



## Antimicrobial activity of *Pleurotus eryngii* var. *ferulae* grown on various agro-wastes

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### Abstract

In this study, the antimicrobial activity of *Pleurotus eryngii* var. *ferulae* grown on various agro-wastes were investigated. The antimicrobial activity from the extract of *P. eryngii* var. *ferulae* which was obtained from various culture medium was evaluated according to the disk diffusion method by using *Bacillus megaterium* DSM 32, *Staphylococcus aureus* COWAN 1, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* FMC 5, *Candida albicans* FMC 17, *Candida glabrata* ATCC 66032, *Trichophyton* spp., and *Epidermophyton* spp. At the end of the experimental studies, the methyl alcohol extracts of *P. eryngii* var. *ferulae* were shown to inhibit to different degrees the growth of microorganisms to (7.7-10.3 mm) also, mushroom extracts have a lower antimicrobial activity as to a comparison antibiotic (13.0-18.0 mm).

**Keywords:** Antimicrobial activity, pathogen microorganisms, *Pleurotus eryngii* var. *ferulae*.

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### INTRODUCTION

Macrofungi have long been used as a valuable food source and as traditional medicines around the world since ancient times, especially in Japan and China. A number of medicinal mushrooms, such as *Aleurodiscus*, *Coprinus*, *Clitocybe*, *Daedalea*, *Marasmius*, *Merulius*, *Pleurotus*, *Polyporus*, *Poria*, *Psathyrella*, and *Tricholoma* spp. are rich sources of  $\beta$ -glucan, proteoglycan, lectin, phenolic compounds, flavonoids, polysaccharides, triterpenoids, dietary fibre, lentinan, schizophyllan, lovastatin, pleuran, steroids, glycopeptides, terpenes, saponins, xanthenes, coumarins, alkaloid, purin, purimidin, kinon, fenil propanoid, kalvasin, volvotoksin, flammotoksin, porisin, AHCC, maitake D-fraction, ribonucleas, eryngeolysin, and also have been used extensively in traditional medicine for curing various types of diseases such as antimicrobial, antiviral, anticancer, antitumor, antiinflammatory, cardiovascular diseases, immunomodulating, central activities etc. (Benedict and Brady 1972, Conchran 1978, Chihara 1993, Karacsonyi and Kuniak 1994, Francia et al. 1999, Gunde-Cimerman 1999, Wang et al. 2000, Bobek and Galbavy 2001, Jose et al.

2002, Wasser 2002, Wang and Ng 2004, Periasamy 2005, Carbonero et al. 2006, Iwalokun et al. 2007).

Both fruiting body and the mycelium of mushrooms contain compounds with wideranging antimicrobial activity. 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 (Benedict and Brady 1972, Suzuki et al. 1990, Collins et al. 1997).

The effects of different mushroom extracts on pathogens and microorganisms are studied by a very large number of researchers in different parts of the world (Jonathan and Fasidi 2003, Rosa et al. 2003, Uzun et al. 2004, Gbolagade et al. 2005, Gezer et al. 2006, Solak et al. 2006, Turkoglu et al. 2006, Barros et al. 2007, Demirhan et al. 2007, Gbolagade et al. 2007, Turkoglu et al. 2007). Turkey is rich in mushrooms diversity, as well as medicinal plants. Turkish people

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have a tradition of using various types of mushrooms for food, instead of using them for the treatment of infectious diseases and various ailments.

*Pleurotus eryngii* (DC. ex Fr.) Quel. var. *ferulae* Lanzi is known locally as "çaksir, çarçur, heliz, mendik, göbek, göbelek, or kirkor mantari" because it grows naturally very close to or on the old stem of the *Ferulae* spp. It was successfully cultivated in 2007 in Elazig-Turkey (Kirbag and Akyuz 2008). It is frequently consumed and distributed from Elazig, Adiyaman, Malatya, Tunceli, Bingol etc. in the Eastern Anatolia Region of Turkey.

The purpose of this study was to evaluate the potential antimicrobial activities of *P. eryngii* var. *ferulae*.

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## MATERIAL AND METHODS

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### Macrofungal materials

The samples (*Pleurotus eryngii* (DC. ex Fr.) Quel. var. *ferulae* Lanzi) used in this study were obtained from previous culture work (Kirbag and Akyuz 2008). The 1:1 rate of wheat straw and cotton stalks were prepared in 2 different compost. All compost were also supplemented with rice bran at a rate of 10 and 20% respectively and in this way six types of compost were prepared see Table 1. The samples, obtained after culture and were harvested in sterile conditions and labeled. The samples were dried at room temperature for 15 days then placed in locked bags and stored at 25°C in lab. These samples were used in this study.

### 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, Oxoid).

### Test microorganisms

A total of 4 bacteria, 2 yeast, and

dermatofit species (*B. megaterium*, *S. aureus*, *E. coli*, *K. pneumoniae*, *C. albicans*, *C. glabrata*, *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.

### Antimicrobial activity

The antimicrobial tests were carried out by the disc diffusion method (Collins and Lyne 1987) using 100 µL of suspension containing 10<sup>6</sup> per/mL of bacteria, 10<sup>4</sup> per/mL yeast, and 10<sup>4</sup> per/mL dermatofit fungi 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 dermatofit fungi. At the end of the incubation period, the inhibition zones were measured (Collins and Lyne 1987).

### 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.

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## RESULTS

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The in vitro antimicrobial activities of *P. eryngii* var. *ferulae* grown on various agro-wastes are shown in Table 1. The antimicrobial activity of mushroom extracts are changeable as seen in Table 1 (7.7-10.3 mm diam.). The most active extracts of *P. eryngii* var. *ferulae* were obtained on WS (10.3 mm), followed by the wild sample (10.0 mm) and WS-CS (1:1)+10% RB (9.3 mm) as seen in Table 1.

The extract sample which was obtained from WS+20% RB, WS-CS (1:1), WS-CS (1:1)+10% RB, WS-CS (1:1)+20% RB, and the wild sample did not show any activity of

*B. megaterium*, while the WS and WS+10% RB did (8.3-8.7 mm inhibition zone) see Table 1. In Table 1, the extract of *P. eryngii* var. *ferulae* which was obtained from WS, WS+10%, WS-CS (1:1), WS-CS (1:1)+20% RB and wild sample did not show any activity of *E. coli*, but was observed to be very high in WS+20% RB (10.0 mm) and WS-CS (1:1)+10% RB (8.0 mm). The extract of *P. eryngii* var. *ferulae* which was obtained from various culture mediums did not show any activity against *K. pneumoniae*, but was observed in the wild samples of *P. eryngii* var. *ferulae* (10.0 mm) see Table 1. The extract of *P. eryngii* var. *ferulae* which was obtained from WS and WS+20% RB showed the maximum activity against *S. aureus*, 10.3 and 8.3 mm, respectively (Table 1).

The extract of *P. eryngii* var. *ferulae* which was obtained from WS and WS-CS (1:1)+10% RB showed the maximum activity against *C. albicans* (7.7 mm). In Table 1, the extract of *P. eryngii* var. *ferulae* which was obtained from WS, WS+20% RB, WS-CS (1:1) and wild samples did not show any activity against *C. glabrata*, but was observed to be very high in WS-CS (1:1)+20% RB (7.7 mm), WS+10% RB (8.3 mm), and WS-CS (1:1)+10% RB (9.3 mm) (Table 1).

The extract of *P. eryngii* var. *ferulae* which was obtained from WS+10% RB, WS-CS (1:1)+20% RB, and wild samples was observed to be very similar statistically against *Epidermophyton* spp. (7.7-8.0 mm). The extract of *P. eryngii* var. *ferulae* which was obtained from WS, WS+10% RB, WS+20% RB, and wild sample was observed to be very similar statistically against *Trichophyton* spp. (7.7-8.7 mm), but extracts of *P. eryngii* var. *ferulae* which were obtained from WS-CS (1:1), WS-CS (1:1)+10% RB, and WS-CS (1:1)+20% RB did not show any activity against *Trichophyton* spp. as seen in Table 1. And also, mushroom extracts have a lower antimicrobial activity as to comparison antibiotic (13.0-18.0 mm) (Table 1).

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## DISCUSSION

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Many medicinal mushrooms may be used as a response to specific health problems. As can be seen in Table 1, the extract of *P. eryngii* var. *ferulae* grown on various agro-wastes and obtained from wild sample

showed activity on other test microorganisms (7.7-10.3 mm).

The result of a previous study (Uzun et al. 2004) on the antibacterial activity of *P. eryngii* which was obtained from a wild sample were shown. They used the aseton extract of *P. eryngii* which did not present an antimicrobial effect against *B. megaterium*, *K. pneumoniae*, and *S. aureus*, but showed activity against *M. luteus* (7-11 mm) and *P. denitrificans* (7-8 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 mm), *K. pneumoniae* (11 mm), and *S. aureus* (11 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* (7-22 mm), *M. luteus* (13-18 mm), *K. pneumoniae* (11-17 mm), and *S. aureus* (17-19 mm) at the concentrations used. Moreover, the ethanol extract of *P. eryngii* showed that the activity against *B. megaterium* (12 mm), *M. luteus* (15-16 mm), *K. pneumoniae* (8-18 mm), *P. denitrificans* (13 mm), and *S. aureus* (7-12 mm) in different ratios, but was not as effective as the control antibiotic (Uzun et al. 2004). In this study, the maximum activity against *B. megaterium* (8.3-8.7 mm), *E. coli* (8.0-10.0 mm), *K. pneumoniae* (10.0 mm), *S. aureus* (8.3-10.3 mm), *C. albicans* (7.7 mm), *C. glabrata* (7.7-9.3 mm), *Epidermophyton* spp. (7.7-8.0 mm), and *Trichophyton* spp. (7.7-8.7 mm) can be seen in Table 1. The antimicrobial activity of *P. eryngii* var. *ferulae* showed activity against *B. megaterium* (8.3-8.7 mm), *K. pneumoniae* (10.0 mm), and *S. aureus* (8.3-10.3 mm) are low compared to an earlier published report (Uzun et al. 2004), 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.

Also, the extracts of different Basidiomycetes showed activity against *B. cereus*, *E. coli*, *K. pneumoniae*, *S. aureus*, *P. vulgaris*, *P. aeruginosa*, *Micrococcus* sp., *A. niger*, *A. flavus*, *C. albicans* and *M. bouliardii* as reported earlier (Jonathan and Fasidi 2003, Rosa et al. 2003, Gbolagade et al. 2005, Gezer et al. 2006, Solak et al. 2006, Turkoglu et al. 2006, Barros et al. 2007, Demirhan et

**Table 1.** The antimicrobial activity of *P. eryngii* var. *ferulae* grown on various agro-wastes.

| Composis                                | 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. |
| WS                                      | 8.7 ± 0.6 <sup>a</sup> | - <sup>a</sup>          | - <sup>a</sup>          | 10.3 ± 0.6 <sup>a</sup> | 7.7 ± 0.6 <sup>a</sup> | - <sup>a</sup>         | - <sup>a</sup>             | 8.7 ± 0.6 <sup>a</sup>   |
| WS + 10% RB                             | 8.3 ± 0.6 <sup>a</sup> | - <sup>a</sup>          | - <sup>a</sup>          | - <sup>b</sup>          | - <sup>b</sup>         | 8.3 ± 0.6 <sup>b</sup> | 7.7 ± 0.6 <sup>b</sup>     | 7.7 ± 0.6 <sup>a</sup>   |
| WS + 20% RB                             | - <sup>b</sup>         | 10.0 ± 1.0 <sup>b</sup> | - <sup>a</sup>          | 8.3 ± 0.6 <sup>c</sup>  | - <sup>b</sup>         | - <sup>a</sup>         | - <sup>a</sup>             | 8.0 ± 1.0 <sup>a</sup>   |
| WS-CS (1:1)                             | - <sup>b</sup>         | - <sup>a</sup>          | - <sup>a</sup>          | - <sup>b</sup>          | - <sup>b</sup>         | - <sup>a</sup>         | - <sup>a</sup>             | - <sup>b</sup>           |
| WS-CS (1:1) + 10% RB                    | - <sup>b</sup>         | 8.0 ± 1.0 <sup>c</sup>  | - <sup>a</sup>          | - <sup>b</sup>          | 7.7 ± 0.6 <sup>a</sup> | 9.3 ± 0.6 <sup>c</sup> | - <sup>a</sup>             | - <sup>b</sup>           |
| WS-CS (1:1) + 20% RB                    | - <sup>b</sup>         | - <sup>a</sup>          | - <sup>a</sup>          | - <sup>b</sup>          | - <sup>b</sup>         | 7.7 ± 0.6 <sup>b</sup> | 8.0 ± 1.0 <sup>b</sup>     | - <sup>b</sup>           |
| <i>P. eryngii</i> var. <i>ferulae</i> * | - <sup>b</sup>         | - <sup>a</sup>          | 10.0 ± 1.0 <sup>b</sup> | - <sup>b</sup>          | - <sup>a</sup>         | - <sup>a</sup>         | 8.0 ± 1.0 <sup>b</sup>     | 8.0 ± 1.0 <sup>a</sup>   |
| Comparison antibiotic                   | 17.0 <sup>oo</sup>     | 13.0 <sup>oo</sup>      | 16.0 <sup>oo</sup>      | 17.0 <sup>oo</sup>      | 18.0 <sup>o</sup>      | 14.0 <sup>o</sup>      | -                          | -                        |

WS: wheat straw, CS: cotton stalks, RB: rice bran

\*: wild (Icme-Elazig), (-): not determined

Comparison antibiotic: <sup>o</sup>Nystatin, <sup>oo</sup>Streptomycin sülfat (Nystatin and Streptomycin sülfat: 100 mg)

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)

al. 2007, Gbolagade et al. 2007, Turkoglu et al. 2007). Results like these herald an interesting promise of constructing a potentially active antimicrobial additive agent from different mushrooms origins. They have great potential as antimicrobial compounds against microorganisms and can be used in the treatment of infectious disease caused by resistant microorganism. It seems that the antimicrobial activity of *P. eryngii* var. *ferulae* (in study 7.7-10.3 mm) are changeable as reported (4.0-29.0 mm) by other researchers (Jonathan and Fasidi 2003, Rosa et al. 2003, Gbolagade et al. 2005, Gezer et al. 2006, Solak et al. 2006, Turkoglu et al. 2006, Barros et al. 2007, Demirhan et al. 2007, Gbolagade et al. 2007, Turkoglu et al. 2007), which may arise from the genetic structure of mushroom species and physical, bioactive-biochemical constituents, and chemical differences of mushroom extracts, solvents and test microorganisms that other research shows clearly when it's compared to the other mushroom species (Benedict and Brady 1972, Conchran 1978, Suzuki et al. 1990, Chihara 1993, Karacsonyi and Kuniak 1994, Collins et al. 1997, Francia et al. 1999, Gunde-Cimerman 1999, Wang et al. 2000, Bobek and Galbavy 2001, Jose et al. 2002, Wasser 2002, Wang and Ng 2004, Periasamy 2005, Carbonero et al. 2006, Iwalokun et al. 2007). This study indicated that there are differences in the antimicrobial effects of mushroom groups, due to phytochemical differences

among species. They claimed that the sensitivity of microorganism to chemotherapeutic compounds change even against different strains. In similar studies (Jonathan and Fasidi 2003, Rosa et al. 2003, Gbolagade et al. 2005, Gezer et al. 2006, Solak et al. 2006, Turkoglu et al. 2006, Barros et al. 2007, Demirhan et al. 2007, Gbolagade et al. 2007, Turkoglu et al. 2007), the extracts of various mushrooms inhibited the growth of some microorganisms at different ratios. Different mushroom species possess 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 metabolites of various chemical types in mushrooms compounds.

At the end of the study, we have found that the extracts of *P. eryngii* var. *ferulae* prepared with methyl alcohol revealed antimicrobial activities against some bacteria, yeasts, and dermatofit, but also they had no antagonistic effect against some test microorganisms used in the study.

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## Çesitli Tarımsal Atıklar Üzerinde Yetistirilen *Pleurotus eryngii* var. *ferulae*'nin Antimikrobiyal Aktiviteleri

### Ozet

Bu çalışmada; değişik tarımsal atıklar üzerinde yetistirilen *Pleurotus eryngii* var. *ferulae*'nin antimikrobiyal aktiviteleri araştırıldı. Farklı kültür ortamlarından sağlanan *P. eryngii* var. *ferulae* ekstraktları disk difüzyon yöntemi ile *Bacillus megaterium* DSM 32, *Staphylococcus aureus* COWAN 1, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* FMC 5, *Candida albicans* FMC 17, *Candida glabrata* ATCC 66032, *Trichophyton* spp. ve *Epidermophyton* spp. üzerindeki antimikrobiyal aktiviteleri değerlendirildi. Deneysel çalışmalar sonunda; *P. eryngii* var. *ferulae*'nin metil alkol ekstraktlarının, mikroorganizmaların gelişmelerini farklı oranlarda engellemislerdir (7.7-10.3 mm). Bununla beraber; mantar ekstraktlarının, antimikrobiyal aktivitesi standart antibiyotiklerden düşük bulunmuştur (13.0-18.0 mm).

**Anahtar Kelimeler:** Antimikrobiyal aktivite, patojen mikroorganizmalar, *Pleurotus eryngii* var. *ferulae*.