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Bioactive Potential of Six Edible Mushrooms: Antimicrobial, Antioxidant, and Cytotoxic Properties

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Abstract

In the present study, antimicrobial, antibacterial, and cytotoxic effects of methanol and acetone extracts obtained from the edible mushroom species of *Boletus edulis, Chanterellus cibarius, Craterellus cornucopioides, Agaricus bisporus, Pleurotus ostreatus*, and *Morchella esculenta* were determined in vitro. The *B. edulis* methanol extract showed the highest 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging effect at 1mg mL^{-1} concentration with 34.68 ± 1.56 %. It was determined that the total phenolic content of the extracts varied between 27.22 ± 2.29 - 16.67 ± 0.88 µg GAE mg^{-1} extract. The findings suggest that the extracts have varying antimicrobial effects on pathogenic bacteria. 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) cell viability test was performed to investigate the cytotoxic effects of the extracts. Cytotoxicity studies demonstrated that *C. cibarius* selectively decreased cancer cell viability, while *C. cornucopioides, P. ostreatus,* and *A. bisporus* exhibited proliferative effects on cancer cells.

Keywords

Edible mushrooms, Antioxidant, Antimicrobial, Cytotoxicity, Extract

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Altı Yenilebilir Mantarın Biyoaktif Potansiyeli: Antimikrobiyal, Antioksidan ve Sitotoksik Özellikler

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Öz

Bu çalışmada, yenilebilir mantar türleri olan *Boletus edulis*, *Chanterellus cibarius*, *Craterellus cornucopioides*, *Agaricus bisporus*, *Pleurotus ostreatus* ve *Morchella esculenta*'dan elde edilen metanol ve aseton ekstraktlarının antimikrobiyal, antibakteriyel ve sitotoksik etkileri in vitro olarak belirlenmiştir. *B. edulis* metanol ekstraktı, 1 mg mL^{-1} konsantrasyonda %34.68 ± 1.56 ile en yüksek 1,1-difenil-2-pikrilhidrazil (DPPH) süpürücü etkisini göstermiştir. Ekstraktların toplam fenolik içeriğinin 27.22 ± 2.29 - 16.67 ± 0.88 µg GAE mg^{-1} arasında değiştiği belirlenmiştir. Bulgular, ekstraktların patojenik bakteriler üzerinde farklı antimikrobiyal etkilere sahip olduğunu göstermektedir. Ekstraktların sitotoksik etkilerini araştırmak için 3-(4,5-Dimetiltiazol-2-il)-2,5-Difeniltetrazolium Bromür (MTT) hücre canlılığı testi yapılmıştır. Sitotoksisite çalışmaları, *C. cibarius*'un seçici olarak kanser hücresi canlılığını azalttığını, *C. cornucopioides*, *P. ostreatus* ve *A. bisporus*'un ise kanser hücreleri üzerinde proliferatif etkiler sergilediğini göstermiştir.

Anahtar kelimeler

Yenilebilir mantarlar, Antioksidan, Antimikrobiyal, Sitotoksisite, Ekstrakt

1. INTRODUCTION

As heterotrophic eukaryotic organisms, fungi are essential ecosystem components contributing to carbon and nitrogen cycles. They obtain nutrients through saprotrophic or parasitic modes of nutrition, playing a crucial role in organic matter decomposition and nutrient recycling [1-3]. Fungi encompass numerous species with both edible and medicinal significance. The dry matter of mushrooms comprises 20–35% protein, making them a valuable dietary source. A high content of unsaturated fatty acids with low total lipid content characterizes them. Additionally, mushrooms are highly digestible and provide all nine essential amino acids, making them a nutritionally complete protein source [3]. Consequently, fungi are a nutrient-rich food source that can be incorporated into a balanced and healthy diet [4]. Additionally, their bioactive compounds have been shown to reduce low-density lipoprotein (LDL) cholesterol levels in the bloodstream while promoting satiety, thereby contributing to overall metabolic health [5].

Only 10% of the estimated 1.5 million fungal species worldwide have been scientifically identified. Among these, 5.020 species are classified as edible mushrooms, while 1.820 species exhibit medicinal properties [6,7]. More than 2,600 macrofungal species have been documented in Türkiye, and approximately 300 are recognized as edible [8,9]. However, despite this diversity, many edible and medicinal mushrooms remain poorly studied, with limited research on their biological activities. In recent years, increasing awareness of mushrooms' nutritional and medicinal value has led to a growing interest in their research. Studies have demonstrated that consuming dietary antioxidants from external sources, including antioxidant-rich foods such as mushrooms, reduces the risk of disease development and slows or delays disease progression. With the rising demand for natural functional food sources, the investigation of mushrooms has gained significant importance, mainly due to their anti-inflammatory, antitumoral, immunomodulatory, cardioprotective, hepatoprotective, and neuroprotective properties [10-14].

Research has demonstrated that numerous mushroom species possess therapeutic properties in addition to their role as a nutritious food source. Their biological activities are attributed to diverse bioactive compounds, including β-glucans in polysaccharide form, ergosterol (a steroid-derived precursor of vitamin D), and ganoderic acids in the triterpene class. Furthermore, mushrooms are rich in essential vitamins, such as thiamine (B1), riboflavin (B2), folic acid, and niacin (B3), contributing to their nutritional and medicinal significance [15].

Previous research has demonstrated that mushrooms exhibit significant pharmacological properties due to their high antioxidant capacity attributed to bioactive compounds such as phenols, flavonoids, ascorbic acid, β-carotene, and lycopene [16]. These antioxidant molecules are crucial in neutralizing free radicals, mitigating oxidative stress, and preventing cellular damage. Consequently, antioxidants contribute to cellular repair mechanisms, counteracting the harmful effects of oxidative stress [17]. Numerous studies have reported that free radicals are a major contributing factor to various diseases, including cancer, cardiovascular disorders, and other chronic conditions [18, 19]. The increasing research focus on the nutraceutical potential of mushrooms is largely driven by their ability to support healthy metabolism through the regulation of oxidative balance within the body. Given their potent free radical-scavenging capacity, the bioactive phenolic compounds found in mushrooms are recognized as highly effective natural antioxidants [20].

Despite significant advancements in medical research, a definitive cure for many invasive diseases, particularly cancer, remains elusive. Moreover, the misuse and overuse of antibiotics have led to the emergence of drug-resistant microorganisms, necessitating the exploration of alternative therapeutic strategies. In response, scientists have increasingly focused on the potential of plants and fungi as natural antimicrobial agents [21]. Recent research efforts have been directed toward investigating the antimicrobial, antioxidant, and cytotoxic properties of bioactive compounds derived from fungi and plants for therapeutic applications. While substantial progress has been made in this field, viral, fungal, and bacterial infections continue to pose significant public health challenges, particularly in developing countries, where the burden of infectious diseases remains high [22]. Therefore, further research is required to comprehensively identify the bioactive compounds present in fungi and elucidate their mechanisms of action.

The objective of this study was to evaluate the antimicrobial activity of acetone and methanol extracts derived from six edible mushroom species—*Morchella esculenta, Boletus edulis, Chanterellus cibarius, Craterellus cornucopioides, Pleurotus ostreatus,* and *Agaricus bisporus*—belonging to different fungal families. These species were selected due to their limited prior study and underexplored bioactive potential. Their extracts were tested against bacterial strains associated with human pathogenicity. Additionally, this study aimed to contribute to existing research by assessing these mushroom species' antioxidant properties and cytotoxic effects.

2. MATERIALS AND METHODS

2.1. Sample Preparation and Extraction

Edible mushroom species of *Boletus edulis, Cantharellus cibarius, Craterellus cornucopioides, Agaricus bisporus, Pleurotus ostreatus* and *Morchella esculenta* were bought from Gurmenet Sanal Mağazacılık Hizmetleri Ltd. Co. and Kurucum Gıda Ltd. Co.. Materials were stored in dark and at room temperature for extraction.

Two different solvents (acetone and methanol) were used to fractionate the soluble compounds from mushrooms. 10 g of dried and powdered mushroom samples were mixed with 100 mL of each solvent. The mixture was kept in a 25 °C water bath for 24 hours by shaking at 120 rpm. After

24 hours, it was filtered through Whatman No: 1 filter paper, and the filtrate was stored in the dark. The same procedure was used with the residues repeated two more times, and the filtrates were combined. Solvents of the collected filtrates were removed with a rotary evaporator (Scilogex RE 100 Pro) at 40 °C. The obtained extracts were stored at +4 °C for antioxidant and cytotoxic activity analysis [23].

2.2. Microdilution Assay

The Microdilution Method determined fungal species' minimum inhibitory concentrations (MIC) [24]. The following bacteria strains were obtained from the culture collection of the Microbiology Laboratory of Kırklareli University Vocational School of Health Services: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 6538 (2), *Escherichia coli* 0157H7 NCTC 11774, *Enterobacter aerogenes* (clinical isolate), *Listeria monocytogenes* ATCC 13932, *Bacillus cereus* ATCC 14774, *Salmonella enteritidis* ATCC 13076. According to this method, Mueller Hinton Broth (MHB) medium was prepared and sterilized, and 90 µL was dispensed into 96 microplates under sterile conditions. 100 µL (4000 µg\mL) of extract was added to the first column of the microplates. The extracts were diluted twice, and their concentrations were adjusted to 2000-1.95 µg mL^{-1}. Test bacteria cultured on Mueller Hinton Agar (MHA) medium for 24 hours were adjusted to $1.5x10^8$ CFU mL^{-1} according to 0.5 McFarland turbidity. When 10 µL of microorganism was added to each well, the bacterial suspension in the wells was diluted in MHB medium to a final concentration of $5x10^5$ CFU mL^{-1}. Dimethyl Sulfoxide (DMSO) was used as a negative control. As a positive control, 2 mg mL^{-1} Ampicillin and 2 mg mL^{-1} Ciprofloxacin were prepared, and the concentration range was determined as 1000-0.98 µg mL^{-1}. MIC values were determined after the inoculated microplates were kept at 37 °C for 18-24 hours.

2.3. Antioxidant Activity Assays

2.3.1. Scavenging Activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radicals

1,1-diphenyl-2-picrylhydrazy (DPPH) was used to determine the free radical-scavenging activity of the extracts by a minor modification of the Blois method [25]. This method prepared" should be removed and the sentence should be as "Each extract was prepared at five different concentrations as 100,250,500,750 and 1000 µg mL^{-1}. 1 mL of these extracts at different concentrations was mixed with 4 mL of 0.1 mM DPPH and vortexed. Absorbance values were measured at 517 nm after 30 minutes of incubation in the dark. Three replicates were performed for each sample. Butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), and vitamin C were standard. The DPPH radical-scavenging activity of the extracts was calculated as Equation 1:

$$\%DPPHRadicalScavengingActivity = \frac{ControlAbsorbance - SampleAbsorbance}{ControlAbsorbance} x100 \quad (1)$$

2.3.2. Determination of Total Phenolic Content

The total amount of phenolic content present in the extracts was determined by the Folin-Ciocalteau reagent (FCR) [26]. To determine the total phenolic content, each sample was taken as 1 mg mL^{-1} and mixed with 45 mL of water and 1 mL of Folin-Ciocalteau reagent. After incubation for 3 minutes, 3 mL of 2% Na_2CO_3 was added. The mixture was vortexed and incubated at 250 rpm for 2 hours at room temperature, after which its absorbance was read at 760 nm. 100, 200, 300, 400, and 500 µg mL^{-1} concentrations of gallic acid were used as standards.

2.3.3 Reducing Activity

The Oyaizu method was used to determine the reducing power [27]. The Prussian blue color is formed after reducing Fe^{3+} ions to Fe^{2+} ions by reducing agents in the extracts and adding $FeCl_3$. The high absorbance value observed is indicative of high reducing power.

1 mL of samples from extracts were prepared at concentrations of 100, 250, 500, 750, and 1000 µg mL^{-1}, then mixed with 2.5 mL of 0.2 M pH 6.6 phosphate buffer and 2.5 mL of 1% $K_3Fe(CN)_6$. This mixture was incubated for 20 minutes at 50 °C. After incubation, 1 mL sample taken from the mixture was mixed with 1 mL distilled water and 0.2 mL 1% $FeCl_3$, and the absorbance values against blank were measured at 700 nm. BHA and vitamin C standards were used to analyze the results.

2.4. Determination of Cytotoxic Activity

Cytotoxic studies were carried out to observe the effects of acetone and methanol extracts of mushroom species on three different cell lines of AML12 (*Mus musculus* liver normal cell), HepG2 (human hepatocellular carcinoma cell) and Hep3B (human hepatocellular carcinoma cell). 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) cell viability test was used to assess the cytotoxic effect of 3 different concentrations of extracts (0.25 mg mL^{-1}, 0.5 mg mL^{-1} and 1 mg mL^{-1}) by the end of 24 and 48 hours after incubation with cell lines [28].

Cells were cultured with Dulbecco′s Modified Eagle′s Medium (DMEM/F-12) (10% fetal bovine serum) at 37 °C in a 5% CO_2 humidified incubator. Each cell line was suspended at a density of 1-5 × 10^4 cells mL^{-1} into each well of 96-well microliter plates and incubated for approximately 16 hours. The extracts were then applied and incubated for 24 and 48 hours. MTT viability analyses were performed in 4 replicates and DMSO as a control group. MTT solution (20 µl) of 5 mg mL^{-1} was then added to each well and incubated for another 4 h. After the incubation, the media in the wells were removed entirely, and 200 µl of ultra-pure DMSO was added to each well and kept in the dark at room temperature for 2-4 hours to completely dissolve the crystals formed. Plates were read spectrophotometrically at a wavelength of 492 nm

(Multiskan GO, Thermo Scientific, Waltham, MA, USA), and the relative viability was calculated as Equation 2:

$$\%CellViability = \frac{SampleAbsorbance - BlankAbsorbance}{ControlAbsorbance - BlankAbsorbance} x100 \quad (2)$$

2.5 Statistical Analysis

All results were evaluated as the means of three parallel replicates. The results were recorded as mean ± standard deviation; error bars are indicated above the graphs. Antioxidant activities were analyzed by SPSS (version 19.0.0 for Windows, SPSS Inc.). The independent t-test was used for pairwise comparisons, and one-way ANOVA-Tukey-HSD tests were used for multiple comparisons in antioxidant activity. The data obtained from the cytotoxicity analyses were given as mean ± standard deviation, the normality of the data was evaluated with the Shapiro-Wilk test, and the differences between the groups were examined with one-way ANOVA. In addition, Dunnett's multiple comparisons after one-way ANOVA revealed the effect of extract types, and the effect of concentration was shown by Tukey's multiple comparisons after one-way ANOVA. $P<0.05$ value was considered statistically significant in all statistical analyses, and cytotoxicity analyses were performed using GraphPad Prism9 (GraphPad Prism 9.0.2 trial version for MacOS).

3. RESULTS AND DISCUSSION

3.1. Antioxidant Activities

Free radicals, particularly reactive oxygen species (ROS), are highly reactive molecules generated as byproducts of normal cellular metabolism or through external factors such as radiation, pollution, and toxins. While low to moderate levels of ROS play essential roles in cell signaling and immune defense, excessive production can lead to oxidative stress, causing damage to lipids, proteins, and DNA. Antioxidants, which include enzymatic systems like superoxide dismutase and catalase, as well as non-enzymatic compounds such as vitamin C, vitamin E, and polyphenols, neutralize ROS and protect cells from oxidative damage. Maintaining a balance between ROS generation and antioxidant defense is crucial for cellular health and the prevention of various chronic diseases [29-31].

The DPPH radical scavenging activity of acetone and methanol extracts from various mushroom species was evaluated using the method of Blois (1958) with BHA, BHT, and vitamin C as standard antioxidants [25]. The percentage inhibition values of these extracts were compared to those of the standard antioxidants (Figure 1). Among the mushroom extracts, the methanol extracts of *B. edulis* (34.68±1.56%), *A. bisporus* (33.97±1.61%), and *M. esculenta* (24.00±1.22%) exhibited the highest DPPH radical scavenging activity for 1000 µg mL^{-1} concentration compared to other methanol and acetone extracts which show varying weak activities between 3.12±1.10% to 12.50±1.17%, considerably lower than those of the standards. Although there was a concentration-dependent increase in activity, the mushroom extracts' overall DPPH radical scavenging activity remained relatively low compared to standard antioxidants, which exhibited approximately 90% scavenging activity. The high DPPH radical scavenging activities observed in *B. edulis, A. bisporus*, and *M. esculenta* suggest that these mushrooms contain bioactive compounds with antioxidant properties. The results imply that the antioxidant potential of these mushrooms may be due to their phenolic compounds, which have been previously linked to radical scavenging activity [1]. In literature, polyphenols from *B. edulis* exhibited significant antioxidant potential, with a strong DPPH radical scavenging capacity (IC_{50} = 99.35 µg mL^{-1}), highlighting their potential health benefits [32]. Gürgen&Sevindik found the DPPH activity of the *Agaricus bisporus* mushroom in 47.246±0.754 mg Trolox Equi/g [33]. However, the other extracts' overall weak activity highlights that these mushrooms' antioxidant effects may vary depending on the species and solvent used for extraction. These findings align with previous studies that showed a strong correlation between phenolic content and antioxidant activity [34].

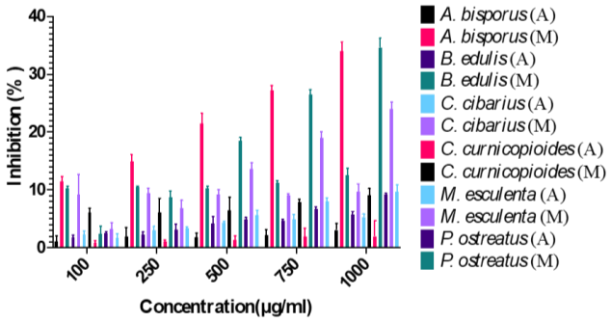

Figure 1. DPPH radical scavenging activity of mushroom extracts

The total phenolic content in acetone and methanol extracts of the mushroom species was determined using the Folin-Ciocalteu reagent [26]. Gallic acid was used to generate a standard curve for calculating the total phenolic content in the extracts. The gallic acid equivalents (µg GAE mg^{-1} extract) ranged from 27.22±2.29 to 16.67±0.88 µg GAE mg^{-1} extract for methanol extracts of *A. bisporus* and acetone extracts of *C. curnicopioides* respectively. The phenolic content in the methanol extracts of all examined mushroom species was higher than in their acetone extracts, except for *C. cibarius* (Table 1).

Table 1. Total phenolic contents of mushroom extracts (µg GAE mg-1 extract)

Mushroom species	Methanol extract	Acetone extract
A. bisporus	27.22±2.29^{ac}	17.33±1.37^{ad}
P. ostreatus	22.22±2.95^{ac}	25.56±4.05^{bc}
C. cibarius	24.33±2.84^{ac}	25.33±4.29^{bc}
C. cornucopioides	23.00±2.79^{ac}	16.67±0.88^{ad}
B. edulis	23.44±3.01^{ac}	17.11±1.30^{ad}
M. esculenta	25.67±3.10^{ac}	25.56±1.95^{bc}

*Letters (a-b) within the same column indicate a statistically significant difference in phenolic compound content among mushroom species ($p<0.05$) (One-way ANOVA, Tukey-HSD), letters (c-d) within the same row indicate a statistically significant difference in phenolic compound content between different solvents for the same mushroom species ($p<0.05$) (T-test).

Previous studies have shown that methanol extracts typically contain higher amounts of phenolics than acetone extracts, which may explain the differences in antioxidant activity between the extracts in this study [23]. In literature, the highest total phenolic content of *A. bisporus* was observed in its methanolic extract, reaching 31.73 µg GAE mg^{-1}, indicating its substantial polyphenol composition and potential antioxidant capacity [35]. The ethanolic and hot water extracts of *B. edulis* demonstrated superior antioxidant effectiveness with naturally occurring antioxidants such as total tocopherols (3.18–6.18 mg g^{-1}) and total phenols (5.67–5.81 mg g^{-1}) in its extracts, which showed a strong correlation ($r = 0.636–0.907$) with the EC_{50} value of its antioxidant activity [36]. The reducing power of acetone and methanol extracts of mushrooms was evaluated using the Oyaizu method and compared with standard antioxidants, BHA, and vitamin C [27]. Although the mushroom extracts exhibited weaker reducing power than the standards, the methanol extracts of *B. edulis* and *M. esculenta* demonstrated higher reducing power, with a value of 0.25 for both, surpassing the other extracts. The low reducing power observed in the mushroom extracts, particularly when compared to standard antioxidants, suggests that while these mushrooms may contain antioxidant compounds, their ability to reduce oxidants is less pronounced than synthetic antioxidants such as BHA and vitamin C. In support of this, the ethanolic extract obtained from B. *edulis* stipe demonstrated higher ferric-reducing antioxidant power (22.14 mg ascorbic acid equivalents g^1 dry weight) compared with aqueous extracts, indicating variability in reducing activity across different extraction methods [37]. The antioxidant capacity of *M. esculenta* has also been reported in the literature, highlighting its potential as a source of antioxidant compounds [38]. Afonso et al. determined that, the total phenolic content of *P. ostreatus* mushroom as 15.80 ± 1.54–16.65 ± 1.01 mg GAEs/g [39].

3.2. Antimicrobial Activities

The antimicrobial activity of acetone and methanol extracts was evaluated by determining MIC values using the microdilution method [24]. The MIC values for different bacterial strains are summarized in Table 2. Notably, the acetone extract of *M. esculenta* exhibited strong activity against *B. cereus, S. enteritidis*, and *L. monocytogenes*, with an MIC value of 0.5 mg mL^{-1}. Similarly, the acetone extracts of *B. edulis* on *B. cereus, P. ostreatus* on *B. cereus*, and *S. aureus* (1), and *C. cornucopioides* on *S. aureus* (1) also demonstrated antimicrobial activity at MIC values of 0.5 mg mL^{-1}.

Table 2. Minimum inhibitory concentration of extracts from mushrooms against test microorganisms

Mushroom extract (mg mL^{-1})	*E. coli*	*E. aerogenes*	*B. cereus*	*S. aureus* (1)	*S. aureus* (2)	*S. enteritidis*	*E. faecalis*	*L. monocytogene*
M. esculenta (M)	1	1	1	>2	>2	1	>2	1
M. esculenta (A)	1	2	0.5	>2	>2	0.5	>2	0.5
B. edulis (M)	2	1	2	>2	2	2	>2	2
B. edulis (A)	1	1	0.5	>2	>2	1	>2	1
C. cibarius (M)	2	2	2	>2	>2	2	>2	2
C. cibarius (A)	1	2	1	2	>2	1	>2	1
A. bisporus (M)	2	2	>2	>2	>2	2	>2	>2
A. bisporus (A)	1	2	1	>2	>2	1	>2	1
P. ostreatus (M)	2	2	2	2	>2	2	>2	2
P. ostreatus (A)	1	>2	0.5	0.5	>2	1	>2	1
C. cornucopioides (M)	2	2	2	1	>2	2	>2	2
C. cornucopioides (A)	1	2	1	0.5	>2	1	>2	1
Amp (µg mL^{-1})	<0.98	<0.98	<0.98	<0.98	<0.98	<0.98	<0.98	<0.98
Cipro (µg mL^{-1})	<0.98	<0.98	<0.98	<0.98	<0.98	<0.98	<0.98	<0.98

*(M) stands for methanolic extracts, and (A) stands for acetonic extracts

The antimicrobial activity of *M. esculenta* mycelium has been demonstrated in a study where it showed varying levels of effectiveness against one Gram-positive bacterium (*S. aureus*) and three Gram-negative bacteria (*E. coli, S. typhimurium, P. aeruginosa*), which are potential pathogens for humans and animals [40]. *B. edulis* exhibited a high content of antioxidant compounds, as demonstrated by DPPH, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and ferric reducing antioxidant power (FRAP) assays [41]. The study reported strong free radical scavenging activity with low IC_{50} values, indicating significant antioxidant potential. Additionally, *B. edulis* extracts effectively inhibited the growth of *S. aureus, E. coli,* and *K. pneumoniae*, suggesting its potential for antimicrobial applications. Other studies have reported similar results

where mushroom extracts demonstrated significant antibacterial activity due to phenolic compounds, terpenoids, and other bioactive substances [42]. Phenolics, organic acids, fatty acids, ergosterols, and low molecular weight volatile compounds found in mushroom extracts can disrupt microbial cell membranes, increase membrane permeability, and inhibit energy metabolism; additionally, polysaccharides/peptides can disrupt quorum sensing or biofilm formation [43]. These findings support the notion that mushrooms can be a valuable source of food preservation and therapeutic purposes.

3.3. Cytotoxic Activities

The cytotoxic effects of acetone and methanol extracts were assessed on three distinct cell lines—AML12 (normal liver cells), HepG2 (human liver cancer cells), and Hep3B (human liver cancer cells)—utilizing the MTT cell viability assay [28]. Cell viability was evaluated at 24 and 48 hours following treatment with extract concentrations of 0.25 mg mL^{-1}, 0.5 mg mL^{-1}, and 1 mg mL^{-1}, with the results presented in Figure 2.

At 24 hours, only the acetone extract of *C. cibarius* resulted in a significant reduction in cell viability (93±7.2%), whereas other extracts, such as those from *M. esculenta,* exhibited no significant effect. In contrast, extracts derived from *C. cornucopioides*, *P. ostreatus*, and *A. bisporus* increased cell viability. After 48 hours, *C. cibarius* continued to exert a cytotoxic effect, while *C. cornucopioides*, *P. ostreatus*, and *A. bisporus* significantly enhanced cell viability. This suggests that *C. cibarius* may contain bioactive compounds with potential anticancer properties, particularly against hepatocellular carcinoma cell lines. Previous studies have reported that various edible and medicinal mushrooms possess cytotoxic effects against cancer cells while sparing normal cells, primarily due to polysaccharides, terpenoids, and phenolic compounds present in these fungi [44].

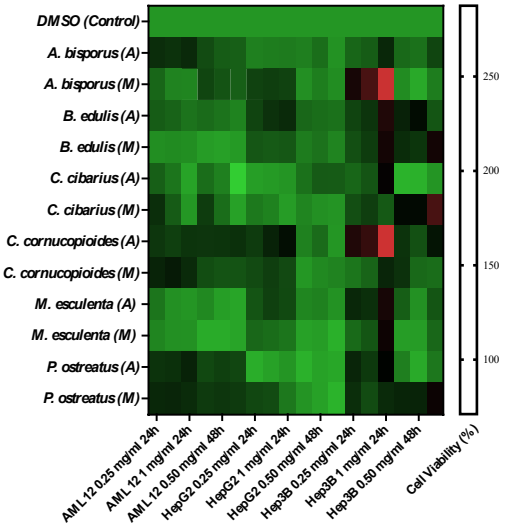

Figure 2. Heat maps illustrate the cytotoxic effects of acetone and methanol extracts from various mushroom species on AML12 (normal liver cells), HepG2 (human liver cancer cells), and Hep3B (human liver cancer cells) at 24 and 48 hours. *The color intensity represents changes in cell viability, with green indicating cytotoxicity (decreased viability) and red indicating increased cell viability.

The methanol extracts of *B. edulis, C. cornucopioides, M. esculenta*, and *A. bisporus* significantly increased cell viability at 24 hours, though no concentration-dependent effects were observed. With a continued effect observed at 48 hours, extracts from *C. cornucopioides, P. ostreatus, M. esculenta*, and *A. bisporus* continued to promote cell viability, with the methanol extract of *M. esculenta* notably reducing viability (88.7±20.3%). This suggests that some mushroom-derived compounds may exert proliferative effects, potentially through antioxidant or immunomodulatory pathways [45]. While such properties may benefit normal cell function, they indicate that these specific extracts may not be suitable for anticancer applications.

Among the 24-hour treatments, the acetone extract of *C. cornucopioides* exhibited the highest increase in cell viability at a concentration of 1 mg mL^{-1} (196±7.5%). At 48 hours, the acetone extract of *C. cibarius* reduced cell viability at higher concentrations but did not significantly affect cell survival at 1 mg mL^{-1}.

The selective cytotoxicity of *C. cibarius* appears to be time- and concentration-dependent, as its acetone extract continued to reduce cell viability at 48 hours, particularly at higher concentrations, suggesting a delayed apoptotic or cytotoxic effect potentially linked to gradual uptake and metabolic activation. In contrast, the increased cell viability observed with *C. cornucopioides, P. ostreatus,* and *A. bisporus* extracts indicates the presence of proliferative or protective compounds. Notably, *P. ostreatus* has been reported to enhance cell survival through antioxidant and anti-inflammatory mechanisms, which may explain its stimulatory effects in this study [46]. In a study conducted in 2025 using the hydroethanolic extract of *B. edulis*, the results showed no significant effect on the loss of cell viability in the HEPG2 cell line [47].

While these properties could have beneficial applications, further investigation is necessary to determine whether they promote healthy cell function or inadvertently support tumor growth, emphasizing the need to evaluate their therapeutic potential in cancer treatment repetitions carefully.

4. CONCLUSION

In conclusion, this study investigates various mushroom species' antioxidant, antimicrobial, and cytotoxic properties, highlighting their bioactive potential. The DPPH radical scavenging assay showed that *B. edulis, A. bisporus,* and *M. esculenta* possess notable antioxidant activity, though weaker than synthetic antioxidants. Future studies should identify phenolic compounds and optimize extraction techniques to enhance efficacy. Antimicrobial analysis demonstrated strong inhibitory effects of *M. esculenta* and *B. edulis* acetone extracts against pathogenic bacteria. However, variations among species suggest further research into isolating active compounds and elucidating their mechanisms of action. Cytotoxicity studies revealed that *C. cibarius* selectively reduced cancer cell viability, whereas *C. cornucopioides,*

P. ostreatus, and *A. bisporus* promoted cell proliferation. Overall, the findings of this study support the potential of mushrooms as sources of bioactive compounds with antioxidant, antimicrobial, and cytotoxic properties. However, significant knowledge gaps remain regarding these compounds' mechanisms of action, bioavailability, and potential therapeutic applications. Future studies should employ advanced analytical techniques such as high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy to identify and characterize the active constituents responsible for these effects. Furthermore, in vivo studies and clinical trials are necessary to establish the efficacy and safety of mushroom-derived compounds in real-world applications. By addressing these gaps, future research can unlock the full potential of mushrooms as functional foods and natural therapeutic agents.

Author Contributions

All the authors equally contributed to this work. They all read and approved the final version of the paper.

Conflict of Interest

All the authors declare no conflict of interest.

Ethical Review and Approval

No approval from the Board of Ethics is required.

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REFERENCES

[1] Stern KR, Jansky S, Bidlack JE. Introductory plant biology. 11th ed. New York: McGraw-Hill Companies; 2008. 346–370 p.

[2] Rost TL, Barbour MG, M. Murphy T, Stocking CR. Kingdom Fungi. In: Plant Biology. 2nd ed. Canada: Thomson Brooks Cole; 2006. p. 336–60.

[3] Kalač P. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. Food Chem. 2009;113(1):9–16.

[4] Losoya-Sifuentes C, Cruz M, del Refugio Rocha-Pizaña M, Loredo-Treviño A, Belmares R. Edible Mushrooms: a Nutrient-Rich Ingredient for Healthier Food Products – A Review. Curr Nutr Rep. 2025 Jan 3;14(1):9.

[5] Kim K, Choi B, Lee I, Lee H, Kwon S, Oh K, et al. Bioproduction of mushroom mycelium of *Agaricus bisporus* by commercial submerged fermentation for the production of meat analogue. J Sci Food Agric. 2011; 91(9):1561–8.

[6] Hawksworth DL. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol Res. 2001;105(12):1422–32.

[7] Anusiya G, Gowthama Prabu U, Yamini N V, Sivarajasekar N, Rambabu K, Bharath G, et al. A review of the therapeutic and biological effects of edible and wild mushrooms. Bioengineered. 2021;12(2):11239–68.

[8] Akata I, Altuntaş D, Kabaktepe Ş. Fungi Determined in Ankara University Tandoğan Campus Area (Ankara-Turkey). Trak Univ J Nat Sci. 2019 ;20(1):47–55.

[9] Sesli E, Denchev CM. Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. Mycotaxon. 2008;106:65–7.

[10] Bowe WP, Pugliese S. Cosmetic benefits of natural ingredients. J Drugs Dermatol. 2014;13(9):1021–5; quiz 26–7.

[11] Falandysz J, Borovička J. Macro and trace mineral constituents and radionuclides in mushrooms: health benefits and risks. Appl Microbiol Biotechnol. 2013 25;97(2):477–501.

[12] Jayachandran M, Xiao J, Xu B. A Critical Review on Health Promoting Benefits of Edible Mushrooms through Gut Microbiota. Int J Mol Sci. 2017 8;18(9):1934.

[13] Mujić I, Zeković Z, Vidović S, Radojković M, Živković J, Gođevac D. Fatty Acid Profiles of Four Wild Mushrooms and Their Potential Benefits for Hypertension Treatment. J Med Food. 2011 Nov;14(11):1330–7.

[14] Zhang JJ, Li Y, Zhou T, Xu DP, Zhang P, Li S, et al. Bioactivities and Health Benefits of Mushrooms Mainly from China. Molecules. 2016 20;21(7):938.

[15] Öztürk A, Çopur ÖU. Mantar Bileşenlerinin Teröpatik Etkileri. Bahçe. 2009;38(1):19–24.

[16] Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? The Lancet. 1994;344(8924):721–4.

[17] Shahidi F. Natural Antioxidants: Chemistry, Health Effects, and Applications. Champagn, Illinois: AOCS Press; 1997.

[18] Aruoma OI. Assessment of potential prooxidant and antioxidant actions. J Am Oil Chem Soc. 1996;73(12):1617–25.

[19] Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, Chen CH, et al. Free radical-scavenging activity of Taiwanese native plants. Phytomedicine. 2003;10(2–3):170–5.

[20] Akyuz M, Onganer A, Erecevıt P, Kırbag S. Antimicrobial Activity of some Edible Mushrooms in the Eastern and Southeast Anatolia Region of Turkey. Gazi University Journal of Science. 2010;23(2):125–30.

[21] Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer Statistics, 2003. CA Cancer J Clin. 2003 1;53(1):5–26.

[22] Atila F, Nadhim Owaid M, Ali Shariati M. The Nutrional and Medical Benefits of *Agaricus Bisporus:* A Review. Journal of microbiology, biotechnology and food sciences. 2017 1;7(3):281–6.

[23] Woldegiorgis AZ, Abate D, Haki GD, Ziegler GR. Antioxidant property of edible mushrooms collected from Ethiopia. Food Chem. 2014;157:30–6.

[24] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing, 28th ed. Wayne PA, USA: CLSI; Performance Standards for Antimicrobial Susceptibility Testing. 2018.

[25] Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 1958;181(4617):1199–200.
[26] Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am J Enol Vitic. 1965;16(3):144–58.
[27] Oyaizu M. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. The Japanese Journal of Nutrition and Dietetics. 1986;44(6):307–15.
[28] Ahmadian S, Barar J, Saei AA, Fakhree MAA, Omidi Y. Cellular Toxicity of Nanogenomedicine in MCF-7 Cell Line: MTT assay. Journal of Visualized Experiments. 2009;3(26).
[29] Kucukler S, Benzer F, Yildirim S, et al. Protective effects of chrysin against oxidative stress and inflammation induced by lead acetate in rat kidneys: a biochemical and histopathological approach. Biol Trace Elem Res. 2021;199:1501–1514.
[30] Caglayan C, Kandemir FM, Darendelioğlu E, Küçükler S, Ayna A. Hesperidin protects liver and kidney against sodium fluoride-induced toxicity through anti-apoptotic and anti-autophagic mechanisms. Life Sci. 2021;281:119730.
[31] Kucukler S, Darendelioğlu E, Caglayan C, Ayna A, Yıldırım S, Kandemir FM. Zingerone attenuates vancomycin-induced hepatotoxicity in rats through regulation of oxidative stress, inflammation and apoptosis. Life Sci. 2020;259:118382.
[32] Guo L, Tan DC, Bao RJ, Sun Q, Xiao KM, Xu Y, et al. Purification and antioxidant activities of polyphenols from *Boletus edulis* Bull.: Fr. Journal of Food Measurement and Characterization. 2020 16;14(2):649–57.
[33] Gürgen A, Sevindik M. Optimization of biological activities of Agaricus species: an artificial intelligence-assisted approach. Sci Rep. 2025 2;15(1):23147
[34] Weier TE, Stocking CR, Barbour M. The higher fungi Botany. In: An Introduction to Plant Biology. New York: John Wiley and sons Inc.; 1970. p. 499–537.
[35] Sezer YÇ, Süfer Ö, Sezer G. Extraction of Phenolic Compounds from Oven and Microwave Dried Mushrooms (*Agaricus bisporus* and *Pleurotus ostreatus*) by Using Methanol, Ethanol and Aceton as Solvents. Indian Journal of Pharmaceutical Education and Research. 2017 30;51(3s2):s393–7.
[36] Tsai SY, Tsai HL, Mau JL. Antioxidant properties of *Agaricus blazei, Agrocybe cylindracea*, and *Boletus edulis*. LWT - Food Science and Technology. 2007;40(8):1392–402.
[37] Novakovic A, Karaman M, Kaisarevic S, Radusin T, llic N. Antioxidant and Antiproliferative Potential of Fruiting Bodies of the Wild-Growing King Bolete Mushroom, *Boletus edulis* (Agaricomycetes), from Western Serbia. Int J Med Mushrooms. 2017;19(1):27–34.
[38] Heleno SA, Stojković D, Barros L, Glamočlija J, Soković M, Martins A, et al. A comparative study of chemical composition, antioxidant and antimicrobial properties of *Morchella esculenta* (L.) Pers. from Portugal and Serbia. Food Research International. 2013;51(1):236–43.
[39] Afonso TB, Marçal S, Vale P, Sousa AS, Nunes J, Pintado M. Exploring the bioactive potential of mushroom aqueous extracts: antimicrobial, antioxidant, and prebiotic properties. Appl Sci. 2025 3;15(3):1551.
[40] Badalyan SM, Gharibyan NG, Iotti M, Zambonelli A. Antimicrobial Activity of Three Italian Strains of *Morchella esculenta* (Ascomycota). Int J Med Mushrooms. 2024;26(2):43–55.
[41] Rosa GB, Sganzerla WG, Ferreira ALA, Xavier LO, Veloso NC, da Silva J, et al. Investigation of Nutritional Composition, Antioxidant Compounds, and Antimicrobial Activity of Wild Culinary-Medicinal Mushrooms *Boletus edulis* and *Lactarius deliciosus* (Agaricomycetes) from Brazil. Int J Med Mushrooms. 2020;22(10):931–42.
[42] Kalač P. A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. J Sci Food Agric. 2013;93(2):209–18.
[43] Ikram A, Ibrahim NA, Arshad MT, Fatima A, Taseer AA, Hussain MF, et al. Mushroom bioactive molecules as anticancerous agents: an overview. Food Sci Nutr. 2025;13(7):e70580.
[44] Kumar A, Devi R, Dhalaria R, Tapwal A, Verma R, Rashid S, et al. Nutritional, Nutraceutical, and Medicinal Potential of *Cantharellus cibarius* Fr.: A Comprehensive Review. Food Sci Nutr. 2025;13(1).
[45] Jakopovic B, Oršolić N, Kraljević Pavelić S. Antitumor, Immunomodulatory and Antiangiogenic Efficacy of Medicinal Mushroom Extract Mixtures in Advanced Colorectal Cancer Animal Model. Molecules. 2020;25(21):5005.
[46] Seifeldin SA, Upadhyay TK, Trivedi R, Rezgui R, Saeed A. Detection of antimicrobial, antioxidant and reactive oxygen species and caspases 3/9 mediated Anticancerous activity of β-Glucan particles derived from *Pleurotus ostreatus* against cervical cancer cells HeLa. J King Saud Univ Sci. 2024;36(11):103577.
[47] Casado-Hidalgo G, Cebollada P, Cano-Lou J, Cardoso RV, Barros L, Rodrígez-Yoldi MJ, López V. *Boletus edulis* as a healthy and prized edible mushroom: analysis of bioactive compounds and in vitro functional properties. Appl Food Res. 2025;101342.