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Biological Activity of *Fomes fomentarius*

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Abstract: In this work the antioxidant and antimicrobial activity of the fractions with different polarity, obtained from water-ethanol dry extracts from *Fomes fomentarius*, has been investigated. Fractions of different polarities, obtained from water-alcohol dry extract from *Inonotus obliquus*, were used as comparison samples. It has been shown, that the most phenol-rich (73.571 ± 21.268 mg E/g) ethyl acetate and ethanol (36.343 ± 10.241 mg E/g) fractions from *Fomes fomentarius* demonstrated the highest radical scavenging activity against DPPH (IC_{50}: 515.6 ± 100.5 μg/ml; 751.2 ± 19.3 μg/ml, respectively) and ABTS (IC_{50}: 229.7 ± 9.6 μg/ml; 423.3 ± 0.66 μg/ml, respectively). It has been found, that chloroform, ethyl acetate and ethanol fractions from *Fomes fomentarius* exhibit antimicrobial activity against *Staphylococcus aureus*.

Key words: *Fomes fomentarius*, *Inonotus obliquus*, antioxidant activity, phenolic compounds, antimicrobial activity, fractions, extracts.

1. Introduction

Medicinal products of natural origin are currently considered promising, as they have significant advantages over synthetic drugs. These advantages include a mild effect on the body, a relatively low frequency of side effects, a variety of biologically active substances and effects, the possibility of using both for the prevention and treatment of diseases. A promising area of pharmacognosy is the study of the chemical composition and pharmacological activity of basidiomycetes, in particular, tinder fungi. Among them, a special place is occupied by *Fomes fomentarius* [1], which is of interest to medicine as a source of phenolic compounds with antioxidant activity. For each organism, the problem of maintaining its integrity and qualitative originality in an aggressive oxidizing environment has become extremely important. Therefore, antioxidants, as compounds that maintain resistance to self-oxidation, are widely distributed in natural objects, including in the plant and animal world. Most natural antioxidants are phenolic compounds, which include functionally substituted phenols and polyphenols, flavonoids, tocopherols, and derivatives of hydroxycinnamic acids [2]. The main part of phenolic antioxidants is of plant origin, but they are also indispensable components of the organism of animals and humans, coming mainly from plant foods [2].

The main group of biologically active substances contained in the present tinder fungus include sterols (ergosterol, fungisterol), polysaccharides (lanophil), organic acids (fumaric, ricinoleic, citric and malic), phenylethanediols, phenolcarboxylic acids (p-hydroxybenzoic, protocatechuic, vanillic, gallic, lilac), flavonoids (glycosides of luteolin and dihydroquercetin) and benzotropolones (purpurogallin). Due to the content of benzotropolones, *Fomes fomentarius* is of interest as a potential source of natural antioxidants. And the known data on its antitumor, immunomodulatory and antimicrobial activity, as well as the experience of its use in Japanese and Korean traditional medicine determine the relevance of this study [3].

The purpose of this work is to determine the antioxidant and antimicrobial activity of fractions of different polarity of *Fomes fomentarius*. The tasks of the work include the establishment of the total content of phenolic compounds, radical-inhibiting (in relation to DPPH and ABTS) and antimicrobial activity of

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fractionated extracts of *Fomes fomentarius*.

2. Material and Methods

To obtain dry water-alcohol extracts, the method of circulating Soxhlet extraction was used. Fruit bodies of tinder fungi (substrate - downy birch), collected in September 2021 in Minsk region, Republic of Belarus, and were extracted for 12 hours with 70% (v/v) ethyl alcohol. Next, the solvent was distilled off under vacuum; the extracts were dried at 50 ℃ and stored in a refrigerator for further use. To study the antioxidant and antimicrobial activity, the extracts were subjected to fractionation. To do this, 1 g of the dry extract was dispersed in 50 ml of water, and then successively shaken with 5 portions of 40 ml of petroleum ether (PE), chloroform (CL), ethyl acetate (EA). The remaining solution was filtered: the filtrate was an aqueous fraction (H_2O) and the precipitate was dissolved in 96% (v/v) ethanol (EtOH-fraction). The organic fractions were separated, dehydrated with anhydrous sodium sulfate, and the solvent was distilled off under vacuum. Figure 1 shows circle charts showing the mass distribution (in %) of dry substances isolated from samples between fractions (PE, CL, EA, H_2O, EtOH).

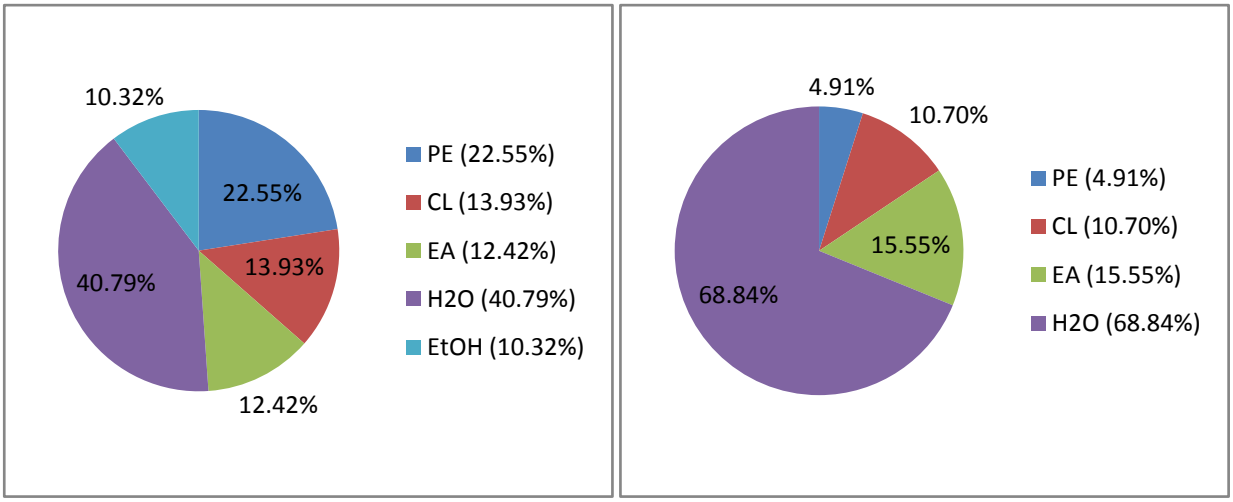

Fig. 1 Distribution between the fractions of dry substances isolated from *Fomes fomentarius* (left) and I*nonotus obliquus* (right), by weight (in%).

The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent [4]. The calibration graph was built using standard solutions of gallic acid. The total phenolic content (TPC) was expressed as the equivalent content of gallic acid (mg) per unit mass of dry extract (g).

The radical-inhibiting activity of the fractions was evaluated by their ability to inhibit the 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH). The method is based on the reduction of the 2,2'-diphenyl-1-picrylhydrazyl radical in a raspberry-colored alcohol solution to diphenylpicrylhydrazine, the solution of which is light yellow in color. Spectrophotometrically at a wavelength of 517 nm, the residual content of the DPPH radical in the solution was determined. The degree of inhibition of extracts was determined by the formula:

$$I = \frac{A_0 - A_1}{A_0} \times 100\%$$

where:

I – the degree of inhibition, %;

A_0 – the optical density of the control experiment (without extract addition);

A_1 – is the optical density of the main experiment (with the addition of extract).

Based on data on the degree of inhibition at different concentrations of extracts, the concentration of half-maximal inhibition (IC_{50}, μg/mL) was calculated [5].

The ability of the extracts to inhibit the radical cation of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was studied using a spectrophotometric method [6].

The antimicrobial activity of 1% fractions of *Inonotus obliquus* and *Fomes fomentarius* was studied by diffusion into agar (diffuse-well method) against gram-positive (*Staphylococcus aureus* ATCC 15442), gram-negative bacteria (*Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 6538) and yeast fungi (*Candida albicans* ATCC 14053). The results were recorded by measuring the diameter of the growth inhibition zone around the wells.

3. Results and Discussion

According to the results of the TPC test (Table 1), the ethyl acetate and ethanol fractions of the tinder fungus contain the maximum amount of phenolic compounds, while phenolic compounds are practically not detected in the non-polar fractions. This is consistent with the ability of phenolic compounds to readily dissolve in ethyl acetate and ethanol. Also, the results of TPC show that the content of phenolic substances in *Fomes fomentarius* is significantly higher than in *Inonotus obliquus*.

Table 1 Comparative table of the TPC test in fractionated extracts of *Inonotus obliquus* and *Fomes fomentarius*.

Sample	Fraction	TPC, mg/g (in terms of gallic acid)
Chaga (*Inonotus obliquus)*	PE	2.672 ±0.77
	CL	–
	EA	3.769 ±0.941
	H_2O	2.395 ±0.566
Tinder fungus (*Fomes fomentarius)*	PE	–
	CL	13.018 ±4.268
	EA	73.571 ±21.268
	H_2O	–
	EtOH	36.343 ±10.241

The radical-inhibiting activity of the fractionated extracts was studied in the DPPH and ABTS radical models. PE fraction of *Fomes fomentarius* practically does not show radical-inhibiting activity, while the ethyl acetate and ethanol fractions demonstrated the highest inhibitory activity against DPPH (Table 2). It is important to note that the fractions of Fomes fomentarius show about 10 times greater inhibitory activity against DPPH chromogen radicals compared to fractions of Inonotus obliquus.

Table 2 Radical-inhibiting activity of Inonotus obliquus and Fomes fomentarius fractions against DPPH radical.

Sample	Fraction	IC_{50}, μg/mL
Chaga (*Inonotus obliquus)*	PE	–
	CL	7011.6 ±204.8
	EA	5447.2 ±1315.5
	H_2O	9608.2 ±953.1
Tinder fungus (*Fomes fomentarius)*	PE	6161 ±249.6
	CL	1601.9 ±356.9
	EA	515.6 ±100.5
	H_2O	417.2 ±284.5
	EtOH	751.2 ±19.3

The data presented in Table 3 are consistent with the data on radical-inhibiting activity against DPPH (Table 2): EA and EtOH fractions show the highest ABTS-inhibitory activity, while the fractions obtained using petroleum ether and chloroform are relatively inactive or do not show significant antioxidant activity.

Table 3 Radical-inhibiting activity of Inonotus obliquus and Fomes fomentarius fractions against ABTS radical.

Sample	Fraction	IC_{50}, μg/mL
Chaga (*Inonotus obliquus)*	PE	–
	CL	–
	EA	75.1 ±7.3
	H_2O	2213.9 ±102
Tinder fungus (*Fomes fomentarius)*	PE	–
	CL	3650.1 ±118.3
	EA	229.7 ±9.6
	H_2O	3280.2 ±28.8
	EtOH	423.3 ±0.66

Experimentally obtained data on antioxidant activity are consistent with the results of determining the content of phenolic substances (table 1). The high antioxidant activity of tinder fungus extracts (EA and EtOH fractions) can be associated with a significant content of benzotropolones (primarily fomentariol) [3].

The results of the agar diffusion test for antimicrobial activity are presented in Tables 4 and 5.

Table 4 Results of zone diameter measurements for chaga (*Inonotus obliquus*).

Fraction / Microorganisms	PE	CL	EA	H_2O
Staphylococcus aureus ATCC 15442	0.9 ±0.1 mm		–	–
Candida albicans ATCC 14053	–	–	–	–
Escherichia coli ATCC 11229	–	–	–	–
Pseudomonas aeruginosa ATCC 6538	0.9 ±0.1 mm	–	–	–

Table 5 Results of zone diameter measurements for tinder fungus (*Fomes fomentarius*).

Fraction / Microorganisms	PE	CL	EA	H_2O	EtOH
Staphylococcus aureus ATCC 15442	0.9 ±0.1 mm		–	–	1.9 ±0.6 mm
Candida albicans ATCC 14053	–	–	–	–	–
Escherichia coli ATCC 11229	–	–	–	–	–
Pseudomonas aeruginosa ATCC 6538	0.9 ±0.1 mm	–	–	–	–

Chaga does not have antimicrobial activity against selected strains of microorganisms. The CL, EA and EtOH fractions of *Fomes fomentarius* suppress the growth of *Staphylococcus aureus* and exhibit insignificant inhibitory activity against yeast-like fungi *Candida albicans*. The studied fungi do not show antimicrobial activity against gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*.

Thus, the ethyl acetate (73.571 ±21.268 mg/g) and ethanol (36.343 ± 10.241 mg/g) fractions of Fomes fomentarius richest in phenolic compounds have the highest radical-inhibiting activity against DPPH (IC_{50}: 515.6 ± 100.5 μg/mL; 751.2 ± 19.3 μg/mL, respectively) and ABTS (IC_{50}: 229.7 ± 9.6 μg/mL;

423.3 ± 0.66 μg/mL, respectively) radicals. In the tests carried out, *Fomes fomentarius* is superior in antioxidant activity to *Inonotus obliquus*. In addition, *Fomes fomentarius* exhibits antimicrobial activity against *Staphylococcus aureus*.

The pronounced antioxidant activity, as well as the presence of antimicrobial activity in the studied extracts of *Fomes fomentarius* testifies to the prospects of its further pharmaceutical development.

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