Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*

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Abstract

This study scientifically examined the photochemistry, antioxidant and antimicrobial potencies of two organic extracts of *Pleurotus ostreatus*. Generally, both extracts were effective against 89.8% of the isolates tested with *Bacillus subtilis* and *Escherichia coli* exhibiting highest gram ± ve and gram –ve susceptibilities by disc diffusion method, respectively. However, petroleum ether extract (PE) exhibited greater anti-gram negative bacterial activity than the acetone extract (AE) and further produced growth inhibition of these isolates in broth. Compared to PE, the acetone extract elicited higher total phenolic content and *in vitro* antioxidant capacity. Phytochemical analyses of the extracts revealed low to moderate levels of terpenoids, tannins, and carbohydrates, while alkaloids were not detected. The results indicate that *P. ostreatus* possesses antimicrobial .

Key words: *Pleurotus ostreatus*, organic extracts, antimicrobial activity, phytochemistry.

Introduction

Edible mushrooms are nutritionally endowed fungi (mostly Basidiomycetes) that grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials (Chang and Miles, 1992; Stamets, 2000; Lindequist et al., 2005). Scientific explorations of Shiitake mushrooms such as *Lentinus edode*s, Maitake mushrooms such as *Grifola frondosa*, chanterelles such as *Chaterellus carius*, white button mushrooms such as *Agaricus bisporus*, and oyster mushrooms have shown that they serve as repositories of B-vitamins such as niacin, flavin and pyridoxine (Solomko and Eliseeva, 1988); organic acids such as ascorbate, shikimate, malate and fumarate;carbohydrates such as the - glucans; monoterpenoid and diterpenoid lipids; proteins such as hydrophobins and trace elements such as selenium (Dikeman et al., 2005; Valentao et al., 2005). These substances have been found through several *in vitro* and *in vivo* studies to be responsible for the antimicrobial, antioxidant, antitumor, antihypertensive and antiaging potentials of edible mush-mushrooms (Lindequist et al., 2005). The recognition of *Garnodema lucidium* also called reshi as an antioxidant mushroom is due to its phenolic, terpenoids and polysaccharide polypeptide contents (Miller et al., 2000; Mau et al., 2002; Valentao et al., 2005).

These bioactive compounds mediate biological activities including stimulation of interleukin-12 production, nitric oxide synthase activetion,free radical scavenging and iron chelating properties (Cui et al., 2005; Acharya et al., 2005).*Pleurotus ostreatus* is among the edible mushrooms consumed in Nigeria. It is used as spice in vegetable soups and also fried to serve as meat (Annon, 2004). The mushroom is credited to be the third largest macrofungus cultivated for food and industial purposes worldwide. Nutritionally, the mushroom has been found to contain vitamins B1 (thiamin), B2 (riboflavin), B5 (niacin), B6 (pyridoxine and B7 (biotin) (Solomko and eliseeva, 1988).The fruiting body of the mushroom is also a potential source of lignin and phenol degrading enzymes (Fountoulakis et al., 2002). While from clinical viewpoint, Bobeck and Galbavy (1999) showed that *P. ostreatus* elicited hypocholesterolemic and antherogenesis inhibition functions in rabbits and rat coutesy of its mycelial secretory products.However, unlike the fruiting bodies of few other edible mushrooms such as *L. edodes, G. fondosa* and *G. lucidium* known for exhibiting antibacterial and antifungal activity *in vitro*, there is lack of information on the microbicidal properties of *P. ostreatus* coupled with inadequate data on its phytochemistry. It is hypothesized that knowledge of the phytoconsitituents of *P. ostreatus* would provide an insight into its biological functions beyond nutrition when consumed. In the present study, organic mycelia extracts of *P. ostreatus* were phytochemically analysed and tested for antioxidant function *in vitro*. The antimicrobial activity of these extracts was also examined.

Materials and methods

Strains of *P. ostreatus MTCC 1801* were grown at Institute from the pure fungal Species procured from IMTECH, Chandigarh The fruiting bodies, carefully removed from the hyphae were weighed then dried at 40oC for 24 h. The dried fruiting body samples were weighed and ground into powder prior to extraction.

Preparation of *Pleurotus ostreatus* extracts

Dried samples of fruiting body powder (3.5 g each) were separately extracted with 100 ml each of petroleum ether (20 – 80oC) and 80% acetone for 2 h using soxhlet apparatus. The residual solvent was removed by evaporation at 40oC for 24 h *in vacuo* using a rotatory evaporator. The resulting organic extracts were further reconstituted to different concentrations (0 – 100% v/v) with 0.1% Tween-20 in phosphate buffered saline (pH 7.2) followed by storage in sterile capped bottles under refrigeration condition (4oC) prior to use for subsequent assays.

Phytochemical analysis of *Pleurotus ostreatus* extracts

The phytoconstituents present in the organic extracts were determined qualitatively according to Sofowora (1993), Trease and Evans (1989) and Harbone (1973). In TLC, the extracts spotted on silica coated plates, were developed using butanol-glacial acetic-water (100 : 10 :10) as the solvent system. The developed plates were then sprayed with with vanillin solution (1% (w/v) in 50% phosphoric acid) for steroid detection, Dragendorff's reagent for alkaloid detection and sodium metaperiodate (0.1%) followed by ethanolic benzidine for glucose detection. Nicotinic acid, cholesterol D-glucose and tannic acid at 1% solution were prepared accordingly and used as standards in the TLC assay. The TLC results were further used to validate the presence of tannins based on positive reaction (brownish green – blue black coloration) with 0.1% FeCl3, alkaloids based on positive reaction (brown coloration) with Dragendorff's reagent (Trease and Evans, 1989; Sofowora, 1993), steroids based on positive reactions (violet to blue or green) with acetic anhydride and H2SO4, steroidal glycosides by Keller-Killani test and cynogenic glycoside based red coloration of picrate paper (Harbone, 1973; Trease and Evans, 1993). The observation of persistent frothing in
distilled water (2 ml) by 1% standard saponin solution (3 ml) followed by formation of emulsion with olive oil (0.5 ml) was used to indicate the presence of saponin in the extract (Trease and Evans,1989).

Microorganisms

The microorganisms to which the antimicrobial properties of the organic extracts of *P. ostreatus* were tested were obtained from IMTECH Chandigarh .The gram-negative organisms include *Escherichia coli* while the gram-positive isolates were *Bacillus subtilis,* were tested to validate the antibiotic susceptibility assays

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Antimicrobial testing of *Pleurotus ostreatus* extracts

P. ostreatus extracts were tested for antimicrobial activity by disc diffusion technique (Akpata and Akinrimisi, 1977) with a little modification. 100 µl of each standard inoculum was then streaked on nutrient agar. Watman filter paper disc were imbibed with 100 µL *P. ostreatus* organic extract. The plates were incubated accordingly as described previously. Growth inhibition was measured as diameters of inhibitory zones in the nearest 0.1 mm.

Results

The present study has revealed the antibacterial activity of petroleum – ether (PE) and acetone (AE) extracts of P. ostreatus fruiting body against bacteria . Phytochemical analysis revealed the presence of terpenoids, tannins, steroidal glycosides and carbohydrates in both extracts but with higher terpenoids contents in the PE fraction. Cyanogenic glycosides, alkaloids, flavonoids and anthraquinones were not detected in both extracts. Among the susceptible gram-negative bacteria tested, inhibitory zones due to petroleum extract were higher than those of the acetone extract. In the gram-positive bacteria tested, *B. subtilis the Inhibition zone* were observed to be the lower [13.0 mm] .In gram-negative bacteria, *E.coli* the inhibition zone was higher [17.6 mm]

S.No.	Name of the drug	Microorganism	Zone of Inhibition (in mm)
1.	P ***ostreatus— Petroleum Ether Extract***	*E.coli*	17.6 mm
		B.Subtillus	13.0 mm
2.	***P ostreatus— Acetone Extract***	*E.coli*	12.22
		B.Subtillus	7.00

Discussion

P. ostreatus, an oyster mushroom is primarily consumed for its nutritive value and used industrially as a bioremediator (Solomko and Eliseeva, 1988; Fountoulakis et al.,2002; Tsioulpas et al., 2002). The present study has further revealed the antimicrobial potency of the oil of the

macrofungus extracted with petroleum ether and acetone. Both extracts were observed to inhibit gram positive and gram-negative bacteria *in vitro* to suggest that *P. ostreatus* has a broad-spectrum antibacterial activity. Similar antimicrobial potentials have been observed in the culture extracts of *Irpex lacteus* (Rosa et al., 2003) *Agrocybe* sp. (Kavanagh et al., 1950; Mavoungou et al., 1987), and juice of *L. edodes* (Kuznetsov et al., 2005). Antimicrobial potencies of the essential oils from mushrooms such as *Cuminum cyminum, carum carvi, Coriandum sativum* and *Foeniculum vulgare* have also been reported (Iacobellis et al., 2005) with activity against bacterial pathogens such as *Pseudomonas, Klebsiella, Salmonella and E. coli* as observed for *P. ostreatus* in this study.The observed disparity in the the susceptibilities of bacteria tested with petroleum ether extract & acetone extract eliciting greater effect provides an indication that the organic solvents used have varying abilities to extract bioactive substances from *P. ostreatus.* This is further evidenced by the different levels of terpenoids and phenolics observed in the two organic extracts.

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