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SOME BIOLOGICAL ACTIVITIES AND ELEMENT CONTENTS OF ETHANOL EXTRACT OF WILD EDIBLE MUSHROOM *MORCHELLA ESCULENTA*

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ABSTRACT

In this study, antioxidant, oxidant, antimicrobial activity and heavy metal contents of *Morchella esculenta* (L.) Pers. mushroom collected from Konya (Turkey) province were determined. Antioxidant and oxidant values were determined by using Rel Assay TAS and TOS kits. Element contents were determined by AAS (atomic absorption spectroscopy). Antimicrobial activities were determined by agar dilution method against bacteria and fungus strains. As a result of the study, TAS (total antioxidant status) value of mushroom was determined as 4.580±0.114 mmoL/L, TOS (total oxidant status) value was determined as 13.549±0.211 μmoL/L and OSI (oxidative stress index) value was found to be 0.296±0.003. Mushroom extracts were found to be effective at concentrations 50-200 μg/mL against the test microorganisms. In addition, the contents of Fe, Cu, Zn, Pb, Ni, Mn, Co, Cd and Cr were determined as 264.57±17.27, 14.77±0.14, 7.82±0.34, 12.59±0.89, 0.53±0.08, 19.45±0.97, 3.82±0.13, 2.45±0.02 and 6.86±0.08 $mg.kg^{-1}$, respectively. It was determined that *M. esculenta* can be one of the antioxidant and antimicrobial sources which can be taken by diet.

Keywords: *Antioxidant, antimicrobial, edible mushroom, heavy metal, Morchella esculenta.*

INTRODUCTION

M. esculenta is a wild mushroom that is economically very expensive. *M. esculenta* is known under different names such as guchi, morel, common morel, real morel, morel mushroom, yellow morel, sponge morel among public [1]. Mushroom hunters often collect morels that can be found more abundant after forest fires. In addition, morels are mostly distributed in the fire-prone conifer forests [2]. Due to the increasing demand of gourmet cooks, the annual large-scale commercial harvest of morels has become a million-dollar industry in the US and other morel-rich countries. In addition, the successful production of commercial morel has made purchasing the fresh morel possible throughout the year [3]. In addition to its nutritional properties, *M. esculenta* mushroom has been reported to have antitumor, immunomodulatory activities, anti-inflammatory effects, antimicrobial activity, antioxidant activity and hepatoprotective activity [4-11]. In this study, it was aimed to determine the antioxidant, oxidant, antimicrobial activity and element contents of the wild edible mushroom *M. esculenta*.

STUDIES

Laboratory Studies

M. esculenta mushroom used in the study was collected under *Pinus* sp. L. from 1390 m, in Çamlık/Derebucak (Konya/Turkey). The mushroom samples collected in the field studies were dried in a desiccator at 40°C. 30 g of the samples were then weighed and extracted with soxhlet apparatus using EtOH (250 mL) at 50°C for approximately 6 hours (Gerhardt EV 14). The extracts condensed with the rotary evaporator under pressure were stored at + 4°C until testing (Heidolph Laborota 4000 Rotary Evaporator).

Antioxidant and Oxidant Tests

TAS and TOS values of the EtOH extract of *M. esculenta* were determined by using Rel Assay kits (Assay Kit Rel Diagnostics, Turkey) The calibrator Trolox was used for the TAS value and the results were shown in mmoL

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Trolox equiv./L. The Calibrator H_2O_2-hydrogen peroxide was used for the TOS value and the results were shown as μmoL H_2O_2 equiv./L [12,13]. The following formula was used to calculate the OSI value (Arbitrary Unit = AU) [13].

$$\text{OSI (AU)} = \frac{\text{TOS, μmoL } H_2O_2 \text{ equiv./L}}{\text{TAS, mmoL Trolox equiv./L X 10}}$$

Antimicrobial Activity Tests

The antimicrobial activity of the EtOH extract of *M. esculenta* were determined using agar dilution method. Bacterial strains (*Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213) were pre-cultured on Muller Hinton Broth (Merck) medium. Fungus strains (*Candida albicans* ATCC 10231 and *C. tropicalis* ATCC 13803) were pre-cultured on RPMI 1640 Broth (Sigma-Aldrich Chemie GmbH Taufkirchen, Germany) [14]. The concentrations of the test compounds were adjusted at the concentrations of 800, 400, 200, 100, 50, 25, 12.5 and 6.25 μg/mL. All dilutions were prepared with distilled water. Fluconazole for fungus, Ampicillin and Ciprofloxacin for bacteria were used as the standard drugs. The lowest concentration inhibiting the proliferation of bacteria and fungi was determined as the minimum inhibitory concentration (MIC) [15,16].

Determination of element contents

The mushroom samples were dried in the oven at 40 °C and pulverized with a mechanical mill. 1 gram of samples prepared in triplicate were placed in 50 mL glass flasks and 10 mL of HNO_3 was added. It was then allowed to stand at room temperature for 24 to 48 hours. The flasks were then heated until the solution was clear with the hot-set hot plate. Incineration was repeated by adding 10 mL of concentrated HCl onto the heated flasks. After the incineration, 20 mL of a dilute solution of HCl was added to the solution prepared and filtered. After filtration, samples were prepared for analysis [17]. Fe, Cu, Zn, Pb, Ni, Mn, Co, Cd and Cr concentrations of prepared solutions were measured using Perkin Elmer (AAnalyst 400).

CONCLUSION

TAS, TOS and OSI Values

Mushrooms have been important nutrients for a long time because of their flavor and texture. Nowadays, it is defined as a nutritious food and it is an important sources of biologically active compounds. Mushrooms have been reported to be one of the natural antioxidant sources [18,19]. In our study, TAS, TOS and OSI values of the EtOH extract of *M. esculenta* were determined.

Table 1. TAS, TOS and OSI Values of *M. esculenta*

	TAS (mmoL/L)	TOS (μmoL/L)	OSI
M. esculenta	4.580±0.114	13.549±0.211	0.296±0.003

Values are presented as mean±SD; Experiments were made in 5 parallels

No studies have hitherto been conducted to determine the TAS, TOS and OSI values of *M. esculenta*. In our study, TAS value of *M. esculenta* was determined as 4.580±0.114 mmoL/L, TOS value was determined as 13.549±0.211 μmoL/L and OSI value was found to be 0.296±0.003. In one study, TAS, TOS and OSI values of *Cyclocybe cylindracea* (DC.) Vizzini & Angelini were reported as 4.325, 21.109 and 0.488 respectively [20]. In a different study, TAS, TOS and OSI values of *Infundibulicybe geotropa* (Bull.) Harmaja were reported as 1.854, 30.385 and 1.639 respectively [21]. In another study, TAS, TOS and OSI values of *Helvella leucopus* Pers. were reported as 2.181, 14.389 and 0.661 respectively [22]. In a different study, TAS, TOS and OSI values of *Cerioporus varius* (Pers.) Zmitr. & Kovalenko were reported as 2.312, 14.358 and 0.627 respectively [23]. In another study, TAS, TOS and OSI values of *Lentinus tigrinus* (Bull.) Fr. were reported as 1.748, 19.294 and 1.106 respectively [24]. In another study, the TAS of *Auricularia polytricha* (Mont.) Sacc. was reported as 0.93 [25]. Compared to these studies, TAS value of *M. esculenta* was found to be higher than *C. cylindracea*, *I. geotropa*, *H. leucopus*, *C. varius*, *L. tigrinus* and *A. polytricha*. High TAS value of *M. esculenta* indicates that the mushroom can be a good source of natural antioxidants. The TOS value shows the oxidant compounds producing and collecting status of a specimen. OSI value shows how much a endogenous antioxidants suppress the oxidant compounds in the specimen. In our study, TOS and OSI values of *M. esculenta* were found to be lower than *C. cylindracea*, *I. geotropa*, *H. leucopus*, *C.*

varius and *L. tigrinus*. These results showed that the mushroom has a low capacity to produce and accumulate oxidant compounds. Also, it's showed that *M. esculenta* suppresses the oxidant compounds better than the other mushrooms. The antioxidant activity of *M. esculenta* was previously reported [5, 26-28]. In our study, the total antioxidant level of *M. esculenta* was determined for the first time and it was determined that it has a high antioxidant potential. As a result, it was determined that *M. esculenta* can be a good antioxidant source that can be taken by diet.

Antimicrobial Activity

Nowadays, antibiotics are very important therapeutic agents used in the fight against diseases of microbial origin. However, with the inadequacy of newly developed antimicrobial drugs, the outbreak of antimicrobial resistance poses a major threat to health. In addition, the ongiong unbalanced use of antibiotics caused the microorganisms to develop resistance against the antibiotics and the research for discovering new antimicrobial drugs has gained importance [6, 29]. In this study, EtOH extract of *M. esculenta* was examined for its antibacterial and antifungal potential. Antimicrobial activity results are shown in Table 2.

Table 2. Antibacterial and Antifungal Activity of *M. Esculenta*

	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*C. tropicalis*
Flukonazole	-	-	-	-	1.56	3.12
Ampicillin	3.12	1.56	3.12	-	-	-
Ciprofloxacin	0.78	0.78	1.56	3.12		
M. esculenta	50	50	50	100	200	200

Extract concentrations 50, 100 and 200 µg/mL

In our study, it was determined that the EtOH extract of *M. esculenta* possesses the highest antimicrobial activity against *S. aureus, E. faecalis* and *E. coli* at a concentration of 50 µg/mL. Mushroom extract was also effective against *P. aeruginosa* at 50 µg/mL concentration. The MIC values regarding the antifungal activity of the mushroom extract against *C. albicans* and *C. tropicalis* was found to be 200 µg/mL. In previous studies, methanol extracts of *M. esculenta* was reported to be effective against bacteria (*S. aureus, Bacillus subtilis, Vibrio cholerae, E. coli, Klebsiella pneumoniae* and *Enterobacter aerogenes*) and fungus strains (*Aspergillus fumigatus* and *A. niger*) at different concentrations [27]. In another study, it was reported that methanol extracts of *M. esculenta* are effective against *S. aureus, Salmonella typhimurium, Listeria monocytogenes, E. coli* and *E. cloacae* at different concentrations [5]. As a result, it was determined that *M. esculenta* can be a natural antimicrobial agent against the tested bacteria and fungi.

Element Contents

Mushrooms are known for their potential to accumulate elements in different levels depending on the content of the substrate [30]. In our study, Fe, Cu, Zn, Pb, Ni, Mn, Co, Cd and Cr concentrations were determined within the body of *M. esculenta*. The results are shown in Table 3.

Table 3. Element Content of *M. Esculenta*

Elements	*M. esculenta* ($mg.kg^{-1}$)
Fe	264.57 ±17.27
Zn	7.82 ±0.34
Cu	14.77 ±0.14
Pb	12.59 ±0.89
Ni	0.53 ±0.08
Mn	19.45 ±0.97
Co	3.82 ±0.13
Cd	2.45 ±0.02
Cr	6.86 ±0.08

Values are presented as mean±S.D.; n=3 (Experiments were made as 3 parallel)

The lowest and highest element levels determined in elemental analysis studies of wild mushrooms were reported as 14.6-835 for Fe, 29.8-158 for Zn, 60.33-95 for Cu, 2.86-16.54 for Pb, 0.67-5.14 for Ni, 18.1-103 for Mn,

0.01-8.27 for Co, 2.71-7.5 for Cr and 9.63-42.7 for Cr mg.kg^{-1} [31-34]. In our study, it was seen that the content of Fe, Mn, Pb and Co of *M. esculenta* is in the literature ranges. It was determined that the contents of Zn, Cu, Ni, Cd and Cr of *M. esculenta* are lower than the literature ranges. In this study, the element contents of *M. esculenta* were found to be normal. In addition, it was observed that there is no problem in terms of element levels when consumption of this mushroom is considered.

In this study, the antioxidant and antimicrobial activity of *M. esculenta* mushrooms collected from Derebucak (Konya/Turkey) were determined. It was determined that the mushroom has high antioxidant and antibacterial activity. In addition, the element contents were found to be normal.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

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