine for the treatment of many diseases are the growths on the birch tree, caused by the fungus Inonotus obliquus, and commonly known as chaga. This fungus grows on birches, growing in conditions of a rather cold climate [Myung-JaYoun et al.2008].

It is a wood-destroying fungus, which occurs in Europe, Asia, North America - usually above 45 degrees of north latitude. Most often grows on the birch, but it also occurs on rowan, willow, alder, oak, hornbeam, beech, elm ...

In nature, we find fruiting bodies of Inonotus obliquus of two types: imperfect or perfect ones.

Sterile fruiting body (anamorph) so called "chaga", typical by irregular outgrowths of dark brown or black colour, with uneven, finely glossy surface, jagged, cracked. Grows for several years (even 10-15), reaching a diameter up to 30 cm. The inner part is solid, corky, red-gold-orange in fresh stage, later woody, dark-brown, odourless.

Sterile, imperfect fruiting body is 10-35 cm large, hemispherical, sometimes elongated, perennial. On the surface there are grey to brown-green fibres in which chlamydospores are formed, which are colourless, later grey-green and brown to brownish brown in the old age. It is formed from January to December on live stems of deciduous trees. Imperfect fruiting bodies are present throughout the year and can live up to 20 years.

Perfect fruiting body (teleomorph) - develops in the last year of life of the tree under the bark, once a year, outspread, up to 1 m long and 1 cm thick - first dark brown soft, then dark hard and brittle. It forms continuous surfaces as well as separate boxes of different shapes and sizes under the bark and the outer layer of sapwood that is lifted and the bark and the part of the wood fall off.

Perfect fruiting bodies occupy 1/3 - ½ circumference of the trunk and reach a length of even several meters. They occur in the period from July to November on a dead deciduous trees at the injury site and in the cavities. Their mycelium has a yellowish colour, which is visible on the edges of the fruiting bodies. Pipes of perfect fruiting bodies are very fragile. Grow parallel to 3 centimetres in length and diagonally from the trunk. It is typical of brown to dark brown. The pores are white to ocher-yellow in the young fruiting bodies, in the older they are brown. The spore powder is sulfur-yellow.

Inonotus obliquus is a parasite, the infection occurs through injuries, broken limbs, and so on. It produces white decay of wood of living trees at the first stage. This is bounded by a red-brown band from healthy wood. After the tree is dead, the wood is coloured in straw yellow colour, it is soft and decaying. In the cracks appears a rusty brown mycelium [Černý A. 1989].

According to Falk's system, the chaga is classified as a wood-decaying fungus, causing white rot, so the wood is destroyed by a corrosive type with simultaneous destruction of both cellulose and lignin. The starting substrates for this fungus are carbohydrates and products of their oxidation, as well as aromatic compounds released during the decay of lignin.

A considerable number of works are devoted to the study of chaga. Particularly relevant are studies of various classes of compounds that can be released into aqueous extracts. Even in the middle ages, the famous doctor Abu Ali ibn Sina (Avicenna) in his works studied the use of chaga as a medicinal product. [Song, H.S. et al.2004]. In Russia Yakimov and Bulatovintroduced a birch mushroom extract known in folk medicine. Shashkina et al. [Inchimura, T.et al. 1999] studied in detail and described the features of several medicinal preparations based on chaga in clinical and preventive practice.

During the last decades, many scientists demonstrate an increasing interest in application of chaga in the treatment of pre-cancerous and cancerous diseases of various etiology. Liang et al. (1998) proved that chaga suspends tumour growth [Ryzhova G.L. et al.1997]. They found out that birch bark contains betulin, a compound that is used in the treatment of cancer, but in the present form it cannot be safely used by human. Fortunately, chaga absorbs it from the birch and modify it into a form suitable for human consumption. Today, betulin and betulinic acid are studied not only to fight against cancer, but also as antiviral agents in the fight against HIV.

Arata et al. [Sysoeva M.A. et al.2003] found that chaga extracts inhibit viruses of hepatitis C and human immunodeficiency and demonstrate strong antioxidant and immunostimulating activities. They also investigated on animals the ability of chaga aqueous extracts influence on lipid metabolism. It was proved that the extracts of Inonotus obliquus demonstrate powerful antitumour activity and thus can be used for the treatment of cancer. They tried to investigate the effect of continuous intake of chaga aqueous extract on tumour suppressing.

Lee et al.[Shashkina M.Ya. et al.2008] also reported about the antitumor activity of the aqueous extract of chaga and found that it suppressed proliferation and induced apoptosis of different carcinoma cells.

Hong et al. [Gubernatorov V.V. et al. 2017] described the hepatoprotective activity of the chaga aqueous extract against the oxidative liver damage induced by peptide hydroperoxide in the primary cultured rat hepatocyte.

Fan et al. [Sysoeva M.A. et al. 2007] isolated and purified a water-soluble polysaccharide (ISP2a) from this fungus. ISP2a not only exhibited antitumor activity in vivo, but also significantly enhanced the immune response of tumourbearing mice. In addition, ISP2a significantly improved the proliferation of lymphocytes and increased the production of TNF-α. Therefore, this polysaccharide has potential applications as a natural antitumor agent with immunomodulating activity.

Park et al. [Sysoeva M.A. et al. 2008] evaluated the protective effect of water extracts from chaga against oxidative damage of DNA in human lymphocytes. An assessment of oxidative damage was performed using one-cell electrophoresis for DNA fragmentation (comet analysis). Cells previously pre-treated with chaga extract showed more than 40% reduction in DNA fragmentation compared to the positive control (treatment with 100 μmol H2O2). Therefore, chaga extracts provides cell protection against damage of endogenous DNA caused by H2O2.

Chaga can act as an antiviral agent. Tian et al.[Sysoeva M.A. et al. 2007] studied the action of chaga extracts against feline viruses. Using cellular models of feline calicivirosis, they demonstrated that chaga extracts demonstrated in vitro antiviral activities in cell assays and also exhibit low cytotoxicity. A study of the mechanism of action of the extracts showed that the treatment of calicivirosis induces its inhibitory effects directly on viral particles by blocking viral binding and absorption. A manifestation of broad-spectrum antiviral activity against the viruses of feline herpes, panleukopenia and infectious peritonitis, which can contribute to the development of respiratory and gastrointestinal diseases in cats, has been detected. It was assumed that polysaccharides from chaga could be potential broad-spectrum antiviral agents not only against feline viruses, but also possibly against viruses that are dangerous to humans.

Chouet al. [Sharikov A.M. et al. 2010] evaluated the therapeutic effect of polysaccharides from chaga on streptozotocin induced diabetic symptoms and potential mechanism of their action. The influence of chaga on body weight, blood glucose level, damaged pancreatic cells, oxidative stresses, anti-inflammatory cytokines and enzymes that metabolize glucose in the liver was investigated. It was found that administration of chaga extract can restore oxidative indices near normal levels.

The effects of various fractions and components of chaga on the viability and apoptosis of colon cancer cells were also investigated [Taek Joon Yoon et al. 2013]. One component of chaga identified as ergosterol peroxide showed the most effective growth inhibition. This compound demonstrated antitumor activity on the human colorectal cancer cell lines.

Glamočlija et al.[XianbinNing et al. 2014] studied the chemical composition and biological properties of aqueous and ethanol extracts from chaga obtained from Finland, Russia and Thailand. Antioxidant and antimicrobial properties of these extracts were evaluated, and their cytotoxicity was tested on various tumour cell lines. It was found that the extract of chaga contains oxalic acid.

The effect of 70% ethanol extract of the fungus on food allergy was examined [Liuping Fan et al. 2012]. This extract significantly reduced allergy symptoms.

Ma et al. [YooKyoung Park et al. 2004] studied the influence of various drying methods (sublimation, convection and vacuum drying) on the physicochemical and antioxidant properties of polysaccharides isolated from chaga. The results showed that these characteristics differ significantly depending on the drying method. Compared to convective and vacuum drying, freeze drying resulted in a lower molecular weight distribution of polysaccharides, which makes it possible to increase the efficiency of their use for medical purposes.

Zheng et al. [Satoru Arata et al. 2016] tried to obtain phenolic compounds from chaga by fermentation. The process was carried out in a continuously stirred reactor to find out how it accumulates phenolic compounds in different cultural media and whether these compounds have antioxidant activity. The phenolic compounds produced by this fungus in the control medium included melanins, flavonoids, polyphenols and small phenols. It was found that their accumulation was influenced by the addition of H2O2 into the medium, which resulted in an increase in the content of total intracellular phenols and melanins, changes in the content of extracellular phenols were less pronounced. 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 concluded that the production of phenolic compounds by chaga fermentation is enhanced by the introduction of an oxidizer, which allows using this fungus as a reliable source of pharmaceutically important phenolic compounds.

Melanin is one of the most important compounds of chaga. It is a phenolic pigment and a powerful antioxidant found in the surface layer of the fungus, which is responsible for its distinctive coloration. Melanin compounds were found in many organisms, including chaga and the human body as well, have photoprotection properties. They absorb harmful light at ultraviolet frequencies and convert it into harmless heat through a process called "ultrafast internal transformation". This process allows melanin to scatter 99.9% of the absorbed UV radiation as heat. Melanin compounds are able to protect all types of organisms, including bacteria and fungi, from stresses of solar UV radiation and aggressive free radicals. The influence of melanin on the nervous system makes it one of the most important biological compounds for maintaining mental health and even the development of deeper levels of consciousness and intelligence.

Thus, studies on birch wood-decaying fungus chaga are quite broad, versatile, in view of its vital properties for humans. In this regard, studies to determine the quality characteristics of the fungal materials (pulps, extracts etc.), depending on the terrain and conditions of growth, are topical for timber manufacturers.

MATERIALS AND METHODS

Four samples of fungus chaga taken from two regions of the Eurasian continent, i.e. from the forests of the Czech Republic and the forests of Western Siberia of the Russian Federation. These samples were used for the study of the content of valuable components in fungus chaga, depending on the terrain and conditions of growth.

A classical method of infusion was used to obtain aqueous extracts of chaga. Distilled water was poured into a flask with chopped chaga in a ratio of 1: 6 m/v and put in a chamber for 5 hours at a constant temperature of 70 ° C. Then the extract was separated from the solids, which is then filled with distilled water in a ratio of 1: 4 m/v and extracted for 5 hours at the same constant temperature [Sysoeva M.A. et al. 2008]. Thus, the extraction was carried out in two stages for more complete isolation of valuable substances. The extracts of the first and second stages were combined and further filtered, followed by isolation of melanin from them (Fig.1).

Figure 1. Samples of milled chaga and dried extract from it.

The composition analysis of the fungal preparations was carried out by a quantitative evaluation of melanin content, since this compound is one of the most valuable components of this fungus currently in demand, providing therapeutic activity of the preparations.

The powder samples were used for FTIR spectroscopic analysis. FTIR spectra of KBr pellets containing samples (spectral region of 4000 – 400 cm-1, 64 scans, resolution 2 cm-1) were recorded with FT-IR spectrometer Nicolet 6700 (ThermoScientific, USA). The spectra were exported in CSV format into Origin 6.0 (Microcal Origin, USA) software for preparation of graphical outputs.

Results and discussion

The contents of melanin in four samples of chaga from West Siberian (Russian) and Central European (Czech) forests are represented in Table 1. Obtained results confirmed that the content of melanin in West Siberian chaga was about 1,35 times higher than that in Central European chaga. These results could be explained by a slower growth of the fungus in more severe Russian conditions and, thereby, a longer consumption on birch substrate and accumulation of secondary metabolites including melanin.

Table 1. Yields of melanin in water extracts from chaga obtained from West Siberian and Central European forests

Origin	**No**	**m (g) of raw chaga**	**Extracts**	**V (ml)**	**Yield of melanin**	
					m (g)	**% m/m**
West Siberia	1	10	1st	25	0.4145	4.14
			2nd	32	0.1998	2.00
			Sum	57	0.6143	6.14
	2	10	1st	34	0.3997	4.00
			2nd	31	0.2243	2.24
			Sum	65	0.6240	6.24
	3	10	1st	36	0.5112	5.11
			2nd	32	0.1821	1.82
			Sum	68	0.6933	6.90
	4	10	1st	36	0.4765	4.77
			2nd	31	0.2179	2.18
			Sum	37	0.6944	7.00
		Mean±SD		56.75±0.275	0.6565±0.1	6.57±0.1
Central Europe	1	10	1st	25	0.2741	2.74
			2nd	32	0.1704	1.70
			Sum	57	0.4494	4.49
	2	10	1st	34	0.3105	3.10
			2nd	31	0.1449	1.45
			Sum	65	0.4549	4.55
	3	10	1st	36	0.2990	2.99
			2nd	32	0.2297	2.30
			Sum	68	0.4997	5.00
	4	10	1st	36	0.3070	3.07
			2nd	31	0.1047	1.05
			Sum	37	0.4117	4.12
		Mean±SD		56.75±0.275	0.4861±0.2	4.86±0.01

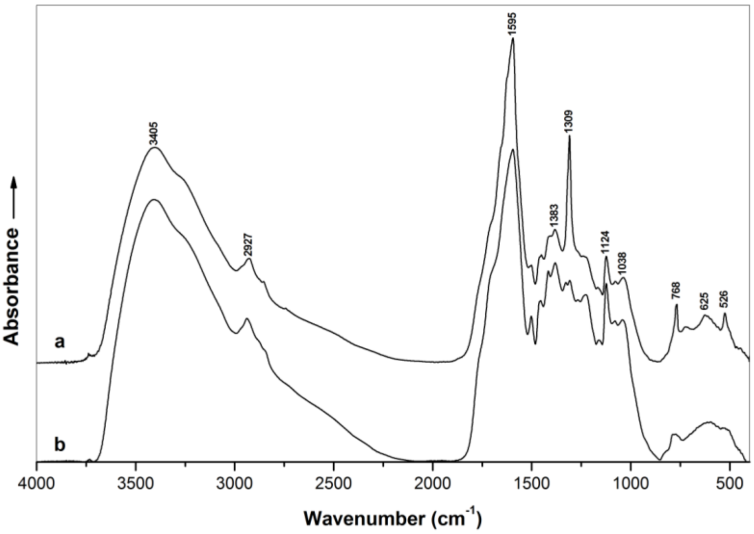

Figure 2. FTIR spectra of dried water extracts obtained from West Siberian (a) and Central European (b) chaga

FTIR spectra of two dried water extracts from West Siberian and Central European chaga samples are shown in Fig. 2. The spectral differences reflect specificities of polyphenoloxicarbonic complex and associated huminic acids according to the number of carboxylic (-COOH), methoxylic (-OCH3) and hydroxylic (-OH) groups. A broad band centred at 3405 cm-1 was found in both spectra. This band was assigned to OH stretching vibration in water and hydroxyls. Weak bands near 2927 and 2854 cm-1 arose from antisymmetric and symmetric vibrations of CH2 groups. The other CH stretching vibrations are located in the region of 2800-3000 cm-1. A very strong band near 1595 cm-1 was assigned to C=C stretching in aromatic compounds. This band was more intense and narrower for West Siberian chaga. Several shoulders at higher wavenumbers corresponded to C=O stretching in ketones or carboxylic groups and their derivatives (amides, esters etc.). The scissoring vibration of CH2 groups was found as a shoulder near 1460 cm-1 overlapped by another band at 1452 cm-1. The latter band has a contribution from antisymmetric C-H bending in OCH3 groups. Several strong to medium bands at 1309, 768 and 526 cm-1 were found only in the spectrum of the extract from West Siberian chaga. The two latter bands were assigned to all-in-phase out-of-plane =CH bending and out-of-plane deformation of aromatic ring; the former band was assigned to COC vibration of aromatic esters. By contrast, the bands at 1502, 1416, 1383, 1227 and 1124 cm-1 were more pronounced for the extract from Central European chaga. The bands at 1124 and 1227 cm-1 was assigned to COC stretching in saturated ethers and esters, respectively. Two bands at 1038 and 1080 cm-1 arose from CO and CC stretching vibrations in polyphenols. Therefore, significant difference in characteristic spectral bands confirmed that the composition of chaga extracts is highly influenced by the location and climatic conditions of growing, and severe conditions lead to increased accumulation of bioactive pigments (polyphenols, melanins).

Conclusion

As a result of many studies it was found that the therapeutic effect of Chaga preparations is due to the presence of melanin in their composition. Therefore, one of the most important characteristics of this fungus is the content of melanin and other pigments. In this connection, a comparative analysis of the melanin content was carried out in mushrooms growing in Central Europe and Western Siberia, which showed an increase in the yield by an average of 1.35 times of the desired substance from fungi that grew under more severe conditions in Russia.

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