



Article

Chemical Composition of Extracts from Fruiting Bodies of Tinder Fungi and Their Effect on the Early Stages of Wheat Development

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Abstract

One of the global challenges is the deficit of food. Food production is highly dependent on the productivity of agricultural plants used by humans and livestock. Various chemical and natural compounds are used to stimulate plant growth and increase their resistance to stress. The aim of our study was to analyze the chemical composition of extracts of the most common Ural tinder fungi and their effect on the early stages of wheat growth. Water–alcohol extracts from five wood-destroying fungi contained biologically active compounds (BACs), such as phenolics, free amino acids and reducing sugars. *F. pinicola* was characterized by the smallest amount of extracted substances. *F. fomentarius* has the largest amount of phenolic compounds and sugars, and *I. obliquus* had the highest concentration of free amino acids. Qualitative analysis revealed alkaloids in *P. betulinus*, and anthraquinones in *F. fomentarius*. Saponins were found in all tested species, except *F. fomentarius*. The extracts stimulated the early stages of wheat development at concentrations of 1.0–0.2 g of fungal biomass per liter. Seed germination rate was comparable to the control samples or exceeded it, and the length of roots and shoots increased. Thus, extracts from fruiting bodies of studied fungi can be recommended for priming wheat seeds, and for biotechnological cultivation.

Keywords: xylotrophic basidiomycetes; biologically active compounds (BACs); antioxidant activity; seed germination

1. Introduction

Modern society faces the challenges of increasing crop yields and environmental protection. Consequently, natural products are being actively introduced into agriculture. These include viable bacteria with plant growth-promoting (PGP) properties [1–3], algae suspensions and extracts [4–6], spores of entomopathogenic fungi [7,8], and so on. These products are ecologically friendly, and their effects can be explained by allelopathic interactions between organisms [9,10]. While plant extracts are widely used as bio-preparations in agriculture [11,12], fungal extracts are mentioned far less frequently. At the same time, fungi produce a wide variety of biologically active metabolites. Despite this, tinder fungi remain understudied in terms of their biologically active compounds. However, their mycelium is easily cultivated using biotechnological methods [13], which is important for the industrial-scale production of fungal metabolites. Polypores are rich in biologically



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active substances [14–17] and are widely used in medicine. We hypothesize that fungal biologically active compounds (BACs) could also regulate plant growth.

It has been shown that the fruiting bodies of wood-decaying fungi contain numerous secondary metabolites with antioxidant and anti-radical activity, such as phenolics, terpenes, reducing sugars, etc. [18–22]. Thus, they could be a promising raw material for bio-preparations that stimulate plant growth.

It is well known that extracts from mold fungi exhibit significant bacteriostatic and fungistatic activities against phytopathogens due to their content of phenolics and anthraquinones [23]. Fungal oligosaccharides can regulate plant growth and stress tolerance [24–26]. Endophytic fungi are capable of producing phytohormones [27] and forming symbiotic relations with plants [28]. As a result, they demonstrate plant growth-promoting characteristics [29,30] and are known as plant growth-promoting fungi (PGPF). Unfortunately, there is a lack of information on the agricultural use of tinder fungi.

Can xylotrophic basidiomycetes contain all BACs known for other fungi and demonstrate a diverse range of biological activities? Are these BACs capable of modifying plant growth? To answer this question, we selected five common tinder species from the Urals. It was necessary to determine which specific fungal metabolites could be responsible for effects on plants.

Thus, the aim of our work was to study the chemical composition of extracts obtained from the fruiting bodies of the most common tinder fungi in boreal forests and to evaluate the effect of the extracts on the early stages of wheat growth.

2. Materials and Methods

Fungal material. Fruiting bodies of common fungal species were collected in forests in the vicinity of Ekaterinburg. The material was heat-treated at 105 °C for 20 min and dried at 70 °C to a constant weight. It was then ground using an electric mill and used for extraction based on a previously described method [31,32], with minor modifications. Extracts were prepared from five species (Table 1).

Table 1. List of studied fungi species.

Species, Family	Growing Substrate	Site
<i>Inonotus obliquus</i> (Ach. ex Pers.) Pil., 1942—Hymenochaetaceae	<i>Betula pendula</i> L.	Biological Station of the Ural Federal University, Dvurechensk (56°35'47" N 61°05'56" E)
<i>Fomes fomentarius</i> (L.) Fr., 1849—Polyporaceae	<i>Betula pendula</i> L.	Biological Station of the Ural Federal University, Dvurechensk (56°35'47" N 61°05'56" E)
<i>Piptoporus betulinus</i> (Bull.) P.Karst., 1881—Fomitopsidaceae	<i>Betula pendula</i> L.	Biological Station of the Ural Federal University, Dvurechensk (56°35'47" N 61°05'56" E)
<i>Fomitopsis pinicola</i> (Sw.) P.Karst., 1881—Fomitopsidaceae	<i>Pinus sylvestris</i> L.	Biological Station of the Ural Federal University, Dvurechensk (56°35'47" N 61°05'56" E)
<i>Ganoderma applanatum</i> (Pers.) Pat., 1889, nom. cons.—Ganoderemaceae	<i>Populus balsamifera</i> L.	Yekaterinburg city (56°50' N 60°35' E)

Extraction. The ground fungal material was subjected to a sequential extraction protocol. It was first treated twice with 10 volumes of 95% ethanol for 2 h at 60 °C with periodic shaking, after which the biomass was separated by filtration. The resulting pellet was then resuspended and extracted twice with 70% ethanol under the same conditions. This procedure was repeated successively with 40% ethanol and finally with distilled water. All filtrates were combined and evaporated to dryness using a rotary evaporator (Hei-VAP Precision, Heidolph, Schwabach, Germany) at 60 °C, 0.2 atm. The dry residue was resuspended in 40% ethanol to create a stock suspension, using a ratio of 10 mL of solvent per 1 g of the original fungal biomass. The use of a graded ethanol series ensured a more complete extraction of metabolites from the fungal material, as we have previously demonstrated significant compositional differences between aqueous and ethanol extracts [33]. Furthermore, resuspension in 40% ethanol helps protect the extract from microbial contamination.

Prior to preparing experimental solutions, the stock suspension was thoroughly shaken, and an aliquot was diluted with distilled water. The following dilutions were studied: 1:10, 1:20, 1:50, 1:200, 1:500, and 1:1000 (*v/v*) (see Table 2).

Table 2. Ethanol concentration in the stock suspension and dilutions.

Dilution	Stock	1:10	1:20	1:50	1:100	1:200	1:500	1:1000
Ethanol concentration, %	40	4	2	0.8	0.4	0.2	0.08	0.04
Equivalent fungal biomass, mg/mL	100	10	5	2	1	0.5	0.2	0.1

Qualitative and quantitative tests. The color of the obtained samples was determined on a white background in a 1 cm layer. The volume of undissolved components was estimated by measuring the sediment size after centrifugation 10 mL of the solution at 3000 rpm for 10 min. Saponins were estimated using a foaming test: 10 mL of the solution were shaken for 30 s, allowed settle for 20 s, and the height of the foam column was determined. Qualitative reactions included tests for alkaloids (precipitation with Dragendorff's reagent, phosphotungstic, and picric acids); phenolic compounds (reaction with FeCl_3); anthraquinones (red coloration with alcoholic magnesium acetate); reducing sugars (with Fehling's reagent); tannins (precipitate with a potassium dichromate); saponins or tannins (precipitation with lead acetate) [31,32].

The quantification of phenolic compounds (using the Folin–Ciocalteu reagent), free amino acids (with ninhydrin) [32], and soluble hydrocarbons (with picric acid) [34] was performed colorimetrically using a Tecan M50 plate reader (Tecan, Grödig, Austria).

Analysis of individual phenolic compounds. Individual phenolic compounds were determined by HPLC-MS. The initial suspension was purified by centrifugation to remove insoluble components. The resulting supernatant was purified using a reverse-phase column (C-18) and a 0.02 μm microfilter. Compound identification was carried out using UHPLC-DAD-MS with an Agilent 1290 UHPLC system coupled to a 6430 triple quadrupole mass spectrometer (Agilent Technologies, Singapore). Separations were performed on an Agilent ZORBAX RRHD Eclipse Plus C18 column (50 $\times$ 2.1 mm, 1.8 μm) (Agilent Technologies, Santa Clara, CA, USA). The mobile phase consisted of 0.1% formic acid (solvent A) and methanol (solvent B). The gradient elution program was as follows: initial ratio 85:15 (A:B); 73:27 at 2 min; 65:35 at 4 min; 50:50 at 7 min; 40:60 at 10 min; 25:75 at 12 min; and 0:100 at 17 min. The flow rate was 0.3 $\text{mL} \cdot \text{min}^{-1}$, the injection volume was 2 μL , and the column temperature was maintained at 30 °C. A list of standards was compiled based on literature data [31,35,36].

Biological tests. For the seed germination test, wheat (*Triticum aestivum* L. var. 'Iren') seeds were washed in tap water followed by distilled water, disinfected for 10 min in a weak potassium permanganate solution, and then rinsed with distilled water until the rinse water became colorless. Finally, the seeds were soaked in distilled water for one hour. After soaking, 50 seeds were placed in Petri dishes on two layers of filter paper. According to the experimental design, 10 mL of the test solutions were added to the dishes. The seeds were germinated in the dark at 25 °C. The seed germination rate (%) and seedling size (root and shoot length) were determined on the 7th day. A control using ethanol at the same concentrations present in the test dilutions was employed (Table 2). Ethanol at a 1:500 dilution did not significantly affect plant size, with root and shoot lengths of 37.5 ± 1.5 mm and 32.8 ± 1.0 mm, respectively, compared to 36.5 ± 2.0 mm and 29.9 ± 1.5 mm in the water control. Therefore, the 1:500 ethanol dilution was deemed to have no negative effect on the seedlings.

To evaluate physiological characteristics, seeds were germinated in 0.5 L plastic pots on filter paper moistened with 10 mL of the test solution under a photosynthetic photon flux density of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a 16/8 h (day/night) photoperiod. The content of photosynthetic pigments was assessed [37] in ethanol extracts on the 5th day of wheat growth.

Statistical analysis. Chemical analyses were performed on five independent samples of fungal biomass. Chlorophyll content was evaluated in five biological replicates (each comprising 5–6 plants). Root and shoot length were measured in 30 individual plants. The seed germination rate was assessed in three replicates.

Statistical data processing was performed using Statistica 13 (TIBCO Software Inc., Tulsa, OK, USA). The Mann–Whitney U-test was used to evaluate differences between samples. ANOVA followed by the Kruskal–Wallis test was used to compare the content of individual metabolites. Figures show mean values and standard errors.

3. Results

3.1. Chemical Composition

The original stock suspension was prepared by extracting 1 g of fungal biomass with 10 mL of solvent. Even at a 1:1000 dilution, the solutions contained undissolved particles. The extracts exhibited coloration up to a 1:200 dilution for two species, and to lower dilutions for the others.

Qualitative chemical tests indicated the presence of saponins in all studied fungi, evidenced by the formation of stable foam upon shaking with water. For three out of the five species, foaming was observed even at a 1:200 dilution. The highest foaming capacity was observed in *P. betulinus*, with a foam column of approximately 1 cm persisting in the 1:500 dilution. The *F. pinicola* suspension contained a slightly lower saponin content, yielding a 3 cm foam column at a 1:10 dilution and 0.5 cm at 1:200. The lowest saponin content was found in *F. fomentarius*, with a 0.5 cm foam column in the stock suspension and only 0.2 cm in the 1:10 and 1:50 dilutions.

In addition to saponins, reducing sugars were detected in all extracts and remained detectable even at the highest dilution of 1:1000.

Phenolic compounds were also present in all stock suspensions but became undetectable by qualitative methods upon dilution. An exception was *F. fomentarius*, where phenolics were detected up to a 1:50 dilution. Several individual phenolic compounds were identified via HPLC-MS in the liquid phase of the stock suspensions. Diluted samples were not analyzed, as the phenolic content fell below the detection limit of the mass spectrometer (Table 3).

Table 3. The content of phenolic compounds in fungal extracts (µg/mL).

Metabolite	<i>F. fomentarius</i>	<i>F. pinicola</i>	<i>G. applanatum</i>	<i>I. obliquus</i>	<i>P. betulinus</i>
Caffeic acid	0.46	0.10	0.02	3.16	0.01
Catechin	0.04	0.67	0.05	0.85	0.24
Chlorogenic acid	0.01	nd	0.03	0.04	nd
Cinnamic acid	nd	nd	0.59	nd	0.47
Epicatechin	nd	0.76	0.04	0.15	0.34
Ferulic acid	0.04	0.03	nd	0.08	nd
Gallic acid	nd	0.05	0.01	nd	0.20
<i>p</i> -coumaric acid	0.22	0.04	0.02	0.25	0.01
Protocatehic acid	nd	0.38	0.44	0.22	nd
Quercetin	nd	0.10	0.01	0.13	nd
Rutin	nd	nd	0.04	0.04	0.04

Note: nd—not detected.

None of the extracts contained all 11 of the detected phenolic compounds. The maximum number of individual phenolics (10) was found in *G. applanatum*, while the fewest (5) were identified in *F. fomentarius*. All detected phenolics were present in small quantities, no more than 1% of the total phenolic content determined spectrophotometrically. Caffeic acid, *p*-coumaric acid, and catechin were detected in all five species studied. In contrast, resveratrol, luteolin, salicylic acid, and syringic acid, which are typical in plants, were not found in any of the fungal species. The *I. obliquus* extract was the richest in caffeic acid and catechin.

Anthraquinones were detected only in the *F. fomentarius* stock suspension, and alkaloids were found solely in *P. betulinus* (Table 4). Since most soluble compounds were detectable within the dilution range of 1:50 to 1:200, further biological testing of the extracts was limited to dilutions up to 1:200.

Table 4. Qualitative analysis of fungal extracts. The values (1:200–1:1000) indicate the limiting dilution at which a positive analytical result was observed.

	Saponins	Reducing Sugars	Phenolic Compounds	Tannins	Anthraquinones	Alkaloids	Dilution, Saving the Color	Dilution, Saving the Sediment
<i>Inonotus obliquus</i>	1:200	1:1000	1:20	nd	nd	nd	1:200	1:1000
<i>Fomes fomentarius</i>	1:50	1:1000	1:50	Stock	1:10	nd	1:200	1:1000
<i>Piptoporus betulinus</i>	1:500	1:1000	stock	1:10	nd	1:10	1:20	1:1000
<i>Fomitopsis pinicola</i>	1:200	1:1000	stock	nd	nd	nd	1:50	1:1000
<i>Ganoderma applanatum</i>	1:200	1:1000	1:10	nd	nd	nd	1:100	1:1000

nd—not detected.

The undissolved sediment likely contained other groups of biologically active compounds (BACs), such as proteins, polysaccharides, and high-molecular-weight or lipophilic isoprenoids. The largest sediment volume was observed in the *P. betulinus* suspension, constituting 10% of the total volume, which decreased to approximately 1% at a 1:50 dilution. The *F. fomentarius* suspension showed 6% sedimentation, while the other three species were characterized by lower levels of 2–3%. As these sediments consist of insoluble compounds, they are not expected to contribute to the biological activity of the soluble extracts.

We proceeded to a quantitative assessment of the soluble compounds. Although phenolic compounds became undetectable by qualitative methods upon dilution, they remained quantifiable by spectrophotometric analysis (Figure 1). The highest concentration of phenolic compounds was found in *F. fomentarius*: 0.55 mg/mL in the stock solution (Figure 1a) and 0.014 mg/mL at a 1:200 dilution. In contrast, the lowest concentration was found in *F. pinicola*, with 0.15 mg/mL in the stock suspension. The other three species contained intermediate and similar amounts, ranging from 0.37 to 0.43 mg/mL in the stock solution. Phenolics were quantifiable up to dilutions of 1:100 and 1:200 (Figure 1b). Consequently, if the biological effects of the fungal extracts are related to their phenolic concentration, the most pronounced effects would be expected for *F. fomentarius* and the least for *F. pinicola*.

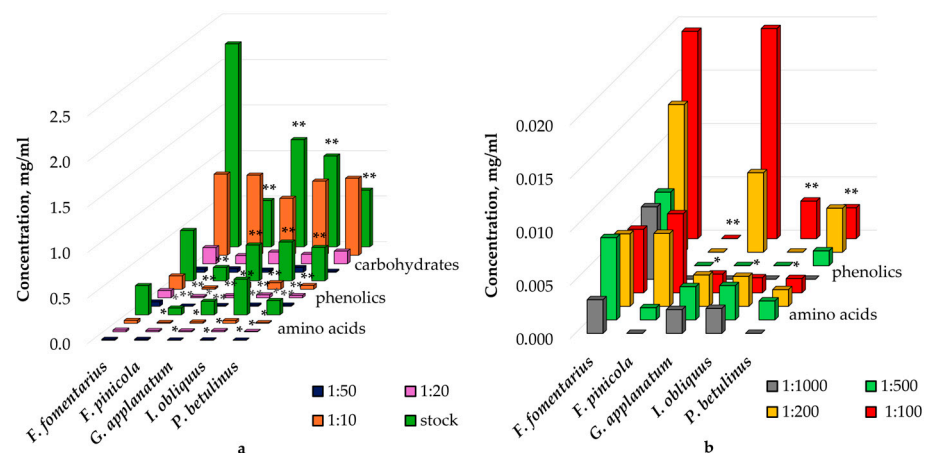


Figure 1. The chemical composition of fungal fruiting body extracts: (a) stock suspension and dilutions 1:10–1:50, (b) dilutions 1:100–1:1000. Note—An asterisk (*) indicates significant differences from the maximum value for each dilution ($p < 0.05$, $n = 5$); a double asterisk (**) indicates significant differences from the maximum value for each dilution ($p < 0.01$, $n = 5$).

The total content of free amino acids in the stock suspensions ranged from 0.39 mg/mL in *I. obliquus* (chaga) to 0.07 mg/mL in *F. pinicola*. Amino acids were also detected in dilutions up to 1:200–1:500 (Figure 1b), albeit at low concentrations. The concentration of soluble carbohydrates in the stock solutions was higher than that of phenolic compounds, which is consistent with carbohydrates being primary metabolites, whereas phenolics are secondary metabolites. The quantification method enabled the detection of carbohydrates in dilutions of up to 1:50 (Figure 1a).

F. fomentarius extracts exhibited the highest antiradical capacity across all tested dilutions, while *F. pinicola* showed the lowest activity (Figure 2). At a 1:10 dilution, the *F. fomentarius* extract inhibited ABTS⁺ radical formation by 77%, an activity equivalent to a 0.1 mg/mL rutin standard solution. The 1:20 dilution of the same extract showed 60% inhibition, which decreased to 11% and 4% at the 1:50 and 1:100 dilutions, respectively. Antioxidant activity was also observed in extracts from the other fungal species up to a 1:20 dilution.

3.2. Biological Tests

To evaluate the effect of fungal extracts on plants, four dilutions were selected: 1:50, 1:100, 1:200, and 1:500. The more concentrated extracts (1:10 and 1:20) were excluded due to the phytotoxicity of their high ethanol content. Similarly, the 1:1000 dilution was omitted because the concentration of biologically active compounds (BACs) was negligible in most chemical assays.

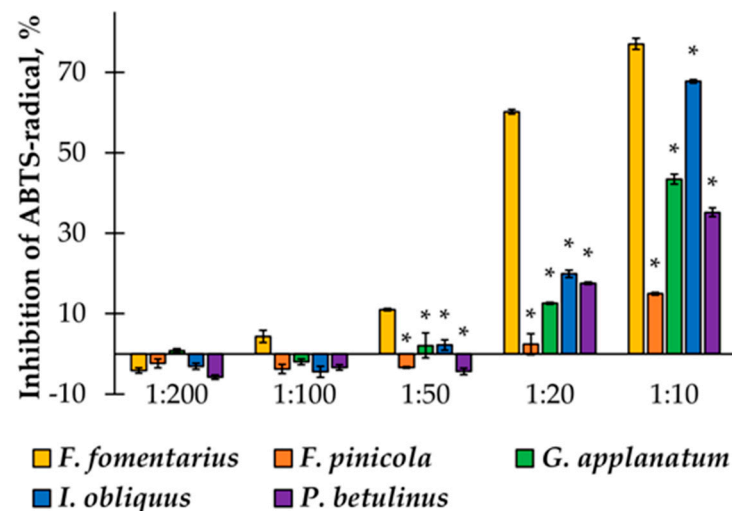


Figure 2. Antioxidant activity of extracts. * indicates significant differences from the maximum value in each dilution ($p < 0.05$, $n = 5$).

Wheat germination tests confirmed that ethanol concentration affected seed germination. For instance, in a control solution of 1:500 (0.08%) ethanol without fungal extracts, the seed germination rate was 86%. When the ethanol concentration was increased tenfold (as in the 1:50 dilution), the germination rate decreased by 10%.

Extracts from *I. obliquus* and *G. applanatum* at 1:50 and 1:100 dilutions stimulated seed germination by 15–20% compared to the ethanol control. At other dilutions, no significant differences from the control were observed (Table 5). These results indicate that the fungal extracts mitigated the negative effect of ethanol but did not significantly influence seed germination at higher dilutions where ethanol toxicity was minimal.

Table 5. Germination rate of wheat seeds, %.

Dilution	Control ¹	<i>F. fomentarius</i>	<i>F. pinicola</i>	<i>G. applanatum</i>	<i>I. obliquus</i>	<i>P. betulinus</i>
Water	88 ± 2	-	-	-	-	-
1:500	85 ± 3	85 ± 1	84 ± 3	87 ± 3	86 ± 4	92 ± 1
1:200	86 ± 1	85 ± 2	83 ± 3	92 ± 3 *	85 ± 3	86 ± 3 ^x
1:100	79 ± 4 ^x	82 ± 4	75 ± 4 ^x	91 ± 2 *	89 ± 2 *	85 ± 1 ^x
1:50	71 ± 2 ^x	87 ± 3 *	82 ± 2	87 ± 2 *	85 ± 5 *	82 ± 4 ^x

Note—¹ Ethanol concentration according to Table 2. An asterisk (*) indicates significant differences from the control ($p < 0.05$, $n = 3$); ^x indicates significant differences from the maximum dilution for each species ($p < 0.05$, $n = 3$).

To evaluate the effect of fungal extracts on plants, four dilutions were selected: 1:50, 1:100, 1:200, and 1:500. The more concentrated extracts (1:10 and 1:20) were excluded due to the phytotoxicity of their high ethanol content. The 1:1000 dilution was also omitted, as the concentration of biologically active compounds (BACs) was negligible in most chemical tests.

Wheat germination tests confirmed that ethanol concentration itself affected seed germination. For example, in a control with a 1:500 dilution (0.08% ethanol) and no fungal extracts, the seed germination rate was 86%. When the ethanol concentration was increased tenfold (as in the 1:50 dilution), the germination rate decreased by 10%.

Extracts from *I. obliquus* and *G. applanatum* at 1:50 and 1:100 dilutions stimulated seed germination by 15–20% compared to the ethanol control. No significant differences from the control were observed at other dilutions or with other species (Table 5). These

results indicate that the fungal extracts mitigated the negative effect of ethanol but did not significantly affect seed germination at higher dilutions where ethanol toxicity was minimal.

Measurements of wheat seedling roots and leaves on day 7 revealed that ethanol concentrations $\geq 0.4\%$ (1:100 and 1:50 dilutions) in control samples inhibited growth. However, the application of fungal extracts markedly affected seedling development.

The application of fungal extracts significantly influenced root growth, with effects ranging from a 12% increase to a 150% increase in length compared to the ethanol control. *F. fomentarius* extract exhibited neither inhibitory nor strong stimulatory effects on root development. In contrast, extracts from *I. obliquus* and *G. applanatum* consistently promoted root growth. *F. pinicola* extracts mitigated the negative effect of ethanol at 1:50 and 1:100 dilutions. However, at higher dilutions where ethanol was not inhibitory, these extracts either had no effect or reduced root length by up to 22%. *P. betulinus* (birch sponge) extract compensated for ethanol's negative effect at a 1:50 dilution and stimulated root growth by 29% at the 1:500 dilution. However, its 1:100 and 1:200 dilutions were toxic, reducing root length by 13–17% (Figure 3a). Overall, the fungal extracts counteracted the negative impact of ethanol and promoted root growth, with the most pronounced effect observed for the *G. applanatum* extract.

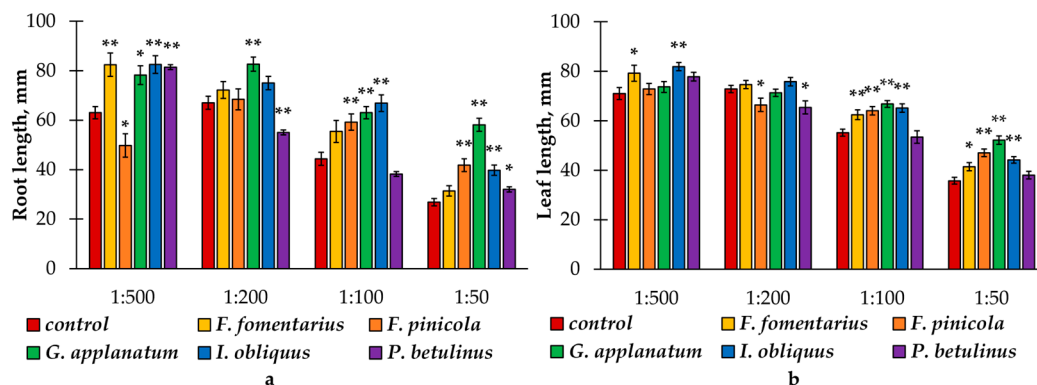


Figure 3. The length of roots (a) and leaf (b) of the 7th day wheat seedlings under the treatment with fungal extracts. An asterisk (*) indicates significant differences from the control at $p < 0.05$ ($n = 30$); ** indicates significant differences from the control at $p < 0.01$ ($n = 30$).

The toxic effect of ethanol was also evident on leaf growth (Figure 3b). In the control, leaf length was 71 mm with 1:500 ethanol, but only 35 mm with 1:50 ethanol. Extracts from *I. obliquus* and *F. fomentarius* at a 1:500 dilution increased leaf length by 9–12%. Extracts from other species did not affect leaf growth at this dilution but showed a stimulatory effect at 1:100. The *P. betulinus* extract did not demonstrate any positive effect on leaf length. In general, wheat seedlings responded to the application of fungal extracts in both roots and leaves, with *I. obliquus*, *G. applanatum*, and *F. fomentarius* extracts demonstrating the most consistent growth-promoting effects.

Chlorophyll concentration, which reflects photosynthetic activity and leaf viability, was assessed using three dilutions (1:100, 1:200, 1:500). The 1:50 dilution was excluded due to its high ethanol content and strong inhibitory effect on leaf growth (Figure 3b). Chlorophyll content remained unchanged at the 1:500 dilution. At 1:200, the chlorophyll concentration increased compared to the control. The *I. obliquus* (chaga) extract at 1:100 was toxic and strongly inhibited growth, whereas extracts from *F. fomentarius*, *F. pinicola*, *G. applanatum*, and *P. betulinus* at this dilution stimulated chlorophyll accumulation (Figure 4).

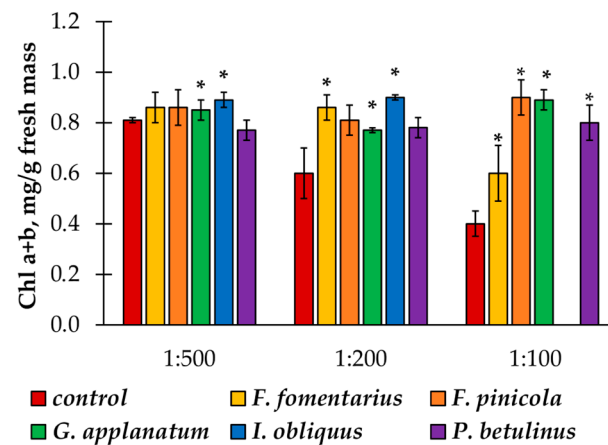


Figure 4. The chlorophyll content in wheat seedlings. An asterisk (*) indicates significant differences from the control at $p < 0.05$, $n = 5$.

4. Discussion

Five of the most common and abundant polypore (tinder) fungus species were studied as potential natural raw materials for agricultural use. Fruiting bodies of *Inonotus obliquus*, *Fomes fomentarius*, *Piptoporus betulinus*, *Fomitopsis pinicola*, and *Ganoderma applanatum* were collected in the forests of the Middle Urals.

A universal protocol for water–ethanol extraction of biologically active compounds (BACs) was developed and applied to all species. This protocol successfully extracted phenolics, reducing sugars, amino acids, and other BACs from the fungal fruiting bodies. Qualitative analysis revealed that alkaloids were unique to *P. betulinus*, while anthraquinones were found only in *F. fomentarius*. Saponins were present in all tested species except *F. fomentarius*, with the highest content found in *P. betulinus* extracts. This qualitative composition is consistent with previous reports [31,32,38,39]. Overall, *F. pinicola* was characterized by the lowest yield of extractive substances. BACs were reliably detected in extracts diluted up to 1:100–1:500, equivalent to 1.0–0.2 g of fungal biomass per liter of the final solution (Figure 1).

Phenolic compounds, known for their multifunctional biological activities such as antioxidant, antiradical, and antibacterial effects, were a key focus. In plants, phenolics act as stress protectors [40–42], lignin precursors [43], and signaling molecules in plant-microbial interactions [44–47]. Given their significant quantities in polypore fungi, we hypothesized they contribute to biological activity of fungal effects. Our HPLC-MS analysis showed that the profile of phenolic compounds in fungi studied differs from that in plants. Typical plant phenolic acids were present only in trace amounts or were absent entirely, collectively accounting for no more than 1% of the total phenolic content. Among the detected compounds, ferulic and *p*-coumaric acids are known as intermediates in humic acid metabolism and as plant growth stimulators [48], while caffeic acid can inhibit plant growth [49,50]. Cinnamic and gallic acids stimulate symbiotic relationships between soil microorganisms and plant roots and enhance resistance to fungal pathogens [47]. Protocatechuic acid can increase auxin production, thereby promoting plant growth [49,50]. Phenolic compounds also exhibit antiradical and antioxidant activity [51–53] and participate in plant hypersensitivity reactions [46]. Thus, the phenolic compounds in fungal extracts likely underpin their diverse biological effects. In our study, the highest concentration of phenolics was found in *F. fomentarius* extracts and the lowest in *F. pinicola*. These compounds were quantifiable up to dilutions of 1:100 and 1:200 (Figure 1). Flavonoids, which are common in plants, were virtually absent.

Fungi are known for their high protein content [38,42,54]. The high temperature and ethanol used for extraction caused protein denaturation and precipitation. However, free amino acids were successfully extracted into the water–ethanol solutions. Amino acids are recognized for their biological activity and ability to regulate plant growth [55,56], and commercial amino acid preparations are often used in agriculture to enhance seed germination and plant development [57]. In our extracts, the concentration of free amino acids ranged from 0.07 mg/mL to 0.39 mg/mL, with the highest amount in *I. obliquus* (chaga) and the lowest in *F. pinicola*. While these concentrations (Figure 1b) are insufficient as a significant nitrogen source for plants, they can still regulate plant growth [58,59]. Our previous study [32] identified 4 different free amino acids (including 2 essential) in *I. obliquus*; 8 (5 essential) in *F. fomentarius*; and 7 (4 essential) in both *F. pinicola* and *P. betulinus*. We therefore propose that the specific composition of amino acids in the fungal extracts contributes to the regulation of seed germination and early plant development, as demonstrated by the priming effect on wheat seeds (Table 5) and the regulatory effects on seedlings (Figures 3 and 4).

Carbohydrates in the extracts can serve as energy sources and also function as growth regulators. For instance, oligosaccharins can exert hormone-like effects on plants [30,60,61], and fungal cell wall glucans can modulate plant immunity by inducing hypersensitivity reactions [62,63]. Sugars also act as common nutrients for rhizosphere and phyllosphere microorganisms [64], therefore can regulate plant life. The concentration of soluble carbohydrates in *F. pinicola* and *P. betulinus* extracts was lower in the original stock suspensions than at the 1:10 dilution. This is attributed to the differential solubility of oligo- and polysaccharides; their solubility in ethanol is low but increases in water. Thus, diluting the stock with water increased the solubility of these carbohydrates, preventing their precipitation.

Low-molecular-weight compounds such as phenolics, reducing sugars, and amino acids are known antioxidants. Our study confirmed that the fungal extracts possess antiradical activity, effectively suppressing ABTS⁺ radical formation. A strong correlation was found between antiradical activity and the concentration of phenolics ($r = 0.98$, $p < 0.05$). High correlations were also observed with the concentration of amino acids ($r = 0.72$, $p < 0.05$) and reducing carbohydrates ($r = 0.85$, $p < 0.05$).

As these water–ethanol extracts were intended as potential biopreparations, we assessed the effect of ethanol itself on wheat. A toxic effect was observed at 1:50 (0.8% *v/v*) and 1:100 (0.4% *v/v*) ethanol dilutions, which the fungal BACs in these preparations could not fully counteract. Therefore, we do not recommend these concentrations for practical agricultural use. In contrast, the 1:200 and 1:500 dilutions generally had a positive effect on seed germination and early growth and can be recommended for priming wheat seeds. As these results were obtained on a single wheat variety, future work will involve testing on other varieties and crop species. Subsequent research will focus on the effects of fungal extracts on plants under stress, with the first results on barley already published [33].

5. Conclusions

Water–ethanol extracts from five wood-decaying fungi (*Inonotus obliquus*, *Fomes fomentarius*, *Piptoporus betulinus*, *Fomitopsis pinicola*, and *Ganoderma applanatum*) were found to contain biologically active compounds, including phenolics, free amino acids, and reducing sugars. This study is the first to systematically evaluate the efficacy of fungal extracts across a wide concentration range (1:10 to 1:1000). At concentrations equivalent to 1.0–0.2 g of fungal biomass per liter, the extracts enhanced seed germination and seedling growth compared to the control. Both leaves and roots of wheat seedlings responded positively to treatment. The extracts also mitigated the negative effects of ethanol on seedlings. Conse-

quently, extracts from these widespread polypore fungi can be recommended for priming wheat seeds and for the development of biopreparations to regulate plant growth.

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