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A Bioactive Compounds Profile Present in the Selected Wood Rot

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Abstract: Wood rot fungi are an essential link in the forest ecosystem. The presented study aimed to determine the content of selected antioxidant active compounds of selected saprobionts commonly found in the European forests: *Hypholoma fasciculare* (Huds.) P. Kumm, *Bjerkandera adusta* (Willd.) P. Karst., *Inonotus obliquus* (Fr.) Pilát, *Kuehneromyces mutabilis* (Schaeff.) Singer & AH Sm., *Trametes versicolor* (L.) Lloyd, *Pleurotus ostreatus* (Jacq.) P. Kumm., *Pholiota squarrosa* (Vahl) P. Kumm. Chemical methods (HPLC determination, $ABTS^{+}$ and FPA methods, and a saponification method by Acquity UPLC) were used to analyze active compounds. In the tested isolates, the presence of 13 phenolic acids has been observed, including *p*-coumaric, ferulic, chlorogenic, and sinapic acid, in high concentrations. The antioxidant activity was from 2.5 to 3.5 times higher in the isolates of *I. obliquus*, *P. ostreatus*, and *H. fasciculare* in comparison to *P. squarrosa* and *B. adusta*. All isolates were tested for β-carotene, lutein, zeaxanthin, and astaxanthin. High concentrations of flavonoids were observed in *H. fasciculare* and *P. squarrosa*. In addition, the observed concentration of naringenin, quercetin, and kaempferol above 21 mg/kg DM was found. The ergosterol was quantified in the saprobiont fungi cultures. A diversified content of bioactive compounds characterized the tested fungi, and the Chaga (*I. obliquus*) reported the highest content of tested compounds.

Keywords: wood rot; *Hypholoma fasciculare*; *Bjerkandera adusta*; *Inonotus obliquus*; *Kuehneromyces mutabilis*; *Trametes versicolor*; *Pleurotus ostreatus*; *Pholiota squarrosa*

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1. Introduction

Wood decay fungi are crucial links in the ecosystems of forests. As saprobionts, they decompose dead organic matter and release elements into circulation in nature. They are the main group of organisms—and often the only ones—capable of decomposing the high-molecular components of wood (cellulose, hemicellulose, lignin), which are the most complex and slowest natural substances to deteriorate in nature [1]. As a result of their decomposition, humus is formed. The direct way the soil humic compounds form is their synthesis from fragments, such as polyphenols, with the involvement of organically originated nitrogen. The polyphenol source may be the lignin decomposition processes and the transformation of the carbohydrates; many polyphenols are formed as the metabolites of various microorganisms [2].

The fungi lead to the polyphenols' oxidation and the formation of the chinoid compound in subsequent stages. The aforementioned processes are observed in various fungi biological systems, indicating interesting studies using the saprotrophs fungi mycelium as an alternative source of polyphenols and other bioactive compounds [3–5].

Common edible wood-decay fungi include *Kuehneromyces mutabilis* (Schaeff.) Singer & A.H. Sm. and *P. ostreatus* (Jacq.) P. Kumm. *Kuehneromyces mutabilis* grows in large numbers in deciduous and mixed forests [6,7]; *Pleurotus ostreatus* is a fungus found in the wood of the deciduous species [8,9]. As edible mushrooms, they provide easily digestible protein, folic acid, vitamins (thiamin, riboflavin, and niacin) [7,10–13], minerals (potassium, magnesium, copper, calcium, phosphorus, iron, selenium) [14,15], and substances that lower blood cholesterol level (lovastatin) [16]. In addition, antioxidant, antidiabetic, antimicrobial, immunostimulating, and antitumor substances have been detected in the fruiting bodies of *P. ostreatus* [17,18].

Poisonous fungi commonly found in the forest environment include *H. fasciculare* (Huds.) P. Kumm. [19]. A compound with an antibiotic effect against *Staphylococcus aureus* was isolated from its fruiting bodies. The antagonistic activity of *H. fasciculare* against other soil fungi and antibacterial activity were also found in the case of *Paraphaeosphaeria minitans* (W.A. Campb.) Verkley, Göker & Stielow [20], *Penicillium citrinum* Thom [21], and *Pleurotus tuber-regium* (Fr.) Singer [21–27]. Another common saprobiont throughout Europe is *T. versicolor* (L.) Lloyd. The fruiting bodies can be found on the trunks or branches of dead shrubs and angiosperm and gymnosperm tree [28], which cause white rot in the wood [29]. In Europe, it is considered an inedible mushroom [19]; however, the mushrooms list prepared for the F.A.O. listed the mushroom as edible in China, Hong Kong, Laos, and Mexico [30]. Mycological and chemical research has proven the presence of many compound groups in *T. versicolor* fruiting bodies, which are responsible for many therapeutic effects, including polysaccharides, protein-polysaccharide complexes, polyphenolic compounds, and terpenes [31,32].

Another mushroom with healing properties is *I. obliquus* (Fr.) Pilát, which also causes white rot in wood. In Poland, *I. obliquus* occurs infrequently, and it is mainly found on birch and rarely on other deciduous trees; therefore, it is under partial species protection [6]. In Poland, the mushroom is considered inedible, while according to the F.A.O. list, it is an edible mushroom in Canada and Russia (Journal of Laws No. of 2014, item 1408) [33]. *I. obliquus* sclerotia (black, cracked, lumpy formations) from birch trunks are used for medicinal purposes. The results of contemporary studies of the metabolites isolated from *I. obliquus* sclerotia [34] indicate their broad biological activity and potential properties, including hypoglycemic [35], antiviral [36,37], antimutagenic [38], anticancer, and cytostatic characteristics [35,39–46], as well as strong antioxidative properties [45,47–51]. Common white rot fungi are: *B. adusta* (Willd.) P. Karst. and *P. squarrosa* (Vahl) P. Kumm.

The presented study aimed to determine the content of selected antioxidant active compounds (phenolic acids, flavonoids, carotenoids) of selected fungi (saprobionts) commonly found in the European forests: *H. fasciculare*, *B. adusta*., *I. obliquus*, *K. mutabilis*., *T. versicolor*, *P. ostreatus*., and *P. squarrosa.* The antioxidant activity measured by the ABTS radical and the free phenolic acids (FPA) content was also tested. In addition, the content of ergosterol, the main component of the fungal cell membrane, was determined.

2. Materials and Methods

2.1. Fungal Isolates

The saprobiont fungi came from the Department of Forest Entomology and Pathology collection at the Poznań University of Life Sciences (Table 1).

Table 1. Fungal isolates.

No	Species	Isolate No.	Isolate Code
1.	Sulfur tuft (*Hypholoma fasciculare*)	96,032	Hf
2.	Smoky polypore (*Bjerkandera adusta*)	322	Ba
3.	Chaga (*Inonotus obliquus*)	110,421.3	Io
4.	Sheathed woodtuft (*Kuehneromyces mutabilis*)	96,040	Km
5.	Turkey tail (*Trametes versicolor*)	230,318	Tv
6.	Oyster mushroom (*Pleurotus ostreatus*)	20,080	Po
7.	Shaggy scalycap (*Pholiota squarrosa*)	206,021	Ps

2.2. The Extracts Preparation

Samples of 5 g the fungal mycelia from each wood decay fungus were collected for analyses of phenolic acids. The samples of all species were homogenized. Total phenolic acids were extracted with 80% MeOH (Sigma-Aldrich,Inc., St. Louis, MO, USA). After flooding with 100 mL MeOH, samples were placed in an ultrasound bath for 30 min in special flasks, and subsequently, the precipitate was collected in distillation flasks. The extraction process was repeated three times. Next, the combined extracts were evaporated to dryness in an evaporator (Laboratory Evaporators Cole-Parmer Rotary,Vernon Hills, IL, USA). Finally, phenolic acids were transferred quantitatively to a vial using MeOH and dried in a stream of nitrogen. An automated N1 system was used, which specializes in the rapid concentration of samples by PERLAN Technologies.

2.3. Chemical Analyzes

2.3.1. Determination of the Phenolic Compounds

Alkaline Hydrolysis

For analysis, the extracts were redissolved in 1 mL 80% MeOH. Next, they were placed in sealed 17 mL culture test tubes, where first alkaline and then acid hydrolyses were run. 1 mL distilled water and 4 mL 2 M aqueous sodium hydroxide (Sigma-Aldrich, Inc., St. Louis, MO, USA) were added to the test tubes to run alkaline hydrolysis. Tightly sealed test tubes were heated in a water bath at 95 °C for 30 min. After cooling (approx. 20 min), the test tubes were neutralized with 2 mL 6 M aqueous hydrochloric acid solution (Sigma-Aldrich, Inc., St. Louis, MO, USA) (pH = 2). Next, samples were cooled in water with ice. The flavonoids were extracted from the organic phase using diethyl ether (Sigma-Aldrich, Inc., St. Louis, MO, USA) (2 × 2 mL). The formed ether extracts were continuously transferred to 8 mL vials.

Acid hydrolysis.

Next, acid hydrolysis was run. For this purpose, the aqueous phase was supplemented with 3 mL 6 M aqueous hydrochloric acid solution. The tightly sealed test tubes were heated in a water bath at 95 °C for 30 min. After being cooled in water with ice, the samples were extracted with diethyl ether (2 × 2 mL for approx. 5 min). The produced ether extracts were continuously transferred to 8 mL vials, after which they were evaporated to dryness in a stream of nitrogen. An automated N1 system was used, which specializes in the rapid concentration of samples by PERLAN Technologies. Next, samples were dissolved in 1 mL methanol (Sigma-Aldrich, Inc., St. Louis, MO, USA). A phenolic compound analysis was performed using an Acquity H class UPLC system equipped with an Acquity P.D.A. detector (the ACQUITY UPLC Photodiode Array (P.D.A.) Detector) (Waters Corp, Milford, MA, USA). Chromatographic separation was performed on an Acquity UPLC®® BEH C18 column (100 mm × 2.1 mm, particle size—1.7 µm) (Waters, Dublin, Ireland). The concentrations of phenolic compounds were determined using an internal standard at wavelengths of λ = 280 nm (phenolic acids), whereas the wavelength of flavonoids was λ = 320 nm, and finally expressed as mg/100 g DW of samples. The compounds were identified by comparing the analyzed peak retention time

with the standard (Sigma-Aldrich, Inc., St. Louis, MO, USA) retention time, which added a specific standard to the analyzed samples, and repeating the analysis. The detection level was 1 μg/g [52].

2.3.2. Determination of the Free Phenolic Acids (FPA) and Antioxidant Activity (ABTS Method)

The free phenolic acids analysis was determined as described by Przybylska-Balcerek et al. [53].

For the ABTS generation from ABTS salt, 3 mM of $K_2S_2O_8$ (Sigma-Aldrich, Inc., St. Louis, MO, USA) was reacted with 8 mM ABTS salt in distilled, deionized water for 16 h at room temperature in the dark. The ABTS solution was then diluted with a pH 7.4 phosphate buffer solution containing 150 mM NaCl (PBS) (Sigma-Aldrich, Inc., St. Louis, MO, USA) to obtain an initial absorbance of 1.5 at 730 nm. The fresh ABTS solution was prepared for each analysis. The kinetic reaction was determined over a 2 h period with readings every 15 min; the reactions were complete in 30 min. The samples and standards (100 μL) were reacted with the ABTS solution (2900 μL) for 30 min.

Trolox was used as a standard. The results were expressed in the ABTS (μmolTROLOX/g DM) sample [52].

2.3.3. Determination of the Carotenoids

Carotenoid isolation and quantification were performed in the fungi samples by the saponification method by an Acquity UPLC (Waters, Milford, MA, USA). First, the carotenoid extracts were obtained from the mycelia (0.4 mg) combined with an acetone and petroleum ether (1:1) mixture. Then, after separating the mycelia, the acetone and the hydrophilic fraction were removed from the extract by washing with water; as a result, the ether extract was obtained with a mixture of carotenoid pigments. The extract prepared was concentrated in a vacuum evaporator at 35 °C until an oily residue was obtained, then digested in 2 mL of methanol (Merck) and subjected to chromatographic analysis. The total carotenoids were determined using an Acquity UPLC (Waters, Milford, MA,USA) with a Waters Acquity P.D.A. detector (Waters, Milford, MA, USA). The chromatographic separation was performed on an Acquity UPLC BEH C18 column (100 mm × 2.1 mm, particle size 1.7 μm) (Waters, Dublin, Ireland). The column and samples were thermostated, the column temperature was 30 °C, and the test temperature was 10 °C. During the analysis, the solutions were degassed in a Waters device. The injection volume was 10 μL. The registration was carried out at a wavelength λ = 445 nm [54]. The compounds were identified by comparing the analyzed peak retention time with the standard (Sigma-Aldrich, Inc., St. Louis, MO, USA) retention time, adding a specific standard to the analyzed samples, and repeating the analysis.

2.3.4. Determination of the Ergosterol (ERG)

The ergosterol was determined by UPLC as described by Perkowski et al. [55] with some modifications. The samples (mycelia) were analyzed by an Acquity H class UPLC system equipped with a Waters Acquity P.D.A. detector (Waters, Milford, MA, USA). The chromatographic separation was performed on an Acquity UPLC®® BEH C18 column (100 mm × 2.1 mm, particle size 1.7 μm) (Waters, Dublin, Ireland) and eluted with methanol/acetonitrile/water (85:10:5) at a 0.4 mL/min flow rate. The ergosterol was detected with a Waters Acquity P.D.A. detector (Waters, Milford, MA, USA) set at 282 nm. Ergosterol was identified by comparing the analyzed peak retention time with the standard (Sigma-Aldrich, Inc., St. Louis, MO, USA) retention time, adding a specific standard to the analyzed samples, and repeating the analysis.

2.4. Statistical Analysis

The chemical analyses were analyzed statistically using the STATISTICA v.8.0 software (Tibco software Inc., Palo Alto, USA). Tukey's multiple comparison procedure was used to compare the contents of individual metabolites: phenolic acids, flavonoids, carotenoids, antioxidant activity, and ergosterol content in the fungal isolates. The identical letters in rows or columns denote the lack of differences at a significance level of $\alpha = 0.05$.

3. Results

3.1. Concentration of Phenolic Compounds

The highest sum of phenolic acid content was found in *I. obliquus* (Io) (435.52 mg/kg DM) and the lowest in *P. squarrosa* (Ps) (Table 2 and Figure 1). Among the analyzed acids, chlorogenic and sinapic acids were found in the highest concentrations in *B. adusta* (Ba) and *P. ostreatus* (Po), respectively. Conversely, pyroglutamic and pyruvic acids were present in low concentrations in all tested samples. Ferulic and coumaric acid in the three studied fungi (*H. fasciculare* (Hf), *I. obliquus* (Io), and *K. mutabilis* (Km) were present at a level above 84 mg/kg DM.

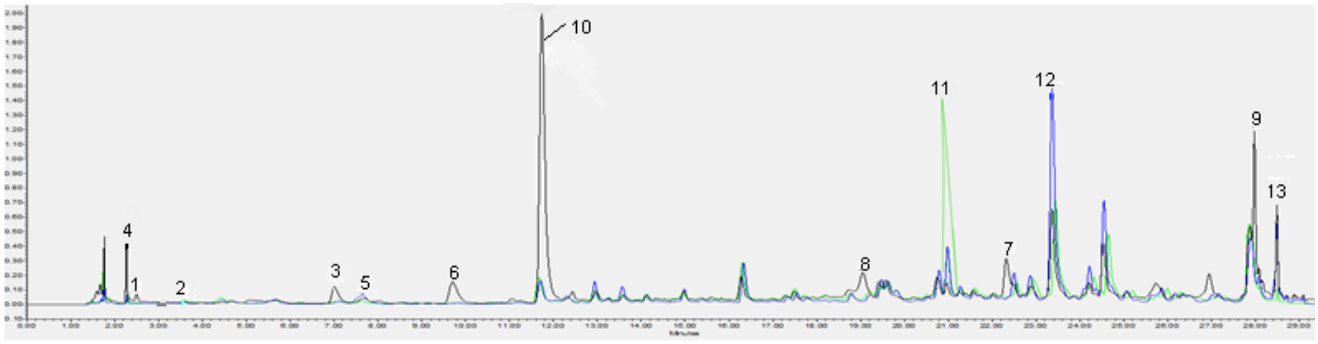

Figure 1. Comparison of phenolic acid (1—protocatechuic, 2—pyroglutamic, 3—pyruvic, 4—gallic, 5—2.5-dihydroxybenzoic, 6—4-hydroxybenzoic, 7—caffeic, 8—syringic, 9—*p*-coumaric, 10—ferulic, 11—chlorogenic, 12—sinapic, 13—t-cinnamic) UPLC chromatograms: Ps (green line), Km (black line), Io (blue line).

The research on the content of the selected flavonoids showed that the highest contents of these metabolites were observed in *I. obliquus* (Io)—114.5 mg/kg DM (sum of flavonoids), and the lowest in *B. adusta* (Ba) –0.07 mg/kg DM (sum of flavonoids) (Table 3 and Figure 2). High concentrations were also observed in *H. fasciculare* (Hf) and *K. mutabilis* (Km) (sum of flavonoids). In addition, high concentrations of naringenin, quercetin, and kaempferol (above 21 mg/kg DM) were found. Conversely, *B. adusta* (Ba) was characterized by the lowest concentration of flavonoids (0.07 mg/kg DM). *P. squarrosa* (Ps) and *T. versicolor* (Tv) were also poor in flavonoids.

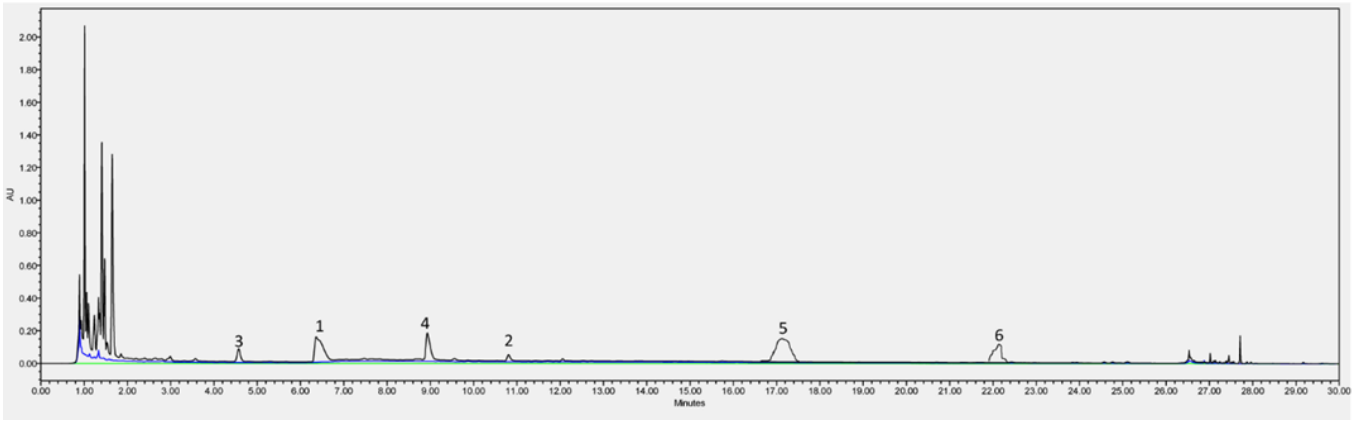

Figure 2. Comparison of flavonoids (1—naringenin, 2—vitexin, 3—rutin, 4—quercetin, 5—apigenin, 6—kaempferol) UPLC chromatograms: Ps (green line), Io (black line), Ba (blue line).

Table 2. The phenolic acids concentration of 7 fungal isolates (mg/kg DM in mycelium).

Phenolic Acids	Hf	Ps	Ba	Io	Po	Tv	Km
Protocatechuic	1.21a	0.12c	0.5b	1.08a	0.95a	0.5b	1.69a
Pyroglutaminic	0.27ab	0.09b	nd	0.77a	nd	nd	0.45a
Pyruvic	1.34c	0.12d	nd	7.2b	nd	10.95a	6.56b
Gallic	26.57a	9.55b	9.82b	32.13a	8.43b	9.77b	28.73a
2.5-dihydroxybenzoic	9.46a	2.07b	3.0b	9.76a	2.8b	2.9b	8.98a
4-hydroxybenzoic	11.22a	1.45b	2.0b	9.45a	1.71b	1.78b	12.69a
Caffeic	31.73a	3.31b	0.01c	31.62a	0.02c	0.01c	32.58a
Syryngic	28.84a	2.92b	0.09c	30.64a	0.08c	0.06c	29.82a
p-coumaric	92.75a	6.12b	6.92b	84.12a	6.45b	6.27b	94.79a
Ferulic	87.87a	10.7b	10.39b	98.43a	9.95b	9.14b	98.38a
Chlorogenic	18.63b	6.56c	108.49a	27.91b	9.55c	9.13c	19.83b
Sinapic	53.01b	1.24c	0.14c	51.56b	100.72a	0.12c	56.31b
t-cinnamic	43.07a	2.16b	2.16b	50.85a	1.16b	1.98b	41.5a
Sum of an identified phenolic acids	405.97a	46.41c	143.52b	435.52a	141.82b	52.61c	432.31a

nd—not detected. a,b,c —the same letters in the columns indicate lines of significant differences at the significance level $\alpha = 0.05$.

Table 3. The flavonoids concentration of 7 fungal isolates (mg/kg DM in mycelium).

Isolate Code	Flavonoids					
	Naringenin	Vitexin	Rutin	Quercetin	Apigenin	Kaempferol
Hf	25.66a	5.84a	16.72a	22.99b	16.35a	20.88a
Ps	0.32c	nd	nd	nd	nd	nd
Ba	0.07c	nd	nd	nd	nd	nd
Io	27.11a	5.18a	19.05a	24.03ab	17.92a	21.21a
Po	0.14c	nd	nd	0.33c	6.99b	nd
Tv	0.18c	0.03b	nd	nd	nd	nd
Km	16.82b	6.62a	19.02a	27.50a	19.57a	24.63a

nd—not detected, a,b,c—the same letters in the columns indicate lines of significant differences at the significance level $\alpha = 0.05$.

3.2. Antioxidant Activities of Mushroom Isolate Extracts

The $ABTS^{+}$ radical neutralizing activity was measured for the methanol-water extracts obtained from the seven fungal mycelium isolates. Among the tested fungi, *T. versicolor* (Tv) was characterized by the highest antioxidant activity with a TEAC value of

908.56 μM/g and the highest concentration of FPA (195.52 mg GAE/g DM). Conversely, *H. fasciculare* (Hf) and *B. adusta* (Ba) had a radical scavenging capacity of 2 and 9 times lower, respectively, compared to *T. versicolor* (Tv). The mean values of the antioxidant activity were shown by the isolates of *K. mutabilis* (Km), *P. ostreatus* (Po), and *I. obliquus* (Io) (TEAC from 534.27 to 351.41 μM/g). The lowest antioxidant activity was observed in *P. squarrosa* (Ps). The lowest concentration of FPA was found for the *B. adusta* (Ba) isolate (102.97 mg GAE/g DM) (Table 4).

Table 4. The antioxidant activities of methanol-water extracts of the obtained mushroom isolates measured by reaction with the radical ABTS cation and the free phenolic acids (FPA) content.

Isolate Code	FPA (mg GAE/g DM)	ABTS (TEAC) μM Trolox/g
Hf	147.10b	467.87b
Ps	162.21c	104.20a
Ba	102.97a	108.28a
Io	188.99d	351.41b
Po	104.81a	417.71b
Tv	195.52d	908.56c
Km	171.62c	534.27b

a,b,c—the same letters in the columns indicate lines of significant differences at the significance level $\alpha = 0.05$.

3.3. Concentration of Carotenoids

The highest carotenoid content was found in *T. versicolor* (Tv) and the lowest in *P. squarrosa* (Ps). In all tested isolates, β-carotene was present in the highest concentration. Similarly, zeaxanthin was found in the trace amounts in *H. fasciculare* (Hf), *P. squarrosa* (Ps), and *K. mutabilis* (Km) (Table 5 and Figure 3).

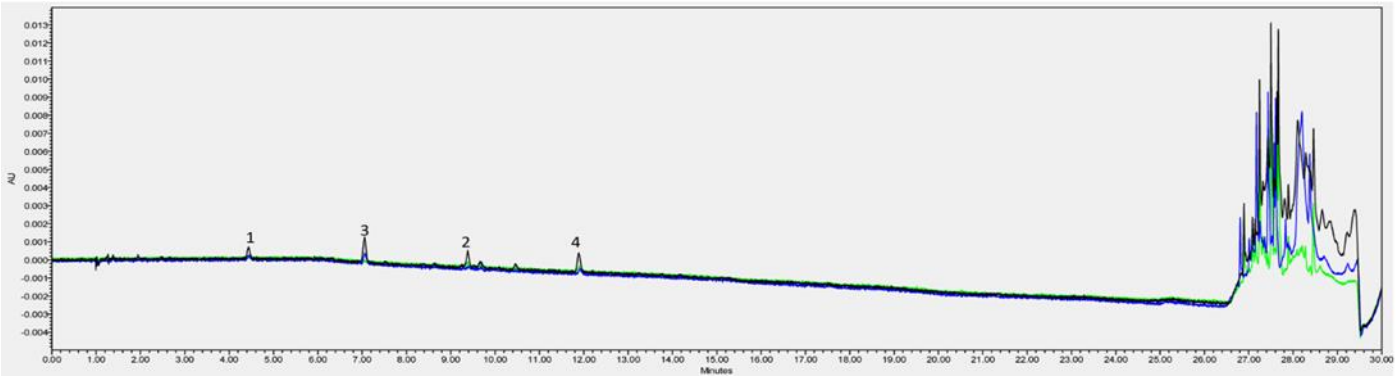

Figure 3. Comparison of carotenoids (1-lutein, 2-zeaxanthin, 3-β-carotene, 4-astaxanthin) UPLC chromatograms: Io (green line), Po (black line), Ba (blue line).

Table 5. The carotenoids concentration (mg/kg) in 7 fungal isolates.

Isolate Code	Lutein	Zeaxanthin	β-Carotene	Astaxanthin	Sum of Identified Carotenoids
Hf	0.15a	0.05a	0.37a	0.10a	0.90a
Ps	0.11a	0.04a	0.19a	0.05a	0.65a
Ba	0.11a	0.08a	0.30a	0.10a	0.99a
Io	0.31b	0.24b	0.51ab	0.21ab	1.65b
Po	0.57b	0.37b	0.70b	0.21ab	1.99b
Tv	0.70c	0.21b	0.99b	0.37b	2.66c
Km	0.03a	0.07a	0.26a	0.10a	0.46a

a,b,c—the same letters in the columns indicate lines of significant differences at the significance level $\alpha = 0.05$.

3.4. Concentration of Ergosterol

The ergosterol (ERG) was quantified in the saprobiont fungi cultures. The highest ERG content was found in *I. obliquus* (Io) (863.33 mg/kg) and the lowest in *T. versicolor* (Tv) (223.30 mg/kg). The high concentration of this metabolite was also observed in *H. fasciculare* (Hf). The ERG content in the remaining isolates was above 300 mg/kg (Table 6).

Table 6. The ergosterol (ERG) concentration (mg/kg) in 7 fungal isolates.

Isolate Code	Ergosterol
Hf	772.50c
Ps	300.63a
Ba	317.92a
Io	863.33c
Po	342.18a
Tv	223.30a
KM	610.89b

a,b,c—the same letters in the columns indicate lines of significant differences at the significance level $\alpha = 0.0$.

4. Discussion

The studied saprobiont fungi occurring in the various Polish forest habitats were characterized by a diversified content of the biologically active compounds. The first group of compounds analyzed in this study was phenolic acids, which included *p*-coumaric, ferulic, chlorogenic, and sinapic acids as the major components. Gallic, p-hydroxybenzoic, protocatechuic, vanillic, syringic, caffeic, ferulic, *p*-coumaric, and sinapic acids are the most common acids in the plant world [56]. In the tested isolates, the presence of 13 acids was observed, including the highest concentrations of *p*-coumaric, ferulic, chlorogenic, and sinapic acid. The greatest antioxidant capacity (protection of cells against hydrogen peroxide) is shown by the following acids: vanillic and caffeic acid [57]. The latter was present in all tested isolates, and the highest concentration was observed in *H. fasciculare* (Hf), *I. obliquus* (Io), and *K. mutabilis* (Km). The other acids determined in the isolates tested, which were characterized by in vitro and in vivo antioxidant, antibacterial, antiviral, and antifungal properties, are p-hydroxybenzoic, gallic, and protocatechuic acid [58].

A rich profile of phenolic acids in *I. obliquus* was presented in this study (Table 2) Numerous reports on the presence of phenolic acids in *I. obliquus* (Io) confirmed the presence of the following acids: gallic, protocatechuic, and p-hydroxybenzoic acid [34,50,59–61]. Ju et al. [62] isolated vanillic, protocatechuic, and 2.5-dihydroxyterephthalic acids.

Similar results were obtained by Janjušević et al. [63], who identified ten phenolic acids in *T. versicolor* (Tv) harvested in northwest Romania, six of which were confirmed in this study, among others: quinic, malic, vanillic, and caffeic acid. Other researchers found that the methanol extract of *T. versicolor* fruiting bodies showed the presence of three free phenolic acids: gallic (73 μg/g DM), protocatechuic (48 μg/g DM), and caffeic acid (154 μg/g DM) [58].

In the conducted analyses, six phenolic acids were determined in *P. ostreatus* (Table 1). Sinapic acid was present in a high concentration (100.72 mg/kg DM) with 141.82 mg/kg DM total acids content. Muszyńska et al. [64] found the presence of five acids in *P. ostreatus*: protocatechuic, p-hydroxybenzoic, *p*-coumaric, sinapic, vanillic, ferulic (1.28–21.38 mg/kg DM), and cinnamic acid (from 1.09 to 8.73 mg/kg DM). Similar results were obtained by Gąsecka et al. [65], but they did not measure sinapic acid. However, Kim et al. [66] noted a higher concentration of chlorogenic acids in the methanol extract of *P. ostreatus*. The study also quantified two other HBA derivatives and protocatechuic acids.

Badalyan et al. [67] determined the following acids in *P. squarrosa* and *P. ostreatus*: 4-hydroxybenzoic, 4-hydroxycinnamic, 4-hydroxy-3-methoxybenzoic, and 3,4-dihydroxyphenylacetic. Woldegiorgis et al. [68] also confirmed the presence of caffeic, gallic, and p-hydroxybenzoic acids in the methanolic extract of *P. ostreatus* cultivated in Ethiopia and determined that caffeic acid had the highest concentration among the phenolic acids. A different result was obtained by Palacious et al. [69], who found a low level of caffeic acid in *P. ostreatus*. Barros et al. [70] isolated the following acids from *H. fasciculare*: protocatechuic, p-hydroxybenzoic, and *p*-coumaric acids. In our research, we also quantified thirteen phenolic acids, and the highest concentration was observed for *p*-coumaric acid.

The carotenoids were another compound detected from the tested fungi. They belong to the group of chemicals that are synthesized in the organisms of bacteria, fungi, algae, and plants. Carotenoids containing 4-keto groups, monocyclic, and 13 double bonds in the structure, are characteristic of fungi [71,72]. The conjugated bonds are responsible for the distinctive color of these compounds (yellow, orange, or purple). These compounds protect against oxidative stress and exposure to visible light or UV radiation [73]. Moreover, carotenoids are intermediates in the biosynthesis of physiologically active apocarotenoids and their derivatives [74]. A large amount of reactive, conjugated double bonds present in the carotenoids causes the high activity of these compounds as antioxidants active against free radicals. These compounds stabilize cell membranes and act as photoreceptors.

All isolates were tested for β-carotene, lutein, zeaxanthin, and astaxanthin. The latter metabolite is characterized by 10-fold higher antioxidant activity than the other carotenoids: β-carotene, zeaxanthin, and lutein [75,76]. In our research, the highest concentration of this metabolite was observed in the *T. versicolor* (Tv) isolate. Other researchers confirmed the presence of β-carotene in *P. ostreatus* [77]. Jayakumar et al. [78] determined β-carotene in a 5-fold higher concentration than the present study. Robaszkiewicz et al. [79], Jaworska et al. [80], and Turfan et al. [81] found lycopene presence in addition to β-carotene. The latter was present at a much lower concentration in *P. ostreatus*. Mushroom species that contain β-carotene are *Cantharellus cibarius* Fr., *Agaricus bisporus* (J.E. Lange) Imbach, *Boletus edulis* Bull., *Suillus bovinus* (L.) Roussel, and *Tricholoma equestre* (L.) P. Kumm. Lycopene was found in the fruiting bodies of *C. cibarius*, *A. bisporus*, *B. edulis*, *S. bovinus*, and *T. equestre*. Lutein, α-carotene, and xanthotoxin were found in the fruiting bodies of *C. cibarius*, while γ-carotene, auroxanthin, and neurosporin was found in *B. edulis*. Barros et al. [82,83] spectrophotometrically proved that among the six *Basidiomycota* (Whittaker ex R.T. Moore) species collected in northeast Portugal, *C. cibarius* contains the highest amount of β-carotene, which was equal to 13.56 mg/g DM. For the other species, the content of this metabolite ranged from 1.95–12.77 mg/g DM [82,83].

Another group of active compounds isolated from the analyzed fungi is flavonoids. Today, more than 9000 different flavonoids are known, and the number continues to grow [84]. Flavonoids are polyphenolic chemical compounds of plant-based origins on the flavone skeleton. Their name comes from the Latin word flavus, meaning yellow. They occur mainly in the form of glycosides in the higher plants' tissues and fungi. The antioxidant activity of the flavonoids depends on the conjugated double bonds in the C-2 and C-3 position, hydroxyl groups, and the carboxyl group in the C-4 position [85,86]. In the conducted analyses, the highest content of these metabolites was observed in *I. obliquus* (Io)—114.5 mg/kg DM. High concentrations were also observed in *H. fasciculare* (Hf) and *P. squarrosa* (Ps). In addition, a high concentration of naringenin, quercetin, and kaempferol above 21 mg/kg DM was found. Similar results were obtained by Zheng et al. [34], who also isolated quercetins, naringenins, and kaempferol from *I. obliquus* (Io). Conversely, *B. adusta* (Ba) (0.07 mg/kg DM) was characterized by containing the lowest concentration of flavonoids. *P. squarrosa* (Ps) and *T. versicolor* (Tv) were poor in flavonoids in our research. Janjušević et al. [63] obtained different results with *T. versicolor* (Tv) flavonoids content harvested from northwest Romania. The obtained profile was rich

and included: flavones (6 compounds, e.g., apiin, vitexin, coumarins (2 compounds), flavanols (6 compounds, e.g., quercetin and rutin), isoflavonoids, and biflavonoids (amentoflavone). The flavonoid content was 67 mg/100 g DM. Other species with significant flavonoid amounts influencing their antioxidant value were: *Lactarius deterrimus* Gröger, *Boletus edulis* Bull. and *Xerocomellus chrysenteron* (Bull.) Šutara.

The compounds commonly found in mushrooms are sterols. The first reports of the presence of sterol in mushroom fruiting bodies date back to 1887. The typical mushroom sterols are distinguished by a high degree of unsaturation. Ergosterol is the main component of the fungal cell membrane and is strongly associated with cytoplasm; it is also a precursor to vitamin D2. Ergosterol can be converted to vitamin D2 through UV radiation. Finnish scientists determined the total sterol content in species of large-fruited mushrooms in the range of 625–774 mg/100 g DM [87]; a different ERG content characterized the analyzed fungi species. The highest ERG content was observed in *I. obliquus* (Io) (863.33 mg/kg). Kim et al. [66] and Shin et al. [88] determined ERG in *I. obliquus,* but not quantitatively. Alternatively, our research found that *H. fasciculare* (Hf) was characterized by a four-times lower ergosterol concentration compared with *I. obliquus*. Chemical composition studies of the edible mushroom species have shown that they are a rich source of ergosterol. After several years of storing the dried fruiting bodies, vitamin D2 content was high, averaging 1.43 μg/g DM in *C. cibarius* Fr. The differences in the ERG content were the results of the different sites of insolation from which the fruiting bodies came from [89]. *Agaricus bisporus* (J.E. Lange) Imbach contains an average of 61.5 mg/100 g of ergosterol. Research on the influence of UV-C radiation on the formation of vitamin D2, carried out by the Center for Plant and Food Science at the University of Western Australia, showed a high conversion rate of ergosterol to vitamin D2 after short-term exposure to the UV-C radiation of these species' fruiting bodies during development. After UV-C radiation irradiating for 2.5, 5, and 10 min, the ergosterol concentrations were 6.6, 15.6, and 23.1 μg/g DM, respectively. Based on the *Boletus edulis* Bull. chemical analysis, it has been shown that the entire fruiting body contains approx. 200 mg of vitamin D2 per 100 g of DM 89].

In the conducted analyses, it was found that the antioxidant activity measured with the ABTS radical was 2.5–3.5 times higher in the isolates of *I. obliquus* (Io), *P. ostreatus* (Po), and *H. fasciculare* (HF) compared to *P. squarrosa* (Ps) and *B. adusta* (Ba). The highest antioxidant activity and the highest total phenolic acid content was found in *T. versicolor* (Tv) when compared to the other isolates. This relationship corroborates that the phenolic compounds' content may affect the level of antioxidant activity. Matijašević et al. [90] investigated the total polyphenol content of the *T. versicolor* (Tv) isolate collected near Belgrade. The concentration of the FPA in the mushroom ethanol extract was 25.8 mg GAE/g. Those results were almost five times lower than the results determined in this study (Table 4). However, these results align with Vamanu and Voica's [91] research, who investigated the total phenolics and antioxidant activity of the several mushrooms harvested from the Moldova region of Romania. In the different isolates of *T. versicolor* (Tv) variegated growths, significant differences were noted in FPA content, flavonoids, and antioxidant capacity. These significant differences can be explained by the genetic factors (different fungal isolates), harvest site and time, type of solvent, and extraction conditions. These compounds' direct antioxidant mechanism captures the free oxygen radicals and reactive oxygen forms and limits their cell production by inhibiting the activity of the oxidizing enzymes (e.g., lipoxygenase) due to the easy hydrogen donation from the carboxyl group, which reduces peroxides and hydroxides.

5. Conclusions

In conclusion, it should be stated that differences in the qualitative and quantitative contents of active compounds may be the result of the different genetic properties and locations from which mushroom fruiting bodies are obtained. Significant qualitative and

quantitative differences were found in the content of selected phenolic acids, flavonoids, sterols, and carotenoids.

Chaga (*Inonotus obliquus*) was characterized by the highest tested compound content among the studied mushrooms.

The antioxidant activity measured by the ABTS radical and the content of free phenolic acids (FPA) was also tested. It was found that the highest antioxidant activity and the highest total phenolic acid content were found in *T. versicolor* (Tv) compared to the other isolates. Chaga was also characterized by a high antioxidant value and a high total phenolic acid content. This dependence confirms that the content of phenolic compounds may affect the level of antioxidant activity. Results show that mushrooms are an excellent source of valuable antioxidant compounds, and the results presented in this study show the enormous potential of fungi as a potential source of bioactive compounds.

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