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Research Article

ANTIOXIDANT POTENTIAL AND ANTI-BACTERIAL ACTIVITIES OF *PLEUROTUS* EXTRACTS

***Gagandeep Kaur[1], Anu Kalia[2] and Harpreet S. Sodhi[1]**
[1]Department of Microbiology, College of Basic Sciences and Humanities,
[2]Electron Microscopy and Nanoscience Laboratory, College of Agriculture, Punjab Agricultural University, Ludhiana, Punjab-141004, India
**Author for Correspondence*

ABSTRACT
The genus *Pleurotus* comprises a group of edible ligninolytic mushrooms with medicinal properties and important biotechnological and environmental applications. The present study reports phytochemical analysis, antioxidant potential and anti-bacterial activities of extracts of three *Pleurotus* species: *P. florida*, *P. ostreatus* and *P. sajor-caju*. Cultivation of *Pleurotus* species was carried out on wheat straw substrate and yield of *P. sajor-caju* was observed to be maximum. Total proteins, peroxidase enzyme activity and ascorbic acid content were estimated on fresh fruit bodies while radical scavenging activity, total phenol content, flavonoid content and antibacterial activity were measured using extracts from dried and powdered mushrooms. *P. florida* had highest ascorbic acid content while *P. sajor-caju* had most radical scavenging potential due to flavonoid content. *P. ostreatus* showed maximum peroxidase activity and total phenol content and also had maximum antibacterial efficiency. Thus, the present study establishes *Pleurotus* species as a functional food and nutraceutical.

Keywords: *Pleurotus, Antioxidants, Antimicrobial, Nutraceutical*

INTRODUCTION
Edible mushrooms are saprophytic macrofungi belonging to class Basidiomycetes. They have delicate flavor and texture, and are recognized as a nutritious food providing biologically active compounds of greater medicinal value. Mushrooms have been an integral part of the fungal diversity for around 300 million years. Their use as food and medicine in Asia began after the Chinese pioneered the cultivation of *Auricularia auricular* on wooden logs in 600 A.D. (Chang and Miles, 2004; Subramanian, 1995).
Mushrooms are rich sources of antioxidants such as vitamin A, C, E, carotenoids, polyphenolic compounds and flavonoids (Radzki *et al.*, 2014; Asatiani *et al.*, 2010), which prevent oxidative stress or free radical damage. Oxidative stress arises due to imbalance in production and elimination of free radicals and reactive metabolites i.e. oxidants or reactive oxygen species (ROS). Oxidative damage to DNA, proteins, and other macromolecules has been postulated to be a one of the major types of endogenous damages leading to aging (Halliwell, 2012). Continued oxidative stress can lead to chronic inflammation, which in turn could mediate most chronic diseases including cancer, diabetes, cardiovascular, neurological and pulmonary diseases (Reuter *et al.*, 2010). Antioxidant compounds in mushrooms are useful to reduce risk of chronic diseases.
Mushrooms contain bioactive compounds with potential applications as anti-bacterial, anti fungal and anti-viral agents (Giri *et al.*, 2012; Alves *et al.*, 2012; Teplyakova *et al.*, 2012; Pan *et al.*, 2013). Over the last decade, it has become clear that antibiotics are losing their effectiveness as pathogens are evolving resistance against them in a multitude of ways.
This situation has generated the need, and has provided the necessary impetus for a continuous search for novel antimicrobial agents from different natural biological sources (Cordell, 2000). Natural antimicrobials can be derived from different plant parts, various animal tissues or from microorganisms (Saleem *et al.*, 2010; Natarajan *et al.*, 2014; Gyawali and Ibrahim, 2014). The antimicrobial activity of mushrooms may be attributed to the presence of various bioactive secondary metabolites, volatile compounds, some phenols, gallic acids, free fatty acids and their derivatives (Bala *et al.*, 2012; Ramesh and Pattar, 2010).

Pleurotus species are a group of oyster shaped mushrooms (basdiocarps), cultivated over a wide range of tropical and temperate regions worldwide. Various *Pleurotus* species have been shown to possess a number of medicinal properties, such as antitumour, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolaemic, antihypertensive, antiplatelet-aggregating, antihyperglycaemic, antimicrobial and antiviral activities (Patel *et al.*, 2012; Khan and Tania, 2012).

The present study aims at comparing the phytochemical composition of methanolic extracts of *Pleurotus florida*, *Pleurotus ostreatus* and *Pleurotus sajor-caju* in order to ascertain their antioxidant potential. Bactericidal activity of the three *Pleurotus* species was also studied against major human pathogenic gram positive and negative bacteria.

MATERIAL AND METHODS

Chemicals

All the chemicals used in these present experiments were of analytical grade and purchased from HiMedia Laboratories Pvt Ltd, Mumbai, India.

Cultivation of Mushrooms

The cultures for three species of *Pleurotus*; *P. florida*, *P. ostreatus* and *P. sajor-caju* were procured from Directorate of Mushroom Research, Solan, Himachal Pradesh, India. The three species were cultivated on wheat straw as substrate using the standard methodology (Chang and Miles, 2004) at Mushroom Research Complex, Punjab Agricultural University, Ludhiana, Punjab, India.

Preparation of the Samples

The fruit bodies of *Pleurotus* species were harvested and cleaned. Fresh fruit bodies were used for estimation of total protein content, ascorbic acid content and peroxidase enzyme activity. For radical scavenging activity and total phenol content estimation, fruit bodies were dried at 40ºC and grinded to fine powder.

Dried powdered fruit bodies (10 g) were extracted with 100 ml ethanol (150 rpm for 24 h). Residues were re-extracted twice and filtrates were combined. The extracts were evaporated to dryness at 40ºC, re-dissolved in the solvent and then stored at 4ºC for further experimentation. For flavonoid content, dried powder of fruit bodies was extracted with 10 ml methanol for 24 h.

For antimicrobial activity, mushrooms dried at 55ºC in the oven and later powdered. The dried powders (1 g) were mixed with 10 ml methanol and kept for shaking at 250 rpm for 24 h, filtered with Whatman filter paper No.1, dried and re-dissolved in 2ml of DMSO. The prepared samples were kept at 4ºC.

Total Protein Content

Total proteins were extracted from biomass of three *Pleurotus* species using 0.5M Tris HCl buffer (pH 6.8). Precipitation of proteins was done using ammonium sulphate. Precipitated proteins were then re-dissolved in minimum amount of buffer. Total protein content was determined using Folin's reagent for reaction (Lowry *et al.*, 1951) and Bovine Serum Albumin as standard.

Peroxidase Enzyme Activity

Fresh fruit bodies (2 g) of *Pleurotus* species were crushed with 6 ml of 0.1 M phosphate buffer (pH 7.0), centrifuged at 10,000 rpm at 4ºC for 10 minutes to obtain clear supernatant containing the enzyme.

Peroxidase activity was determined spectrophotometrically based on the oxidation of guaiacol in the presence of H_2O_2 (Reuveni *et al.*, 1992).

The assay mixture contained 3 ml of 0.05 M guaiacol as donor, 0.1 ml of 0.8 M H_2O_2 as substrate, and 50µl crude enzyme extract. The reaction mixture was placed in a quartz cuvette and the optical density was recorded at 15 seconds intervals for 3 min at 420 nm wavelength. The level of enzyme activity was determined by measuring the difference in optical density and expressed in mol $min^{-1}g^{-1}$ of tissue.

Ascorbic Acid Content

Two grams of fresh mushroom sample was crushed in 5% Trichloroacetic acid, centrifuged at 8,000 rpm for 15 minutes and supernatant was obtained for ascorbic acid estimation. Pure ascorbic acid at 0.5 mg ml^{-1} was used as standard. The extracts were treated with DNPH-thiourea mixture and dichloroindophenol reagent and analysed spectrophotometrically at 505 nm.

Free Radical Scavenging Assay

The hydrogen atoms or electron donation ability of the corresponding extracts was measured from the bleaching of the purple colored DPPH methanol solution (Sánchez-Moreno *et al.*, 1999). Various samples (0.05 ml) were added to 1.95 ml of methanolic DPPH (0.1 mM) and mixed thoroughly. The mixture was left to stand for 45 min in the dark at room temperature and the absorbance was measured at 515 nm. A lower absorbance represented a higher DPPH scavenging activity. The scavenging effect was expressed as scavenging activity (1 −Ac/Ad), where Ac is the absorbance of solution when the extract is added at a particular concentration, and Ad is the absorbance of the DPPH solution.

Total Phenolic Content

The total phenolic content was determined by Folin–Ciocalteu method (Minussi *et al.*, 2003). Sample solution of 100 µl was added to 2 ml of 2% sodium carbonate, mixed thoroughly and allowed to stand for 2 min. Then, 100 µl of Folin–Ciocalteu reagent (Folin: Methanol, 1:1, v/v) was added and the mixture was mixed well. After incubation for 30 min, the absorbance was measured at 750 nm. A calibration curve was obtained using various concentrations of gallic acid. The total phenolic content of the sample was expressed as mg of gallic acid equivalents (GAEs) per gram of dry sample.

Total Flavonoid Concentration

Quercetin dissolved in methanol (12.5-100 µg/ml) was used to prepare the standard curve. Methanol extracts (0.5 ml) of *Pleurotus* species were taken in test tubes and 1.5 ml methanol, 0.1ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water were added separately to each tube. All the tubes were incubated at room temperature for 30 min optical density was measured at 415 nm by using spectrophotometer (Woisky and Salatino, 1998).

Antimicrobial Activity

The live cultures slants of the test pathogenic bacteria, *Escherichia coli, Yersinia enteroclolitica, Aeromonas* spp., *Klebsiella pneumoniae* and *Staphylococcus aureus* were collected from Department of Microbiology, Punjab Agricultural University, Ludhiana and culture of *Salmonella typhi* was procured from Department of Microbiology, Christian Medical College and Hospital, Ludhiana, Punjab, India. The extracts from *P. florida*, *P. ostreatus* and *P. sajor-caju* were tested for their antibacterial activity against these pathogens using agar well diffusion technique. The bacteria were multiplied by cultivating on Nutrient Agar plates using spread plate technique. Wells of 0.5 mm diameter were prepared in five different spots on the media containing petri plate using a sterile cork-borer. These wells were loaded with 100 µl of mushroom extracts. Gentamicin, tetracycline, kanamycin and ampicillin (100 µg ml^{-1}) and DMSO were used as control. The plates were incubated at 37ºC for 24 hrs and the zones of clearance were measured for each sample.

Statistical Data Analysis

All experiments were carried out in triplicates. Data obtained were analyzed by one way analysis of variance and differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Cultivation of *Pleurotus* species was carried out on wheat straw substrate and fruit bodies were harvested and weighed to calculate the yield potential of the three species.

The yield of *P. ostreatus* and *P. sajor-caju* was found to be at par and more than *P. florida*; while number of fruiting bodies was maximum for *P. sajor-caju* followed by *P. ostreatus* and *P. florida*. In a cultivation study conducted by Moyson and Verachtert (1991), *P. sajor-caju* was found to reduce the lignin content in wheat straw to half its initial value by digestion.

The phytochemical studies showed that ascorbic acid content was maximum in *P. florida* (1.035mg g^{-1}) among the three species (Table 1).

Peroxidase activity (0.340 mol $min^{-1}g^{-1}$) and total phenol content (89.617 µg GAE g^{-1}) were maximum in *P. ostreatus* while *P. sajor-caju* had maximum radical scavenging activity (.557) and flavonoids content (439.66 µg QE g^{-1}) among the three species. Traditional medicinal plants like *Mellilotus officinalis* and *Adiantum capillusveneris* were found to have flavonoid content of 57±5.4 mg g^{-1} and 78.3±4.5 mg g^{-1} and

total phenols 289.5±5 and 22.3±3 mg g^{-1}, respectively while their scavenging power was found to be 0.94 and 0.44, respectively (Pourmorad *et al.*, 2006).

Even the flavanoid rich extracts of garlic were observed to contain 470 µg QE g^{-1}, which is invariably quite close to the flavonoid contents in mushroom samples (Chun *et al.*, 2005). Mohamed and Farghaly (2014) performed Gas Chromatography/Mass Spectrometry (GC/MS) analysis of fresh and dried *Pleurotus ostreatus* fruit bodies reported the occurrence of 107 metabolites including diverse functional groups like alcohols, alkanes, amides, esters, fatty acids, terpenoids, heterocyclic compounds and phenols.

Table 1: Total phytochemical analysis of *Pleurotus* species

Species	Yield (kg/ 100 kg dry substrate)	Number of Fruit Bodies/ 100 kg dry substrate	Total Protein Content (mg g^{-1})	Peroxidase activity (mol $min^{-1}g^{-1}$)	Ascorbic Acid content (mg g^{-1})	Radical Scavenging Activity	Total Phenol Content (µg GAE g^{-1})	Flavonoid Content (µg QE g^{-1})
P. florida	50.204	3,733	1.644	0.160	1.035	0.513	85.191	427.334
P. ostreatus	57.120	5,797	1.728	0.340	0.835	0.418	89.617	219.338
P. sajor-caju	57.653	7,917	2.494	0.280	0.897	0.557	77.276	439.66
Critical Difference at p=0.05	3.016	24	0.011	0.004	0.006	0.006	2.087	3.705

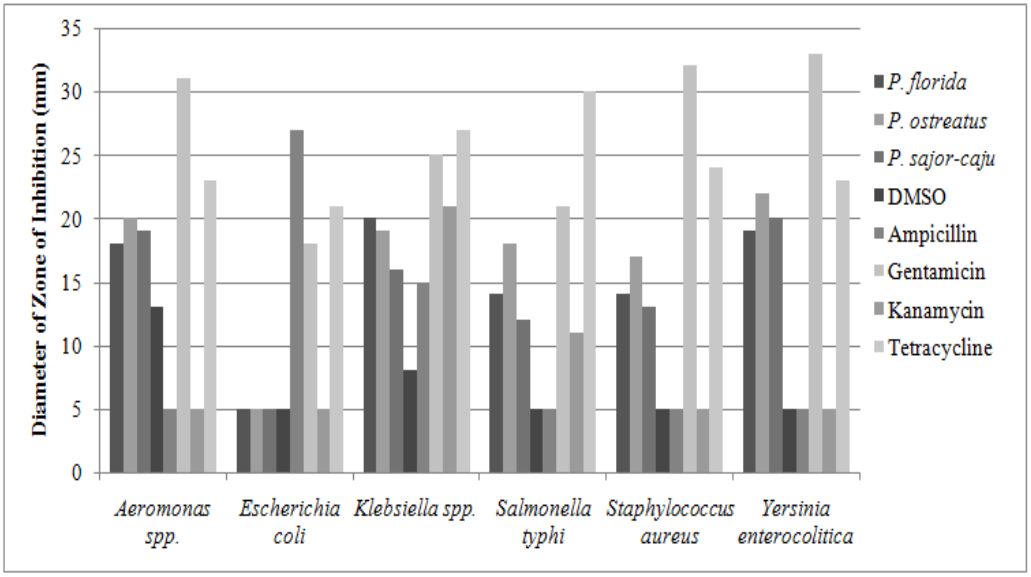

Figure 1: Antibacterial activity of extracts of *Pleurotus* species against various pathogens

Antimicrobial activity of *Pleurotus florida*, *Pleurotus ostreatus* and *Pleurotus sajor-caju* extracts was tested (Fig 1) against a variety of gram positive and negative pathogenic bacteria. All the *Pleurotus* species showed strong inhibitory effects towards most of the test cultures. *P. ostreatus* showed highest bactericidal potential against maximum pathogens. *Yersinia enterocolitica* was found to be the most susceptible pathogen while *E. coli* was the least. A similar report by Kunjadia *et al.*, (2014) also

advocated significant antimicrobial activities of *Pleurotus ostreatus* ethanolic extract against gram-positive and negative bacteria, and also against some fungi. Thus, the present study conclusively establishes *Pleurotus* species as a source of natural antioxidants in food and nutraceutical industries as well as a significant antimicrobial agent and a protein source.

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