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Original Article

ANTIOXIDANT, ANTIMICROBIAL ACTIVITIES, TOTAL PHENOLIC AND ELEMENT CONTENTS OF WILD EDIBLE MUSHROOM *BOVISTA NIGRESCENS*

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ABSTRACT

Mushrooms are natural materials that spread in cosmopolitan manner. In this study, antimicrobial and antioxidant properties of wild mushroom *Bovista nigrescens* Pers were determined. In addition, the total phenolic contents and element levels of the mushroom were determined. The mushroom was extracted with ethanol and methanol in a soxhlet apparatus. The antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) were measured using Rel Assay Diagnostics (Mega Tıp/Türkiye) kits. The agar dilution technique was used to test for antimicrobial activity. To quantify total phenolic content, the Folin-Ciocalteu reagent was utilised. A device that uses atomic absorption to measure element concentrations was used. The results showed that the TAS value was the greatest in the ethanol extract. Methanol extract was found to have the highest values of both TOS and OSI. Ethanol extracts were shown to be effective against bacterial strains at concentrations of 50-200 µg/mL and against fungal strains at 100 µg/mL. The methanol extract was shown to be effective against bacteria at concentrations between 100 and 400 µg/mL, and against fungi at concentrations between 100 and 200 µg/mL. The ethanol extract and the methanol extract had total phenolic contents of 46.83±4.77 mg/g and 39.22±4.86 mg/g, respectively. Overall, element concentrations were determined to be consistent with what is reported in the literature. However, it was found that Pb content was rather high. Therefore, *B. nigrescens* was shown to possess antioxidant and antibacterial properties.

KEYWORDS: Antimicrobial, Antioxidant, Black bovist, Brown puffball, Medicinal mushroom, *Bovista nigrescens*

1. Introduction

Mushrooms have many cultural and religious uses, including as a food, medicine, spice, and even a natural resource [1]. Mushrooms contain important components in terms of many nutrients such as vitamins, minerals and protein [2]. In many parts of the world, they are a constant element of diet due to their pungent aroma and nutritional properties [3]. In addition to their nutritional properties, many mushroom species have medicinal potential. In many studies conducted by different researchers, it has been reported that fungi have biological activities such as antioxidant, anticancer, antibacterial, DNA protective, antiproliferative, anti-inflammatory and hepatoprotective [4-9]. In this context, the determination of the biological activities of mushrooms are very important in terms of their use as a natural resource.

In this study, *Bovista nigrescens* Pers was used as material. This mushroom species, known as brown puffball or black bovist, is an edible mushroom species [10]. This fungus is usually abundant in lawns and pastures from late summer to autumn. Mushroom ethanol and methanol extracts were tested for their antioxidant and antimicrobial properties. In addition, the element levels accumulated in the fruiting body were determined. Also, total phenolic contents were determined.

2. Matherials and Methods

Mushroom samples were taken from Trabzon (Turkey) province. Distilled water was used to wash the dust off of the parts of the samples that had been collected. The samples were then put in a fruit drier to dry. Then, 30 g of powdered mushroom sample was extracted with 250 mL of ethanol at 50 °C for about 6 hours. Following the same procedures, an extract was made using methanol. The solvents of the obtained extracts were removed with a rotary evaporator.

2.1 Elemental Analysis

To learn how much of each element had accumulated in the mushrooms, samples were dried at 80 °C. Next, a micro-

wave solubilizer (Milestone Ethos Easy) was used to mineralize 0.5 g of mushroom sample in a combination of 9 mL of HNO_3 and 1 mL of H_2O_2. The concentrations of Fe, Mn, Ni, Cr, Cd, Cu, Zn, and Pb were then measured using an atomic absorption spectrophotometer (Agilent 240FS AA) [11].

2.2 Total Phenolic Content Tests

First, 1 mL stock solution was prepared from 0.1 mL mushroom extract and Folin-Ciocalteu reagent (1 mL, 1:9, *v/v*) was added. Then, this solution was vortexed and 0.75 mL of 1% Na_2CO_3 was added and the whole solution was incubated at room temperature for 2 hours. The resulting 760 nm absorbance reading was recorded. From the gallic acid standard solution calibration curve, the total phenolic content was calculated and represented as mg.GAE/g [12].

2.3 Antioxidant Activity Test

Extracts from the mushroom sample were analysed for their antioxidant capacity using Rel Assay Diagnostics (Mega Tıp/Türkiye) TAS (Total antioxidant status) and TOS (Total oxidant status) kits. Hydrogen peroxide was utilised as an oxidant standard and Trolox as an antioxidant standard. The TAS value was shown as mmoL Trolox equiv./L, and the TOS value as µmoL H_2O_2 equiv./L [13,14]. OSI (Arbitrary Unit: AU) was calculated by dividing the sum of TOS values by the sum of TAS values [15]. The results of the research, which were presented as mean ± SD, were based on analyses of three replicates of mushroom samples.

2.4 Antimicrobial Activity Test

To find out whether ethanol and methanol extracts of the mushroom sample were effective against bacteria and fungi, an agar dilution test was conducted. Muller Hinton Broth medium was used to cultivate the bacterial strains. To cultivate the fungi, RPMI 1640 Broth medium was used. Stock solutions were created from the mushroom extracts at concentrations between 12.5-800 µg/mL and the lowest extract concentrations that prevent the growth of bacteria and fungi were determined.

Bacterial strains: *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606.

Fungi strains: *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 and *C. glabrata* ATCC 90030 [16-18].

3. Results and Discussion

3.1 Element Contents

Mushrooms are very important in the ecosystem because of their role in the breakdown of organic matter. Due to this role, they accumulate elements at different levels in their bodies, depending on the element levels and type of the material they decompose [19]. In this study, the levels of elements in *B. nigrescens* were determined. The obtained results are shown in Table 1.

Table 1. Element Contents of *Bovista nigrescens* (mg/kg).

Sample	Fe	Mn	Ni	Cr	Cd	Cu	Zn	Pb
B. nigrescens	778.15±26.63	36.29±2.85	2.63±0.24	10.90±1.17	1.19±0.36	98.24±2.32	69.57±2.70	23.05±1.98

Element levels of wild mushrooms have been reported in different studies in the literature. These are: Fe (14.6-835), Mn (5.25-103), Ni (0.67-5.14), Cr (9.63-73.01), Cd (0.16-8.96), Cu (1.90-109.95), Zn (29.8-158.00), and Pb (0.68-16.54 mg/kg) [20-22]. Fe, Mn, Ni, Cr, Cd, Cu, and Zn concentrations in the fruiting body of *B. nigrescens assessed* in our study were found to be within the range reported in the literature. The Pb levels were also determined to be greater than those reported in the literature. In this context, it is thought that *B. nigrescens* may be an indicator of Pb levels. In addition, based on the literature data [20-22] of *B. nigrescens*, it was determined that the element levels were generally at normal levels.

3.2 Total Phenolic contents and Antioxidant Activity

As a result of their defense mechanisms, mushrooms can form many phenolic compounds in their fruting bodies. These phenolic compounds have very important medicinal potential [23,24]. In this study, the total phenolic content of ethanol and methanol extracts of *B. nigrescens* was analyzed. The findings are shown in Table 2. In the study, it was found that the total phenolic content of the ethanol extract of the mushroom was higher. It was previously reported that the total phenolic content of the methanol extract of *B. nigrescens* collected from Portugal was 26.50 mg/g [25]. Compared to this study, it was found that both ethanol and methanol extracts of *B. nigrescens* samples used in our study had higher phenolic content. The main reason for this is thought to be due to the fact that the material used in our study was young and the region where it was collected was different.

As a result of the metabolic processes of living organisms, reactive oxygen species, which are oxidant chemicals, are produced. These oxidizing chemicals can be helpful as catalysts at low concentrations, but can be harmful at higher concentrations [26]. The antioxidant defense system suppresses or eliminates the effects of these harmful oxidant compounds. On the other hand, in the presence of high level of oxidant compounds, the antioxidant defense system is insufficient [27]. As a result, oxidative stress occurs. As a result of oxidative stress, serious diseases such as cancer, heart diseases, multiple sclerosis, Alzheimer's and Parkinson's diseases may occur [28,29]. Supplementation with antioxidants can be used to reduce the effects of oxidative stress [30]. In this context, the antioxidant potential of *B. nigrescens* was determined in this study. The obtained results are shown in Table 2. It has been reported in the literature that *B. nigrescens* has antioxidant potential [25]. TAS value of *B. nigrescens* was reported for the first time using Rel Assay Diagnostics kits (Mega Tıp/Türkiye). TAS, TOS and OSI values of different wild edible mushrooms have been reported in the literature. In this context, TAS, TOS and OSI values of *Cyclocybe cylindracea* (DC.) Vizzini & Angelini (TAS: 4.235 mmol/L, TOS: 21.109 µmol/L, OSI: 0.488), *Lactifluus rugatus* (Kühner & Romagn.) Verbeken (TAS: 3.237 mmol/L, TOS: 8.178 µmol/L, OSI: 0.254), *Leucoagaricus leucothites* (Vittad.) Wasser (TAS: 8.291

mmol/L, TOS: 10.797 µmol/L,

Table 2. Total antioxidant, oxidant, fenolic contents of *Bovista nigrescens*

Sample	TAS (mmol/L)	TOS (µmol/L)	OSI (TOS/(TASx10))	Total phenolic (mg GAE/g)
Ethanol	4.140±0.172	8.860±0.140	0.215±0.010	46.83±4.77
Methanol	3.712±0.093	9.655±0.160	0.261±0.011	39.22±4.86

Values are presented as mean±SD, n=3

OSI: 0.130), *Lepista nuda* (Bull.) Cooke (TAS: 3.102 mmol/L, TOS: 36.920 µmol/L, OSI: 1.190), *Laetiporus sulphureus* (Bull.) Murrill (TAS: 2.195 mmol/L, TOS: 1.303 µmol/L, OSI: 0.059), *Terfezia boudieri* Chatin (TAS: 2.332 mmol/L, TOS: 26.945 µmol/L, OSI: 1.156) have been reported [31-36].

Our research found that the TAS values of *B. nigrescens* ethanol and methanol extracts were higher than those of *L. rugatus*, *L. nuda, L. sulphureus*, and *T. boudieri*, and lower than those of *C. cylindracea* and *L. leucothites*. The TAS value represents all of the antioxidant chemicals present in the product. The antioxidant capacity of a natural substance is best represented by a high TAS value [37]. *B. nigrescens*, which was employed in this research, was shown to have a strong antioxidant potential.

The total oxidant potential (TOS) of a product is an indication of the presence of endogenous oxidant chemicals [37]. Our research found that the TOS values of *B. nigrescens* ethanol and methanol extracts were lower than those of *C. cylindracea*, *L. leucothites, L. nuda*, and *T. boudieri*, and higher than those of *L. rugatus* and *L. sulphureus*. It has been noted that the oxidant compound production capability of the fungus is within typical ranges in this setting.

The level of oxidant compound suppression by the antioxidant defence system is represented by the OSI value. It is recommended that natural products with high OSI values should not be consumed or their consumption should be limited [37]. In our study, the OSI value of the ethanol extract of *B. nigrescens* was found to be lower than *C. cylindracea*, *L. rugatus*, *L. nuda* and *T. boudieri*, and higher than *L. leucothites* and *L. sulphureus*. Methanol extract of *B. nigrescens* had a higher OSI than *L. rugatus*, *L. leucothites*, and *L. sulphureus*, but a lower OSI than *C. cylindracea, L. nuda*, and *T. boudieri*. In this setting, the mushroom has been found to have significant potential in inhibiting oxidant chemicals.

3.3 Antimicrobial Activity

Microorganisms are among the main causes of many diseases today. Antimicrobial drugs are used in the treatment of microbial diseases [38]. However, due to the possible side effects of the synthetic drugs used and the emergence of resistant microorganisms due to the carelessly used antibiotics, the antimicrobial drugs used today are insufficient [39,40]. This result has led researchers to search for new antimicrobial drugs. In our study, the antimicrobial potential of ethanol and methanol extracts of *B. nigrescens* against bacterial and fungal strains was determined. The obtained results are shown in Table 3.

Table 3. MIC values of different extracts of *Bovista nigrescens*

	S. aureus	*S. aureus* MRSA	*E. faecalis*	*E. coli*	*P. aeruginosa*	*A. baumannii*	*C. albicans*	*C. glabrata*	*C. krusei*
EtOH	50	50	100	100	200	100	100	100	100
MeOH	100	100	200	200	400	100	200	100	200

50, 100, 200 and 400 µg/mL extract concentrations

It has been reported in the literature that the ethanol extract of *B. nigrescens* is effective at different concentrations against *Bacillus subtilis, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Staphylococcus carnosus, Staphylococcus epidermidis* and *Salmonella typhimurium* [41]. In our study, antimicrobial activity of *B. nigrescens* was investigated by using ethanol and methanol extracts. According to the findings, it was determined that the ethanol extract generally showed higher activity than the methanol extract. Ethanol extract was found to be effective against *S. aureus* and *S. aureus* MRSA at 50 µg/mL concentration, against *E. faecalis, E. coli, A. baumannii, C. albicans, C. glabrata* and *C. krusei* at 100 µg/mL concentration, and against *P. aeruginosa* 200 µg/mL concentration. On the other hand, methanol extracts were effective against *S. aureus, S. aureus* MRSA, *A. baumannii* and *C. glabrata* at 100 µg/mL concentration, against *E. faecalis, E. coli, C. albicans* and *C. krusei* at 200 µg/mL concentration. In addition, it was determined that the mushroom extract was effective against *P. aeruginosa* at 400 µg/mL concentration. It was determined that the mushroom ethanol extract exhibited the highest activity against *S. aureus* and *S. aureus* MRSA. In this context, it has been determined that the mushroom can be used as a natural antimicrobial resource.

4. Conclusions

In this study, antimicrobial and antioxidant potentials of wild edible mushroom *B. nigrescens* were determined. Total phenolic and elemental content of the mushroom was also calculated. As a result of the study, it was determined that the mushroom has antioxidant and antimicrobial properties. It was also found to have a high total phenolic content. In addition, it has been determined that the mushroom can be used as an indicator due to its high Pb content.

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