Assessment of antimicrobial and antioxidant components of Inonotus obliquus extract as a food ingredient

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Abstract. Inonotus Obliquus or Tinder fungus is widely used in folk medicine for the treatment of gastrointestinal diseases, for the prevention of cancer. It is widely known to be rich in polyphenolic substances. This study was designed to determine antioxidant and antibacterial potential. The objects of research are the aqueous extracts of chaga obtained by remaceration and microwave radiation in concentrations of 5, 50, 100, 200 g / dm³. It has been proven that aqueous extracts obtained using microwave radiation have a more pronounced antioxidant and antimicrobial effect in comparison with samples obtained only by remaceration.

1. Introduction
The composition of plant and natural objects contains various chemical substances that play an important role in their life support [1]. In recent years, much attention has been paid to polyphenols, including flavonoids, anthocyanins, and tannins. Research is being conducted on the health benefits of these substances and their important role in disease prevention in many studies [2–4]. Their positive impact was noted. This is due to their high antioxidant properties. In addition, polyphenols have a pronounced antimicrobial activity [5], which makes them a good alternative to some food preservatives.

Inonotus obliquus (I. obliquus) belongs to the Hymenochaetaceae family and is a black-brown plant-type parasitic fungus [6]. Known among the common people under the name of the mushroom "chaga". The genus Inonotus is widespread in North America, Asia and Europe and has about 100 species. I. obliquus is the primary tree parasite that causes decomposition of living stems. It can be found on many tree species, such as alder, ash, maple, mountain ash, poplar, oak, willow, chestnut and walnut, but mainly I. obliquus is found on birches [7].

There are studies that the melanin complex obtained from Inonotus obliquus contains a strong antioxidant and exhibits gene-protective activity [8]. In addition, it has been shown that it protects against the effects of gamma radiation [9] and increases catalase activity in HeLa S3 tumor cells. Mushrooms usually contain a wide range of molecules that scavenge free radicals, such as polysaccharides and polyphenols [10]. It was found that the composition of chaga includes water-soluble pigments called melamins, as well as some organic acids, polysaccharides, flavones, a significant part of mineral compounds, vitamins, etc. It is this complex of substances that is responsible for the medicinal properties of chaga.
Studies have found that chaga has a noticeable antitumor, anti-inflammatory and immunostimulating effect. It has been proven that preparations from chaga help lower blood sugar and can be effectively used to prevent various forms of diabetes.

Chaga is usually harvested in early spring and late autumn - in summer it is simply not visible among the foliage, and in winter, snow interferes with harvesting. Chaga outgrowths are cut from the trunk, cleaned of bark and wood residues, chopped into small pieces and dried at 40-50 °C [7].

This study evaluated the antioxidant activity of extracts obtained from chaga by remaceration and using microwave radiation and compared that of dihydroquercetin. This study aims to assess the antioxidant and antimicrobial activity of chaga.

2. Materials and methods

2.1. Materials and preparation of plant extracts

For this study, a patented method of obtaining aqueous extracts of chaga by maceration with microwave treatment was chosen, which made it possible to reduce the duration of the extraction process of chaga by 3.5 times compared to obtaining aqueous extracts of chaga by remaceration [11].

The study used dry crushed chaga from FITOFARM LLC, purchased from a pharmacy. The extracts were prepared by adding sterile distilled water to dry crushed raw materials (chaga), covered with cling film, and treated for 2 min using microwave radiation (180 W, 2450 MHz). Then the extracts were infused for 3 h at 105 °C. Then the extract was filtered and filtered through a CHROMAFIL Xtra pa-20/25 sterilizing filter. The extracts were prepared in the following concentrations:

- extract E5MWR - extract with a concentration of plant materials 5 g / dm³;
- extract E50MWR - extract with a concentration of plant materials 50 g / dm³;
- extract E100MWR - extract with a concentration of plant materials 100 g / dm³;
- extract E200MWR - concentrated extract with a concentration of 200 g / dm³.

Obtaining a control sample, the mushroom extract was carried out by the remaceration method. The remaceration process is carried out in two stages of infusion at a temperature of 70 °C; at the first stage, the ratio of raw materials and extractant is 1: 6, at the second 1: 4. The total extraction time is 10 hours. Distilled sterile water acts as an extractant. The extracts were prepared in the following concentrations:

- extract E5 - extract with a concentration of plant materials 5 g / dm³;
- extract E50 - extract with a concentration of plant materials 50 g / dm³;
- extract E100 - extract with a concentration of plant materials 100 g / dm³;
- extract E200 - concentrated extract with a concentration of 200 g / dm³.

2.2 Preparation of culture of microorganisms

The bacterial strains used in this study were S. aureus (NCIMB 8625), Enterococcus spp, V. Parahaemolyticus, E. coli (ATCC25922) and Proteus spp. All bacterial strains were grown and maintained on nutrient agar slants. Fresh bacterial cultures were obtained by reseeding the original bacterial cultures on freshly prepared nutrient agar and incubating at 37 °C for 24 hours prior to testing.

2.3 Determination of antibacterial activity

The antibacterial activity of extracts of different concentrations was assessed by diffusion in agar [12] against five bacterial strains. Bacterial strains were first grown on nutrient agar for 24 hours at 37 °C before use to achieve a stationary phase. Then, the bacterial cell suspension (10⁶ CFU / ml) was spread into Petri dishes (using sterile swabs) containing Mueller-Hinton agar. Discs (3 mm in diameter) were applied to the surface of the inoculated agar and soaked in 50 microliters of various concentrations of the plant extract. Standard chemical food preservatives such as sodium benzoate and potassium sorbate were used as controls for antimicrobial activity. The concentration of the solutions was 1 g / ml. Control disks were moistened with sodium benzoate and potassium sorbate solutions. All Petri dishes were preincubated in a refrigerator (4 °C) for 1 hour to ensure complete diffusion of the samples, and then incubated at 37 °C for 24 hours.
After incubation, antibacterial activity was determined by measuring the diameter of the zone of inhibition in millimeters (mm).

2.4 Measurement of antioxidant activity
Antioxidant activity was measured using the DPPH method. The antioxidant activity of the samples was measured in accordance with the method [13]. The technique is based on the ability of antioxidants of the feedstock to bind the stable chromogen radical 2,2-diphenyl-1-picrylhydrozyl (DPPH). DPPH (4 mg) was dissolved in 100 ml ethanol. Aliquots of the test extract were dissolved in 100 ml of distilled water. Then 2.0 ml of each solution was added to 2.0 ml of DPPH solution at 20 °C and kept in the dark for 30 minutes. Determined the transmittance at 517 nm.

All experiments were carried out in three repetitions.

3. Results and discussion

3.1 Antioxidant activity
Due to unfavorable environmental factors, such as exhaust gases from cars, waste emissions from enterprises, changing climatic conditions, irreversible changes can occur in the human body. Eating foods fortified with functional ingredients have been shown to provide preventive health benefits and may reduce the risk of disease. The risk of some diseases is reduced due to the presence of antioxidants in plants and natural objects [14]. The human body naturally produces free radicals to counteract these harmful effects. However, in most cases, the levels of free radicals are much higher than the levels of natural antioxidants.

The results of determining the total content of phenolic substances are shown in Figure 1. Based on the data on the content of phenolic substances, it is possible to isolate into extracts with high rates. Extracts of the following concentration and method of preparation (see Figure 1) are (in mg of gallic acid per 100 g of substance): E200MWR (552), E200 (403), E100MWR (407). Extracts with average values include E100 (298), E50MWR (122). Extracts in E50 (78) and E5 MWR (57) and E5 (21) have low values for the total phenolic index.

![Figure 1. The content of phenolic substances in chaga extracts.](image-url)
The effective role of antioxidants is in their interaction with free oxidative radicals. During the DPPH process, antioxidants react with stable free radicals α, α-diphenyl-β-picyrylhydrayzyl and gradually change or adapt them for the production of DPPH derivatives, which leads to a color change [15]. The results obtained showed that the aqueous extraction of chaga obtained in two ways neutralized the free radicals present in the solution. Differences in antioxidant concentrations in different extracts prepared by different methods were compared with the concentration of dihydroquercetin, one of the main natural antioxidants in plants, widely used as a standard measure for determining antioxidant values. The percentage of antioxidant capacity was measured to determine the absorption activity. However, this value could include all types of antioxidants present in the sample, so the change in antioxidant properties could not be accurately measured [16].

### 3.2 Antimicrobial activity

The zones of inhibition of aqueous extracts of chaga, obtained in different ways against five microorganisms of food spoilage, were determined using the analysis of diffusion in agar [15].

The chemical preservatives sodium benzoate and potassium sorbate were used to control the effect of the chaga extract. After 24 hours of incubation, the diameters of the zones of inhibition were measured for each type of extract (Table 1).

| Processing type | Extract concentration (g/dm³) | S. aureus (mm) | V. parahaemolyticus (mm) | Enterococcus spp (mm) | Proteus spp (mm) | E. coli (mm) |
|----------------|-------------------------------|----------------|--------------------------|----------------------|-----------------|-------------|
| Remaceration   | 5                             | -              | -                        | -                    | -               | -           |
|                | 50                            | 3 ± 0.5        | -                        | -                    | 3.5 ± 0.7       | 3 ± 0.4     |
|                | 100                           | 5 ± 0.5        | 4.7 ± 0.5                | 4 ± 0.5              | 3.6 ± 0.4       | 4 ± 0.5     |
|                | 200                           | 5 ± 0.15       | 5 ± 0.12                 | 5 ± 0.17             | 3.6 ± 0.4       | 4.8 ± 0.2   |
| Maceration + microwave radiation | 5                             | -              | -                        | -                    | -               | -           |
|                | 50                            | 3 ± 0.6        | 4.5 ± 0.5                | 4.1 ± 0.1            | 3.9 ± 0.1       | 4 ± 0.2     |
|                | 100                           | 10 ± 0.8       | 7 ± 0.3                  | 9.5 ± 0.5            | 5.5 ± 0.15      | 12.5 ± 0.5  |
|                | 200                           | 12 ± 0.4       | 9 ± 0.3                  | 10.3 ± 0.3           | 6.5 ± 0.5       | 13.7 ± 0.3  |
| Potassium sorbate (control) | 1                             | 30 ± 0.5       | 32 ± 0.6                 | 35 ± 0.5             | 32 ± 0.8        | 40 ± 0.5    |
| Sodium benzoate (control) | 1                             | 33 ± 0.3       | 30 ± 0.4                 | 35 ± 0.5             | 28 ± 0.17       | 32 ± 1.5    |

It is supposed to use the obtained aqueous extract of chaga as a food ingredient for fortification of food products. The aim of the work was also to reveal the ability of the extract to act as a preservative of natural origin to replace chemical preservatives. A certain native microflora is characteristic of different types of food products. It was for this purpose that certain spoilage microorganisms were used.

According to the results obtained, it can be seen that the aqueous extracts of chaga obtained by remaceration and by remaceration and subsequent treatment with microwave radiation at a concentration of 5 g/dm³ did not show any activity in relation to microorganisms. At the same time, all extracts with a concentration of 50 to 200 g/dm³ showed antimicrobial activity. The activity of extracts obtained by remaceration without microwave radiation treatment in relation to microorganisms is much less than the same extracts obtained using the same radiation. However, these data are significantly inferior to the standard widespread chemical preservatives acting as control samples.

Thus, the use of ultrahigh frequency radiation increases the content of phenolic substances by 1.36 times, and also significantly increases the activity against microflora. This can be explained by the difference in the arrangement of melanins in the resulting objects. When microwaves act on the colloidal system of
aqueous extracts of chaga, the protein-polysaccharide component of melanin can undergo various changes, which leads to a change in the size of melanin particles and their content in aqueous extracts.

4. Conclusion
In this study, the antioxidant and antimicrobial properties of aqueous extracts of Inonotus obliquus obtained by remaceration and using microwave radiation were evaluated. It was determined that antibacterial activity at low concentrations of the extract was not observed, while with an increase in the concentration of the extract, their antimicrobial activity also slightly increases. The greatest value is observed in extracts obtained using microwave radiation. There is also an observation in the determination of antioxidant activity. With an increase in the concentration of the extract, its antioxidant activity also increased. Moreover, for aqueous extracts irradiated with ultrahigh frequencies, this value is 1.36 times higher.

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