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In vitro studies on phytochemical evaluation and antimicrobial properties of *Pleurotus florida*

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Abstract

The present study scientifically examined the phytochemistry and antimicrobial potencies of two organic extracts of *Pleurotus florida*. Generally, both extracts were effective against 89.8% of the isolates tested with *Bacillus subtilis* (10-10.2mm) and *Escherichia coli* (8.6-9 mm) exhibiting highest gram +ve and gram -ve by agar well diffusion method, respectively. However, petroleum ether extract (PE) exhibited greater anti-gram -ve bacterial activity than the acetone extract (AE) and further produced growth inhibition of these isolates in broth. The phytochemical analyses of the extracts revealed the presence of terpenoids, tannins, steroidal glycosides, carbohydrates and Flavonoids. While the presence of alkaloids, carboxylic acids, Volatile oils, Cardinolides, Resins, Quinones, fatty acids and cynogenic glycosides were not detected. The results indicate that *P. florida* possesses antimicrobial activity.

INTRODUCTION

Modern scientific studies on medicinal mushrooms have expanded exponentially during the last two decades not only in Japan, Korea and China but also in USA and scientific explanation to show mushrooms derived compounds function in human system are increasingly being established [1] A number of medicinal mushrooms, such as *Aleurodiscus, Coprinus, Clitocybe, Daedalea, Marasmius, Merulius, Pleurotus, Polyporus, Poria, Psathyrella,* and *Tricholoma* spp are rich sources of ß-glucan, proteoglucan, lectin, phenolic compounds, flavonoids, polysaccharides, triterpenoids, diatery fibre, lentinan, schizophyllan, lovastatin, pleuran, steroids, glycopeptides, terpenes, saponins, xanthones, coumarins, alkaloid, purin, purimidin, kinon, fenil propanoid, kalvasin, volvotoksin, flammutoksin, 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, anti-inflammatory, cardiovascular diseases, immunomodulating, central activities 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 stipe 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 antibacterial activity *in vitro*, there is lack of information on the microbicidal properties of *P. florida* coupled with inadequate data on its phytochemistry. It is hypothesized that knowledge of the phytoconsitituents of *P. florida* would provide an insight into its biological functions beyond nutrition when consumed. In the present study, organic mycelia extracts of *P. florida* were phytochemically analysed and tested for antimicrobial function *in vitro*.

MATERIALS AND METHODS

Pleurotus florida

P. florida sold as edible mushrooms in oil palm area at different locations in Hyderabad were collected. They were identified at the Department of microbiology, Osmania University, Hyderabad. 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 florida* 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 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 (4ºC) prior to use for subsequent assays.

Phytochemical analysis of *Pleurotus florida* extracts

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

Microorganisms

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

Antimicrobial testing of *Pleurotus florida* extracts

P. florida extracts were tested for antimicrobial activity by agar well diffusion technique[14] with a little modification. An overnight culture of each microbial isolate was emulsified with nutrient broth to a turbidity that was equivalent to 0.5 McFarland (10^8 CFU/ml). 100 µl of each standard inoculum was then streaked on nutrient agar and PDA at 106 CFU/ml per spot to

Table 1. : Fruiting body details

Parameter	Amount
Fruiting body wet weight(g)/ strain	1.34-1.55(1.44±0.045)
Fruiting body dry weight (g)/ strain	0.145-0.187(0.166±0.0035)

Table 2. : Extractive values of petroleum ether and acetone extracts of *Pleutrotus florida.*

S.No	Type of extract	Extractive Value (in mg/g dry weight)
1	Petroleum ether	3.2
2	Acetone	3.4

Table 3. : Extractive values of petroleum ether and acetone extracts of *Pleutrotus florida.*

S.No	Plant constituents	Petroleum ether extract	Acetone 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 flavonods	+	+
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 Cardinolides	-	-
15	Test for Cyanogenic Glycosides	-	-
16	Test for steroidal glycosides	+	+

+ Indicates the presence of the constituents.
- Indicates the absence of the constituents

attain a confluent growth[15] . Wells were made on the agar using a sterile cork borer and filled with 100 µL *P. florida* 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 µl of ciprofloxacin at 5 µg per well. 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 antimicrobial florida fruiting body against multi drug resistant bacterial pathogens presented in Table 4. Data presented in Table 1showed the fruiting body wet and dry weights per *P. florida* strain analyzed ranged from 1.34-1.55 g (mean weight 1.44 ± 0.045 g) and 0.145-0.187 g (mean weight 0.166 ± 0.0035 g) , respectively. The petroleum ether and acetone 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 antimicrobial testing of petroleum ether and acetone extract of *P. florida* by agar-well diffusion produced zones of growth inhibition which are shown in table 4. On the whole, 89.8% of the isolates tested showed sensitivity to acetone and petroleum ether extracts of *P. florida.* (Table 4). The petroleum ether extract of *P. florida* further showed stronger inhibition of these organisms in broth compared to the acetone extract.

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

Isolates	*Pleurotus florida*		Ciprofloxacin
	Petroleum ether	Acetone	
Staphylococcus aureus	8.3	7.6	27.4
Staphylococcus epidermidis	8.0	7.8	29
Bacillus subtilis	10.2	10.0	28.2
Salmonella typhi	7.9	7.4	28.5
Escherichia coli	9.0	8.6	28.3
Proteus vulgaris	7.9	7.5	27.6
Klebsiella pneumoniae	8.1	7.9	28.0
Clostridium botulinum	8.5	7.4	24.8

DISCUSSION

Pleurotus species have high medicinal value. Compounds extracted from these mushrooms exhibit activity against various chronic diseases including hypertension, hypercholesterolemia [16,17] The medicinal beneficial effects of *Pleurotus* species were discovered independently in different countries. 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 the growth of gram positive and gram-negative bacteria tested *in vitro* to suggest that *P. florida* has a broad-spectrum antibacterial activity. Similar antimicrobial 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 petroleum ether extract eliciting greater effect provides an indication that the organic solvents used have varying abilities to extract bioactive substances from *P. florida.* Further the bioactive compounds are to be extracted and there chemical structures are to be well analyzed to construct and synthesis an artificial compound which may save human life.

CONCLUSION

Based on the results of this study, it is concluded that *P.florida,* an edible oyster mushroom has antimicrobial potentials and possess 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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