

Antimicrobial Activity of some Edible Mushrooms in the Eastern and Southeast Anatolia Region of Turkey

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ABSTRACT

In this study, the antimicrobial activity of *Pleurotus eryngii* var. *eryngii*, *P. eryngii* var. *ferulae*, *P. ostreatus*, *P. sajor-caju*, *Terfezia boudieri* and *Agaricus bisporus* were investigated. The antimicrobial activity from the methyl alcohol extract of *Pleurotus* spp., *T. boudieri* and *A. bisporus* were evaluated according to the disk diffusion method by using *Bacillus megaterium*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans*, *C. glabrata*, *Trichophyton* spp. and *Epidermophyton* spp. At the end of the experimental studies, the methyl alcohol extracts of *Pleurotus* spp., *T. boudieri* and *A. bisporus* were shown to inhibit to different degrees the growth of microorganisms to (7.5-15.5 mm) also, mushrooms extract have a lower antimicrobial activity as to a comparison antibiotic (13.0-18.0 mm).

Key Words: *A. bisporus*, Antimicrobial Activity, Pathogen Microorganism, *Pleurotus* spp., *T. boudieri*.

1. INTRODUCTION

Infections diseases remain one of the major threats to human health. Although a number of natural-synthetic antimicrobial agents have been isolated-developed to killed pathogenic microorganisms effectively, global antimicrobial resistance is an increasing public health problem. A various spesific plant have continued to be an important therapeutic aid for alleviating the ailments of humankind. Therefore, novel antimicrobial agents from diffent biological sources are continuously sought [1]. There is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects [2]. This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants [3] and also mushrooms. Nowadays, there is a renewed interest in traditional medicine and increasing demand for more drugs from fungi source.

Macrofungi have long been used as a valuable food source and as traditional medicines around the world since ancient times [4-5]. Both fruiting body and the mycelium of mushrooms contain compounds with wideranging antimicrobial activity and their compounds could be isolated from many mushrooms species and could be of benefit for human. A number of medicinal mushrooms, such as *Aleurodiscus*, *Coprinus*, *Clitocybe*, *Daedalea*, *Marasmius*, *Merulius*, *Pleurotus*, *Polyporus*, *Poria*, *Psathyrella*, and *Tricholoma* spp., are rich sources of β -glucan, lectin, phenolic compounds, flavonoids, polysaccharides, triterpenoids, diatery fibre, lentinan, schizophyllan, lovastatin, pleuran, steroids, glycopeptides, terpenes, saponins, xanthones, coumarins, alkaloid, purin, purimidin, kinon, fenil propanoid, kalvasin, volvotoksin, flammütoksin, porisin, eryngeolysin etc. [6-13]. They are rich sources of natural antibiotics, where the cell wall glucans are well known for their immunomodulatory properties, and many of the externalized secondary metabolites combat bacteria, fungi, and viruses [6, 10, 14-16], and also have been used extensively in traditional medicine for curing various types of disases such as

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antimicrobial, antiviral, anticancer, antitumor, antiinflammatory, cardiovascular diseases, immunomodulating, central activities etc. [17-23].

Turkey is rich in mushrooms diversity, as well as medicinal plant. Turkish people have a tradition of using a number of mushrooms for food, instead of the treatment of infectious diseases and various ailments. Therefore, it is necessary to know the levels of antimicrobial activity in mushrooms before using them. The purpose of this study was to evaluate the potential antimicrobial activities of *Pleurotus* spp., *T. boudieri* and *A. bisporus* on the some bacteria, yeast and dermatophytes.

2. MATERIALS AND METHOD

2.1. Macrofungul Materials

The samples (*Pleurotus eryngii* (DC. ex Fr.) Quel. var. *eryngii*, *Pleurotus eryngii* (DC. ex Fr.) Quel. var. *ferulae* Lanzi, *Pleurotus ostreatus* (Jacq. ex Fr.) Kumm., *Pleurotus sajor-caju* (Fr.) Singer) used in this study were obtained from previous culture work (Mushroom Cultured Laboratory, Firat University, Elazig-Turkey), *Agaricus bisporus* (Lange) Sing. was purchased from mushroom farm in Gezin-Elazig. In addition, *Pleurotus ostreatus* (Jacq. ex Fr.) Kumm. was collected from Elazig and Diyarbakir, and also *Terfezia boudieri* (Chatin) was collected from Baskil-Elazig, Turkey. The samples were dried at room temperature for 15 days then placed in locked bags and stored at 25°C. These samples were used in this study.

2.2. Extraction Procedure

The dried and powdered mushroom materials were dried at 55°C in the oven for 1 h. Then, 1 g of these powdered materials were mixed with 10 mL methyl alcohol solvent in a beaker and then placed on a rotary shaker for 24 h. The aqueous solutions were then filtered using Whatman filter paper (No 1) and then concentrated in vacuo for 15 min at 37°C using a Rotary evaporator. The concentration was then dissolved in 15 min of dimethylsulfoxide and stored at 4°C for further study. Then, 100 µL (100 µg) extracts were injected into an antibiotic disc having a diameter of 6 mm (Antimicrobial susceptibility test disc, CT 0998B Oxoid).

2.2. Test Microorganisms

A total of 4 bacteria (*Bacillus megaterium* DSM 32, *Staphylococcus aureus* COWAN 1, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* FMC 5), 2 yeast (*Candida albicans* FMC 17 and *Candida glabrata* ATCC 66032), and 2 dermatophytes (*Trichophyton* spp. and *Epidermophyton* spp.) were used in this study. Microorganisms were provided by the Microbiology Research Laboratory, Department of Biology, Faculty of Science and Arts, Firat University, Elazig-Turkey.

2.3. Antimicrobial Activity

The antimicrobial tests were carried out by the disc diffusion method [24], using 100 µL of suspension containing 10⁶ per/mL of bacteria, 10⁴ per/mL yeast, and 10⁴ per/mL dermatophytes inoculated into Mueller Hinton Agar (Difco), Malt Extract Agar (Difco), and Glukoz Sabouroud Agar (Difco), respectively. The discs (6 mm) were then impregnated with 100 µL of mushroom extract and then placed on the inoculated agar. Petri dishes were prepared at 4°C for 2 h. Then, the inoculated plates were incubated at 37±0.1°C for 24 h for bacterial strains and also 25±0.1°C for 72 h for yeast and dermatophytes. At the end of the incubation period, the inhibition zones were measured [24].

3. STATISTICAL ANALYSIS

Experimental values are given as means ± standard deviation (SD). Statistical significance was determined by one way variance analysis (ANOVA). Differences at P<0.05 were considered to be significant. A Tukey HSD's multiple comparison test for comparison of multiple means was used with the SPSS 13.0 computer programs (SPSS, Chicago, Illinois, USA). The experiments were repeated three times.

4. RESULTS

The *in vitro* antimicrobial activities of *Pleurotus* spp., *A. bisporus* and *T. boudieri* are shown in Table 1. The antimicrobial activity of mushroom extracts are changeable as seen in Table 1 (7.5-15.5 mm diam.). The extract sample which was obtained from *T. boudieri*, *P. ostreatus*^{o*}, *P. ostreatus*^{**}, *P. ostreatus*^{**}, *A. bisporus* and *P. sajor-caju* did not show any activity of *B. megaterium*, while the *P. ostreatus*^{o*}, *P. ostreatus*^{**}, *P. eryngii* var. *eryngii* and *P. eryngii* var. *ferulae* did (7.5-9.0 mm inhibition zone) see Table 1. In Table 1, the extract of *T. boudieri*, *P. ostreatus*^{o*}, *P. ostreatus*^{**} and *P. sajor-caju* did not show any activity of *E. coli*, but was observed to be very high in *P. ostreatus*^{o*} and *P. ostreatus*^{**} (9.5 mm), *P. eryngii* var. *eryngii* (8.5 mm), *P. eryngii* var. *ferulae* (8.0 mm), *P. ostreatus*^{**} and *A. bisporus* (7.5 mm). The extract of *P. ostreatus*^{o*}, *P. ostreatus*^{**}, *P. ostreatus*^{**}, *A. bisporus*, *P. sajor-caju*, *P. eryngii* var. *eryngii* and *P. eryngii* var. *ferulae* did not show any activity against *K. pneumoniae*, but was observed in *T. boudieri* (7.5 mm), *P. ostreatus*^{**} (8.5 mm) and *P. ostreatus*^{**} (8.0 mm) see Table 1. The extract of *P. eryngii* var. *eryngii* and *P. ostreatus*^{**} showed the maximum activity against *S. aureus*, 12.0 and 10.5 mm, respectively (Table 1).

The extract of *P. eryngii* var. *ferulae* (8.5 mm) and *P. eryngii* var. *eryngii* (7.5 mm) showed the maximum activity against *C. albicans* (7.7 mm) as seen Table 1. The extract of *T. boudieri*, *P. ostreatus*^{o*} and *P. ostreatus*^{**} did not show any activity against *C. glabrata*, but was observed to be very high in *P. ostreatus*^{o*} (15.5 mm), *P. eryngii* var. *eryngii* (11.5 mm), *A. bisporus* (9.5 mm), *P. sajor-caju* and *P. eryngii* var. *ferulae* (8.5 mm), *P. ostreatus*^{o*} and *P. ostreatus*^{**} (8.0 mm) (Table 1).

Table 1. Antimicrobial activity of some edible mushrooms in the Eastern and Southeast Anatolia Region of Turkey.

Mushrooms	Inhibition zone (mm)							
	<i>B. megaterium</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>C. glabrata</i>	<i>Epidermophyton</i> spp.	<i>Trichophyton</i> spp.
<i>T. boudieri</i> *	— ^a	— ^a	7.5±0.7 ^c	— ^a	— ^a	— ^a	— ^c	— ^c
<i>A. bisporus</i> **	— ^a	7.5±0.7 ^b	— ^a	8.5±0.7 ^c	— ^a	9.5±2.1 ^c	— ^c	10.0±1.4 ^{ab}
<i>P. ostreatus</i> °*	— ^a	9.5±0.7 ^c	— ^a	— ^a	— ^a	8.0±0.0 ^c	— ^c	8.5±0.7 ^a
<i>P. ostreatus</i> **	— ^a	— ^a	8.5±0.7 ^b	— ^a	— ^a	15.5±2.1 ^d	8.0±0.0 ^a	8.0±0.0 ^b
<i>P. ostreatus</i> * *	— ^a	— ^a	— ^a	— ^a	— ^a	— ^a	8.5±0.7 ^a	9.0±1.4 ^{ab}
<i>P. ostreatus</i> * *	8.5±0.7 ^{bc}	9.5±0.7 ^c	— ^a	9.0±1.4 ^b	— ^a	8.0±0.0 ^c	— ^c	8.5±0.7 ^b
<i>P. ostreatus</i> **	7.5±0.7 ^c	7.5±0.7 ^b	8.0±0.0 ^{bc}	10.5±0.7 ^{bd}	— ^a	— ^a	— ^c	11.5±2.1 ^a
<i>P. sajor-caju</i> **	— ^a	— ^a	— ^a	7.5±0.7 ^c	— ^a	8.5±0.7 ^c	— ^c	— ^c
<i>P. eryngii</i> var. <i>eryngii</i> **	9.0±1.4 ^b	8.5±0.7 ^{bc}	— ^a	12.0±1.4 ^d	7.5±0.7 ^b	11.5±2.1 ^b	10.0±1.4 ^b	9.5±2.1 ^{ab}
<i>P. eryngii</i> var. <i>ferulae</i> **	8.5±0.7 ^{bc}	8.0±0.0 ^b	— ^a	7.5±0.7 ^c	8.5±0.7 ^c	8.5±0.7 ^c	8.0±0.0 ^a	— ^c
Control group	17.0 ^{oo}	13.0 ^{oo}	16.0 ^{oo}	17.0 ^{oo}	18.0 ^o	14.0 ^o	— ^c	— ^c

*: wild, **: culture, °vicinity of Dicle University Campus-Diyarbakır, * Kesik Agac Village-Diyarbakır, *Tekevler-Elazığ, °Center of Elazığ,

Comparison antibiotic: °Nystatin, °°Streptomycin sülfat (Nystatin and Streptomycin sülfat: 100 µg), (-): not detected,

Each value is expressed as mean ± SD of three replicates,

Values with different small letters in the same column are significantly different at the level of 0.05 (P<0.05)

The extract of *P. ostreatus*°, *P. ostreatus** and *P. eryngii* var. *ferulae* was observed to be very similar statistically against *Epidermophyton* spp. (7.7-8.0 mm), but changeable in *P. eryngii* var. *eryngii* (10.0 mm) as seen in Table 1. The extract of *T. boudieri*, *P. sajor-caju* and *P. eryngii* var. *ferulae* did not show any activity against *Trichophyton* spp., but was observed to be very high in *P. ostreatus*** (11.5 mm), *A. bisporus* (10.0 mm), *P. eryngii* var. *eryngii* (9.5 mm), *P. ostreatus** (9.0 mm), *P. ostreatus*°* and *P. ostreatus*°* (8.5 mm), *P. ostreatus* ** (8.0 mm) see Table 1.

And also, mushroom extracts have a lower antimicrobial activity as to comparison antibiotic (13.0-18.0 mm) (Table 1).

5. DISCUSSIONS AND CONCLUSION

Many medicinal mushrooms may be used as a response to specific health problems. As can be seen in Table 1, the extract of *Pleurotus* spp., *T. boudieri* and *A. bisporus* showed activity on the other test microorganisms (7.5-15.5 mm).

The result of a previous study [17] on the antibacterial activity of *Terfezia* and *Tirmania* sp. which was obtained from a wild sample were shown. They used the methyl alcohol and ethyl acetate extract of *Terfezia* and *Tirmania* sp. showed activity against *B. subtilis* and *S. aureus*. The result of Gücin and Tamer [18] on the antimicrobial activity of *T. boudieri* which was obtained from a wild sample were shown. They used the various extracts of *T. boudieri* showed activity against *S. aureus*, *B. subtilis*, *M. luteus*, *M. smegmatis*, *C. utilis*, *E. coli* and *S. thyphimurium* at different ratios. In this study, the extract of *T. boudieri* did not show any activity of *B. megaterium*, *E. coli*, *S. aureus*, *C.*

albicans, *C. glabrata*, *Epidermophyton* spp. and *Trichophyton* spp., but was observed to be very high in *K. pneumoniae* (7.5 mm) see Table 1. It seems that the antimicrobial activity of *T. boudieri* are changeable as reported by other researchers [17-18]. This may be indicative of the use of different solvents and test microorganisms.

The extracts of *A. bisporus* showed activity against *B. subtilis* (12 mm), *S. aureus* (18-22 mm), *K. pneumoniae* (15 mm), *P. aeruginosa* (12-16 mm) and *C. albicans* (12-14 mm), but not activity against *E. coli* as reported earlier [19]. In this study, the extract of *A. bisporus* did not show any activity of *B. megaterium*, *K. pneumoniae*, *C. albicans* and *Epidermophyton* spp. but was observed to be very high in *Trichophyton* spp. (10.0 mm), *C. glabrata* (9.5 mm), *S. aureus* (8.5 mm) and *E. coli* (7.5 mm) see Table 1. It seems that the antimicrobial activity of *A. bisporus* (in this study 7.5-10.0 mm) are low compared to an earlier published report (12.0-18.0 mm) [19].

The result of a previous study [20] on the antibacterial activity of *P. ostreatus* were reported. They used the aseton extract of *P. ostreatus* did not present an antimicrobial effect against *E. coli*, *S. aureus* and *P. aeruginosa*. An ethyl acetate extract of *P. ostreatus* showed activity against *E. coli* (8.7 mm), *S. aureus* (10.0 mm) and *P. aeruginosa* (11.3 mm). The chloroform and ethanol extract of *P. ostreatus* did not show any activity against *P. aeruginosa*, but was observed to be very active against *E. coli* (9.3 mm) and *S. aureus* (8.0 mm) at the concentrations used [20]. The petroleum ether and acetone extracts of *P. ostreatus* showed activity against *Staphylococcus* sp. (7.0-7.6 mm), *Bacillus* sp. (7.1-7.8 mm), *S. thyphi* (7.0-7.5 mm), *E. coli* (7.0-8.2 mm), *K. pneumoniae* (7.0-7.1 mm) and *Candida* sp. (8.0-8.3 mm) as reported earlier [21]. In this study, the extract sample which was obtained from

P. ostreatus^{o*}, *P. ostreatus*^{**} and *P. ostreatus*^{**} did not show any activity of *B. megaterium*, while the *P. ostreatus*^{v*} and *P. ostreatus*^{**} did (7.5-8.5 mm) see Table 1. The extract of *P. ostreatus*^{**} and *P. ostreatus*^{v*} did not show any activity of *E. coli*, but was observed to be very high in *P. ostreatus*^{o*} and *P. ostreatus*^{v*} (9.5 mm) and *P. ostreatus*^{**} (7.5 mm) as seen in Table 1. The extract of *P. ostreatus*^{o*}, *P. ostreatus*^{**} and *P. ostreatus*^{v*} did not show any activity against *K. pneumoniae*, but was observed in *P. ostreatus*^{**} (8.5 mm) and *P. ostreatus*^{**} (8.0 mm) see Table 1. The extract of *P. ostreatus*^{**} showed the maximum activity against *S. aureus*, 10.5 mm, respectively (Table 1). The extract of *P. ostreatus* which was obtained from wild sample and culture medium did not show any activity against *C. albicans*. Moreover, the extract of *P. ostreatus*^{**} and *P. ostreatus*^{**} did not show any activity against *C. glabrata*, but was observed to be very high in *P. ostreatus*^{**} (15.5 mm), *P. ostreatus*^{o*} (8.0 mm) and *P. ostreatus*^{v*} (8.0 mm) see Table 1. The extract of *P. ostreatus*^{**} and *P. ostreatus*^{v*} was observed to be very similar statistically against *Epidermophyton* spp. (8.0-8.5 mm), but extract of *P. ostreatus*^{o*}, *P. ostreatus*^{v*} and *P. ostreatus*^{**} did not show any activity against *Epidermophyton* spp. And also, the ethanol extract of *P. ostreatus* showed that the activity against *Trichophyton* spp. in different ratios (8.0-11.5 mm) as seen in Table 1. The antimicrobial activity of *P.ostreatus* showed activity against test microorganism are similar to those reported by several researchers [20-21], but some values (15.5 mm in *P. ostreatus*^{**}) are variable. Moreover, the extract of *P. ostretus* was found to be higher than those reported by Iwalokun et al. [21].

The ethanol extract of *P. sajor-caju* did not show any activity against *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa* and *C. albicans*, but was observed to be very active against *S. aureus* (20.0 mm), *S. mutans* (18.0 mm), *M. luteus* (20.0 mm), *B. subtilis* (10.0 mm), *E. coli* (14.0 mm) and *S. abony* (14.0 mm) at the concentrations used [22]. They used the ethanol extract of *P. florida* and *P. aureovillosus* did not present an antimicrobial effect against *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa* and *C. albicans*, but showed activity against *S. aureus* (16.0 and 20.0 mm), *S. mutans* (14.0 and 17.0 mm), *M. luteus* (16.0 and 19.0 mm), *B. subtilis* (9.0 and 14.0 mm) and *E. coli* (12.0 and 14.0 mm), respectively [22]. In this study, the extract of *P. sajor-caju* did not show any activity of *B. megaterium*, *E. coli*, *K. pneumoniae*, *C. albicans*, *Trichophyton* spp. and *Epidermophyton* spp., but was observed to be very high in *C. glabrata* (8.5 mm) and *S. aureus* (7.5 mm) see Table 1. It seems that the antimicrobial activity of *P. sajor-caju* (in this study 7.5-8.5 mm) are low compared to an earlier published report (10.0-20.0 mm) [22].

The result of a previous study [23] on the antibacterial activity of *P. eryngii* which was obtained from a wild sample were shown. They used the aseton extract of *P. eryngii* did not present an antimicrobial effect against *B. megaterium*, *K. pneumoniae* and *S. aureus*, but showed

activity against *M. luteus* (7.0-11.0 mm) and *P. denitrificans* (7.0-8.0 mm). An ethyl acetate extract of *P. eryngii* showed no activity against *M. luteus* and *P. denitrificans*, but did show activity against *B. megaterium* (7.0 mm), *K. pneumoniae* (11.0 mm), and *S. aureus* (11.0 mm). The chloroform extract of *P. eryngii* did not show any activity against *P. denitrificans*, but was observed to be very active against *B. megaterium* (7.0-22.0 mm), *M. luteus* (13.0-18.0 mm), *K. pneumoniae* (11.0-17.0 mm), and *S. aureus* (17.0-19.0 mm) at the concentrations used. Moreover, the ethanol extract of *P. eryngii* showed that the activity against *B. megaterium* (12.0 mm), *M. luteus* (15.0-16.0 mm), *K. pneumoniae* (8.0-18.0 mm), *P. denitrificans* (13.0 mm), and *S. aureus* (7.0-12.0 mm) in different ratios, but was not as effective as the control antibiotic [23]. In this study, the maximum activity against *B. megaterium* (8.5-9.0 mm), *E. coli* (8.0-8.5 mm), *S. aureus* (7.5-12.0 mm), *C. albicans* (8.5-7.5 mm), *C. glabrata* (8.5-11.5 mm), *Epidermophyton* spp. (8.0-10.0 mm) and *Trichophyton* spp. (0.0-9.5 mm) for *P. eryngii* var. *ferulae* and *P. eryngii* var. *eryngii* can be seen in Table 1. The antimicrobial activity of *P. eryngii* var. *ferulae* and *P. eryngii* var. *eryngii* showed activity against *B. megaterium*, *K. pneumoniae* and *S. aureus* are low compared to an earlier published report [23], but some values are variable. This may be indicative of the presence of the broad spectrum antibiotic compounds in the mushroom and due to the use of different solvents and test microorganisms.

It seems that the antimicrobial activity of *Pleurotus* spp., *T. boudieri* and *A. bisporus* are changeable as reported by other researchers [17-23], which may arise from the genetic structure of mushroom species, physical, biochemical constituents, chemical differences of mushroom extracts, solvents and test microorganisms that other research shows clearly when it's compared to the other mushroom species [6-16]. This study indicated that there are differences in the antimicrobial effects of mushroom groups, due to phytochemical differences among species. They claimed that the sensivity of microorganism to chemoterapeutic compounds change even against different strains. In similar studies [17-23], the extracts of various mushrooms inhibited the growth of some microorganisms at different ratios. Mushroom species posses different constituents and in different concentration, which account for the differential antimicrobial effect, as suggested. The broad spectrum of antimicrobial activity may be attributed to the presence of bioactive metabolities of various chemical types in mushrooms compounds.

At the end of the study, we have found that the extracts of *Pleurotus* spp., *T. boudieri* and *A. bisporus* prepared with methyl alcohol revealed antimicrobial activities against some bacteria, yeasts, and dermatophytes (7.5-15.5 mm), but also they had no antagonistic effect against some test microorganisms used in the study.

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