

Research Article

## ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF MYCELIAL EXTRACTS OF DIFFERENT PLEUROTUS SPECIES

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### ABSTRACT

Mushrooms could be used as a potential means of producing natural antioxidants and antimicrobials. To obtain fungal biomass in submerged culture is an easy and rapid method. For this reason, biomasses of *Pleurotus* species which grown on liquid media were used to prepare hot water extracts and their antioxidant and antibacterial properties were determined. The highest total phenolic content was determined in *P. ostreatus* extract (9.14 mg.g<sup>-1</sup> dry weight of extract) whereas *Pleurotus sajor-caju* gave highest reading of total flavonoid content (3.10 mg.g<sup>-1</sup> dry weight of extract). In the scavenging effect of DPPH radical test, *P. sajor-caju* showed the highest activity potential (69.67%). Mycelia extracts from *Pleurotus* species showed the antibacterial activity against the Gram negative and Gram positive bacteria (plant and human pathogens). Based on the results obtained, each extract from the five species *Pleurotus* (*P. florida*, *P. citrinopileatus*, *P. sajor-caju*, *P. ostreatus* and *P. eryngii*) showed antioxidant and antibacterial properties, and could be used in the formulation of nutraceuticals. Furthermore, the results presented in this work demonstrated that extracts were capable of inhibiting the *in vitro* growth of *Helicobacter pylori*.

**Keywords:** Antioxidant, Antibacterial, Mycelial extract, *Pleurotus*

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## Introduction

Mushrooms synthesis a range of secondary bioactive molecules (phenols, polysaccharides, pigments, tocopherols, terpenes and steroids) with high therapeutic value. These molecules have pharmacological activities such as antimicrobial, antiviral, antioxidant, antiinflammatory, antitumor, anti-allergic, antiaging, antidiabetic, anti-Alzheimer and hypocholesterolemic (De Silva et al., 2013, Canli et al., 2016). Among the metabolites, phenolic and flavonoid compounds show excellent antioxidant capacity (Barros et al., 2007, De Silva et al., 2013, Yildiz et al., 2015). These metabolites can be extractable (with water or different organic solvents) from both mycelial biomass and fruit body of mushrooms (Lee et al., 2007, Han et al., 2015) and they are sold as a capsule or tablet for diseases prevention (De Silva et al., 2013).

*Pleurotus* species are found throughout world and are among the most widely-cultivated. It well known that *Pleurotus* species produce bioactive molecules such as phenolic, pigment, polysaccharide, and terpenoid (Dündar et al., 2013, Corrêa et al., 2016). Bioactive metabolites are usually produced with little efficiency and productions of these metabolites depend on the species and growth parameters. These metabolites, obtained from *Pleurotus*, shows antioxidant and antimicrobial activities (Carvajal et al., 2012, Reis et al., 2012, Younis et al., 2015). *Pleurotus* species are mostly grown on solid substrates to obtain fruit body. It is known that the production of fruiting bodies take several months. However, mushroom mycelia/biomass can be produced in a short-time (a few weeks) using submerged fermentation.

Moreover, submerged fermentation facilitates compounds extraction and purification, higher production of biomass by reducing growth time and contamination. These species are quite easily cultivated artificially in submerged medium (Reis et al., 2012, Mukhopadhyay and Guha, 2015). Thus mycelial is a cheap alternative and constant source to fruit bodies. *Because of their potential benefits of health to human body, mushrooms products (mushrooms extracts and tablets) are available in market.* There are growing demands for natural antioxidants and antimicrobials due to restriction in the use of synthetic ones. Because of these reasons, the aim of this work is to investigate and compare the antibacterial and antioxidant properties of the hot water extracts obtained from mycelia of *Pleurotus* species.

## Materials and Methods

### *Fungal Cultures and Storage Conditions*

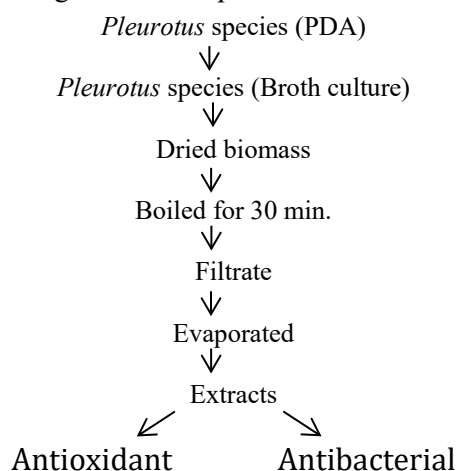
The *Pleurotus citrinopileatus*, *P. eryngii*, and *P. ostreatus* were obtained from Erkel Gıda A.Ş., İzmir, Turkey. *P. sajor-caju* and *P. florida* were obtained from Dr. Abdurrahman Dündar, Mardin Artuklu University. The mushrooms are stored on potato dextrose agar (PDA) at 4°C.

### *Media Preparation and Fermentation Conditions*

The *Pleurotus* mushrooms were firstly grown on PDA petri plates for at 25°C 10 days. Submerged fermentation was carried out in 250 mL Erlenmeyer flasks, containing 100 mL of liquid medium (Glucose 20 g.L<sup>-1</sup>, peptone 2 g.L<sup>-1</sup>, yeast extract 3 g.L<sup>-1</sup>, KH<sub>2</sub>PO<sub>4</sub> 1 g.L<sup>-1</sup>, MgSO<sub>4</sub> 0.5 g.L<sup>-1</sup>). Each flask was inoculated with five 5-mm agar plugs. Each flask was maintained at 25°C and 150 rpm for 15 days. After growth, the fungal biomasses were obtained from the aqueous medium by filtration. The obtained biomasses were washed 4 times with sterilized distilled water, and then dried in the oven.

### *Preparation of Pleurotus Extracts*

To obtain hot water extraction, 5 g dry and powdered mycelial biomass was boiled with 100 mL deionized water for 30 min. and then cooled. The cooled solution was filtered through Whatman filter paper (No. 4). To obtain dried extract, filtered solution evaporated in the oven (55 °C). The dried extracts were dissolved in sterilized distilled water as 100 mg.mL<sup>-1</sup> and kept at 4°C for further studies (Figure 1).



**Figure 1.** Obtaining the fungal extracts

### Determination of Total Phenolic and Flavonoid Content

Total phenolic and flavonoid amounts of hot water extracts were estimated with using gallic acid (GAE) (Vamanu, 2012) and quercetin (QE) (Turkoglu et al., 2007) as standards, respectively.

### Scavenging Activity of DPPH Radical

The radical scavenging activity of mycelia extracts was calculated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Reis et al., 2012; Barros et al., 2008). An aliquot of 270  $\mu\text{L}$  of  $6 \times 10^{-5}$  mol.L<sup>-1</sup> DPPH radical in methanol was added to a test tube with 30  $\mu\text{L}$  of mycelia extract of various concentrations. The solution was mixed at room temperature and after incubated for 30 min. in dark conditions. The absorbance of the mixture was performed at 515 nm using Micro plate Reader. The antioxidant capacity was calculated in following way:

$$\text{Antioxidant activity} = \frac{(\text{Abs}_{\text{sample}} - \text{A}_{\text{control}})}{\text{Abs}_{\text{sample}}} \times 100$$

### Microorganisms

*Bacillus cereus* BC-On (Ozidal et al., 2016a), *Arthrobacter agilis* A17 (Ozidal et al., 2017), *Pseudomonas aeruginosa* OG1 (Ozidal et al., 2016b), *Xanthomonas campestris* MO-03 (Genbank accession number KF939142), *Klebsiella oxytoca* (clinical isolate), *Helicobacter pylori* (ATCC 43629) were used in the study.

### Antimicrobial Activity

The antimicrobial properties of the extracts were determined by disk diffusion technique. One hundred milliliters ( $10^8$  cfu.mL<sup>-1</sup>) of the tested bacterial suspensions were spread on agar plates. Mueller–Hinton agar containing 5% sheep blood plates for *H. pylori*, and Tryptic Soy Agar (TSA) for other bacteria were used for antibacterial activities. *H. pylori* plates were incubated under microaerophilic conditions in anaerobic jars (Oxoid). Sterile paper disks (6 mm diameter) impregnated with 2 mg, 4 mg and 6 mg of each extract (60, 120 and 180  $\mu\text{L}$  of stock solutions) were placed onto agar medium, and then incubated at 37°C for 48 hours. The discs were dried for 24 hours before use. At the end of the time, the diameter of the inhibition zone formed around each disc was measured in millimeters.

## Results and Discussion

### Yield of Dried Mycelial Biomass and Crude Extracts

Maximum biomass yields were obtained from the species of *P. ostreatus* (11.2 g.L<sup>-1</sup>) and *P. citrinopileatus* (9.8 g.L<sup>-1</sup>). The lowest biomass production was observed by *P. eryngii* with 6.1 g.L<sup>-1</sup> (Table 1). Rosado et al. (2003) showed that

production of mycelia biomass for *P. ostreatoroseus* was 16.8 g.L<sup>-1</sup> and for *P. florida* was 22.8 g.L<sup>-1</sup> in polysaccharide production medium, after a 9-day incubation. Confortin et al. (2008) obtained 8.18 g.L<sup>-1</sup> of mycelial biomass when cultivating *P. sajor-caju* PS-2001. Sartori et al. (2015) reported that the production of mycelial biomass for *Pleurotus sajor-caju*, *P. ostreatus*, *P. albidus* and *P. flabellatus* were 12.73 g.L<sup>-1</sup>, 13.27 g.L<sup>-1</sup>, 16.27 g.L<sup>-1</sup> and 8.20 g.L<sup>-1</sup> in vinasse for 15 days, respectively. As seen in Table 1, *P. citrinopileatus* provided a higher extract yield (340 mg.g<sup>-1</sup>) and *P. eryngii* provided a lower extract yield (180 mg.g<sup>-1</sup>).

**Table 1.** Dry weight of mycelia biomass and hot water extraction yield of *Pleurotus* species

<i>Pleurotus</i> species	Mycelial biomass (g.L <sup>-1</sup> )	Yield of extract (mg.g <sup>-1</sup> )	Appearance
<i>P. eryngii</i>	6.1±1.5	180±5	Dark brown
<i>P. sajor-caju</i>	8.8±1.6	260±3	Dark orange/brown
<i>P. citrinopileatus</i>	9.8±1.9	340±4	Dark brown
<i>P. ostreatus</i>	11.2±2.1	300±4	Dark brown
<i>P. florida</i>	7.6±2.4	210±3	Dark orange/brown

The appearance/consistency of the five extracts was similar for *P. sajor-caju*, *P. ostreatus* and *P. eryngii* (dark brown powder), however for *P. florida* and *P. citrinopileatus*, the extracts were dark brown to orange powder.

### Total Phenol and Total Flavonoids Contents

The maximum phenol content was obtained from *P. ostreatus* (7.1 mg.g<sup>-1</sup> extract) and this was followed by *P. sajor-caju* (7 mg.g<sup>-1</sup> extract), *P. citrinopileatus* (5 mg.g<sup>-1</sup> extract), *P. florida* (4.5 mg.g<sup>-1</sup> extract) and *P. eryngii* (3.6 mg.g<sup>-1</sup> extract). The maximum flavonoid content was obtained from *P. sajor-caju* and this was followed by *P. florida*, *P. eryngii*, *P. citrinopileatus*, *P. ostreatus* (Figure 2). In this study, total phenols of various *Pleurotus* species varied from 3.6 to 7.1 mg GAE.g<sup>-1</sup> compared to the reported amounts in other *Pleurotus* species such as *P. eryngii* (21.67 mg tannic acid g<sup>-1</sup>), *P. djamor* (18.88 mg tannic acid g<sup>-1</sup> of mycelium) (Mishra et al. 2013), *P. eryngii* (4.45 mg GAE.g<sup>-1</sup>), *P. ostreatus* (4.37 mg GAE.g<sup>-1</sup>), *P. florida* (4.56 mg GAE.g<sup>-1</sup>), *P. sajor-caju* (3.97 mg GAE.g<sup>-1</sup>) (Dundar et al. 2013). González-Palma et al. (2016) reported in aqueous mycelium extracts (obtained by boiling) of *P. ostreatus* 4.09 mg GAE.g<sup>-1</sup> and 0.192 mg QE.g<sup>-1</sup>. Lee et al. (2007) showed that hot water extract from mycelia of *P. citrinopileatus* as 7.85 mg GAE.g<sup>-1</sup>. The production of extractable metabolites produced by *Pleurotus* species may alter due to different

growth media, different strains of mushroom and extraction solvents.

### Antioxidant Activity of Extracts

The hot water extracts were screened for antioxidant activity. For this purpose, DPPH free radical scavenging technique was applied (Figure 3). In the presence of 10 mg.mL<sup>-1</sup> extract, *P. sajor-caju* (69.67%) showed the highest radical scavenging effect and this followed by *P. ostreatus* (66.12%), *P. citrinopileatus* (64.12%), *P. florida* (56.42%) and *P. eryngii* (48.85%), respectively. As the extract concentration increased, the scavenging activity was also increased. This means that there is a dose dependent DPPH

scavenging efficiency of the mycelium extract. Similar results have been reported in previous studies. The obtained results have been reported similarly in previous studies. Dundar et al. (2013) mentioned that the ethanolic extracts (obtained from mycelia) from *P. eryngii*, *P. ostreatus*, *P. florida* and *P. sajor-caju* scavenged DPPH radicals with 68.01%, 71.29%, 61.97% and 62.82% at 10 mg.mL<sup>-1</sup>, respectively. Antioxidant activity of the hot water mycelial extract of *P. salmoneo-stramineus*, *P. ostreatus*, *P. eryngii* and *P. citrinopileatus* was determined between 22% and 75% at 10 mg.mL<sup>-1</sup> (Smith 2014).

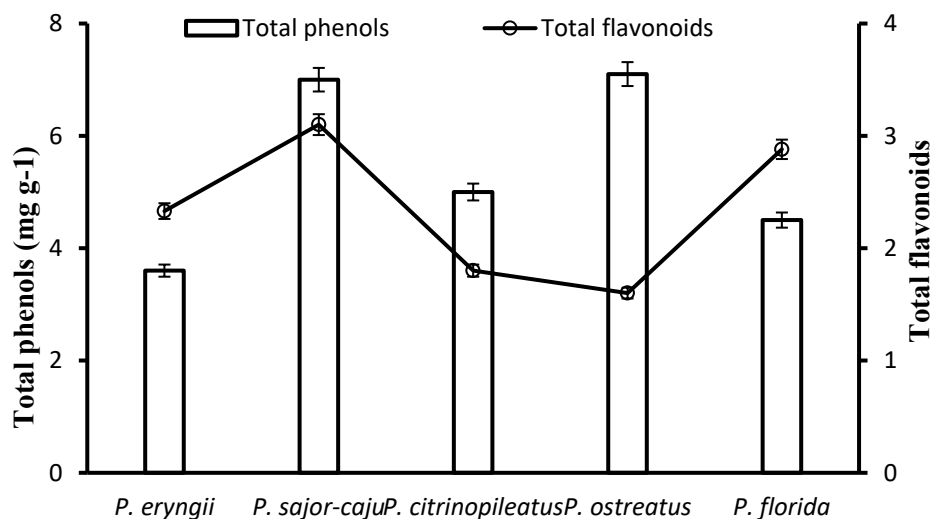


Figure 2. The total phenolic and flavonoids content in mushroom extracts

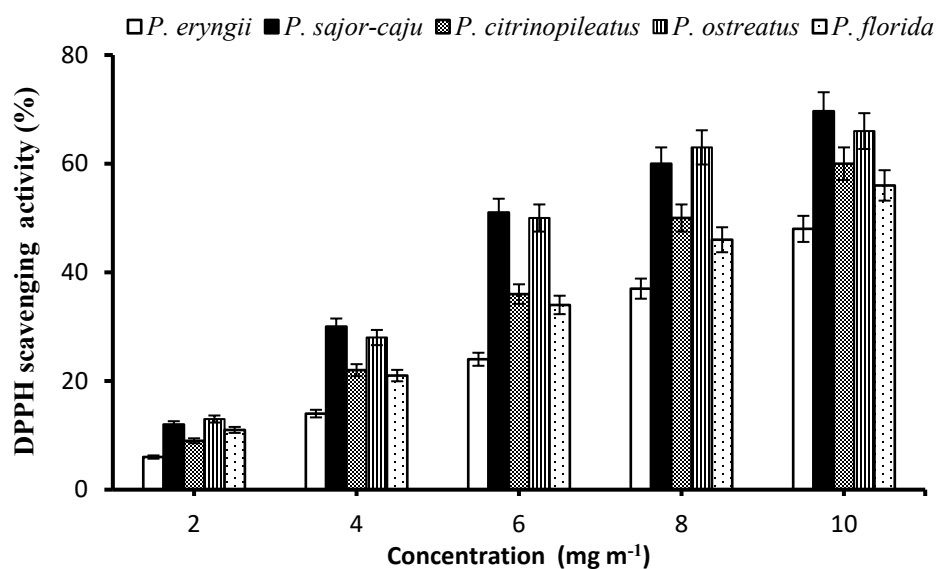


Figure 3. DPPH• scavenging capacity of *Pleurotus* species mycelial extracts

*Antimicrobial Activity*

The hot water extracts (2 mg.disc<sup>-1</sup>, 4 mg.disc<sup>-1</sup> and 6 mg.disc<sup>-1</sup>) of the mycelia cells of five different *Pleurotus* species were performed against two Gram positive (*B. cereus* and *A. agilis*) and four Gram-negative (*P. aeruginosa*, *X. campestris*, *K. oxycota* and *H. pylori*) bacteria by the disc diffusion technique. As shown in Table 2, the concentration of the extracts changes the antibacterial effect. Among all bacteria, *Xanthomonas campestris* was the most sensitive to extracts. The extracts of *P. ostreatus* and *P. florida* were highly antibacterial against *A. agilis* and *H. pylori*. The extract of *P. eryngii* was highly antibacterial against *K. oxycota* and *X. campestris*.

Mushrooms contain different natural antimicrobial compounds such as anthraquinones, aromatic organic compounds, benzoic acid derivatives, fatty acids, organic acids, ribonuclease, peptides, polysaccharides, proteins, quinolines, steroids, terpenes, (Alves et al. 2012). Antibacterial effects of mushroom extracts largely dependent on the mushroom species, their strains and vegetative forms, cultivation conditions, method of extract preparation, methods of evaluation and interpretation of the results (Yamaç and Bilgili, 2006, Vamanu, 2012, Heleno et al., 2013, Dogan et al., 2013, Canli et al., 2015). *Pleurotus* species have a variety of compounds at different concentrations, and this explains the difference of antibacterial activity. Many researchers reported that extracts from the fruiting body or mycelial biomass of *Pleurotus* species exhibited antibacterial activity against different microorganisms (Table 3).

**Table 2.** Inhibition zones (mm) of crude extracts of *Pleurotus* species against bacteria

Bacteria	Concentration of crude extracts of <i>Pleurotus</i> species (mg.disc <sup>-1</sup> )														
	<i>P. eryngii</i>			<i>P. sajor-caju</i>			<i>P. citrinopileatus</i>			<i>P. ostreatus</i>			<i>P. florida</i>		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
<i>H. pylori</i>	-	7.1	8.4	-	6.4	7.3	-	6.2	7.3	7.5	9.8	12.4	8.1	10	13
<i>X. campestris</i>	7.2	8.3	12.1	-	7.4	9.7	7.4	8.7	11.8	7	8.3	10.7	6.3	7.4	9.8
<i>K. oxycota</i>	11.2	13.4	15.2	-	8.2	10.3	-	7.8	9.8	-	8.2	10.8	-	8.6	11.6
<i>P. aeruginosa</i>	-	7	8.8	-	7.2	9	-	6.8	8.1	-	7.3	9.4	6.3	8	9.7
<i>B. cereus</i>	-	7.4	9.8	-	7.1	10.2	-	7.3	9.6	-	7	9.4	-	7.2	8.8
<i>A. agilis</i>	-	8	10.4	-	8.4	11.2	-	7.2	9.5	11	15	18	7.4	9.3	12.1

a. 2 mg.disc<sup>-1</sup>, b. 4 mg.disc<sup>-1</sup>, c. 6 mg.disc<sup>-1</sup>; “-“: no activity observed.

**Table 3.** *Pleurotus* extracts with antimicrobial activity against microorganisms

<b>Pleurotus species</b>	<b>Bacterial species</b>	<b>Extract-Solvent</b>	<b>References</b>
<i>P. ostreatus</i> <i>P. sajor-caju</i> <i>P. eryngii</i>	<i>B. megaterium</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>Candida glabrata</i> , <i>S. aureus</i> <i>B. megaterium</i> , <i>C. albicans</i> , <i>C. glabrata</i> , <i>E. coli</i> , <i>S. aureus</i> ,	Fruit body- Methanol	Akyuz et al. 2010
<i>P. ostreatus</i>	<i>K. pneumoniae</i> , <i>S. aureus</i> , <i>S. pyogenes</i> , <i>S. dysenteriae</i> , <i>B. subtilis</i> , <i>K. pneumoniae</i> , <i>S. aureus</i> , <i>Shigella dysenteriae</i> , <i>Salmonella enterica</i> , <i>P. aeruginosa</i>	Mycelial biomass-water Fruit body- water	Younis et al. 2015
<i>P. eryngii</i> , <i>P. ostreatus</i>	<i>H. pylori</i>	Fruit body- Ethanol	Shang et al. 2013
<i>P. eryngii</i> <i>var. ferulae</i>	<i>B. megaterium</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>C. albicans</i> , <i>C. glabrata</i> , <i>Trichophyton spp.</i> , <i>Epidermophyton spp.</i>	Fruit body- Methanol	Akyuz and Kirbag 2009



## Conclusion

In conclusion, this study showed the presence of antimicrobial and antioxidant activities in different species of *Pleurotus*. The obtained mushroom extracts can be used in food supplement products and medicinal products for health promotion. The uses of antibiotics are known to be the main control method for diseases but they are detrimental to the human health and the ecosystem, and may contribute to the development of chemical-resistant microorganisms. It is well known that *H. pylori* which cause the ulceration of stomach and then cancer. In this study, we found that *P. sajor-caju* can be used as a medical food for recovering and treatment of ulcer.

## Compliance with Ethical Standard

**Conflict of interests:** The authors declare that for this article they have no actual, potential or perceived conflict of interests.

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