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Study of the Composition of Biologically Active Compounds in Chaga Meal. Perspectives of Application of Chaga Meal in Pharmaceutical Industry

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Abstract—Methods of utilization of chaga meal are reviewed. Extraction of chaga meal with ethanol, chloroform, and *tert*-butyl methyl ether gives compounds possessing a high antioxidant activity. A wide spectrum of other biologically active compounds was found in these extracts. These compounds can be used for production of drugs and biologically active additives.

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INTRODUCTION

Aqueous extract from the chaga fungus (*Inonotus obliquus*) and drugs on their basis are widely used in traditional and official medicine for prevention and therapy of gastroenteric and oncologic diseases [1–6]. Compounds contained in chaga exhibit a broad-spectrum biological activity. They possess antioxidant, radioprotector, gene protector, adaptogen, immunomodulating, antiviral, and antioxidant properties and control activity of blood enzymes and functioning of the cardiovascular, nervous, and respiratory systems [6, 11–20].

At present the chaga fungus and preparations on its basis attract a great interest [7–10]. In this connection, a problem has arisen of rational use of chaga raw material, even though the distribution area of this birch fungus is quite extensive [21–25].

Natural renewal of the raw material is a fairly long process (5–7 years) [26, 27], whereas a biotechnologically cultured chaga differs from natural in the composition of aqueous extracts and the nature of the principal component [27–37].

About half of the pharmaceutical companies in Russia produce preparations from chaga. In particular, Befungin is produced at such major pharmaceutical factories as Marbiofarm, Tomskkhimfarm, Vifiteks, Tatkhimfarmpreparaty OAO, Tver pharmaceutical factory, and Yaroslavl pharmaceutical factory [38]. Befungin is standardized by the content of chaga chromogens (melanin) [39].

The initial stage of the technology of production of chaga preparations, such as таких как Befungin and such biologically active additives as Chagovit and Chagolux, and some others, involves aqueous extraction from the raw material of the most part of biologically active compounds [39]. The principal waste product of this technology is meal which is further not used. The aqueous extract from chaga is a hydrophilic colloid system. Its dispersion phase and therapeutically active components are chromogens [11, 40, 41] or the so-called polyphenol hydroxycarboxylic complex [42–45]. Chaga chromogens are classed with melanins [46–48]. Structurally, melanins are granules comprising amorphous microparticles (20 nm) which are organized into aggregates and subaggregates of different shapes and sizes. Such molecular structure predetermines the biochemical and biological properties of melanins [49–51].

Water extracts from chaga 17–30% of compounds [52]. Therewith, the fraction of melanin in the aqueous

Table 1. Composition and antioxidant activity of the aqueous extract and alcoholic tinctures of chaga

System	Dry substances, g/100 ml	Carbohydrates, mg ml^{-1}	Phenolic compounds, mg ml^{-1}	Melanins, g/100 ml	Antioxidant activity of the extract, Cl ml^{-1} [a]
Aqueous extract	2.1336	2.80	0.470	1.690	1.80
Alcoholic tincture					
Producer "Moscow Pharmaceutical Factory" OAO	0.629	0.45	0.044	0.29	1.36
Producer "Fitofarm-NN"	0.533	0.72	0.060	0.28	2.18

[a] The antioxidant activity was measured by coulometric titration with electrically generated bromine [9].

extract is 9–16% [52–55]. It was shown that by extracting chaga meal one can obtain an additional amount of biologically active compounds, including melanin. In this connection, quite an important problem arises to develop procedures for extraction of biologically active compounds from the meal, which would favor the most effective use of the raw material (chaga).

In what follows we present the research on extraction of chaga meal, whose results can be used for developing a technology for production of pharmaceutica preparations from this secondary raw material.

Extraction of Biologically Active Compounds from Chaga Meal

Chaga meal look like a loose grit with the particle size of 2–10 mm. The meal as the waste product at pharmaceutical factories is suitable for processing for a maximum of 7 days. Then oxidation processes and microflora develop, which leads to microbiological damage of the material [55]. Therefore, the meal belonging to a single commercial batch is difficult to conserve. In our study we used the laboratory meal samples obtained by a technology analogous to the commercial technology.

The solvents used for extraction of chaga meal were ethanol, chloroform, and methyl *tert*-butyl ether. Ethanol extracts both hydrophilic and hydrophobic compounds; chloroform extracts lipophilic compounds; methyl *tert*-butyl ether completely extracts phenolic compounds [56, 57].

To assess the quality of chaga meal to find out whether it is a useful source of biologically active compounds, we studied the compositions of the aqueous extract from chaga and commercial alcoholic tinctures (Table 1).

As seen from Table 1, water extracts much more compounds than alcohol. The aqueous extract contains an order-of-magnitude more phenols and about 6 times more melanins.

Extraction with Aqueous Ethanol

Two procedures were used for obtaining alcoholic extracts from chaga meal: infusing and refluxing in ethanol.

The extraction of chaga meal by infusion in aqueous ethanol was performed at 70°C in two stages [58]. At an optimal alcohol concentration (50%), up to 17% of dry substances are extracted, and the ash content of the extracts is about 5% of that of the aqueous extracts from the raw material (chaga) [58, 59]. In terms of the carbohydrate content, ethanolic extracts from chaga meal rank only slightly below than commercial alcoholic tinctures of chaga and are double as rich in phenolic compounds. The melanin content of the ethanolic extract from chaga meal (6.8%), too, is higher compared to commercial tinctures.

The antioxidant activity of the alcoholic extract from chaga meal is 1.6 Cl ml^{-1}, which is generally lower than that of the aqueous extracts and compares with that of the alcoholic extracts from chaga. The antioxidant activity of melanin isolated from the alcoholic extract (67 Cl/100 g melanin) is double as high as that of melanin contained in the aqueous extract from chaga.

By treatment with aqueous ethanol of chaga meal one can additionally extract 6.8% of melanin, 23–27% of phenolic compounds, and 8–22% of carbohydrates. Taking into account the content of melanin and its activity in the alcoholic extract, for preparing melanin-based drugs it makes sense to isolate melanin from the extract, rather than to use the extract itself [60, 61].

The meal was also extracted by refluxing in aqueous ethanol (30, 50, and 70% solutions) for 2 h [60–62]. It was found that as the alcohol concentration in the extract decreases, the contents of the dry residue, ash, and melanin decrease, whereas the contents of cabohydrates and phenolic compounds increase. Thus, the optimal concentration of ethanol for the extraction of chaga meal by this procedure is 70%. Such solutions extract from the meal up to 15% of dry substances and increase the contents of phenolic compounds in the extracts 3−4 times. The content of melanin in the alcoholic extract is 5 times lower than in the aqueous extract from chaga and about 2 times lower than in the alcoholic extracts from the meal, obtained by infusion.

The antioxidant activity of the alcoholic extracts from chaga meal (3.92 Cl ml^{-1}) [63] is 1.5 times higher compared to the aqueous extracts from chaga and almost double as high as that of the alcoholic tincture of the meal. These results are well consistent with the data in [28, 64], which showed that the antioxidant activity of the alcoholic extracts from chaga, obtained under reflux, is more contributed by phenols rather than melanins.

Refluxing chaga meal in 70% aqueous ethanol makes it possible to additionally extract 2.7% of melanin, 75% of phenolic compounds, and 26% of carbohydrates [60, 61]. The content of phenolic compounds in the alcoholic extract from chaga meal, obtained under reflux (0.351 mg ml^{-1}) is 6 times higher than their content in commercial chaga tinctures, and the carbohydrate content is at the same level (0.585 mg ml^{-1}).

The antioxidant capacity of melanin in the alcohol extract obtained under reflux is double as high as that of melanin in the aqueous extract from chaga and compares with that of melanin isolated from the alcoholic extract obtained by infusion. Compared with commercial tinctures, the antioxidant activity of the alcoholic extracts from chaga meal is, on average, double as high. Thus, we can conclude that the 70% alcoholic extract from chaga meal, obtained under reflux, is a promising starting material for drugs and biologically active additives.

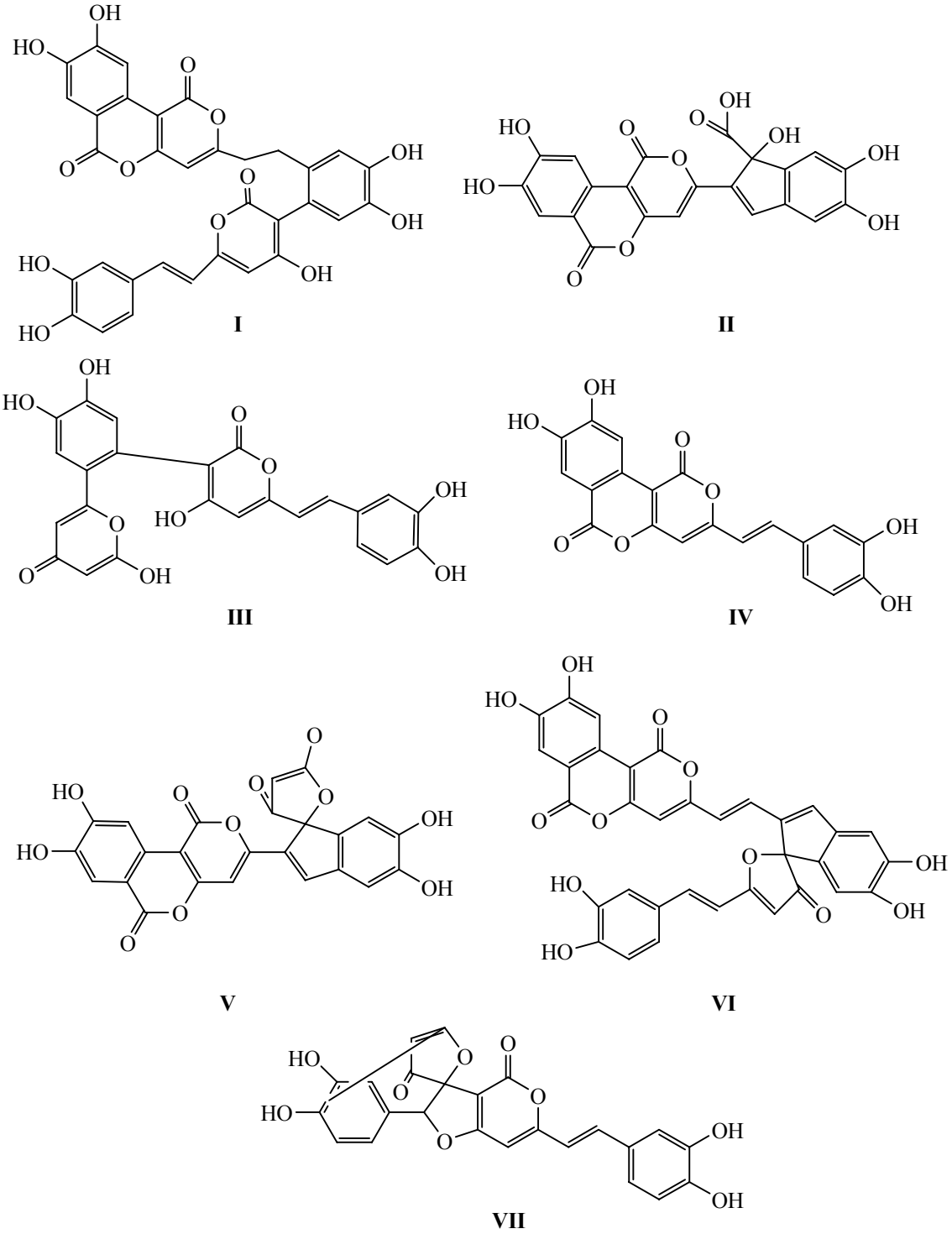
To gain insight into the reasons for the high antioxidant activity of the alcoholi extract from chaga meal, we determined the particle size of melanin and measured IR and electronic absorption spectra. It was shown than the size of the melanin particles isolated from alcoholic extracts of the meal and from aqueous extracts from chaga falls in the nano range [65, 66]. However, if the effective radius of the melanin particles isolated from the aqueous chaga extract is 75 nm, then the melanin particles isolated from the alcoholic extract from chaga meal are much larger, R_{eff} 225 and 112 nm. According to recent findings [67–72], the antioxidant activity of melanin isolated from the aqueous extract from chaga increases as the particle size in the disperse phase decreases. This is probably explained by the fact that increased size of the melanin particles isolated from the alcoholic extracts of chaga meal favor formation of more active centers due to the effect of the dispersion medium containing ethanol [71]. The IR and electronic absorption spectra of melanin isolated from the alcoholic extract of chaga meal are anal;ogous to those of melanin isolated from the aqueous extract from chaga, implying similar molecular organizations [61].

The antioxidant activity of the material precipitated during distillation of ethanol from the extract is fairly high (20 Cl/100 g melanin). Apparently, the precipitate is a mixture of compounds. This mixture was impossible to separate into phenolic compounds and carbohydrates by direct chromatography of the precipitate. The chemical composition of the precipitate was determined after its hydrolysis in 15% HCl followed by column chromatography (preliminarily we removed hemicelluloses whose content in the precipitate was 6% [61].)

It was found that the hydrolysate contains almost equal amounts of glucose, xylose, and arabinose. This finding provides evidence showing that the alcoholic extract from chaga meal contains, apart from hemicelluloses, higher polysaccharides, like xylanes, glucanes, and arabinogalactons which were previously detected [73, 74] in the aqueous and alcoholic extracts from chaga. Paper chromatography of the hydrolysate revealed such flavonoids as apigenin, quercetine, naringenin, and robinetin [75]. Previously these compounds were detected in the aqueous extract from chaga [76], and apigenin was found in aqueous extracts [77]. Furthermore, chromatography [78] and IR spectroscopy of the precipitate revealed a large fraction of aromatic acids (hydroxybenzoic, vanillic, and syringic), in agreement with published data [60]. These compounds should contribute much into the oxidant activity of the alcoholic extracts from chaga meal.

The separation of the mixture of compounds in the precipitate by successive extraction with organic solvents of increasing polarity [79] and subsequent analysis (HPLC, paper and thin-layer chromatography,

and IR and electronic absorption spectroscopy) revealed seven terpenes (structures **I**–**VII**), eleven lactons and their glycosides (two of which are stereoisomers), two carboxylic acids, a little of polar lipids, as well as analogs of hispidin [6-(3,4-dihydroxystyrl)-4-hydroxy-2-pyrone] and their glycosides, for example, inobelins and phelligridins which were found in chaga previously [80].



I

II

III

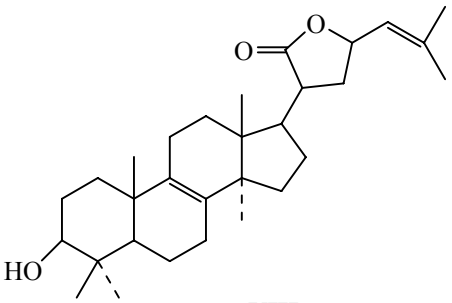
IV

V

VI

VII

The principal component of the precipitate is structurally similar to compound **VIII** [61].



VIII

The components of the precipitate could not be fully separated by the methods in hand. On this basis we can conclude that the material precipitated duding distillation of ethanol from the extract is formed by melanin particles. This part of the dispersion phase of the colloid system of the alcoholic extract is unstable and precipitates, when ethanol has been is removed. Actually, the R_{eff} of particles in the precipitate remaining after consecutive organic extractions is 104 nm against R_{eff} = 163 nm in the starting precipitate [71].

It was found that the contents of melanin, carbohydrates, and phenols in the alcoholic extracts from the meal remaining after processing of different batches of the raw material (chaga) vary only slightly. This circumstance is an important prerequisite for the development of production technologies for drugs on the basis of alcoholic extracts and melanins from chaga meal.

Extraction with Chloroform

Extraction with chloroform under reflux for 1 h allows one to additionally extract from chaga meal up to 0.12% of dry substances. Paper chromatography revealed in the chloroform extracts a broad spectrum of phenolic compounds, which explains the high antioxidant activity of such extracts (3.41 Cl ml^{-1}) [81]. The chloroform extract from the meal is 1.5 times more active than the commercial chaga tincture and double as active as the aqueous extract from chaga.

The chromatograms and IR and electronic absorption spectra of the chloroform extract from chaga meal established the presence of neutral and polar lipids, lactones, and terpenes (di-, tri-, and tetraterpenes) [61]. Furthermore, HPLC relealed an individual compounds (98%) structurally similar to compound **VIII** and ~2% of a structurally different lactone [61].

Extraction of Methyl tert-Butyl Ether

Methyl *tert*-butyl ether is used for extraction of simple phenols from natural matrices. Compared with other aliphatic ethers, this solvent possesses a higher extractive power and does not form peroxides [56, 82, 83]. We resorted to methyl *tert*-butyl ether extraction in view of the fact that the alcoholic extracts from chaga meal contain much phenolic compounds.

Using this solvent, we could extract from the raw material additionally up to 0.8% of dry substances. The absorption spectrum showed that the extract contains a complex mixture of compounds. Chromatography revealed in the ethereal extract a lot of phenols (phenolcarboxylicacids), terpenes (tri-, tetra, and sesquiterpenes), and iridoids (iridoid glycosides), as well as a little of polar lipids [61]. However, while containing a great diversity of compounds, the extract exhibits a low antioxidant activity (Table 2). This fact can be explained by a low content of extractive compounds in this extract.

Table 2. Yield of extractive substances and antioxidant activity of chaga meal extracts

Compounds	Content of extractive substances		Antioxidant activity, Cl ml^{-1}
	g/100 ml	%	
Alcooholic extract from chaga meal (infusion)	0.3640	7.34	1.06
Alcooholic extract from chaga meal (reflux)	0.1970	3.92	3.92
Chlorofom extract from chaga meal	0.0058	0.12	3.41
Methyl *tert*-butyl extract from chaga meal	0.0041	0.08	0.09
Commercial tinctures	0.6290	–	2.18
Befungin	1.5100	–	2.66
Aqueous extract from chaga	2.1336	21.34	1.80

Extraction of Impoverished Chaga Meals

The extraction of chaga with aqueous organic solvents (with the organic contents varying from 0.1 to 1.0%) makes it possible to extract much more extractive substances: up to 18% of dry substances and 14.2% of melanin. [86, 87]. Water extracts from the same raw material 15% of dry substances and 11.7% of melanin.

Aqueous organic extraction of chaga leads to a meal impoverished in extractive compounds and changes its color and moisture absorption. At the same time, such meal can be stored up to two weeks, undergoing no microbiological decay and drying. The antioxidant activity of the aqueous organic extract from chaga is 7.97 Cl ml^{-1}, and the activity of melanin from such extracts is 52 kCl/(100 g melanin) [86–88].

For the most efficient extraction from the impoverished chaga meal, we used 70% ethanol. The content of extractive compounds in the alcoholic extracts is lower by 5–10% and the melanin content is higher by 10% compared with the alcoholic extract of the meal obtained by aqueous extraction of the raw material [89–91]. The antioxidant activity of the alcoholic extract from the impoverished meal is 1.31 Cl ml^{-1}, i.e. it compares with that of commercial alcoholic tinctures. The antioxidant activity of melanin is 28.8 kCl/100 g melanin, which compares with the activity of melanin isolated from the aqueous extract from chaga [90, 91].

Treatment of the impoverished chaga meal with 70% ethanol allowed additional extraction of 4.36% melanin. Based on the high activity of the alcoholic extract, as well as the high yield and activity of melanin from this extract we conclude that both the extract and melanin are suitable starting materials for drug production.

CONCLUSIONS

Chaga meal can be used as a secondary raw material for drugs or biologically active additives.

The most promising substances in terms of the development of potent antioxidant drugs are melanin isolated by infusion of a 50% aqueous alcoholic extract from chaga meal, 70% alcoholic extract obtained under reflux, and chloroform extract. These samples are superior in the antioxidant activity to alcoholic tinctures of chaga and Befungin. The melanins isolated from the aqueous chaga extract and from the alcoholic meal extract, obtained by infusion can be combined. Such a melanin-based drug can be used in the therapy of oncological diseases, radiation disease, atherosclerosis, and other pathologies induced by free radicals [60, 84, 85].

The chloroform and ethereal extract can be used for preparing drugs and biologically active additives with a target-specific effect associated with the presence of such biologically active compounds as terpenes, lactones, phenols, and lipids.

The suggested scheme of complex processing of chaga can be applied not only for utilization of chaga meal, a waste product in the process of production of traditional chaga-derived drugs, but also for innovative development of highly efficient antioxidant drugs. The complex processing of the chaga birch fungus will ensure the rational use of the natural raw material due to the maximal extraction from it of biologically active compounds and will serve for extending the range of phyto preparations with diverse therapeutic activity. This will favor reduced production costs due to their distribution for the production of several preparations.

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