

An Edible Mushroom With Medicinal Significance; *Auricularia polytricha*

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ABSTRACT

Auricularia polytricha, also known as wood ear mushroom, is a macrofungus. The aim of study was to determine the antioxidant and antimicrobial activities of *A. polytricha* extracts with two different solutions. We used ethanol and distilled water as a solvent in order to prepare mushroom extracts. The water and ethanol extracts were evaluated for the total antioxidant status according to the procedures described in TAS Assay Kit (Rel Assay Diagnostics®, Turkey). Antimicrobial activity was investigated with disc diffusion method against two gram positive bacteria, two gram negative bacteria and one yeast. TAS values of ethanol extracts were determined higher than that of in distilled water extracts. In addition, it was observed that ethanol extracts have antimicrobial activity whereas water extracts have no antimicrobial activity. Ethanol extracts were more effective against *Candida albicans* (15.6 ± 1.5 mm) than other microorganisms. Significantly, ethanol extracts of mushroom showed antifungal activity. Among other four test microorganisms, *Pseudomonas aeruginosa* (13.1 ± 1.9 mm) had higher antimicrobial activity. In conclusion, the investigation of antioxidant and antimicrobial activities of edible mushrooms have become important for the discovery of new antimicrobial agents. *A. polytricha* has both antioxidant and antimicrobial properties against to *C. albicans*, *E. coli*, *E. faecalis*, *P. aeruginosa*, and *S. aureus*. And also, other positive contributions to health of this mushroom will be determined by means of the other studies planned.

Keywords:

Auricularia polytricha; Total Antioxidant Status; Antimicrobial Activity; extraction.

INTRODUCTION

Mushrooms play an important role in the carbon-nitrogen cycle by breaking down organic substance in nature [1]. In addition to its existence for centuries, fungi have been used as nutritious food in daily meal and therapeutic agent in medicine because they are rich in protein, vitamins, and minerals [2].

Auricularia polytricha is a macrofungus that is a member of Basidiomycota class and belongs to Auriculariaceae family [3]. They grow in Asia, tropical America, and other regions of the world [4]. The spread of fungal spores of *A. polytricha* are present in high amount during late July [5]. Culturing of this fungus is not difficult but slow like some other fungi [6]. *A. polytricha* is edible mushroom.

When the mushroom is fresh, the structure becomes rubbery, gelatinous, and ear-like. In contrast, it becomes shapeless and brittle if it is dried [7, 8]. As a consequence of its nutrient and medical value, the consumption and cultivation of *A. polytricha* have increased rapidly [9-12].

Up to now, some studies showed that lots of different mushroom species have antioxidant, cytotoxic, anti-proliferative, anti-diabetic, antimicrobial, and anti-inflammatory effects [13-18]. In addition, since cell wall glucans of fungi indicates immunomodulatory properties, their secondary metabolites are active against bacteria and viruses [19, 20]. The aim of the present study is to evaluate the total antioxidant status and antimicrobial effects of *A. polytricha*.

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MATERIALS AND METHODS

Preparation of Mushroom Extract

Auricularia polytricha was obtained commercially (Agroma Food, Turkey). Dried mushroom samples were grounded in a grinder using a 2 mm diameter mesh. Then, they (10 g) were extracted in a Soxhlet extractor sequentially with 250 ml of distilled water and ethanol for 12 h. All the solvent extracted fractions were subjected in a rotary vacuum evaporator (Stuart Rotary Evaporator, RE300P). The residues were incubated a bit more of dryness for a while in an oven at 40°C. The test residues were prepared as stocks using ultra-pure water and ethanol (1.5 mg/ml). After that, the extracts were filtered with 0.45 micrometer pore diameter to sterilize and tested for antimicrobial and the total antioxidant works. Extracts were kept in dark at 4°C until executing the experiments.

Determination of Total Antioxidant Status (TAS)

The water and ethanol extracts were evaluated for the total antioxidant status according to the procedures described in TAS Assay Kit (Rel Assay Diagnostics, Turkey). The data were calculated as a novel automated measurement method developed by Erel in 2004 [28]. In this method, the Fenton reaction forms a hydroxyl radical which was reacted with colorless substrate o-dianisidine. As a result of this reaction, a bright yellowish-brown dianisyl radical was obtained. The results were expressed as micromolar Trolox equivalents per liter ($\mu\text{mol Trolox Eq/L}$).

Determination of Antimicrobial Activity

Microorganisms and Growth Conditions

Enterococcus faecalis (ATCC-29212), *Staphylococcus aureus* (ATCC-25923) as gram-positive bacteria, *Pseudomonas aeruginosa* (ATCC-27853) and *Escherichia coli* (ATCC-25922) as gram-negative bacteria, and *Candida albicans* (ATCC-10231) as fungus were grown in order to investigate the antibacterial and antifungal effects of *A. polytricha*. Nutrient broth and agar (Difco) were used for all microorganism culture. All strains were obtained from culture collection at -20°C in an appropriate medium containing 10% glycerol at Hitit University, Faculty of Science and Arts, Department of Molecular Biology and Genetic, Microbiology Research Laboratory Culture Collection.

Disc Diffusion Method

The antimicrobial activity of mushrooms extracts was evaluated through disc diffusion method. Microorganisms were activated two times in nutrient broth and incubated at 37°C for 16-24 hours. After the activation for two times, optical density (OD) was

adjusted to approximately 0.600 ($\text{OD}_{600} \approx 600$) for all microorganisms. 100 μL of culture suspensions were inoculated on Mueller Hinton Agar (MHA). Sterile prepared discs from Whatman Filter paper were placed into petri dishes. 10 μL of extracts dissolved previously within respective solvents (ultra-pure water and ethanol) were poured and incubated at 37°C for 24 hours. All inhibition zones were measured.

Statistical Analysis

Statistical analysis was performed on the data by SPSS 20.0 Bivariate Correlation Analysis (SPSS Inc., Chicago) with statistical significance determined at $P < 0.05$. All experiments were done in duplicate, and mean values are presented. The results were expressed as means \pm standard deviations (SD).

RESULTS AND DISCUSSION

Determination of Total Antioxidant Status (TAS) of Extracts

In recent years, the antioxidant properties of mushrooms have been widely reported by many authors [22-25]. Researchers reported that *Auricularia polytricha* has lowering blood-fat, antioxidant, antitumor, and immunomodulatory activities [26-28]. In the present study, antioxidant activity of *A. polytricha* mushroom extracts with ethanol and distilled water investigated by TAS method is presented in Table 1. The antioxidant activity of distilled water extract was found to be 0.91 $\mu\text{mol Trolox Eqv./L}$. In contrast, ethanol extract was found as 0.73 $\mu\text{mol Trolox Eqv./L}$. When the antioxidant capacities of the extracts were compared, the distilled water extract of *A. polytricha* displayed higher capacity than ethanol extract.

In the literature, several studies related to the antioxidant activities of *A. polytricha* are present. In these studies, both ethanol and water extracts of *A. polytricha* have significant antioxidant activities determined using different methods such as in vitro free radical scavenging assays, DPPH assay and TAS assay [29-30]. In a previously reported work, *A. polytricha* had high DPPH scavenging activity [31]. Another study showed that *A. polytricha*, which is among the edible mushrooms, had the strongest radical scavenging and metal chelating activities in addition to the highest polyphenolic and flavonoid contents [32].

Table 1. Antioxidant activities of *A. polytricha* extracts in water and ethanol solvents.

	Ethanol extract	Water extract
Total Antioxidant Status*	0.73	0.91

*Total Antioxidant Status were calculated as $\mu\text{mol Trolox Eqv./L}$.

Determination of Antimicrobial Activity of *A. polytricha* Extracts

Until now, antimicrobial activities of *A. polytricha* have been studied in different solvents such as ethanol and methanol. In a study, it was reported that *A. polytricha* has moderate inhibition against gram-positive *S. aureus* and gram-negative *P. aeruginosa* [32]. When using methanol as solvent for extraction process, antifungal activities of mushroom extract were inactive against *C. albicans* whereas *A. polytricha* inhibited the growth of *E. coli* and *S. aureus* [33]. In our study, antimicrobial activity of distilled water and ethanol extracts of *A. polytricha* was investigated against the test organisms. Antimicrobial activity results of mushroom extracts are summarized at Table 2. Ethanol extracts of *A. polytricha* had antimicrobial activity while no antimicrobial activity of distilled water extracts was observed against any of the organisms. The diameters of inhibition growth zones were measured as mm. As tested organisms, two species of gram positive bacteria (*S. aureus* and *E. faecalis*), two species of gram negative bacteria (*P. aeruginosa* and *E. coli*) and one species of fungi (*C. albicans*) were used. In ethanol mushroom extracts, the maximum inhibitory zone was determined against *C. albicans* (15.6 ± 1.5 mm) followed by *P. aeruginosa* (13.1 ± 1.9 mm), *E. coli* (10.6 ± 1.9 mm), and *E. faecalis* (10.3 ± 0.5 mm). In contrast, the minimum zone of inhibition was obtained against *S. aureus* (9.6 ± 0.8 mm). All microorganisms used in this study were found to have various degrees of zone. Additionally, the inhibition zones of tested

microorganisms are shown in Figure 1 and 2. It could be concluded that *A. polytricha* had a significant antifungal activity and also was very active especially against Gram-negative bacteria in contrast to Gram-positive bacteria. All these results indicated that different extracts prepared in various solvents including ethanol, water, and methanol can have various degree of antimicrobial activity of *A. polytricha*. This situation could stem from different antimicrobial activity on the microorganisms of each solvent.

CONCLUSIONS

Currently, the investigation of antioxidant and antimicrobial activities of edible mushrooms has become significant. We studied the antioxidant and antimicrobial properties of ethanol and water extracts of *A. polytricha*. Results showed that both water and ethanol extracts have antioxidant activity. In addition, ethanol extracts had antimicrobial activity on tested organisms; but, antimicrobial properties were not determined in the distilled water extracts. In conclusion, the investigation of antioxidant and antimicrobial activities of edible mushrooms have become important for the discovery of new antimicrobial agents. *A. polytricha* has both antioxidant and antimicrobial properties against to *C. albicans*, *E. coli*, *E. faecalis*, *P. aeruginosa*, and *S. aureus*. And also, other positive contributions to health of this mushroom will be determined by means of the other studies planned.

Table 2. Antimicrobial effects of ethanol extract obtained from *A. polytricha*

Mushrooms Extracts		<i>S. aureus</i> ATCC 25923	<i>E. faecalis</i> ATCC 29212	<i>P. aeruginosa</i> ATCC 27853	<i>E. coli</i> ATCC 25922	<i>C. albicans</i> ATCC 10231
Ethanol extract	IZ	9.6 ± 0.8	10.3 ± 0.5	13.1 ± 1.9	10.6 ± 1.9	15.6 ± 1.5
	AI	1.0	1.01	1.13	1.08	1.15
Water extract		ND	ND	ND	ND	ND
Antibiotic		7.0 ± 1.0	9.5 ± 2.0	8.7 ± 2.2	10.5 ± 2.5	ND
Antifungal		ND	ND	ND	ND	10.5 ± 1.5

Values are mean of duplicate readings (mean \pm S.D).

Antibiotic: Gentamycin; Antifungal: Flucanazole; ND: Not Determinate

IZ= Inhibition zone (in mm) includes the diameter of disc (6 mm); AI (activity index) = IZ of test sample / IZ of standard. Standards: Distilled Water (10 μ l/disc), Sodium hypochloride 3% (10 μ l/disc), Hydrogen peroxide 1% (10 μ l/disc)

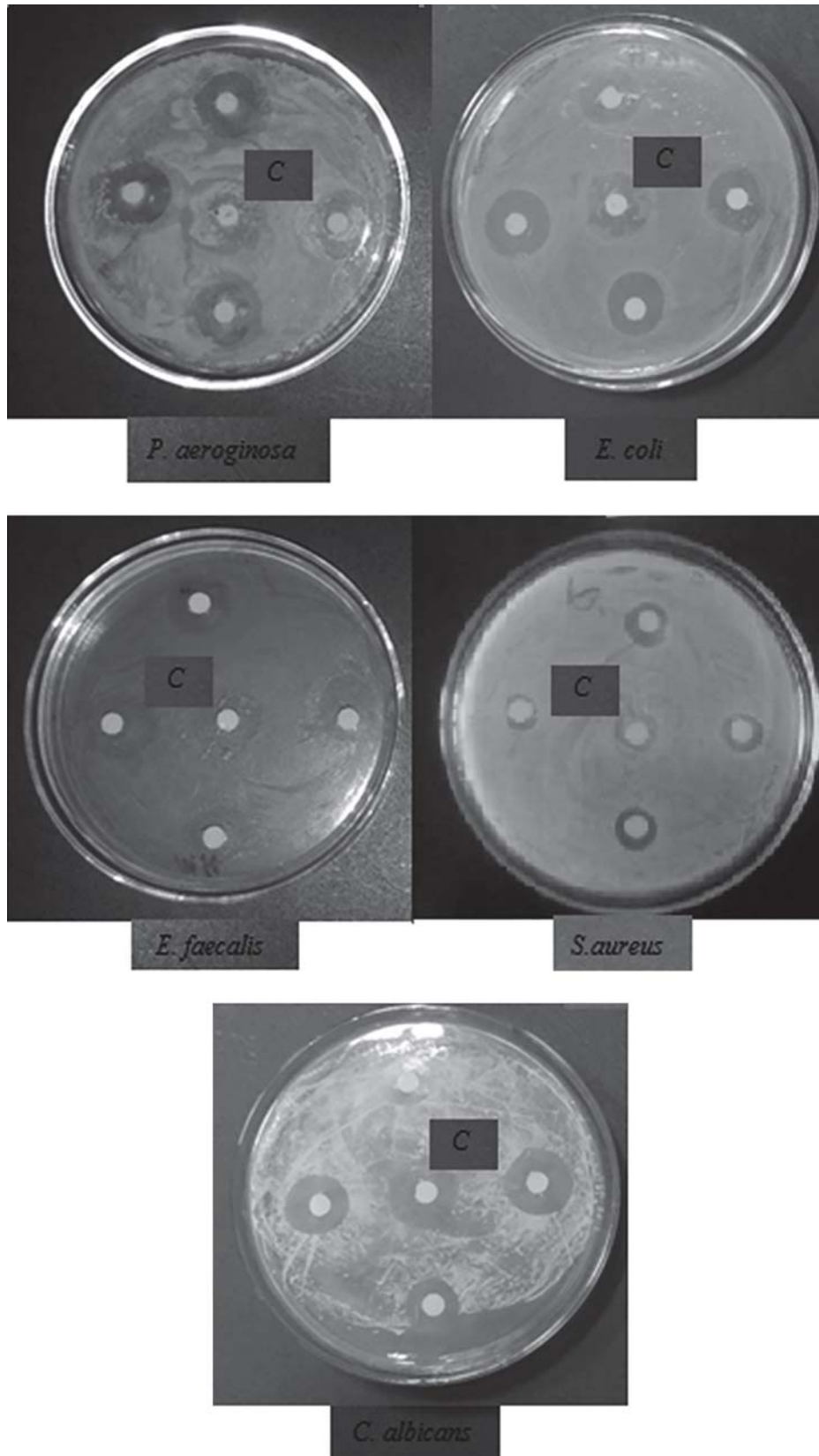


Figure 1. Zones of inhibition of ethanol extract (10 μ l) which is obtained from *A. polytricha*. C; Control- ethanol without extract.

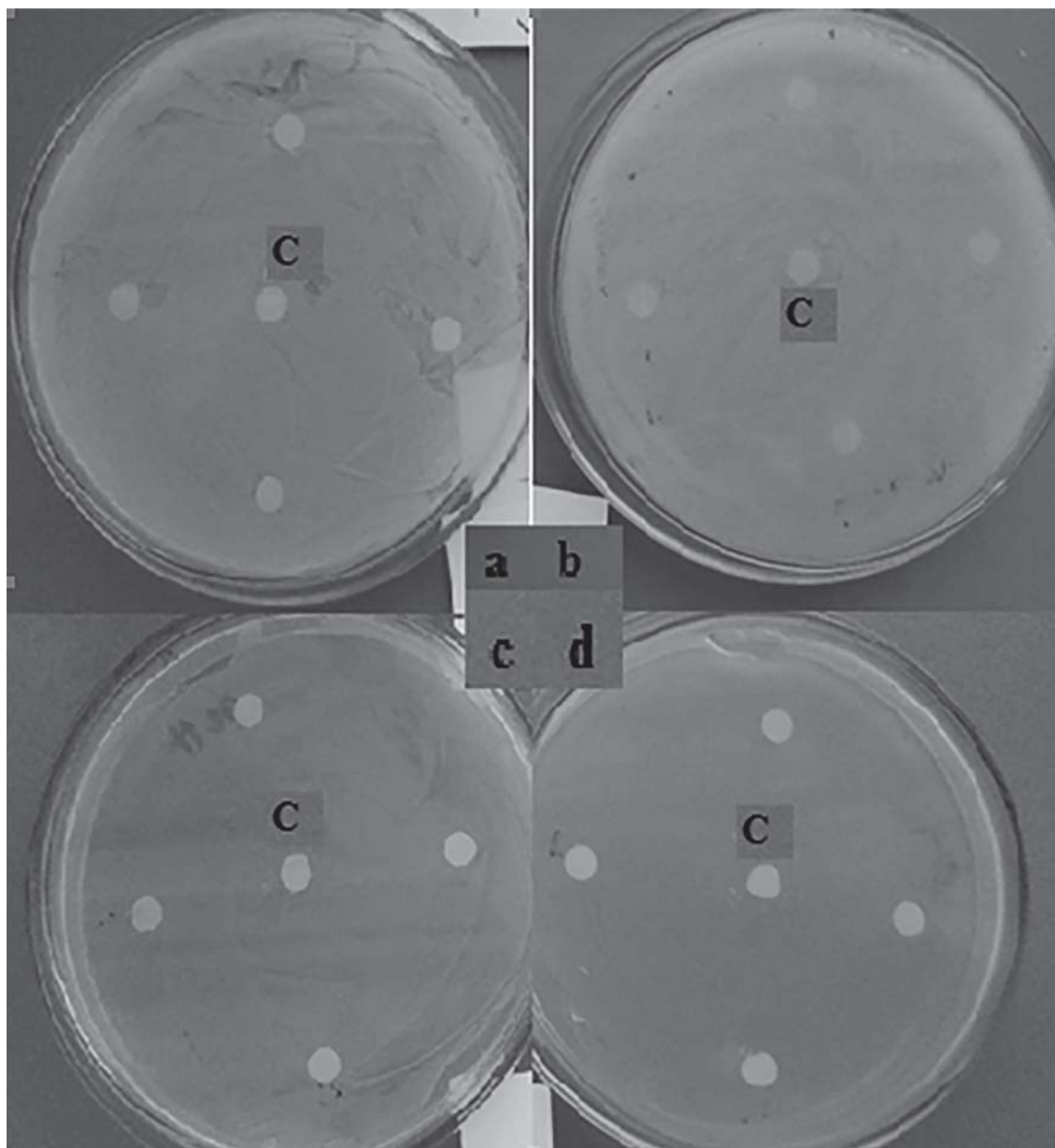


Figure 2. Zones of inhibition of water extract (10 μ l) which is obtained from *A. polytricha*. a; *S. aureus* (ATCC-65389), b; *E. faecalis* (ATCC-29212), c; *P. aeruginosa* (ATCC-27853) and d; *E. coli* (ATCC-25922), C; Control- ethanol without extract.

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