



COMPARATIVE *IN VITRO* STUDIES ON PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF ETHANOLIC EXTRACTS OF *PLEUROTUS FLORIDA* AND *PLEUROTUS OSTREATUS*

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ABSTRACT

The present investigation scientifically examined the phytochemistry and antibacterial potencies of ethanolic extracts of *Pleurotus florida* and *Pleurotus ostreatus*. Phytochemical analyses of the ethanolic extracts of the above pleurotus species revealed that there was moderate levels of terpenoids, tannins, steroidal glycosides and carbohydrates, flavonoids while alkaloids, carboxylic acids, Volatile oils, Cardinolides, Resins, Quinones, fatty acids and cynogenic glycosides were not detected. Generally, both extracts were effective against the isolates tested by agar well diffusion method, respectively. However, ethanolic extract of *P.florida* exhibited greater antibacterial activity than that of *P.ostreatus* and further produced growth inhibition of these isolates in broth. The results indicate that *P. florida* possesses antibacterial activity.

KEYWORDS: *Pleurotus florida*, *Pleurotus ostreatus*, organic extracts, antibacterial activity, photochemistry.



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INTRODUCTION

Recent scientific examination on medicinal mushrooms have prolonged exponentially during the last two decades not only in Japan, Korea and China but also in USA and scientific elucidation to show mushrooms derived compounds role in human system are progressively more being established [1]. A number of medicinal mushrooms, such as *Aleurodiscus*, *Coprinus*, *Clitocybe*, *Daedalea*, *Marasmius*, *Merulius*, *Pleurotus*, *Psathyrella*, and *Tricholoma* spp. Are rich sources of β -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, porisin, AHCC, maitake D-fraction, ribonucleas, eryngeolysin, and also have been used widely in traditional medicine for curing a variety of types of diseases such as antibacterial, antiviral, anticancer, antitumor, anti-inflammatory, cardiovascular diseases, immunomodulating, central actions etc. [2-9]

Pleurotus florida is an edible and highly nutritious mushroom which is a common species in tropical west Africa and southern parts of Asia. This edible fruit bodies develop in large number as a group on fallen trees, logs of wood and wooden poles. The cap measurement may range from 1.5 to 7.5 cm diameter while the stripe is 0.5cm to 2.5 cm long and the spore print is cream –white in colour [10]. However, unlike the fruiting bodies of few other edible mushrooms such as *L. edodes*, *G. fondosa* and *G. lucidium* known for exhibiting antimicrobial activity *in vitro*, there is lack of information on the microbicidal properties of *P. florida* and *P.ostreatus* coupled with inadequate data on its phytochemistry aspects. It is hypothesized that acquaintance of the phytoconstituents of *P. florida* and *P.ostreatus* would provide an insight into its biological functions beyond nutrition when consumed. In the present investigation, organic mycelia extracts of *P. florida* and *P.ostreatus* were phytochemically analysed and tested for

antibacterial function *in vitro* with some known common bacterial species.

MATERIALS AND METHODS

Pleurotus .Sp collection

P. florida and *P.ostreatus* are edible mushrooms in oil palm area at different locations in Hyderabad were collected. They were identified at the Department of botany, Osmania University, Hyderabad. The fruiting bodies, carefully removed from the hyphae were weighed then dried at 40°C for 24 h. The dried fruiting body samples were weighed and ground into powder prior to extraction (Table 1).

Preparation of Pleurotus.Sp ethanolic extracts

Dried samples of fruiting body powder (3.5 g each) were separately extracted with 100 ml each of ethanol for 2 h using soxhlet apparatus. The residual solvent was removed by evaporation at 40°C for 24 h *in vacuo* using a rotator evaporator. And its extract yield is calculated (Table 2). 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 (4°C) prior to use for subsequent assays.

Phytochemical analysis of Pleurotus.Sp ethanolic extracts

The phytoconstituents present in the organic extracts were determined qualitatively according to Sofowora (1993), Trease and Evans (1989) and Harbone (1973) [11,12,13,14,15]. (Table 3).

Microorganisms

The microorganisms to which the antibacterial properties of the organic extracts of *P. florida* and *P.ostreatus* were tested were obtained from Microbial Type Culture and collection, Chandigarh, India.

Antibacterial testing of *P. florida* and *P. osteratus* ethanolic extracts

P. florida extracts were tested for antibacterial activity by agar – well diffusion technique [16] with a little modification. An overnight culture of each bacterial isolate was emulsified with nutrient broth to a turbidity that was equivalent to 0.5 McFarland (10^8 cfu/ml). 100 μ L of each standard inoculum was then streaked on nutrient agar and PDA at 106 cfu/ml per spot to attain a confluent growth [17]. Wells were made on the agar using a sterile cork borer and filled with 100 μ L *P. florida* and *P. osteratus* organic extract. The plates were incubated accordingly as described previously. Standard strain of *E. coli* was used as control organism for bacterial assays. Bacteria control wells contained 100 μ L of ciprofloxacin at 5 μ g per well. Growth inhibition was measured as diameters of inhibitory zones in the nearest 0.1 mm.

RESULTS

The present experiment has exposed the antibacterial activity of ethanolic extracts of *P.*

florida and *P. osteratus* fruiting body against multi drug resistant bacteria pathogens presented in Table 4. Data presented in (Table 1) showed the fruiting body wet and dry weights per *P. florida* and *P. osteratus* strain analyzed ranged from 1.34-1.55 g and 1.32-1.47 g, respectively. The ethanolic extract yields ranged from 3.2 – 3.4 mg / g dry mycelial weight (Table 2). Phytochemical analysis revealed the presence of low to moderate levels of terpenoids, tannins, steroidal glycosides and carbohydrates, flavonoids while alkaloids, carboxylic acids, Volatile oils, Cardinolides, Resins, Quinones, fatty acids and cynogenic glycosides were not detected (Table 3). Preliminary antibacterial testing of ethanolic extract of *P. florida* and *P. osteratus* by agar-well diffusion produced zones of growth inhibition which are shown in table 4. On the whole, of the known common bacterial isolates tested showed sensitivity to ethanolic extracts of both the species (Table 4). The ethanolic extract of *P. florida* further showed stronger inhibition of these organisms in broth compared to that of *P. osteratus*.

Table 1
Fruiting body details

Parameter	Amount in gms
Fruiting body wet weight(g)/ strain of <i>P. florida</i>	1.34-1.55
Fruiting body dry weight (g)/strain of <i>P. florida</i>	0.145-0.187
Fruiting body wet weight(g)/ strain of <i>P. osteratus</i>	1.32-1.47
Fruiting body dry weight (g)/strain <i>P. osteratus</i>	0.140-0.180

Table 2
Extractive values of ethanolic extracts of *Pleurotus .Sp*

S.No	Type of extract	Extractive Value (in mg/g dry weight)
1	<i>Pleurotus florida</i>	3.2
2	<i>Pleurotus osteratus</i>	3.4

Table 3
Phytochemical analysis of ethanolic extracts of *Pleurotus florida* and *Pleurotus osteratus*

S.No	Plant constituents	<i>Pleurotus florida</i> Ethanol extract	<i>Pleurotus osteratus</i> Ethanol extract
1	Test for Alkaloids	-	-
2	Test for Volatile oils	-	-
3	Test for Carboxylic acids	-	-
4	Test for Fixed oils	+	+
5	Test for Saponins	+	+

6	Test for Tannins	+	+
7	Test for flavonoids	+	+
8	Test for Phenols	+	+
9	Test for Carbohydrates	+	+
10	Test for Fatty acids	-	-
11	Test for Resins	-	-
12	Test for Quinones	-	-
13	Test for Terpenoids	+	+
14	Test for Cardiolides	-	-
15	Test for Cyanogenic Glycosides	-	-
16	Test for steroidal glycosides	+	+

+ Indicates the presence of the constituents.

- Indicates the absence of the constituents

Table 4
Preliminary antimicrobial testing of *Pleurotus florida* and *Pleurotus ostreatus* extracts. Inhibition zone diameter (mm)

Test Bacteria	Zone of inhibition (mm)		Ciprofloxacin
	<i>P.ostreatus</i>	<i>P.florida</i>	
<i>Escherichia coli</i>	12	14	28.3
<i>Staphylococcus aureus</i>	10	12	27.4
<i>Bacillus subtilis</i>	09	08	28.5
<i>Proteus vulgaris</i>	07	08	28.3
<i>Klebsiella pneumoniae</i>	05	07	28.0
<i>Clostridium botulinum</i>	09	10	24.8

DISCUSSION

The present study has further revealed the antibacterial potency of the oil of the macrofungus extracted with ethanol. Both the ethanolic extracts were observed to inhibit gram positive and gram-negative bacteria tested *in vitro* to suggest that *P. florida* has a broad-spectrum antibacterial activity. Similar antibacterial potentials have been observed in the culture extracts of *Irpex lacteus* [18] *Agrocybe* sp. [19], and juice of *L. edodes* [20]. The observed disparity in the the susceptibilities of gram-ve bacteria tested with ethanoilc extract eliciting greater effect provides an indication that the organic solvents used have varying abilities

to extract bioactive substances from *P. florida* and *P.ostreatus*.

CONCLUSION

Based on the results of this study, it can be concluded *P.florida*, an edible oyster mushroom has antibacterial potentials and possesses a broad-spectrum antibacterial activity, optimisable by multiple organic mycelia extraction. Further investigations that would identify the bioactive compounds and determine the optimal mycelia growth conditions of *P.florida* are necessary.

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