



Polyphenols of *Hericium erinaceus* and *Inonotus obliquus* Mushrooms and their Antioxidant Potential

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Abstract

Mushrooms of the genera *Hericium* and *Inonotus* are valuable sources of bioactive polyphenols with notable antioxidant and antimicrobial properties. This study examined the total polyphenol content (TPC), antioxidant potential, and antimicrobial activity of ethanol and methanol extracts obtained from submerged mycelial cultures of *Hericium erinaceus* 2530 and *Inonotus obliquus* 2512. The total polyphenol content (TPC) was quantified using the Folin–Ciocalteu method, and antioxidant capacity was assessed by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. Fourier Transform Infrared Spectroscopy was applied to identify the principal functional groups of phenolic constituents. Marked strain-dependent variations were observed in both polyphenol content and antioxidant performance, with *Inonotus obliquus* 2512 showing the highest activity and *Hericium erinaceus* 2530 demonstrating moderate but significant effects. Both species exhibited measurable antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. These findings underscore the potential of submerged mushroom cultures as sustainable sources of natural antioxidants and antimicrobial agents for prospective nutraceutical and pharmaceutical applications.

Keywords: *Hericium erinaceus*; *Inonotus obliquus*; Polyphenol; antioxidant and antimicrobial properties; Bioactive compounds.

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1. Introduction

Mushrooms are widely recognized as valuable natural sources of bioactive metabolites with diverse pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, and neuroprotective effects.^[1–3] Among these metabolites, phenolic compounds play a central role due to their ability to neutralize free radicals, chelate metal ions, and protect biological systems from oxidative stress.^[4,5] These natural antioxidants have attracted increasing attention as alternatives to synthetic compounds traditionally used in food and pharmaceutical industries.

Species of the genera *Hericium* and *Inonotus* (Basidiomycota) are among the most promising medicinal mushrooms due to their high content of biologically active compounds.^[6,7] *Hericium erinaceus* (Lion's Mane mushroom) produces a variety of bioactive substances such as erinacines and hericenones, known for their neuroprotective and

immunomodulatory effects.^[8–10] *Inonotus obliquus* (Chaga mushroom), a parasitic fungus growing on birch trees, is rich in polyphenolic compounds, melanins, and triterpenes that exhibit strong antioxidant, antiviral, and antitumor properties.^[11–13] The composition and concentration of these metabolites can vary depending on the part of the fungus, growth conditions, and extraction solvent.^[14–16] While most previous studies have focused on the fruiting bodies of *Hericium* and *Inonotus*, submerged mycelial cultures offer a sustainable and controllable alternative for producing bioactive metabolites.^[17–19] Submerged cultivation ensures high biomass yields, short production cycles, and consistent metabolite profiles under defined environmental conditions.^[20] Moreover, this method allows for scalable production of antioxidant-rich extracts without the need for seasonal harvesting of fruiting bodies.^[21,22]

Polyphenols are key contributors to the antioxidant capacity of mushrooms, and their biosynthesis in submerged cultures is influenced by strain genetics, nutrient composition, and environmental factors.^[23–25] Understanding the variability in polyphenol content and antioxidant potential among different strains can help to identify the most productive and biologically active cultures.

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Therefore, the present study aimed to evaluate and compare the total polyphenol content, antioxidant activity, and antimicrobial properties of ethanol and methanol extracts obtained from submerged mycelial cultures of *Hericium erinaceus* 2530 and *Inonotus obliquus* 2512. In addition, Fourier-transform infrared (FTIR) spectroscopy was employed to characterize functional groups associated with phenolic compounds. The results contribute to a better understanding of the bioactive potential of submerged-cultured medicinal mushrooms and demonstrate their prospective use as natural sources of antioxidants and antimicrobial agents for nutraceutical and pharmaceutical applications.

In this study, we hypothesize that variations in solvent polarity and strain-specific metabolic capacity significantly influence the phenolic composition and biological activity of fungal extracts. The novelty of this work lies in the direct comparative evaluation of *Hericium erinaceus* 2530 and *Inonotus obliquus* 2512 strains, combining quantitative analysis of polyphenols with functional antioxidant and antimicrobial assays. This integrated approach provides new insights into strain-dependent bioactivity relationships in medicinal mushrooms cultivated under submerged conditions.

2. Materials and methods

2.1 Materials

All chemicals and reagents used in this study were of analytical grade. Ethanol (95v/v%) was purchased from local pharmacy store and methanol (99v/v%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin-Ciocalteu reagent and sodium carbonate 99%; DPPH (2,2-diphenyl-1-picrylhydrazyl) 99% were obtained from Merck (Darmstadt, Germany). Gentamicin sulfate 99.5% Gallic acid 99% and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) 99% were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as reference standards for polyphenol and antioxidant assays, respectively. Standard nutrient medium components—D-(+)-glucose 99%, peptone, and yeast extract were obtained from Sigma-Aldrich (St. Louis, MO, USA). Nutrient broth and Mueller-Hinton agar were supplied by HiMedia Laboratories (Mumbai, India). Distilled and deionized water were used throughout all experiments.

2.2 Samples

Hericium (five strains) *Inonotus obliquus* (four strains) were

obtained from the Collection of Cultures of *Agaricomycetes* (IBK), M. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine. The strains had been previously identified based on both morphological characteristics and molecular analysis of the ITS region. Mycelial biomass was cultivated in 500 mL Erlenmeyer flasks containing 200 mL of standard nutrient medium (glucose-peptone-yeast extract; D-(+)-glucose, peptone, yeast extract; Sigma-Aldrich, USA) adjusted to pH 6.2. Cultivation was carried out at 28 °C under stationary conditions for 10 days, until maximum biomass accumulation was achieved. After cultivation, the mycelium was separated from the culture broth by filtration, washed twice with distilled water, and dried at 40 °C to a constant weight.

2.3 Extraction procedure

Extraction of phenolic compounds was conducted using 70% ethanol (Sigma-Aldrich, USA) and 99% methanol (Sigma-Aldrich, USA) to evaluate solvent effects on extraction efficiency. These solvents were selected because their polarity allows efficient dissolution of a wide range of phenolic acids and flavonoids from fungal biomass. Aqueous ethanol (70%) provides an optimal balance between polarity and extraction yield, enabling recovery of both hydrophilic and moderately lipophilic compounds, whereas pure methanol effectively extracts more polar metabolites. Dried and powdered mycelium (particle size < 1 mm) was mixed with solvent at a ratio of 1:10 (w/v) and incubated at 30 °C for 24 h with continuous agitation at 150 rpm to improve solvent penetration and prevent degradation of thermolabile compounds. Extracts were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 40 °C. The resulting crude extracts were dried to constant weight and stored at 4 °C in airtight vials until analysis. Prior to antioxidant and antimicrobial assays, all dried extracts were tested for residual solvent by weighing before and after vacuum drying and confirmed to contain negligible ethanol or methanol. This ensured that observed bioactivities were attributable to fungal phenolic compounds rather than solvent artifacts. All extractions were performed in triplicate to ensure reproducibility, and variability between independent extractions did not exceed 5%, confirming consistency of the extraction procedure. Extraction yield (%) was calculated as the ratio of dried extract weight to initial dry mycelium weight and is reported in Table 1 for both ethanol and methanol extracts.

2.4 Determination of polyphenol content

The total polyphenol content of the extracts was determined using the Folin–Ciocalteu colorimetric method (Merck, Germany) with minor modifications. Briefly, 0.1 mL of extract was mixed with 0.5 mL of 10% (v/v) Folin–Ciocalteu reagent and 0.4 mL of 7.5% (w/v) sodium carbonate (Merck, Germany). The mixture was incubated in

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the dark at room temperature for 30 min, after which the absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan). Gallic acid (Sigma-Aldrich, USA) was used as the reference standard to construct a calibration curve ($R^2 > 0.99$). The results were expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM). All measurements were conducted in triplicate, and data are presented as mean $\pm$ standard deviation (SD). Independent extraction replicates showed consistent TPC values (coefficient of variation $<5\%$), confirming good reproducibility of the extraction and analytical procedures.

FTIR spectra of fungal ethanol and methanol extracts were recorded using a Shimadzu IRTracer-100 FTIR spectrometer (Japan) in the range of 4000–500 cm^{-1} with a resolution of 4 cm^{-1} . Dried extracts (≈ 2 mg) were mixed with spectroscopic-grade KBr (1:100, w/w) and pressed into pellets under vacuum. Spectral data were processed using IR-resolution software for baseline correction and normalization.

2.5 Determination of antioxidant activity

Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay (Merck, Germany). A 0.1 mL aliquot of extract at various concentrations (0.05–2.0 $\text{mg}\cdot\text{mL}^{-1}$) was mixed with 3.9 mL of 0.1 mM DPPH solution prepared in methanol. The reaction mixtures were vortexed and incubated in the dark for 30 min at room temperature to allow the reaction to reach equilibrium. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800). Methanol was used as the blank, and a control sample containing only the DPPH solution (without extract) was measured to determine the initial absorbance (A_0). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich, USA) served as the reference standard in the concentration range of 10–200 μM . The calibration curve for Trolox was constructed by plotting absorbance against concentration, and the resulting regression Eq. (1) was:

$$y = 0.0123x + 0.034, R^2 = 0.998 \quad (1)$$

Antioxidant capacity was expressed as micromoles of Trolox equivalents per gram of dry matter ($\mu\text{mol TE/g DM}$), based on the Trolox calibration curve ($R^2 > 0.99$). The percentage of DPPH radical inhibition was calculated using the Eq. (2):

$$\text{Inhibition (\%)} = ((A_0 - A) / A_0) \times 100 \quad (2)$$

where A is the absorbance of the control and A is the absorbance of the sample. IC_{50} values, representing the extract concentration required to inhibit 50% of DPPH radicals, were calculated by nonlinear regression analysis. All determinations were performed in triplicate.

2.6 Determination of antimicrobial activity

The antimicrobial potential of fungal extracts was evaluated

using the agar well diffusion method against Gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633) and Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883) bacteria. Test cultures were obtained from the Collection of the Department of Microbial Synthesis Biotechnology, National University of Food Technologies, Ukraine. Overnight bacterial cultures were grown in nutrient broth (HiMedia, India) and adjusted to 0.5 McFarland standard ($\sim 1 \times 10^8$ CFU/mL). Cultures were evenly spread on Mueller–Hinton agar plates (HiMedia, India). Wells of 6 mm diameter were filled with 50 μL of fungal extract (10 mg/mL in 70% ethanol, Sigma-Aldrich, USA). 70% ethanol served as a negative control, and gentamicin (10 $\mu\text{g/mL}$, Sigma-Aldrich, USA) was used as a positive control. Plates were incubated at 37 $^{\circ}\text{C}$ for 24 h, and inhibition zones were measured in millimeters (mm).

All assays were performed in triplicate. Results were expressed as mean $\pm$ standard deviation (SD), and statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test, with significance accepted at $p < 0.05$.

2.7 Statistical analysis

All experiments were conducted in triplicate, and results are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using GraphPad Prism version 10.0.2 (GraphPad Software, San Diego, CA, USA) and OriginPro version 2023b (OriginLab Corporation, Northampton, MA, USA). Prior to ANOVA, data normality and homogeneity of variance were verified using the Shapiro–Wilk and Levene's tests, respectively. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied to determine significant differences between means ($p < 0.05$ was considered statistically significant). Pearson's correlation coefficient (r) was used to evaluate the relationship between total polyphenol content and antioxidant activity. Linear regression models were applied to assess the relationship among extraction solvent, polyphenol content, and IC_{50} values.

3. Results & discussion

3.1 Polyphenol content in extracts of *hericium* and *inonotus obliquus* species

The total polyphenol content was determined in aqueous-alcoholic extracts of mycelial biomass obtained from five *Hericium* strains and four *Inonotus obliquus* strains. The results are summarized in Tables 1 and 2.

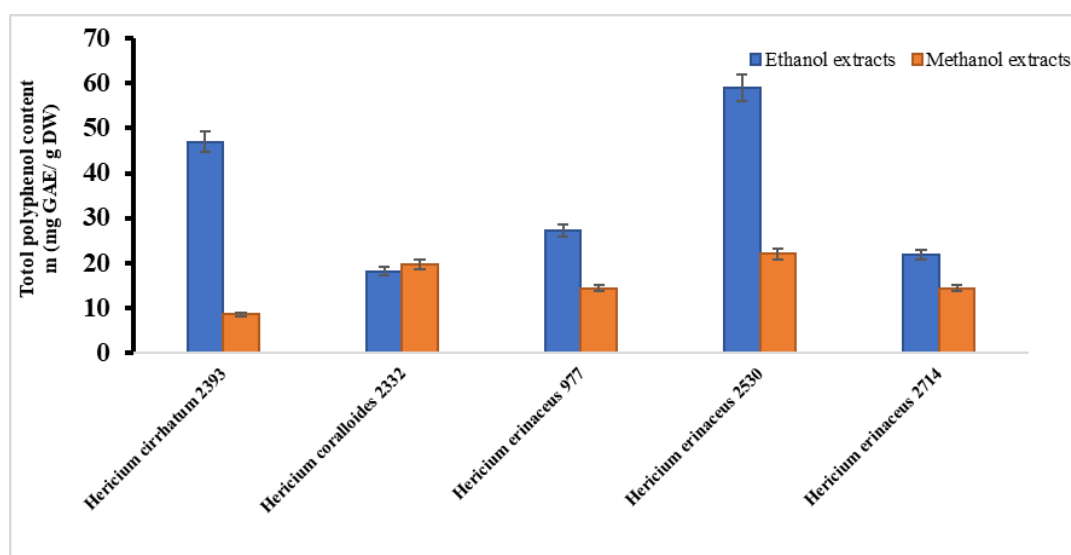
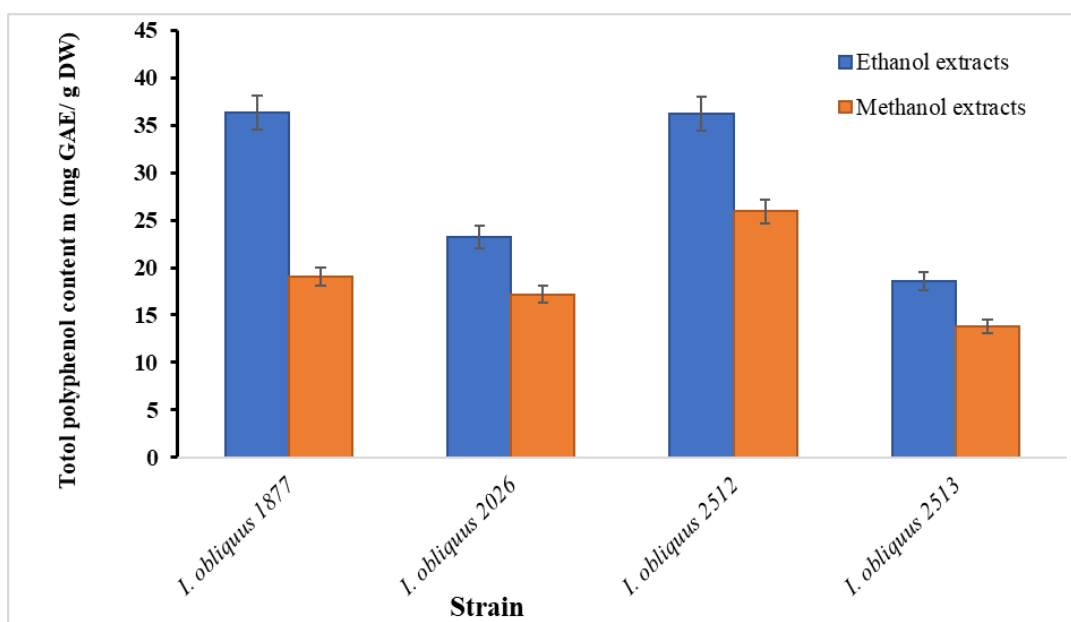
In *Hericium* species, the highest TPC values were observed in the ethanolic extracts of *H. erinaceus* 2530 (59.03 ± 0.28 mg GAE/g DW), while for *H. cirrhatum* 2393 and *H. erinaceus* 977 contained 46.98 ± 0.33 mg GAE/g DW , and 27.17 ± 0.12 mg GAE/g DW , respectively. Methanolic extracts revealed lower phenolic concentrations, except for *H. coralloides* 2332, whose methanolic extract exhibited a relatively higher content (19.69 ± 0.12 mg GAE/g DW),

Table 1: Total polyphenol content and extraction yield of *Hericium* species mycelial extracts.

Strain	Ethanol extracts (mg GAE/g DW)	Methanol extracts (mg GAE/g DW)	Extraction yield (g extract/g DW)
<i>H. cirrhatum</i> 2393	47.0 ± 0.33	8.59 ± 0.05	0.062 ± 0.003
<i>H. coralloides</i> 2332	18.2 ± 0.23	19.7 ± 0.12	0.058 ± 0.002
<i>H. erinaceus</i> 977	27.2 ± 0.12	14.4 ± 0.03	0.065 ± 0.004
<i>H. erinaceus</i> 2530	59.0 ± 0.28	22.0 ± 0.71	0.072 ± 0.002
<i>H. erinaceus</i> 2714	21.9 ± 0.06	14.4 ± 0.03	0.060 ± 0.003

Table 2: Total polyphenol content and extraction yield of *Inonotus obliquus* mycelial extracts.

Strain	Ethanol extracts (mg GAE/g DW)	Methanol extracts (mg GAE/g DW)	Extraction yield (g extract/g DW)
<i>I. obliquus</i> 1877	36.3 ± 0.8	19.1 ± 0.2	0.069 ± 0.004
<i>I. obliquus</i> 2026	23.2 ± 0.4	17.2 ± 0.2	0.060 ± 0.003
<i>I. obliquus</i> 2512	36.2 ± 0.2	25.9 ± 0.2	0.075 ± 0.002
<i>I. obliquus</i> 2513	18.6 ± 0.2	13.8 ± 0.2	0.057 ± 0.003

**Fig. 1:** Total phenolic content in extracts of *Hericium* mycelial biomass (ethanol and methanol extracts).**Fig. 2:** Total phenol content in extracts of *Inonotus obliquus* mycelial biomass (ethanol and methanol extracts).

suggesting species-specific solvent affinities and differential localization of polyphenolic compounds within the fungal cell matrix (Fig. 1).

These findings align with previous reports indicating substantial variability in the phenolic content of *H. erinaceus* depending on cultivation parameters, substrate composition, and extraction method. Valu *et al.*^[21] reported TPC values ranging from 11.1 to 23.2 mg GAE/g DW under submerged fermentation, while ultrasonic-assisted extraction markedly enhanced polyphenol yield and antioxidant capacity.^[22,23] Likewise, Yu *et al.*^[26] demonstrated that nutrient modulation and growth stage significantly influence the profile of phenolic acids and flavonoids. The higher TPC values detected in *H. erinaceus* 2530 and 977 in the present study point to strong strain-specific metabolic potential and corroborate the high intraspecific variability characteristic of *Hericium* species.^[27–29]

According to available data, this work provides the first quantitative assessment of polyphenol content in *H. cirrhatum* and *H. coralloides* mycelium. The high phenolic accumulation observed in these species underscores the chemical diversity and adaptive biosynthetic capacity of the genus *Hericium*.^[30,31] Moreover, pronounced differences among strains cultivated under identical conditions emphasize the importance of genetic regulation in secondary metabolite biosynthesis.^[32,33] Consequently, strains *H. erinaceus* 2530, *H. cirrhatum* 2393, and *H. coralloides* 2332 may be regarded as promising candidates for the biotechnological production of phenolic-rich extracts with potent antioxidant potential.^[34,23]

A comparable trend was observed among *Inonotus obliquus* strains. The highest polyphenol concentrations were recorded in the ethanolic extracts of strains 1877 and 2512, reaching 36.3 ± 0.8 and 36.2 ± 0.2 mg GAE/g DW, respectively (Table 2). These values were significantly higher than those of strains 2026 and 2513 (18–25 mg GAE/g DW). Ethanol consistently produced extracts with greater phenolic content than methanol, corroborating previous findings that ethanol exhibits superior solubilizing capacity for complex phenolic matrices due to its intermediate polarity and ability to disrupt hydrogen bonding networks.^[35–37] Methanol extracts contained approximately 25–35% less total phenols on average (Fig. 2).

However, methanol appeared to preferentially extract lower-molecular-weight phenolic acids and simple flavonoids, which may contribute differently to antioxidant mechanisms.^[37,38] The most phenolic-rich methanolic extracts were obtained from *I. obliquus* 2512 (25.92 ± 0.2 mg GAE/g DW) and 1877 (19.12 ± 0.2 mg GAE/g DW). These data collectively indicate that solvent polarity and extraction conditions substantially influence the recovery efficiency and composition of polyphenol metabolites.

Polyphenolic compounds from *I. obliquus* are widely recognized for their antioxidant, anti-inflammatory, and antitumor activities.^[39–41] The elevated TPC values detected in strains 2512 and 1877 highlight their potential as efficient producers of bioactive metabolites suitable for pharmaceutical and nutraceutical applications. Overall, both *Hericium* and *Inonotus obliquus* species demonstrate pronounced strain-dependent variability in polyphenol accumulation, reinforcing the importance of careful strain selection, culture optimization, and solvent system choice in the development of standardized functional fungal bioproducts.

Thus, ethanol proved to be a more effective solvent for polyphenol extraction from both *Hericium* and *Inonotus obliquus* species, yielding significantly higher TPC values than methanol. The strains *H. erinaceus* 2530, *H. cirrhatum* 2393, *H. coralloides* 2332, *I. obliquus* 1877, and *I. obliquus* 2512 exhibited the highest polyphenol levels and are therefore of particular biotechnological interest. The results obtained confirm a strong influence of both genetic and environmental factors on phenolic biosynthesis, highlighting the potential of these fungal strains as valuable sources of natural antioxidants for pharmaceutical and functional food applications.

3.2 Analysis of polyphenol compounds by IR spectroscopy

For FTIR analysis, only two representative strains of *Hericium erinaceus* 2530 and *Inonotus obliquus* 2512 were selected. This choice was based on their highest total polyphenol content values among the tested isolates, which ensured a sufficient concentration of phenolic compounds for accurate detection and interpretation of characteristic functional groups in the FTIR spectra. In addition, these strains demonstrated stable growth and consistent metabolite production under controlled culture conditions, making them suitable model

Table 3: Polyphenol compounds identified by IR spectral analysis in ethanol extracts of *H. erinaceus* 2530 and *I. obliquus* 2512.

Compound identified	Main IR absorption bands (cm ⁻¹)	Chemical class	Relative intensity (<i>Hericium</i>)	Relative intensity (<i>Inonotus</i>)
Gallic acid	3390, 1715, 1615, 1210	Phenolic acid	+++	++
Caffeic acid	3360, 1680, 1608, 1270	Phenolic acid	++	+++
p-Coumaric acid	3405, 1655, 1610, 1185	Hydroxycinnamic acid	++	++
Syringic acid	3380, 1705, 1600, 1265	Methoxyphenolic acid	+	++
Catechin	3425, 1620, 1515, 1210	Flavan-3-ol	+++	+
Quercetin	3410, 1650, 1590, 1175	Flavonol	++	++
Vanillic acid	3430, 1695, 1610, 1230	Aromatic acid	+	++
Ferulic acid	3400, 1685, 1605, 1255	Methoxyphenolic acid	++	+++

Note: (+) weak signal, (++) moderate, (+++) strong relative absorption intensity.

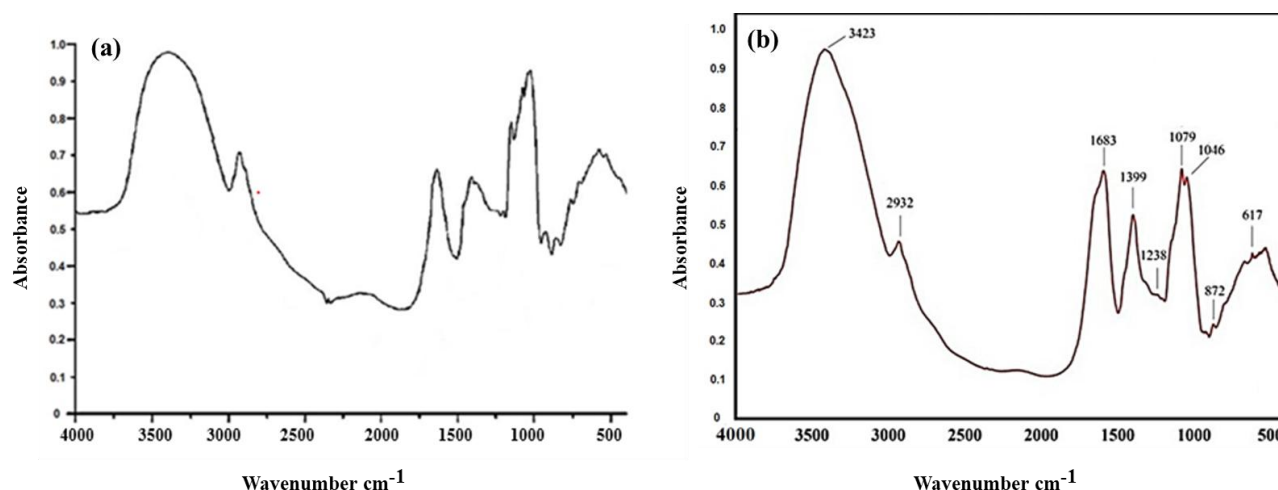


Fig. 3: FTIR spectra of ethanoic extracts of: a) *I. obliquus* 2512 and b) *H. erinaceus* 2530.

representatives for each genus. The selected *H. erinaceus* 2530 and *I. obliquus* 2512 thus reflect the most biochemically active polyphenol producers, allowing for a detailed assessment of the structural features and functional diversity of their polyphenolic metabolites.

Strong broad absorption peaks in the region of 3400–3300 cm^{-1} indicated the presence of multiple hydroxyl phenolic groups (–OH), suggesting a high degree of phenolic hydroxylation. Distinct bands near 1610–1650 cm^{-1} were attributed to aromatic C=C stretching vibrations of aromatic ring presented in flavonoids and phenolic acid structures.^[42] while signals around 1700 cm^{-1} corresponded to C=O stretching vibrations of carboxylic and esterified groups typical of gallic and caffeic acids (Table 3). Weak and moderate intensity peaks in the 1250–1150 cm^{-1} region were assigned to C–O–C stretching in aryl ethers and glycoside linkages, indicating the presence of polyphenol glycosides.^[43]

Analysis of the FTIR spectra (Fig. 3) revealed notable differences between *H. erinaceus* 2530 and *I. obliquus* 2512. Extracts of *H. erinaceus* 2530 showed higher relative intensities of peaks associated with catechin and gallic acid, confirming their enrichment in flavan-3-ols and hydroxybenzoic acids. These compounds are known for their potent antioxidant and neuroprotective activities, consistent with previous reports on *H. erinaceus* 2530 mycelium. In contrast, *I. obliquus* 2512 extracts demonstrated stronger bands corresponding to caffeic and ferulic acids, indicative of a higher proportion of hydroxycinnamic derivatives, which contribute to anti-inflammatory and immunomodulatory properties.^[44] The presence of syringic and vanillic acids in both genera supports the hypothesis that lignin-derived phenolic precursors are incorporated into fungal secondary metabolism pathways^[45] Such diversity in phenolic profiles underscores the adaptive metabolic strategies of basidiomycetes and their potential as sources of multifunctional antioxidant compounds.

Thus, FTIR spectroscopic analysis confirmed the presence of diverse polyphenol compounds in the ethanol extracts of *H.*

erinaceus 2530 and *I. obliquus* 2512. *H. erinaceus* 2530 extracts were enriched in catechin and gallic acid, whereas *I. obliquus* 2512 extracts contained higher levels of caffeic and ferulic acids. These differences reflect strain-specific biosynthetic capacities and suggest distinct antioxidant, neuroprotective, and immunomodulatory potentials, highlighting both species as valuable sources of bioactive phenols.

3.3 Antioxidant Activity of Fungal Extracts

To evaluate the biological significance of the detected polyphenol compounds, the antioxidant capacity of ethanol and methanol extracts from the examined *H. erinaceus* 2530 and *I. obliquus* 2512 strains was determined. The antioxidant activity of the hydroalcoholic extracts was assessed using the DPPH radical-scavenging assay, and results were expressed in micromoles of Trolox equivalents per gram of dry matter ($\mu\text{mol TE/g DM}$). The corresponding IC_{50} values were calculated to determine the extract concentration required for 50% inhibition of the DPPH radical (Fig. 4).

Among all tested samples, the ethanol extract of *I. obliquus* 2512 exhibited the highest antioxidant capacity ($487 \pm 15.8 \mu\text{mol TE/g DM}$) with the lowest IC_{50} value (0.42 mg/mL). These data clearly indicate the high efficiency of the extract in donating electrons and neutralizing free radicals, reflecting the presence of potent redox-active phenolic constituents. Methanol extracts of the same strain also showed strong antioxidant activity ($463 \pm 14.3 \mu\text{mol TE/g DM}$), though slightly lower. According to one-way ANOVA followed by Tukey's post-hoc test, the difference between ethanol and methanol extracts of *I. obliquus* 2512 was not statistically significant ($p > 0.05$), confirming that both solvent systems effectively extract phenolic antioxidants from this strain.

To evaluate the biological significance of the detected polyphenol compounds, the antioxidant capacity of ethanol and methanol extracts from *H. erinaceus* 2530 and *I. obliquus* 2512 was determined. The antioxidant activity of the hydroalcoholic extracts was assessed using the DPPH radical-

scavenging assay, and results were expressed in micromoles of Trolox equivalents per gram of dry matter ($\mu\text{mol TE/g DM}$). The corresponding IC_{50} values were calculated to determine the extract concentration required for 50% inhibition of the DPPH radical (Fig. 4).

A significant positive correlation ($r = 0.86$, $p < 0.01$) was observed between total polyphenol content and DPPH radical-scavenging activity, suggesting that phenolic compounds play a dominant role in determining the redox potential of fungal extracts. To statistically validate this relationship, a Pearson correlation analysis was performed using GraphPad Prism 10.0, confirming a strong linear association between TPC and antioxidant activity across all samples ($R^2 = 0.74$, $p < 0.01$). This observation aligns with previous reports emphasizing that phenolic hydroxyl groups act as hydrogen donors and stabilize free radicals through resonance delocalization.^[14,46]

In contrast, *H. erinaceus* 2530 extracts demonstrated lower antioxidant values compared with *I. obliquus* 2512, though their activity remained within a biologically relevant range (310.4–342.1 $\mu\text{mol TE/g DM}$). Interestingly, the methanol extract of *H. erinaceus* 2530 showed slightly higher antioxidant capacity (342.1 $\mu\text{mol TE/g DM}$) than its ethanol counterpart (310.4 $\mu\text{mol TE/g DM}$), and this difference was statistically significant ($p < 0.05$). This pattern may indicate selective extraction of lower-molecular-weight phenolic acids and other antioxidant constituents with higher polarity. In addition to phenolic compounds, *Hericium* species are known to produce various antioxidant metabolites such as erinacines, hericenones, melanoidins, and triterpenoids that may synergistically contribute to the observed activity.^[23]

Similar solvent-dependent patterns have been reported for other medicinal fungi, including *Ganoderma lucidum*, *Pleurotus ostreatus*, and *Cordyceps militaris*, where methanolic extracts often display superior radical-scavenging capacity compared to aqueous or ethanolic ones.^[47] Such differences arise primarily from variations in solvent polarity, which affect the solubilization efficiency of specific antioxidant molecules. Ethanol, being less polar, preferentially extracts complex polyphenols and triterpenoids, whereas

methanol enhances extraction of low-molecular-weight phenolic acids and flavonoids.

The strong antioxidant response observed in *I. obliquus* 2512 extracts corroborates previous findings on its high phenolic content and the presence of compounds such as inonoblins, hispidin derivatives, and polyphenolic glycosides, all of which exhibit potent radical-scavenging and metal-chelating activities.^[48] These bioactive molecules are thought to protect fungal cells from oxidative stress during growth on lignocellulosic substrates, where exposure to reactive oxygen species is particularly high.

Overall, the results confirm that the antioxidant potential of medicinal mushrooms is highly strain- and solvent-dependent. Extracts from *I. obliquus* 2512 exhibited the most pronounced activity, while *H. erinaceus* 2530 displayed moderate but biologically meaningful radical-scavenging ability. The combination of high total polyphenol content and low IC_{50} values observed for *I. obliquus* 2512 highlights its promising applicability in the development of nutraceutical and pharmaceutical formulations aimed at mitigating oxidative stress, inflammation, and age-related degenerative diseases.

3.4 Antimicrobial activity of selected strains

The antimicrobial potential of the ethanol and methanol extracts of *Hericium erinaceus* 2530 and *Inonotus obliquus* 2512 was evaluated against representative Gram-positive and Gram-negative bacteria. Both fungal species demonstrated moderate to strong inhibitory effects, with *I. obliquus* 2512 extracts exhibiting broader activity spectra. Based on their highest total polyphenol content and antioxidant capacity, the ethanol extracts of *H. erinaceus* 2530 and *I. obliquus* 2512 were selected for detailed antimicrobial assessment.

Antibacterial activity was determined using the agar well diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Table 4, Fig. 5 and 6). All experiments were performed in triplicate, and inhibition zone diameters were expressed as mean $\pm$ SD (mm). Statistical significance was assessed using one-way ANOVA followed by Tukey's

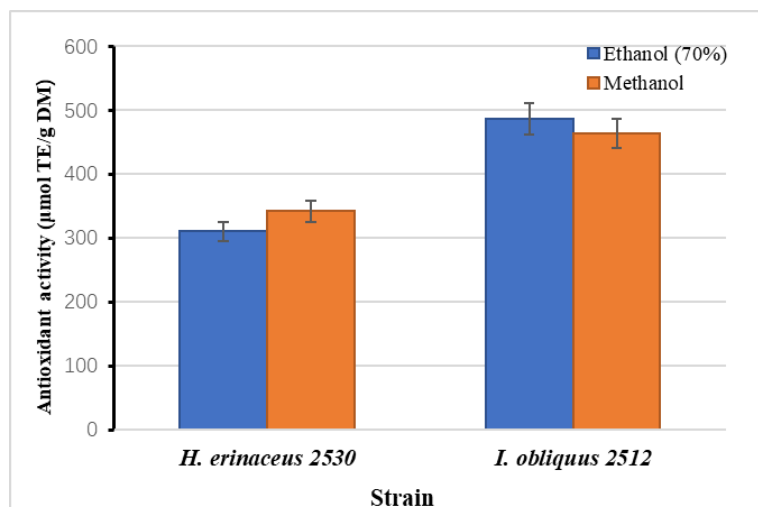
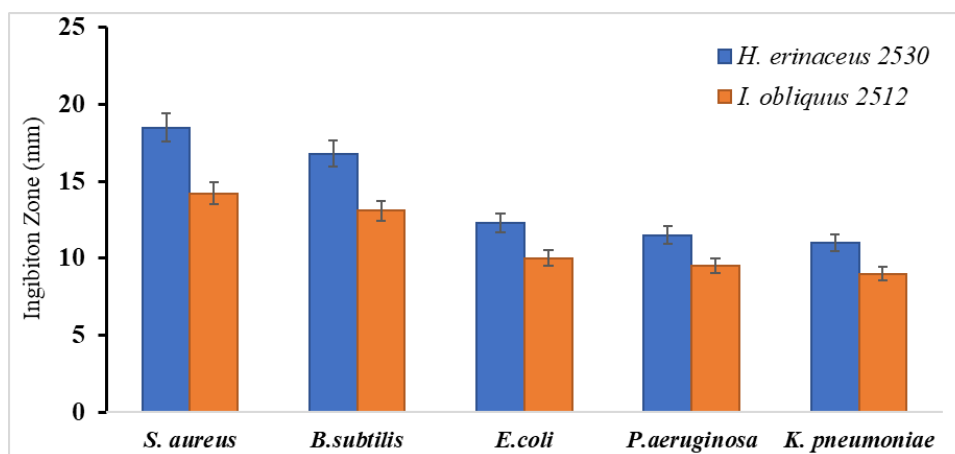
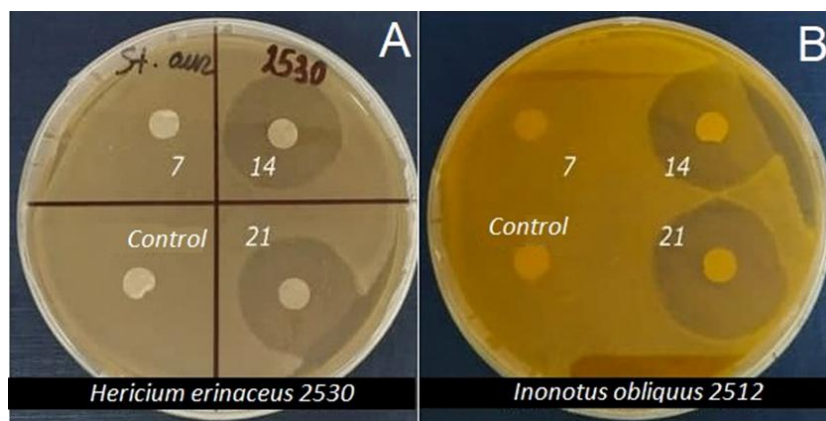


Fig. 4: Antioxidant activity (DPPH assay) of ethanol and methanol extracts of *H. erinaceus* 2530 and *I. obliquus* 2512.

Table 4: Inhibition zones (mm) of ethanolic extracts from *H. erinaceus* 2530 and *I. obliquus* 2512 against selected bacterial strains.

Bacterial Strain	<i>H. erinaceus</i> 2530	<i>I. obliquus</i> 2512
<i>Staphylococcus aureus</i>	18.5 ± 0.5 ^a	14.2 ± 0.4 ^b
<i>Bacillus subtilis</i>	16.8 ± 0.6 ^a	13.1 ± 0.5 ^b
<i>Escherichia coli</i>	12.3 ± 0.4 ^a	10.0 ± 0.3 ^b
<i>Pseudomonas aeruginosa</i>	11.5 ± 0.3 ^a	9.5 ± 0.2 ^b
<i>Klebsiella pneumoniae</i>	11.0 ± 0.4 ^a	9.0 ± 0.3 ^b

Values represent mean ± SD (n = 3). Different superscript letters within a row indicate significant differences (p < 0.05, one-way ANOVA, Tukey's post-hoc).

**Fig. 5:** Antimicrobial activity of ethanol extracts from *H. erinaceus* 2530 and *I. obliquus* 2512. Error bars represent standard deviations (n = 3). Different letters indicate statistically significant differences (p < 0.05; one-way ANOVA, Tukey's test).**Fig. 6:** Representative inhibition zones of ethanol extracts against *Staphylococcus aureus* (p < 0.05, one-way ANOVA).

post-hoc test (p < 0.05). Significant differences among samples are indicated by different superscript letters in Table 4 and Figs. 5-6.

Both strains demonstrated measurable antibacterial activity, with *H. erinaceus* 2530 consistently producing larger inhibition zones across all tested microorganisms (p < 0.05). The most pronounced effects were observed against Gram-positive bacteria (*S. aureus* and *B. subtilis*), whereas Gram-negative strains (*E. coli*, *P. aeruginosa*, *K. pneumoniae*) exhibited comparatively lower sensitivity.

This trend is consistent with previous studies showing that polyphenolic compounds generally exert stronger antibacterial effects on Gram-positive bacteria due to

structural differences in their cell envelopes. The absence of an outer lipopolysaccharide membrane in Gram-positive bacteria facilitates easier penetration of phenolic compounds, which can disrupt membrane integrity, alter enzymatic function, and inhibit protein synthesis.^[49]

The antimicrobial mechanism of action of fungal polyphenols is believed to involve multiple cellular targets, including membrane permeabilization, oxidative stress induction, and interference with nucleic acid and protein synthesis. Compounds such as hispidin derivatives, phenolic acids, and triterpenoids from *Inonotus* and *Hericium* species can generate reactive oxygen species (ROS), chelate essential ions (Fe²⁺, Mg²⁺), and inhibit key bacterial enzymes, leading

to loss of membrane potential and cell death.^[50,36] Such multi-targeted action enhances the likelihood of antimicrobial efficacy and reduces the potential for resistance development.

The pronounced inhibitory effect of *H. erinaceus* 2530 against *S. aureus* suggests that specific phenolic acids, flavonoid derivatives, and related metabolites act synergistically to induce oxidative stress and destabilize the bacterial membrane. Several reports indicate that such polyphenols can chelate essential metal ions or interfere with bacterial quorum-sensing pathways, thereby enhancing antimicrobial efficacy.^[50]

The ethanol extract of *I. obliquus* 2512 also displayed significant antibacterial activity, though of slightly lower magnitude. This moderate activity may be attributed to the complex composition of the extract, in which polyphenols interact with other bioactive constituents, including triterpenoids, melanin-like pigments, and polysaccharides characteristic of *I. obliquus*. These compounds may act through multiple mechanisms, such as enzyme inhibition, reactive oxygen species generation, and membrane destabilization.^[36]

Overall, the results confirm that both *H. erinaceus* 2530 and *I. obliquus* 2512 possess dual bioactivity—antioxidant and antimicrobial—making them promising candidates for multifunctional fungal bioproducts. Their natural extracts can potentially be applied in pharmaceutical formulations, food preservation systems, and cosmetic products to reduce microbial contamination, extend shelf life, and mitigate oxidative degradation.

4. Conclusion

In this research we discussed the comparative evaluation of the polyphenolic composition, antioxidant, and antimicrobial potential of *Hericium erinaceus* 2530 and *Inonotus obliquus* 2512 extracts. Significant strain- and solvent-dependent variability in total polyphenol content was observed, with *H. erinaceus* generally exhibiting higher accumulation. These variations reflect the influence of genetic background, cultivation conditions, and extraction solvent polarity on the biosynthesis and recovery efficiency of bioactive metabolites. Antioxidant activity illustrated a strong positive correlation with total polyphenol content ($r = 0.86$, $p < 0.01$) comparable to the commercial positive control, confirming the central role of phenolic compounds in free radical neutralization and redox regulation. *I. obliquus* 2512 extracts demonstrated the highest radical-scavenging capacity, whereas *H. erinaceus* 2530 exhibited moderate but consistent activity.

This suggests that diverse phenolic profiles, together with other metabolites such as flavonoids, triterpenoids, and melanoidins, may synergistically enhance antioxidant effects.

Methanolic extracts of *H. erinaceus* 2530 were enriched in low-molecular-weight phenolic acids and flavonoids, while ethanolic extracts of *I. obliquus* 2512 contained more complex polyphenols and terpenoids with pronounced antioxidant potential. Both extracts exhibited measurable antimicrobial

activity against Gram-positive and Gram-negative bacteria, with *H. erinaceus* 2530 showing stronger inhibition zones overall. The antimicrobial mechanism likely involves membrane permeabilization, oxidative stress induction, and interference with nucleic acid and protein synthesis, reflecting a multi-targeted action of fungal polyphenols.

Overall, *H. erinaceus* 2530 and *I. obliquus* 2512 represent promising natural sources of polyphenolic antioxidants and antimicrobial agents suitable for functional foods, nutraceuticals, pharmaceutical formulations, and food preservation applications. Their bioactive metabolites may contribute to the prevention of oxidative stress, inflammation, and age-related degenerative disorders. Future studies should focus on identifying individual phenolic constituents and elucidating their specific mechanisms of antioxidant and antimicrobial action in cellular and *in vivo* models, as well as optimizing extraction protocols for maximal recovery of target compounds.

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Conflict of Interest

The authors declare no conflict of interest

Supporting Information

Not applicable.

CRedit Statement

Narmuratova Zhanar: Writing – Original draft, Visualization, Methodology, Conceptualization. **Kairat Mustafin and Dmitriy Berillo:** Writing – Original draft, Visualization, Methodology, Conceptualization. **Nina Bisko:** Writing – Review & editing, Visualization. **Aigerim Zhakipbekova:** Writing – Review & editing. **Zhanara Suleimenova:** Writing – Review & editing, Supervision, Conceptualization. **Sholpan Islam and Nurlan Akhmetsadykov:** Methodology, Writing – Review & editing, Supervision, Funding acquisition, Conceptualization.

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