

Beta-glucan Content, Antioxidant and Antimicrobial Activities of Some Edible Mushroom Species

Özge Özcan^{1*}, Figen Ertan²

¹Vocational College of Health Services, Kırklareli University, Kırklareli, Turkey

²Department of Biology, Edirne, Faculty of Science, Trakya University, Turkey

Copyright©2018 by authors, all rights reserved. Authors agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

Abstract In this study the β -glucan content, antioxidant and antimicrobial activities of *Boletus edulis*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *Hydnum repandum* and *Agaricus bisporus* were studied. For this purpose, methanol and acetone extracts of mushrooms were obtained. The β -glucan content of mushrooms varied between $14.57 \pm 0.80\%$ - $11.26 \pm 0.69\%$. According to the results obtained from the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, the radical scavenging activity was highest in *B. edulis* and *A. bisporus*. The total phenolic content of extracts ranged between 22.75 ± 0.32 - 45.5 ± 0.71 $\mu\text{g}/\text{mg}$ as gallic acid equivalents. Mushroom extracts had low reducing power capacity in comparison to standards. When the guaiacol peroxidase activity of mushrooms was examined, *B. edulis* and *A. bisporus* showed enzyme activities of 0.07 U/ml and 0.13 U/ml, respectively. The antimicrobial activity of acetone and methanol extracts of mushrooms showed varied levels of inhibitory effects on the microorganisms tested.

Keywords Mushroom, Antimicrobial, Antioxidant, β -glucan

1. Introduction

Mushrooms have been utilized for medicinal purposes as well as being consumed as food in many parts of the world for centuries. Mushrooms are food sources that are poor in fat while being quite rich in protein, dietary fibre, vitamins and minerals [1]. In the structure of the mushrooms, some minerals such as calcium, phosphorus, potassium, iron, copper are found apart from thiamine, riboflavin, niacin, biotin, cobalamin, ascorbic acid, vitamin D and K [2]. In addition to their high nutrition content, mushrooms are rich in bioactive metabolites such as lecithin, polysaccharide, phenolic and polyphenolic compounds, polyketides,

terpenoids, glycopeptides, saponin and ergosterol with high medicinal value [3, 4]. Along with the immunomodulatory and antitumour effects of the mushrooms, it is known that they have antioxidant, antihypertensive, cholesterol lowering, blood glucose regulating, antiallergic, hypolipidemic, antiviral, antimicrobial, hepatic tissue protecting and antifibrotic characteristics [5,6]. Some species of mushrooms have been proven to have antitumour effects [7, 8]. The antitumour compounds clavacin, volvotoksin, flammotoksin, lentinan and porisin are isolated only from mushrooms [5].

The most outstanding molecules among the compounds in the structure of mushrooms are the β -glucans. β -glucan is a polysaccharide in the fungal cell wall, displaying a $\beta(1\rightarrow3)$ linkage and may display $\beta(1\rightarrow6)$ branching. It is found in cereals, bacteria and mushrooms. β -glucan has effects on immune system activation as well as antimicrobial, antioxidant, antiviral, antifungal, antitumour, cholesterol lowering, and blood glucose regulation effects [7].

Mushrooms synthesise secondary metabolites such as phenolic compounds, polyketides, terpenes, and steroids. These secondary metabolites, particularly phenolic compounds found inside the mushroom, have strong antioxidant activities. Phenolic compounds function in metal chelation, free radical scavenging and lipid peroxidation inhibition. Thanks to these antioxidant effects, mushrooms are effective treatments for many ailments, especially cancer and cardiac disease [9].

Various antimicrobial compounds isolated from both mycelium and fruiting bodies of mushrooms have been reported. These secondary metabolites function just like antibiotics. Mushrooms need these antibiotics and vitamins to vegetate and reproduce. Synthetic antibiotics and antimicrobial medicines both threaten human health and cause antibiotic resistance. For this reason, new natural antimicrobial substances obtained from nature are necessary. In this respect, mushrooms are important organisms. Along with antibiotics, β -glucan in fungal cell

walls has been determined to have an antimicrobial effect [10].

The aim of this study was to determine the antimicrobial activities and β -glucan content of four different edible wild mushroom species that grow naturally in the Trakya Region of Turkey and to compare them with a cultured mushroom. Mushrooms were studied in the fresh form as eaten in salads, meals and canned food.

2. Materials and Methods

2.1. Mushrooms

The edible mushroom species were *Boletus edulis* (bolet mushroom), *Cantharellus cibarius* (chanterelle), *Craterellus cornucopioides* (trumpet mushrooms) and *Hydnum repandum* (anchusa mushrooms). The cultured mushroom studied was *Agaricus bisporus*. Mushrooms were provided and identified by Vize Mushroom Food and Agricultural Products Marketing Industry and Trade Limited Company and Akya Mushroom Limited Company. Mushroom samples were obtained fresh and stored at -20°C until analysis.

2.2. Preparation of Mushroom Extract

Mushrooms stored at -20°C were allowed to stand at room temperature to thaw. 100 g of fresh mushroom and 400 ml solvent (acetone or methanol) were homogenized and stirred overnight. The mixture was incubated for 24 hours in a 25°C water bath with shaking at 120 rpm. After 24 hours the homogenate was filtered through Whatman No. 1 filter paper and the filtrate was stored in the dark. The same process was repeated on the 2nd and 3rd days by adding 300 ml solvent to the remaining mushroom sediment in Whatman paper. After three days, samples were treated with 1/10 solvent. Supernatants were collected and the solvent was evaporated in the evaporator at 35°C . Obtained extracts were stored at 4°C until use. Although β -glucan content determination was made with fresh mushroom, antioxidant activity and antimicrobial activity assays made with extracts [11].

2.3. Determination of β -glucan Content

β -glucan content determination of the mushroom species was made with fresh mushrooms which milled < 1 mm. To determine the β -glucan content a "Mushroom and Yeast β -glucan K-YBGL 8.4" kit was used [12].

2.4. Antioxidant Activity

2.4.1. Determination of DPPH radical scavenging activity

The free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined using the

method of Blois (1958) [13]. In this method, each extract was prepared at 5 different concentrations from 100-1000 $\mu\text{g/ml}$. 1 ml from the different concentrations of these extracts was mixed with 4 ml of 0.1 mM DPPH and vortexed. After standing for 30 minutes in the dark, the absorbance was measured at 517 nm. Each sample was measured in triplicate. Ethanol and methanol were used as negative controls and butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) as positive controls.

The % DPPH radical scavenging activity was calculated using the formula:

$$\% \text{DPPH radical scavenging activity} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

The concentration by which a substance inhibits 50% of the DPPH is expressed as IC50 [14]. The IC50 values were calculated from the graph obtained from % free radical removal activity values against different concentrations studied.

2.4.2. Determination of total phenolic compounds

The total phenolic content soluble in methanol and acetone extracts of mushrooms was determined using Folin Ciocalteu Reagent (FCR) [15]. From each sample 1 mg/ml was mixed with 45 ml water and 1 ml of Folin-Ciocalteu Reagent. After standing for 3 minutes, 3 ml of 2% Na_2CO_3 was added. After vortexing, the mixture was centrifuged at 250 rpm for 2 hours at room temperature. Absorbance was measured at 760 nm. In this study 50-500 $\mu\text{g/ml}$ concentrations of gallic acid were used as the standard.

2.4.3. Determination of reducing power capacity

Reducing power was analysed using the Oyaizu (1986) method [16]. 1 ml was taken from each extract sample prepared at 100-1000 $\mu\text{g/ml}$ concentrations and mixed with 2.5 ml 0.2 M pH6.6 phosphate buffer and 2.5 ml of 1% $\text{K}_3\text{Fe}(\text{CN})_6$. The mixture was incubated for 20 minutes at 50°C . After incubation, 1 ml of sample was taken from the mixture and was mixed with 1 ml of distilled water and 0.2 ml of 1% FeCl_3 , absorbance values were measured at 700 nm relative to a reagent blank.

2.4.4. Guaiacol peroxidase enzyme activity

Singh's method was used to determine the guaiacol peroxidase enzyme activity of mushroom species. According to the method, reaction mixtures were prepared for samples and for controls. (1.25 ml of 50 mM Potassium phosphate buffer pH7.0, 0.5 ml, 1% Guaiacol 0.5 ml, 0.5 ml 30% Hydrogen peroxide, 0.1 ml filtered mushroom homogenate for enzyme extract) [17]. After preparation of reaction mixtures, absorbance of the samples was measured with a UV spectrophotometer at 470 nm every 60 seconds for 4 minutes (1 Enzyme unit: Amount of enzyme that transforms 1 μmol guaiacol.) ($\epsilon=26.6 \text{ Mm}^{-1}\text{cm}^{-1}$).

2.5. Antimicrobial Activity

2.5.1. Test microorganisms

Test microorganisms used in our study were obtained from Trakya University Faculty of Pharmacy, Department of Pharmaceutical Microbiology Culture Collection and Kırklareli State Hospital Microbiology Laboratory. *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* clinical isolate, *Escherichia coli* ATCC 25922, *Escherichia coli* clinical isolate, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecalis* clinical isolate, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* clinical isolate, *Klebsiella pneumoniae* clinical isolate, *Serratia marcescens* clinical isolate, *Candida albicans* ATCC 10231, *Candida albicans* clinical isolates.

2.5.2. Antibacterial assay

Antimicrobial activity was determined by the disk diffusion method. 50, 100 and 200 mg/ml concentrations were prepared by dissolving methanol and acetone extracts obtained from mushrooms in dimethyl sulfoxide (DMSO) and sterilizing by passage through a Chromafil Xtra PTFE-45 filter. The general media, Mueller Hinton Agar, was used for replication of test organisms. After preparation and sterilization in required amounts, media was poured into 90 mm petri dishes up to 12.5 ml/plate. 6 mm discs prepared using Whatmann No:1 paper were sterilized. Test microorganism cultures incubated for 24 hours were adjusted to a cell density of 1.5×10^8 CFU/ml as per 0.5 McFarland Standard and 100 μ l was taken and spread on prepared media. Blank discs were impregnated with 10 μ l of extract and were placed at certain points within the petri dish. 100% DMSO was used as negative control. Ampicillin 10 μ g, chloramphenicol 10 μ g and ofloxacin 5 μ g antibiotic disks were used as positive controls. The petri dishes were stored at 4°C for 2 hours to achieve absorption, and then incubated at 37°C for 24 hours. Antimicrobial activities of the extracts were determined by measuring the diameter of the resulting zones of inhibition [6].

2.5.3. Minimum inhibitory concentration

Minimum Inhibitory Concentrations of mushroom species (MICs) were determined using the microdilution method. According to this method, Mueller-Hinton Broth and Sabouraud Dextrose Broth media was prepared and sterilized and distributed to 96-well micro plates containing 100 μ l/plate under sterile conditions. 100 μ l of 4 mg/ml extract was added to the first column of the microplates. By performing two-fold dilutions of extracts, concentrations were adjusted to 0.98-2000 μ g/ml. Test bacteria cultured on Mueller Hinton Agar for 24 hours, were adjusted according to 0.5 McFarland turbidity. The bacterial suspension was diluted 1:100 in order to achieve a final concentration of 105 CFU/ml. The cell concentration of yeast suspensions was adjusted to 2.5×10^3 CFU/ml by

first diluting 1:50, then 1:20. 10 ml of microorganisms was added to each well. DMSO was added instead of extract as a negative control. As a positive control 1 mg/ml ampicillin and 2 mg/ml ciprofloxacin were used. Inoculated microplates were stored for 24 hours at 37°C and MIC values subsequently determined according to whether they were breeding in the wells [18,19].

2.6. Statistical Analysis

All results were evaluated as an average of three parallel repetitions. Results were expressed as arithmetical mean \pm standard deviation. Error bars are indicated on the graphs. Independent Student's t test was used for pairwise comparisons and One-way ANOVA-Tukey HSD was used for multiple comparisons. The analysis was performed using SPSS 19.0.0 software.

3. Results and Discussion

3.1. Extract Yield

We determined that the extract yields of mushroom samples ranged from 2.67 to 4.45% for methanol and from 1.81 to 4.83% for ethanol (Table 1). As the mushroom samples are edible, they were used when fresh, in the form to be consumed and were not exposed to any drying process. Previous mushroom extract yields have been reported to be 1.5–4% [19,21] in one study, and 25–35% [9,22] in another. The fact that the fresh mushroom species are rich in water affects extract yield. Additionally, 1 gram of fresh sample is not equal to 1 gram of dried sample in terms of matter content. In a study conducted with fresh mushroom samples, it was shown that the extract efficiency depends on species and differs from 1 to 16% [11]. These findings are consistent with the findings of this study.

When the mushroom sample extract yield percentages are compared, methanol and acetone extracts and the different mushroom species have shown statistically significant differences within themselves ($P < 0.05$).

Table 1. Yields of mushroom extracts by methanol and acetone * (%)

Mushroom Species	Methanol Extracts Yield (%)	Acetone Extracts Yield (%)
<i>B. edulis</i>	2.67 \pm 0.3	1.81 \pm 0.01
<i>C. cibarius</i>	4.70 \pm 0.01	4.83 \pm 0.08
<i>C. curmicopioides</i>	3.28 \pm 0.13	1.90 \pm 0.03
<i>H. repandum</i>	4.45 \pm 0.01	2.97 \pm 0.1
<i>A. bisporus</i>	4.27 \pm 0.11	3.52 \pm 0.01

3.2. β -glucan Content

The highest β -glucan content from wild mushrooms was found in *B. edulis* (13.93 \pm 0.78%). *C. cibarius* with the value of approximately 12.89 \pm 0.84% and *H. repandum* (12.84 \pm 0.11%) were the next highest. The lowest

β -glucan content was found in *C. cornucopoides*. *A. bisporus* (cultivated mushroom), which is cultivated on compost and used for commercial purposes, had the highest overall β -glucan content at $14.57 \pm 0.80\%$. The difference in β -glucan content among the mushroom species was determined to be ($P < 0.05$) as shown in Figure 1.

Synytysya et al. (2008), have previously reported that dried *Pleurotus* mushroom contains between 32.5 and 50% β -glucan when stem and stalk are analysed separately. While the β -glucan content of the stem is between 27.4–

39.2%, the content of the stalk part has been determined to be between 35.5–50.0% [12]. A study comparing the β -glucan content of *Pleurotus abalonus*, *Pleurotus eous* and *Auricularia auricular* species with long and short stalks, found values of 39.78%, 39.15%, 47.11% and 56.44% respectively [24]. In these studies and also this study were made with K-YBGL Kit (MEGAZYME), but Manzi and colleagues determined the β -glucan content of *Pleurotus* species to be 0.22–0.53g/ 100g on dry basis with McCleary and Holmes method (1985) [23].

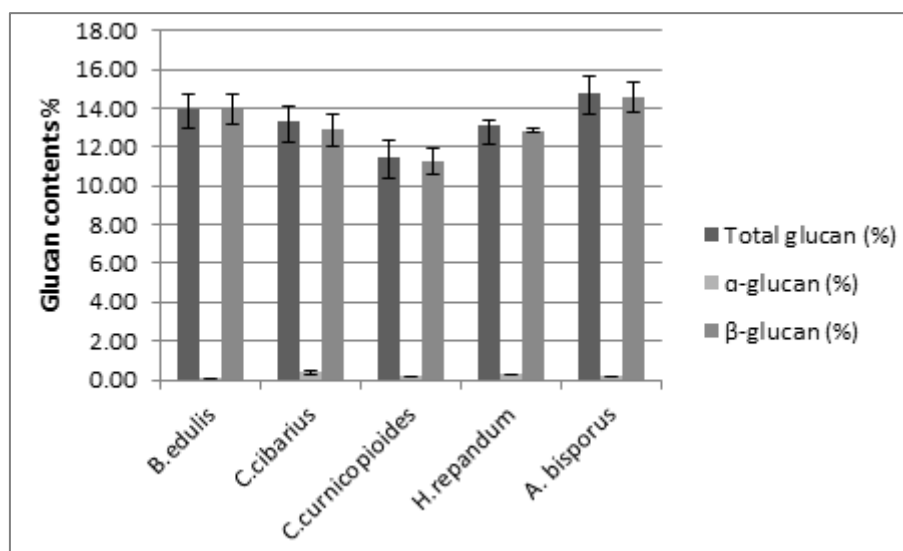


Figure 1. Glucan content of mushroom species (%)

Table 2. Scavenging activities (%) of DPPH radical of the methanol and acetone extracts of mushrooms at different concentrations a,b,c,d

Mushroom species	Solvent	Extract Concentrations ($\mu\text{g/ml}$)				
		100	250	500	750	1000
<i>B. edulis</i>	Methanol	13.41 \pm 0.02af	26.28 \pm 0.23bf	42.76 \pm 0.26cf	64.66 \pm 1.57df	78.16 \pm 0.17ef
	Acetone	10.15 \pm 0.02af	16.20 \pm 0.27bg	27.71 \pm 1.02cg	43.19 \pm 0.99dg	51.66 \pm 0.24eg
<i>C. cibarius</i>	Methanol	4.06 \pm 0.42af	4.94 \pm 0.03af	4.42 \pm 0.48af	9.10 \pm 0.14af	9.20 \pm 0.19af
	Acetone	0.46 \pm 0.66af	1.64 \pm 0.63af	5.59 \pm 1.38ag	6.55 \pm 0.28af	8.36 \pm 0.55af
<i>C. cornucopoides</i>	Methanol	4.55 \pm 0.72af	8.21 \pm 0.39af	10.59 \pm 0.51af	15.89 \pm 0.23af	19.98 \pm 0.67af
	Acetone	4.87 \pm 0.24af	7.50 \pm 0.24af	9.66 \pm 0.12af	12.75 \pm 0.19af	14.45 \pm 0.03af
<i>H. repandum</i>	Methanol	3.41 \pm 0.14af	4.29 \pm 1.24af	4.82 \pm 0.35af	5.83 \pm 0.06af	5.43 \pm 0.39af
	Acetone	3.56 \pm 0.19af	3.84 \pm 0.20af	4.32 \pm 0.38af	5.38 \pm 0.30af	6.15 \pm 0.06af
<i>A. bisporus</i>	Methanol	9.41 \pm 0.35af	19.39 \pm 0.37bf	31.64 \pm 0.02cf	50.78 \pm 0.19df	60.53 \pm 0.43ef
	Acetone	4.19 \pm 0.59af	12.33 \pm 0.17af	20.42 \pm 0.25af	29.84 \pm 0.90af	37.55 \pm 0.09af
Standarts	BHA	95.42 \pm 0.56	98.31 \pm 0	-	-	-
	BHT	63.81 \pm 0	73.55 \pm 0.22	-	-	-
	C vitamin	95.80 \pm 0.52	96.45 \pm 0	-	-	-

a Values are means S.D. of triplicate measurement.

b Means in the same row with different letters (a-e) were significantly different ($P < 0.05$) (One-way ANOVA-, Tukey-HSD)

c A statistically significant difference in concentration between each different fungal species were observed in the same column ($P < 0.05$) (One-way ANOVA-, Tukey-HSD)

d Different letters in the same line (f-g) indicates that the effects of fungal species at different solvents is statistically significant ($P < 0.05$) (Student T test)

e BHA, butylated hidroxyanisole

f BHT, butylated hidroxytoluene

In this study, the DPPH radical scavenging activity of 1 mg/ml of *Boletus edulis* was determined to be $78.16 \pm 0.17\%$ in the methanol extract and $51.66 \pm 0.24\%$ in the ethanol extract. The EC₅₀ values of *Boletus edulis* methanol and ethanol extracts were 0.585 and 0.952 mg/ml. Similarly, in the study of Sarikürkçü et. al. the inhibition value of *Boletus edulis* methanol extract at the same concentration was $80.15 \pm 0.85\%$ [25]. Keles et. al. determined the DPPH scavenging activity of 25 mg/ml of *Boletus edulis* dried sample methanol extract to be 93.18% [26]. In addition to this, the comparison between the extracts of fresh forms and dried forms of mushrooms has been stated to be inconsistent.

Kosanić et. al. determined the EC₅₀ value of *Boletus edulis* to be 212.47 in a methanol extract and 4.72 in an acetone extract [27]. In another study, the EC₅₀ value of *Boletus edulis* has been indicated to be 1.40 ± 0.31 [9].

The DPPH radical scavenging activity of *Agaricus bisporus* was determined to be $60.53 \pm 0.43\%$ in the 1 mg/ml methanol extract. In the study of Elmastaş et al., the DPPH radical scavenging activity of the 180 µg/ml methanol extract obtained from dried samples of cultivated mushroom was determined to be 77.5 [28]. Keleş et al. (2011), found DPPH radical scavenging activity of methanol extracts of dried samples of *Agaricus bisporus* at the concentration of 25 mg/ml to be 67.86% [26].

The EC₅₀ value of cultivated mushrooms used in our study was calculated as 0.744 mg/ml for the methanol extract. In a similar study, the EC₅₀ value of cultivated mushrooms was shown as 0.988 ± 0.13 mg/ml [29].

In this study, the radical scavenging activity decreases between mushrooms as follows *C. curnicopioides* > *C. cibarius* > *H. repandum*. The inhibition values of methanol and acetone extracts of the samples were similar. The findings of Keleş et al. (2011) support the findings of this study; the inhibition value of DPPH radical scavenging activity of a methanol extract of *Hydnum repandum* was shown to be 10.17% (25 mg/ml) [26]. In another study, the EC₅₀ value of *Hydnum repandum* was determined to be 14.50 ± 0.38 [9]. Heleno and colleagues found the EC₅₀ value of *Hydnum repandum* to be 30 ± 3.05 [30] and the fact that *Hydnum repandum* has a low DPPH scavenging effect among the mushroom species has been supported in studies that parallel our findings. Liu et al. (2012) found no DPPH radical scavenging activity in *Craterellus curnucoiodes* [31]. It has been observed in the literature that there have not been enough studies regarding the antioxidant activity of *C. curnucoiodes*.

We found that there is a significant difference between the percentage inhibition values of the different concentrations of methanol and acetone extracts of *B. edulis* and *A. bisporus* ($P < 0.05$). At the same time, for each of the concentrations studied, there was a significant difference in the DPPH radical scavenging activity between the different mushroom species ($P < 0.05$). When methanol and acetone yields of each mushrooms

were compared within themselves, there was a difference observed between the DPPH radical scavenging activity in 250, 500, 750, 1000 µg/ml samples of *B. edulis* and in 500 µg/ml sample of *C. cibarius*. The methanol and acetone extracts of other mushroom species have been observed to have similar activities.

3.3.2. Total phenolic compounds

Using the Folin-Ciocalteu Reactive method we found that the phenolic compound contents of acetone extracts of the mushroom samples varied between $25.75 \pm 0.5 - 45.5 \pm 0.71$ GAE µg/mg and the phenolic contents of the methanol extracts varied between $22.75 \pm 1.08 - 41.25 \pm 0.42$ GAE µg/mg. The phenolic contents of mushroom extracts decreased as follows: *B. edulis* > *A. bisporus* > *C. curnicopioides* > *H. repandum*. When the phenolic compounds of the mushrooms were compared, no significant difference was detected, even though a remarkable difference has been observed in *B. edulis*. Likewise, when phenolic compounds in the methanol and acetone extracts of mushroom species were compared, only in cultivated mushroom was there a significant difference between the methanol and acetone extracts ($P < 0.05$) (Table 3).

Table 3. Yield of phenolics (mg of GAEs µg/mg) in different mushrooms obtained by different solvents b,c,d ($P < 0.05$)

Mushroom Species	Concentrations µg/mg	
	Methanol Extract	Acetone Extract
<i>B. edulis</i>	$41.3 \pm 0.42ac$	$45.5 \pm 0.71ac$
<i>C. cibarius</i>	$25.75 \pm 1.06bc$	$26.25 \pm 0.35bc$
<i>C. curnicopioides</i>	$26.3 \pm 0.28bc$	$27.75 \pm 1.13bc$
<i>H. repandum</i>	$22.75 \pm 0.32bc$	$25.75 \pm 0.50bc$
<i>A. bisporus</i>	$34 \pm 0.71bc$	$30 \pm 0bd$

a GAEs, gallic acid equivalents

b Values are means S.D. of triplicate measurement.

c Different letters (a,b) in the same column and row represents a statistically significant difference in the amount of phenolics between mushroom species ($P < 0.05$) (One-way ANOVA-,Tukey-HSD)

d Different letters in the same line (c,d) indicates it is statistically significant that the amount of phenolic compounds in different solvents mushroom species ($P < 0.05$) (Student T test)

The extracts with highest phenolic compound content between the studied mushroom species were the acetone extract (45.5 ± 0.71 µg/mg) of *B. edulis* and methanol extract (41.25 ± 0.42 µg/mg) of *B. edulis*. The phenolic contents of the methanol extracts of *B. edulis*, *H. repandum* and *C. cibarius* species have been previously reported as 8.4 mg/g, 2.6 mg/g and 2.8 mg/g by the same method but with dried mushrooms [9]. The total phenolic content of an acetone extract of *B. edulis* has been calculated as 8.14 ± 1.211 µg/mg and the total phenolic content of a methanol extract of *B. edulis* has been calculated as 4.64 ± 1.318 µg/mg of pyrocatechol equivalent according to Kosanić et al. [27]. In the study of Sarikürkçü et al., the total phenolic contents of a methanol extract of *B. edulis* were observed to

be $31.64 \pm 0.55 \mu\text{g}/\text{mg}$ with the same method and dried mushrooms [25]. The total phenolic content of dries *B. edulis* was reported as $12.775 \text{ mg}/\text{g}$ [26].

In this study, the total phenolic content of the methanol extract of *C. cibarius* was $25.75 \pm 1.06 \mu\text{g}/\text{mg}$. In the study of Ramesh et. al. , the total phenolic contents of a methanol extract of *C. cibarius* were $3.20 \pm 0.05 \text{ mg}/\text{g}$ [32]. In the study, the total phenolic content of a methanol extract of *C. cibarius* was $0.74 \pm 0.15 \mu\text{g}/\text{mg}$ [31]. The total phenolic content of a methanol extract of *Hydnum repandum* was measured as $22.75 \pm 0.32 \mu\text{g}/\text{mg}$. In the study of Heleno et al., the phenolic content of *H. repandum* mushroom was found to be $0.51 \pm 0.02 \text{ mg}/\text{g}$ [30].

When the DPPH radical scavenging and total phenolic contents of the studied mushroom samples were compared, *B. edulis* and *A. bisporus* had the higher values than the other samples in both methanol and acetone extracts.

3.3.3. Reducing power capacity

When the reducing power of mushroom samples was

compared with the standards, 1 mg/ml samples had lower values than the 100 $\mu\text{g}/\text{ml}$ standards (Table 4). Taking these data into consideration, reducing power was observed in the studied mushroom samples. These results concur with similar studies of the same mushrooms from this region, and other countries [9, 22, 27, 33].

Previous studies of mushroom species dried at 40°C and frozen, found that the antioxidant levels had increased; whereas, in boiled samples, the levels decreased [34]. When the reducing power capacities of mushrooms were compared here, there was no significant difference observed ($P < 0.05$) (Table 4).

With the mushroom species used in our study and several other species such as *Pleurotus*, *Schizophyllum*, *Lentinus*, *Termitomyces*, *Geastrum* species etc., activity studies have been conducted that found antioxidant activities at different ratios depending on the concentration, species and solution type used for the extract preparation [35, 36].

Table 4. Reducing power capacities in different mushrooms obtained by different solvents b,c,d,e (700 nm)

Mushroom species	Solvent	Concentrations ($\mu\text{g}/\text{ml}$)				
		100	250	500	750	1000
<i>B. edulis</i>	Methanol	0.81 ± 0.07	0.79 ± 0.11	0.84 ± 0.20	0.82 ± 0.31	0.86 ± 0.39
	Acetone	0.82 ± 0.13	0.84 ± 0.22	0.85 ± 0.28	0.80 ± 0.40	0.81 ± 0.47
<i>C. cibarius</i>	Methanol	0.77 ± 0.07	0.83 ± 0.10	0.84 ± 0.09	0.82 ± 0.10	0.84 ± 0.10
	Acetone	0.79 ± 0.06	0.74 ± 0.06	0.81 ± 0.07	0.80 ± 0.07	0.83 ± 0.11
<i>C. cornucopioides</i>	Methanol	0.82 ± 0.09	0.82 ± 0.09	0.83 ± 0.09	0.82 ± 0.12	0.82 ± 0.13
	Acetone	0.93 ± 0.27	0.87 ± 0.19	0.88 ± 0.17	0.86 ± 0.16	0.84 ± 0.14
<i>H. repandum</i>	Methanol	0.82 ± 0.09	0.85 ± 0.14	0.85 ± 0.11	0.85 ± 0.13	0.82 ± 0.07
	Acetone	0.87 ± 0.22	0.95 ± 0.30	0.79 ± 0.06	0.80 ± 0.06	0.81 ± 0.08
<i>A. bisporus</i>	Methanol	1.11 ± 0.16	1.02 ± 0.28	1.11 ± 0.10	1.12 ± 0.03	1.12 ± 0.03
	Acetone	0.81 ± 0.07	0.79 ± 0.11	0.84 ± 0.20	0.82 ± 0.31	0.86 ± 0.39
Standarts	BHA	1.82 ± 0.01	-	-	-	-
	BHT	1.72 ± 0.03	-	-	-	-
	C vitamin	1.70 ± 0.01	-	-	-	-

a Absorbance

b Values are means S.D. of triplicate measurement.

c A statistically significant difference between different concentrations of each mushroom species weren't observed on the same line ($P > 0.05$) (one-way ANOVA-Tukey-HSD)

d A statistically significant difference between different concentrations of each mushroom species weren't observed on the same column ($P > 0.05$) (one-way ANOVA-Tukey-HSD)

e A statistically significant difference between different solvents of each mushroom species weren't observed on the same line. ($P > 0.05$) (Student T test)

3.4. Guaiacol Peroxidase Activity

Peroxidases are an important enzyme group, having antioxidant effects and being found in plants, mushrooms and vertebrates. Guaiacol peroxidases are a group of the peroxidase enzyme family that use guaiacol as a substrate [37].

Guaiacol peroxidase activity with an antioxidant effect has been found in *Boletus edulis* and *Agaricus bisporus*. However, in the other mushroom species no guaiacol peroxidase enzyme activity has been detected. According to this studies results, the enzyme activities of *Boletus edulis* and *Agaricus bisporus* are 0.07 and 0.13 U/ml. Furthermore, in a study of *Cantharellus cibarius* and *Pleurotus eryngii*, observed guaiacol peroxidase activity in *Cantharellus* mushrooms at 0.011 ± 0.001 U/mg [38].

3.5. Antimicrobial Activity

In this study, the antimicrobial effects of *Boletus edulis*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *Hydnum repandum* and *Agaricus bisporus* on hospital isolates and standard strains: Gram-positive bacteria; *Staphylococcus aureus*, *Enterococcus faecalis* as well as Gram-negative bacteria; *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Pseudomonas aeruginosa* were searched. Using the methanol and acetone extracts obtained from mushroom samples, agar disk diffusion and micro-dilution methods were implemented. We found that different concentrations of mushroom extracts had antimicrobial effects on several bacteria at different ratios (Table 5).

According to the experiment results, the 200 mg/ml concentration of *B. edulis* methanol extract produced a 15 mm zone of inhibition over *S. aureus* bacteria. For this bacterium, the microdilution method revealed a MIC greater than 2 mg/ml. The MIC value of the acetone extract

of *Boletus edulis* was 2 mg/ml for *E. coli* 25922. In a study conducted by Kosanić et al. (2012), The MIC value of an acetone extract of *Boletus edulis* was 5 mg/ml for *E. coli*. In another study, the same mushroom was found to have an inhibitory effect on *Actinomyces viscosus* and *Staphylococcus aureus* bacteria replication at 50-100 mg/ml concentrations [39].

An 8 mm zone of inhibition over *S. aureus* bacteria was produced by the *Cratarellus cornucopioides* acetone extract. The methanol extract produced a 15 mm zone of inhibition on *K. pneumoniae*. The MIC value for *S. aureus* was 2 mg/ml and for *K. pneumoniae* was greater than 2 mg/ml.

The methanol and acetone extracts of *Cantharellus cibarius*, particularly the 200 mg/ml samples generated 10 mm and 14 mm zones of inhibition for *S. aureus* and *S. marcescens* bacteria. The MIC value for *S. aureus* was 2 mg/ml and for *S. marcescens*, it was greater than 2 mg/ml. Previous studies of *Cratarellus cornucopioides* found that methanol and acetone extracts had antimicrobial effects on several varieties of bacteria [32, 33, 39, 40].

The zone of inhibition produced by the methanol and acetone extracts of *H. repandum* generated for *S. aureus*, *E. faecalis* and *P. aeruginosa* was between 8-15 mm. The MIC values of the extracts was > 2 mg/ml for *E. faecalis* and *P. aeruginosa* and 0.5 mg/ml for *S. aureus*. In previous studies, *H. repandum* extracts produced a zone of inhibition less than 10 mm for these bacteria as well as 50-100 mg/ml MIC values [9, 39].

Agaricus bisporus has been shown to have antimicrobial activity against *P. aeruginosa* bacteria, producing a zone of inhibition of 9-12 mm. The MIC values observed were > 2 mg/ml. In the other antimicrobial activity studies conducted with cultivated mushrooms, different extracts generated zones of inhibition between 15 and 24 mm for the bacteria tested [2, 39].

Table 5. Mushroom species that have antimicrobial activities at different test microorganisms^{a,b,c,d}

Mushroom species	Test microorganisms (clinical isolates)	Methanol extracts			Acetone extracts			Positive control			Negative Control (DMSO)
		50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	Amp (10µg)	Clo (10µg)	OfI (5µg)	
<i>Cantharellus cibarius</i>	<i>S.aureus</i>	-	8	10	-	10	12	19	28	27	-
	<i>S.marcescens</i>	-	-	12	-	10	14	23	26	28	-
<i>Boletus edulis</i>	<i>S.aureus</i>	-	-	15	-	-	-	20	28	29	-
<i>Craterellus cornucopioides</i>	<i>K.pneumoniae</i>	-	-	15	-	-	15	10	30	30	-
<i>Hydnum repandum</i>	<i>S.aureus</i>	-	15	15	14	14	15	20	27	27	-
	<i>E.facealis</i>	-	-	10	-	8	10	26	22	20	-
	<i>S.marcescens</i>	-	-	-	8	10	12	26	29	28	-
<i>Agaricus bisporus</i>	<i>P.aeruginosa</i>	-	-	10	-	-	14	-	20	-	-
	<i>P.aeruginosa</i>	-	9	9	-	10	12	-	15	-	-

a Amp, Ampicilin

b OfI, Ofloxacin

c Clo, Chloramphenicol

d DMSO, dimethyl sulfoxide

(-), Not detected

4. Conclusions

In this study we determined the antimicrobial activities, antioxidant activities and β -glucan content of four different edible wild mushroom species that grow naturally and a cultured mushroom. Based on the results, we suggest that these wild mushroom species are consumed more as a food source and their extracts should be used as an alternative treatment method. As the mushroom samples are edible, they were used when fresh, in the form to be consumed and were not exposed to any drying process. From this point of view, this study can be regarded as a first and it will be able to shed light on future studies. Therefore, further work can be the investigation of anti-carcinogenic effects of these mushroom extracts in cell culture studies in different cancer cell lines.

Acknowledgements

The study was supported by Trakya University Scientific Research Fund, project no: 2013/02.

REFERENCES

- [1] P. Manzi, L. Gambelli, S. Marconi, V. Vivanti, L. Pizzoferrato. Nutrients in edible mushrooms: an inter-species comparative study, *Food chemistry*, Vol.65, 477-482, 1999.
- [2] Ch. Surekha, DSVGK. Kaladhar, J. R. Raju Srikakarlappu Haseena. Evaluation of antioxidant and antimicrobial potentiality of some edible mushrooms, *International Journal of Advanced Biotechnology and Research*, Vol.2, 130-134, 2011.
- [3] E. Pereira, L. Barros, A. Martins, I. C. Ferreira. Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats, *Food Chemistry*, Vol. 130, 394-403, 2012.
- [4] K. A. Hillaimaharani, K. Sharmila, P. Thangaraju, M. Karthick, M. Kalaiselvam. Studies on antimicrobial and antioxidant properties of oyster mushroom *Pleurotus florida*, *Journal of Pharmaceutical Sciences and Research*, Vol.4, 1540-1545, 2013.
- [5] R. Duman, H. Taner, H. H. Doğan. Antimicrobial activities of some macrofungus, *Afyon Kocatepe University Journal of Science*, Vol.7, No.1, 55-65, 2007 (In Turkish)
- [6] M. Yamaç, F. Bilgili. Antimicrobial activities of fruit bodies and/or mycelial cultures of some mushroom isolates, *Pharmaceutical Biology*, Vol.44, 660-667, 2006.
- [7] S. Rahar, G. Swami, N. Nagpal, M. A. Nagpal, G. S. Singh. Preparation, characterization, and biological properties of β -glucans, *Journal of Advanced Pharmaceutical Technology and Research*, Vol.2, 94-103, 2011.
- [8] I. Y. Bae, H. W. Kim, H. J. Yoo, E. S. Kim, S. Lee, D. Y. Park, H. G. Lee. Correlation of branching structure of mushroom β -glucan with its physiological activities, *Food Research International*, Vol.51, 195-200, 2013.
- [9] N. G. Puttaraju, S. U. Venkateshaiah, S. M. Dharmesh. Antioxidant activity of indigenous edible mushrooms. *Journal of Agricultural and Food Chemistry*, Vol.54, 9764-9772, 2006.
- [10] M. Akyuz, A. N. Onganer. Antimicrobial activity of some edible mushrooms in the eastern and southeast Anatolia region of Turkey. *Gazi University Journal of Science*, Vol.23, No.2, 125-130, 2010.
- [11] J. Y. Wong, F. Y. Chye. Antioxidant properties of selected tropical wild edible mushrooms, *Journal of Food Composition and Analysis*, Vol.22, 269-277, 2009.
- [12] A. Synytsya, K. Mičková, I. Jablonský, M. Slukocá, J. Čopíková. Mushrooms of genus *Pleurotus* as a source of dietary fibres and glucans for food supplements, *Czech Journal of Food Sciences*, Vol.26, 441-446, 2008.
- [13] M. S. Blois. Antioxidant determinations by the use of a stable free radical, *Nature*, Vol.181, 1199-1200, 1958.
- [14] S. C. Litescu, S. Eremia, Radu. Methods for the determination of antioxidant capacity in food and raw materials, *Advances in Experimental Medicine and Biology*, Vol.698, 2010.
- [15] V. L. Singleton, J. A. Rossi. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *American Journal of Enology and Viticulture*, Vol.16, 144-158, 1965.
- [16] M. Oyaizu. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine, *Japanese Journal of Nutrition*, Vol.44, 307-315, 1968.
- [17] H. P. Singh, D. R. Batish, R. K. Kohli, K. Arora. Arsenic-induced root growth inhibition in mung bean (*Phaseolus aureus* Roxb.) is due to oxidative stress resulting from enhanced lipid peroxidation, *Plant Growth Regulation*, 53, 65-73, 2007.
- [18] Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS), Performance Standards for Antimicrobial Susceptibility Testing 8th Informational Supplement. CLSI M100-S18, Clinical and Laboratory Standards Institute, 940 West Valley Road, Wayne, Pennsylvania, USA, 2008.
- [19] Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS), Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast Approved Standard, M27-A3, Clinical and Laboratory Standards Institute, 940 West Valley Road, Wayne, Pennsylvania, USA, 2006.
- [20] P. Bobek, L. Ozdín, Š. Galbavý. Dose- and time-dependent hypocholesterolemic effect of oyster mushroom (*Pleurotus ostreatus*) in rats, *Nutrition*, Vol.14, 282-286, 1998.
- [21] N. Ejikeme, O. U. Henrietta. Antimicrobial activity of some local mushrooms on pathogenic isolates, *Journal of Medicinal Plants Research*, Vol.4, No:23, 2011.
- [22] C. Sarıkürkçü. Determination of the antioxidant activity of some edible mushrooms in the Mediterranean, PhD. Thesis, Süleyman Demirel University Institute of Science, Isparta,

- Turkiye, 2009. (In Turkish)
- [23] P. Manzi, L. Pizzoferrato. Beta-glucans in edible mushrooms, *Food Chemistry*, 68, 315-318, 2000.
- [24] T. Inyod, S. Piamvaree, S. Jamjumroon, V. Sasomsaup, S. Verasathiean, S. Sungkarn. A β -glucan from short and long-stalked *Pleurotus abalonus* Han, *Pleurotus eous* and *Auricularia auricula* (Hook.) Underw, *Agricultural Science Journal*, Vol. 42, 650-652, 2011.
- [25] C. Sarikurkcü, B. Tepe, M. Yamaç. Evaluation of the antioxidant activity of four edible mushrooms from the Central Anatolia, Eskisehir-Turkey: *Lactarius deterrimus*, *Suillus collitinus*, *Boletus edulis*, *Xerocomus chrysenteron*, *Bioresource Technology*, Vol.99, No.14, 6651-6655, 2008.
- [26] A. Keleş, İ. Koca, H. Gençlepe. Antioxidant properties of wild edible mushrooms, *Journal of Food Processing and Technology*, Vol.2, 6, 2011.
- [27] M. Kosanić, B. Ranković, M. Dašić. Mushrooms as possible antioxidant and antimicrobial agents, *Iranian Journal of Pharmaceutical Research*, Vol.11, No.4, 1095-1102, 2012.
- [28] M. Elmastas, O. Isildak, I. Turkekul, N. Temur. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms, *Journal of Food Composition and Analysis*, Vol.20, No.3, 337-345, 2007.
- [29] M. Öztürk, M. E. Duru, Ş. Kivrak, N. Mercan-Doğan, A. Türkoglu, M. A. Özler. In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three *Agaricus* species with fatty acid compositions and iron contents: A comparative study on the three most edible mushrooms, *Food and Chemical Toxicology*, Vol.49, No.6, 1353-1360, 2011.
- [30] S. A. Heleno, L. Barros, M. J. Sousa, A. Martins, I. C. Ferreira. Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity, *Food Chemistry*, Vol.119, No.4, 1443-1450, 2010.
- [31] Y. T. Liu, J. Sun, Z. Y. Luo, S. Q. Rao, Y. J. Su, R. R. Xu, Y. J. Yang. Chemical composition of five wild edible mushrooms collected from Southwest China and their antihyperglycemic and antioxidant activity, *Food and Chemical Toxicology*, Vol.50, No.5, 1238-1244, 2012.
- [32] C. Ramesh, M. G. Pattar. Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of western ghats of Karnataka, India, *Pharmacognosy research*, Vol.2, No.2, 107-112, 2010.
- [33] M. Kosanic, B. Rankovic, M. Dasic. Antioxidant and antimicrobial properties of mushrooms, *Bulgarian Journal of Agricultural Science*, Vol.19, No.5, 1040-1046, 2013.
- [34] L. Barros, S. Falcão, P. Baptista, C. Freire, M. Vilas-Boas, I. C. Ferreira. Antioxidant activity of *Agaricus* sp. mushrooms by chemical, biochemical and electrochemical assays, *Food chemistry*, Vol.111, No.1, 61-66, 2008.
- [35] A. Z. Woldegiorgis, D. Abate, G. D. Haki, G. R. Ziegler. Antioxidant property of edible mushrooms collected from Ethiopia, *Food Chemistry*, Vol.157, 30-36, 2014.
- [36] H. H. Arbaayah, Y. U. Kalsom. Antioxidant properties in the Oyster mushrooms (*Pleurotus* spp.) and split gill mushroom (*Schizophyllum commune*) ethanolic extracts, *Mycosphere*, Vol.4, 661-673, 2013.
- [37] L. Ghamsari, E. Keyhani, S. Golkhoo. Kinetics properties of guaiacol peroxidase activity in *Crocus sativus* L. corm during rooting, *Iranian Biomedical Journal*, Vol.11, No.3, 137-146, 2007.
- [38] J. Keyhani, E. Keyhani. Anti-oxidative stress enzymes in golden chanterelle (*Cantharellus cibarius*), *Microbes in Applied Research: Current Advances and Challenges*, Vol.23, 21-27, 2012.
- [39] M. J. Alves, I. C. Ferreira, J. Dias, V. Teixeira, A. Martins, M. Pintado. A review on antimicrobial activity of mushroom (*Basidiomycetes*) extracts and isolated compounds, *Planta Medica*, Vol.78, No.16, 1707-1718, 2012.
- [40] D. A. Aina, S. G. Jonathan, O. J. Olawuyi, D. O. Ojelabi, B. M. Durowoju. Antioxidant, antimicrobial and phytochemical properties of alcoholic extracts of *Cantharellus cibarius*—a Nigerian mushroom. *New York Science Journal*, Vol.5, No.10, 114-120, 2012.