



New Approaches in Traditional and Complementary Alternative Medicine Practices: *Auricularia auricula* and *Trametes versicolor*

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Abstract

Several mushroom species are consumed by collecting from the nature or in cultured form for their nutritional and medical characteristics. The objective of the present study is to determine antioxidant activities, DNA-protective activities, total antioxidant status (TAS), total oxidant status (TOS), oxidative stress indices (OSI) and Fe, Mg, Zn, Cu, Na and Ca content in *Auricularia auricula* (L.) Underw. and *Trametes versicolor* (L.) Lloyd mushroom species. Mushroom ethanol extracts were obtained and antioxidant activities with DPPH method, TAS, TOS and OSI values with Rel Assay Diagnostics kits, and DNA protective activities using pBR322 supercoil DNA were identified. Furthermore, Fe, Mg, Zn, Cu, Na and Ca contents were determined with atomic absorption spectrophotometer. It was determined that antioxidant potential of mushroom ethanol extracts were low compared to the standard and they did not have DNA-protective activities. It was also observed that the mushrooms have variable element content, and have similar TAS, TOS and OSI levels. High OSI values found in both mushroom species showed that these mushroom are unhealthy. Thus, it was recommended to demonstrate caution in consumption of mushrooms collected in these regions. However, it was also considered that the mushroom samples collected from regions with adequate OSI values could be used as antioxidant.

Keywords: *Auricularia auricula*; *Trametes versicolor*; Oxidative stress; Antioxidant; DNA protective

Introduction

The usage of various foods and natural products for therapeutic purposes has a long history [1]. Since early times, mushrooms have been an important nutrient for humans and have had significant medical values [2,3]. The number of mushroom species is estimated to be around 140,000 and only 10% of these (about 14,000) have been designated [4]. Today, along with the increase in molecular studies, their use in biological warfare as well as in the production of antibiotic and other pharmacological products has increased [5]. In addition to immune system booster, antitumor, antibacterial, antiviral and anti-mutagenic effects, it is also known that mushrooms are effective in prevention and treatment of diseases such as hypertension and hypercholesterolemia [6-8]. Macro-fungi are a significant nutritional supplement for humans. However, several fungus species accumulate both important nutritional elements and heavy metals in their fruit bodies [9]. Although living organisms need certain elements such as iron, cobalt, copper, manganese, chrome and zinc in trace amounts, excessive amounts of these elements create toxic effects on these living organisms [10]. Thus, regardless of the purpose of its use, it is of utmost importance to determine metal concentrations in the mushrooms before consumption.

Oxidative stress occurs as a result of the imbalance between reactive oxygen species (ROS) and antioxidant defense produced in living organisms [11]. ROS are highly reactive O₂ metabolites that include superoxideradical, hydrogen peroxide and hydroxyl radical. The ROS causes considerable injury to DNA, protein and lipid and it is claimed that this injury is a main reason to aging and deteriorating disorders of aging [12]. Depending on the level of oxidative stress, quite serious health problems such as Alzheimer's, Parkinson's disease, carcinogenesis, neurological disorders, cancer and several metabolic diseases could arise in humans [13,14]. To reduce or remove oxidative stress, external supplemental antioxidant intake could be necessary when the antioxidant content in the body is insufficient [15]. It is considered that the use of resources with relatively high oxidative

stress index while taking these antioxidants could negatively affect the human health. Thus, it is quite important to examine the consumed mushrooms for their oxidative stress index (OSI).

Turkey has a rich biodiversity for macro-fungi species [16]. In this present study, it was aimed to determine the concentrations of Fe, Mg, Zn, Cu, Na and Ca elements that effect antioxidant activities, DNA-protective activities, total antioxidant and total oxidant levels and oxidative stress status of *Auricularia auricula* (L.) Underw. and *Trametes versicolor* (L.) Lloyd mushroom ethanol extracts.

Material And Method

Mushroom samples

The mushroom samples used in the present study, *A. auricula* (Figure 1) on *Fagus orientalis* Lipsk was obtained from Kemalije-Erzincan and *T. versicolor* (Figure 2) on *Alnus orientalis* DECNEK was collected from Köyceğiz-Muğla. Fungarium samples are stored at Akdeniz University, Faculty of Sciences, Department of Biology (Antalya, Turkey) fungarium (Figures 1 and 2).

Preparation of mushroom extracts

Mushroom samples were dried in the laboratory under 40°C and then pulverized using a mechanical grinder. Pulverized mushroom samples were separated into 30 g batches and samples that were placed

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Figure 1: *Auricularia auricula*.



Figure 2: *Trametes versicolor*.

in cartridges were subjected to extraction process for approximately 6 h at 75°C with ethanol in a soxhlet extractor (BUCHI Extraction System Model B-811). Obtained extracts were condensed in a rotary evaporator (BUCHI Rotavapor Model R-144) under pressure and stored in a refrigerator at +4°C.

Determination of antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma, Aldrich) is a free radical with having a maximum optic absorbance under 517 nm. Reaction between free radical scavenger and DPPH results in a decrease in absorbance at 517 nm. Stock solutions that contain 1 mg/mL compound were prepared in DMSO. 50 μ L solution was incubated in room temperature for 30 min after addition of 160 μ L 0.039% DPPH. Later on, the absorbance was read under 517 nm. The same procedures were repeated for all concentrations and samples. Furthermore, ascorbic acid was used as reference antioxidant [17]. DPPH free radical scavenging percentages were calculated with the formula below:

$$\text{Scavenging activity (\%)} = \frac{(\text{ADPPH} - \text{ASample})}{(\text{ADPPH})} \times 100$$

Determination of DNA-protective activity

pBR322 plasmid DNA (Vivantis) was used to determine the DNA-

protective activity of mushrooms from UV and oxidative damages. For this assay, standard solutions with concentrations of 100 and 200 μ g/mL were prepared from all mushroom extracts. In the experiment, initially 0.5 μ g of pBR322 supercoil plasmid DNA was placed in Eppendorf tubes. Later on, 10 μ L standard mushroom extract solution was added on this plasmid DNA in the Eppendorf tubes. Then, 10 μ L Fenton agent (30 mM H_2O_2 , 50 μ M ascorbic acid and 80 μ M FeCl_3) was added on this solution and incubated for 10 min under ambient temperature. The solution was finally prepared to have a volume of 20 mL and rested for 30 min at 37°C. Later on, DNA was analyzed with electrophoresis using 1% agarose gel that contains ethidium bromide [18].

Determination of TAS, TOS and OSI Values

Total antioxidant status (TAS) of mushroom ethanol extracts was determined using calibrator Trolox and Rel Assay brand commercial kits (Rel Assay Kit Diagnostics, Turkey). Results were expressed in mmol Trolox equiv./L (Erel 2004). Total oxidant status (TOS) was measured using hydrogen peroxide as calibrator and Rel Assay brand commercial kits. Results were expressed in μ mol H_2O_2 equiv./L [19]. Oxidative stress index (OSI) was calculated with the formula below:

$$\text{TOS, } \mu\text{mol H}_2\text{O}_2 \text{ equiv./L}$$

$$\text{OSI} =$$

$$\text{TAS, mmol Trolox equiv./L} \times 10$$

Determination of element content

Mushrooms were dried at 80°C and grinded in a mortar. 1 g samples were placed in 50 mL glass Erlenmeyer flasks. 10 mL of concentrated HNO_3 was added to the Erlenmeyer flasks and they were left to rest under ambient temperature for 24 h. These samples were heated on a hot-plate until sediment is formed. Later on, 10 mL of concentrated HCl was added in Erlenmeyer flasks and firing process was repeated. After this process, 20 mL of diluted HCl was added to the samples and they were filtered (Akgül et al.). Mushroom sample element content was identified using Perkin Elmer (AAAnalyst 400) device.

Results And Discussion

DPPH Free radical scavenging activities

DPPH free radical scavenging activities of *A. auricula* and *T. versicolor* mushroom ethanol extracts were determined and presented in Table 1. It was determined that both mushroom samples have low levels of antioxidant quality compared to the ascorbic acid standard. It was observed that *T. versicolor* DPPH activity is higher than that of *A. auricula*. Furthermore, it was identified that DPPH free radical scavenging success of mushroom ethanol extracts is directly proportional to extract concentration. There are many studies on *Auricularia* sp. and *Trametes* sp. fungi in the literature. From these studies, Acharya et al. [20], identified the antioxidant effect of the ethanolic extract of *Auricularia auricula* in their study. [21] have identified the antioxidant potential of *Auricularia auricula-judae* mushroom in their study. Xu et al. [22], studied the antioxidant potential of *Auricularia auricula*. Hosseinihashemi et al., identified the antioxidant effects of *T. versicolor* in different solvents in their study. In parallel with these studies, it has been determined that antioxidant effects of fungi are involved. However, it has been determined that this activity is not at high levels.

DNA-protective activity

As a result of conducted DNA-protective activity tests, it was

determined that the produced hydroxyl radical is low-dose, but it was able to disintegrate plasmid DNA (negative control) when compared to the groups that contained extract. However, it was identified that all applied doses do not exhibit DNA-protective activity when compared to the negative control.

TAS, TOS and OSI values

Mushroom TAS, TOS and OSI values are presented in Table 2. It could be observed that *A. auricula* has higher TAS and TOS values when compared to *T. versicolor*. However, high TOS values observed in this mushroom is an indicator of oxidative stress factors such as environmental pollution in the region it was collected. It could be said that Köyceğiz where *T. versicolor* was collected has lower oxidative stress index when compared to Kemaliye where *A. auricula* was collected. Hence it can be concluded that Köyceğiz could be considered as more adequate environment in terms of mushroom growth compared to Kemaliye.

In a study conducted by Akgul et al., it was found that TAS value for *Tricholoma terreum* (Schaeff.) P. Kumm gathered in Muğla center is 0.38, TOS value is 16.76, and OSI value is 4.41 and in the same study, TAS value for *Coprinus micaceus* (Bull.) Fr. gathered in Muğla-Ula was found to be 0.46, TOS value was found to be 16.87 and OSI value was determined to be 3.67. Compared to this study, although the oxidant levels of mushroom samples collected at Kemaliye-Erzincan and Muğla center were higher, their oxidative stress indices were found to be low due to the antioxidant levels they have .

Element compositions

Data obtained as a result of element analyses and lowest and highest values (mean \pm SD) for all mushroom species found in the literature were given in Table 3. Although the element content of mushrooms varies based on the mushroom species, since they play a role in organic matter catabolism in the nature, it was considered that the substrate

Concentration (mg/mL)	<i>A. auricula</i> (%)	<i>T. versicolor</i> (%)	Ascorbic Acid (%)
0.25	7.31	5.26	62.35
0.5	14.55	16.25	97.01
1	24.50	26.77	97.21

Table 1: Mushroom samples' DPPH free radical scavenging activities (% inhibition).

	TAS (mmol Trolox equiv./L)	TOS (μ mol H ₂ O ₂ equiv./L)	OSI
<i>A. auricula</i>	1.010 \pm 0.098	23.910 \pm 0.369	2.367 \pm 0.200
<i>T. versicolor</i>	0.820 \pm 0.063	17.760 \pm 0.456	2.166 \pm 0.114

Values are presented as mean \pm S.D.; n=6 (Experiments were made as 5 parallel)

Table 2: Mushroom Extracts' TAS, TOS and OSI values.

Element	Lowest and highest values in literature (mg.kg ⁻¹)	Study data (mg.kg ⁻¹)	
		<i>A. auricula</i>	<i>T. versicolor</i>
Fe	14.6-835	133.52 \pm 2.75	154.34 \pm 6.71
Mg	600-2500	137.8 \pm 4.12	133.54 \pm 8.65
Zn	29.8-158	17.96 \pm 1.71	15.68 \pm 0.94
Cu	71-95	2.52 \pm 0.93	8.94 \pm 0.54
Na	60-920	479.6 \pm 5.32	214.6 \pm 5.09
Ca	18-590	222.6 \pm 1.85	161.62 \pm 1.98

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

Table 3: Lowest and highest values (mean \pm SD) for mushroom species found in the literature and study data.

content that mushrooms use is also a significant factor as well (Gadd et al.,). In the literature, the lowest and highest element content for mushrooms were determined as 14.6-835 for Fe, 600-2500 for Mg, 29.8-158 for Zn, 71 - 95 for Cu, 60 - 920 for Na, and 18-590 mg.kg⁻¹ for Ca [23]. When compared to the determined literature values, it was observed that Fe, Na and Ca content of both mushrooms are within the range specified in the literature, while Cu, Zn and Mg content were lower than the literature value ranges. It was considered that these differences are due to the mineral characteristics of the habitat where the mushrooms were collected from and to the substrate content these mushrooms used.

Conclusion

Mushrooms are one of the natural sources possessing medicinal potentials for the production of alternative drugs, natural preservative substances, natural vitamins and amino acids [24-30]. The fact that antioxidant activities of mushroom samples were identified as a result of conducted studies infers that these mushrooms could be utilized as alternative antioxidant sources. It was determined that mushroom ethanol extracts do not exhibit any DNA-protective activity. However, it was also considered that different effects may be observed in experiments conducted using different solvents.

Mushrooms could be used as pollution indicators based on the element levels they contain. In the present study, the fact that mushroom samples' Fe, Na and Ca levels were within the ranges specified in the literature and Cu, Zn and Mg levels were lower than the ranges depicted in the literature showed that sampling regions have acceptable levels of heavy metal pollution.

Increased oxidant substances in living organisms interact with nucleic acids, lipids, proteins, enzymes and carbohydrates and could cause cellular metabolic disorders and processes that could lead to the death of the organism. High OSI values determined in both mushroom species in the present study demonstrated that consumption of these mushrooms could be unhealthy. Thus, it was concluded that consumption of mushrooms collected in these regions could cause health problems in individuals and it was recommended that *Auricularia auricula* and *Trametes versicolor* mushrooms obtained from these regions should be consumed with caution and in limited amounts. However, mushroom samples collected from regions with adequate OSI values could be utilized as antioxidant alternative.

References

- Acharya K, Samui K, Rai M, Dutta BB, Acharya R (2004) Antioxidant and nitric oxide synthase activation properties of *Auricularia auricula*. Indian J Exp Biol 42: 538-540.
- Akgül H, Nur AD, Sevindik M, Doğan M (2016) *Tricholoma terreum* ve *Coprinus micaceus*' un bazı biyolojik aktivitelerinin belirlenmesi. Artvin Çoruh Üniversitesi Orman Fakültesi Dergisi 17: 158-162.
- Barros L, Falcão S, Baptista P, Freire C, Vilas-Boas M, et al. (2008) Antioxidant activity of *Agaricus* sp. mushrooms by chemical, biochemical and electrochemical assays. Food Chemistry 111: 61-66.
- Cai M, Lin Y, Luo YL, Liang HH, Sun P (2015) Extraction, antimicrobial, and antioxidant activities of crude polysaccharides from the wood ear medicinal mushroom *Auricularia auricula-judae* (Higher Basidiomycetes). International J Medicinal Mushrooms 17: 591-600.
- Chen SC, Lu MK, Cheng JJ, Wang DL (2005) Antiangiogenic activities of polysaccharides isolated from medicinal fungi. FEMS Microbiology Letters 249: 247-254.
- Cheung YC, Siu KC, Wu JY (2013) Kinetic models for ultrasound-assisted extraction of water-soluble components and polysaccharides from medicinal fungi. Food and Bioprocess Technology 79: 214-220.

7. Dias DA, Urban S, Roessner U (2012) A historical overview of natural products in drug discovery. *Metabolites* 2: 303-336.
8. Doğan HH, Şanda MA, Akata I (2012) Mn, Fe, K, Na, and P contents in some *Tricholoma* (Fr.) staude (*Tricholomataceae*) taxa from central Anatolia, Turkey. *Fresenius Environ Bull* 21: 3389-3393.
9. Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry* 37: 277-285.
10. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry* 38: 1103-1111.
11. Gadd GM, Mowll JL, White C, Newby PJ (1986) Methods for assessment of heavy metal toxicity towards fungi and yeasts. *Environmental Toxicology* 1: 169-185.
12. Hosseinihashemi SK, Salem MZ, HosseinAshrafi SK, Latibari AJ (2016) Chemical Composition and Antioxidant Activity of Extract from the Wood of *Fagus orientalis*: Water Resistance and Decay Resistance against *Trametes versicolor*. *BioResources* 11: 3890-3903.
13. Ilahi I, Samar S, Khan I, Ahmad I (2013) In vitro antioxidant activities of four medicinal plants on the basis of DPPH free radical scavenging. *Pak J Pharm Sci* 26: 949-952.
14. Kozarski M, Klaus A, Jakovljevic D, Todorovic N, Vunduk J, et al. (2015) Antioxidants of edible mushrooms. *Molecules* 20: 19489-19525.
15. Lee JC, Kim HR, Kim J, Jang YS (2002) Antioxidant property of an ethanol extract of the stem of *Opuntia ficus-indica* var. *saboten*. *J Agricultural Food Chem* 50: 6490-6496.
16. Lee IK, Kim YS, Jang YW, Jung JY, Yun BS (2007) New antioxidant polyphenols from the medicinal mushroom *Inonotus obliquus*. *Bioorganic & Medicinal Chemistry Letters* 17: 6678-6681.
17. Mallikarjuna SE, Ranjini A, Haware DJ, Vijayalakshmi MR, Shashirekha MN, et al. (2013) Mineral composition of four edible mushrooms. *Journal of Chemistry*, <http://dx.doi.org/10.1155/2013/805284>
18. Obodai M, Ferreira IC, Fernandes Â, Barros L, Mensah DLN, et al. (2014) Evaluation of the chemical and antioxidant properties of wild and cultivated mushrooms of Ghana. *Molecules* 19: 19532-19548.
19. Palacios I, Lozano M, Moro C, D'arrigo M, Rostagno MA, et al. (2011) Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chem* 128: 674-678.
20. Ribeiro B, Lopes R, Andrade PB, Seabra RM, Gonçalves RF, et al. (2008) Comparative study of phytochemicals and antioxidant potential of wild edible mushroom caps and stipes. *Food chemistry* 110: 47-56.
21. Sarikurkcu C, Copur M, Yildiz D, Akata I (2011) Metal concentration of wild edible mushrooms in Soguksu National Park in Turkey. *Food Chem* 128: 731-734.
22. Sevcikova L, Pechova A, Pavlata L, Antos D, Mala E, et al. (2011) The effect of various forms of selenium supplied to pregnant goats on the levels of selenium in the body of their kids at the time of weaning. *Biological Trace Element Research* 143: 882-892.
23. Sevindik M, Akgül H, Bal C (2017) Determination of Oxidative Stress Status of *Ompholatus olearius* Gathered from Adana and Antalya Provinces in Turkey. *Sakarya University J Science* 21: 324-327.
24. Shimada K, Fujikawa K, Yahara K, Nakamura T (1992) Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of agricultural and food chemistry* 40: 945-948.
25. Soares AA, de Sá-Nakanishi AB, Bracht A, da Costa SMG, Koehnlein EA, et al. (2013) Hepatoprotective effects of mushrooms. *Molecules* 18: 7609-7630.
26. Talas ZS, Gogebakan A, Orun I (2013) Effects of propolis on blood biochemical and hematological parameters in nitric oxide synthase inhibited rats by Nω-Nitro-L-arginine methyl ester. *Pak J Pharm Sci* 26: 915-919.
27. Talas ZS, Gok Y, Ozdemir I, Ates B, Gunal S, et al. (2015) Synthesis, antioxidant and anti-microbial properties of two organoselenium compounds. *Pak J Pharm Sci* 28: 611-616.
28. Tsai SY, Huang SJ, Lo SH, Wu TP, Lian PY, et al. (2009) Flavour components and antioxidant properties of several cultivated mushrooms. *Food Chemistry* 113: 578-584.
29. Wong JY, Chye FY (2009) Antioxidant properties of selected tropical wild edible mushrooms. *J Food Composition Analysis* 22: 269-277.
30. Xu S, Zhang Y, Jiang K (2016) Antioxidant activity in vitro and in vivo of the polysaccharides from different varieties of *Auricularia auricula*. *Food and Function* 7: 3868-3879.

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