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STUDIES ON ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF OYSTER MUSHROOM *PLEUROTUS FLORIDA*

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ABSTRACT: The antimicrobial and antioxidant activity of four different solvent extracts of *P. florida* were investigated. The antimicrobial activities of different solvents were tested against different bacteria and fungi using disc diffusion method. Total antioxidant, reducing power and total phenolic content effect of the extracts was determined by phosphomolybdenum assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and Folin-Ciocalteu method. Results indicated that the maximum antibacterial and antifungal activity was observed in ethanol extract of *P. florida* was found at 23 mm and 20mm against *Streptococcus* sp and *E. floccosum* respectively. Ethanolic extract of P. *florida* produced minimum inhibitory concentration (MIC) at 25mg/ml against *E. coli* followed by *K. oxytoca* (25mg/ ml), *P. murabilis* (75mg/ml) and *Streptococcus* sp. (50mg/ml). The ethanol extract exhibited good antioxidant activity (230 µg equivalent of BHT/g), strongest reducing power inhibition (79.24%) and also contain high phenolic content (6.25 mg gallic acid/g of dry extract). The results provided evidence that the ethanolic extracts of *P. florida* might indeed be potential sources of natural antioxidant and antimicrobial agents.

INTRODUCTION: *Pleurotus* species is one of the choice edible mushrooms which can be cultivated in many countries in the subtropical and temperate zones. Generally *Pleurotus* is referred to as 'oyster mushroom' over the world while in China it is known as Abalone mushroom' and 'Dhingri' in India. *Pleurotus* species have been used by the people in all over the world for their nutritional value, medicinal properties and other beneficial effects [1].

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The fruiting body of the mushroom is also a potential source of lignin and phenol degrading enzymes [2]. Both fruiting body and the mycelium of this mushroom contain compounds with wide-ranging antimicrobial activity and their compounds could be isolated from many mushrooms species and could be of benefit for human.

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, 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, eryngeolysin etc. They are rich sources of natural antibiotics, where the cell wall glucans are well known for their immunomodulatory properties and many of the externalized secondary metabolites combat bacteria, fungi and viruses [3, 4, 5, 6, 7, 8, 9] also have been used extensively in traditional medicine for curing various types of diseases such as antimicrobial, antioxidant, antiviral, anticancer, antitumor, anti-inflammatory, cardiovascular diseases, immunomodulating, central activities etc.,[10, 11, 12, 13, 14].

Mushrooms need antibacterial and antifungal compounds to survive in their natural environment. Therefore, antimicrobial compounds could be isolated from many mushroom species and could be of benefit for human's [15]. Not much literature is available with regard to their antimicrobial and antioxidant activities of *Pleurotus florida*. It was therefore the aim of this present study to screen *P. florida* for their antimicrobial and antioxidant activities.

MATERIALS AND METHODS:

Sample Collection: The mushroom *(P. florida*) was obtained from Agalya mushroom foundation, Solinganallur, Chennai. It was dried at 45 -50ºC for 48 h. the dried mushroom was finely ground and kept in a freezer at -20ºC until use.

Preparation of Mushroom Extract: 10g of dried finely crushed mushrooms were extracted for 24 hour in 100 ml of methanol, ethanol, chloroform, and di- ethyl ether at room temperature under dark condition. The extraction was twice repeated, the extracts were filtered through glass funnel and Whatman No.1 filter paper. Each filtrate was concentrated to dryness under reduced.

Pathogen used for the Antimicrobial Assays: *Escherichia coli, Salmonella typhi, Klebsiella pneumonia, Vibrio parahaemolyticus, Klebsiella oxytoca, Vibrio cholera, Proteus murabilus* and *Streptococcus* sp. and the fungal species *Trichophyton rubrum, Epidermophyton floccosum and Microsporum gypseum* were obtained from Department of Microbiology, Raja Muthiah Medical College and Hospital, Annamalai University, Annamalainagar. The pathogens were maintained on Nutrient Agar (NA) and Cezpek Dox Agar (CDA).

Antibacterial Assay: The bioassay was carried out using the agar disc diffusion method with paper disc of 6mm diameter prepared from Whattman No. 1 filter papers The bacterial inoculation were grown in nutrient broth over night and a fixed volume inoculated into 10ml aliquots of nutrient agar mixed and then poured over a nutrient agar base in sterile petridishes. This formed the bacterial lawn. The paper discs of 6mm diameter soaked in 6 micro liter of crude extract and placed on the bacteria lawn after it had solidified. Standard antibiotic discs were used for control. The zones of inhibitions were measured after the 24 hrs incubation and were recorded in mm.

Antifungal Assay: Young fungal cultures were incubated for 2-3 days at room temperature and seeded on Czapex Dox agar plates for bioassay by disc diffusion method. Whattman No. 1 filter paper disc of 6mm containing the crude extracts were placed on the surface of the plates. After 72-h at 30ºC, the plates were observed for the presences of inhibition zones.

Minimum Inhibitory Assay: Dilution assays are standard method used to compare the inhibition efficiency of the antimicrobial agents. The crude extracts are mixed with suitable media that been inoculated with the test microorganism. 5ml of the Nutrient broth, 0.1ml of the 24 hrs growing bacterial cultures and the different concentration (25 – 100 µg/ml) of the drug were inoculated. 0.1ml of the 24 hrs growing bacterial culture was inoculated in 5ml of the Nutrient broth used as control group. The tubes were incubated at 37ºC for 24hrs. After the incubation, optical densities of bacterial culture in each tube were measured in UV- Visible Spectrophotometer at 600nm. The percentages of viable cells were calculated using the following formula. % viable cells = control OD- test OD/ Control OD× 100.

Determination of Total Phenolics: The amount of total phenolics in the extracts was determination by the modified Folin- ciocatleu method Wolfe *et al*[16]. An aliquot of the extracts was mixed with 5ml F-ciocalteu reagent (previously diluted with 1:10 v/v) and 4ml (75g/1) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40cfor color development. Absorbance was then measured at 765nm using the Perkin Elmer Lamda 25 UV- spectrophotometer.

Samples from extract were evaluated at a final concentration of 0.1mg/ml. Total phenolics content was expressed as mg/g gallic acid equivalent.

Total Antioxidant Activity: Total antioxidant activities of crude extracts were determined according to the method of Prieto *et al.,* [17]. Briefly 0.3 ml of samples was mixed with 0.3 ml reagent solution (0.6m sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95c for 90 min under water bath. Absorbance of all the sample mixture was measured at 695nm. Total antioxidant activity is expressed as the number of equivalence of ascorbic acid.

DPPH Radical- Scavenging Activity: The scavenging effect of samples for DPPH radical were monitored according to the method of Yen and Chen[18]. Briefly, a 2.0 ml of aliquot of test sample was added 2.0ml of 0.16mm DPPH methanolic solution. The mixture was vortexed for 1min and then left to stand at room temperature for 30min in the dark and its absorbance was read at 517nm. Synthetic antioxidant, Gallic acid and ascorbic acid were used as positive controls. The ability to scavenge the DPPH radical was calculated using the formula, Radical scavenging effect (%) = Ab- As / Ab× 100 Where, Ab = Absorbance of blank, As = Absorbance of Sample.

RESULTS AND DISCUSSION: The oyster mushroom *P. florida* was purchased from Agalya mushroom foundation, Chennai. This was extracted by using four different solvents like ethanol, methanol, chloroform and ethyl acetate and screened for its antimicrobial and antioxidant activity (**fig. 1**).

FIG. 1: OYSTER MUSHROOM OF *P. FLORIDA*

Antibacterial Activity of Different *P. florida* Extracts: The antibacterial activity of different extract of *P. florida* was tested against 8 human bacterial pathogens such as *E. coli*, *S. typhi*, *K. pneumoniae*, *V. parahaemolyticus*, *K. oxytoca*, *P. murabilus*, *V. cholarae* and *Streptococcus* sp. The specific zone of inhibition against various types of pathogenic bacteria was shown in Fig.2. Among these, the ethanol extract was more effective against bacteria. The maximum antibacterial activity of ethanol extract of *P. florida* was found at 23 mm against *Streptococcus* sp and minimum 4mm against *V. parahaemolyticus.* Minimum antibacterial was observed in chloroform extracts. This showed activity (11mm) against *E. coli* and there was no activity found against *V. cholarae*. This is more or less similar with the results of Akyuz and Kirbag [19] reported the ethanol extracts of *P. eryngii* showed activity against *B. megaterium*, *M. Luteus*, *K. pneumonia*, *P. denitrificans* and *S. aureus* in different ratios. This may be the indication of the broad spectrum of antibiotic compounds present in the mushrooms due to the use of different solvents and test organisms. The antibacterial activity of mushroom sample varied according to the solvents.

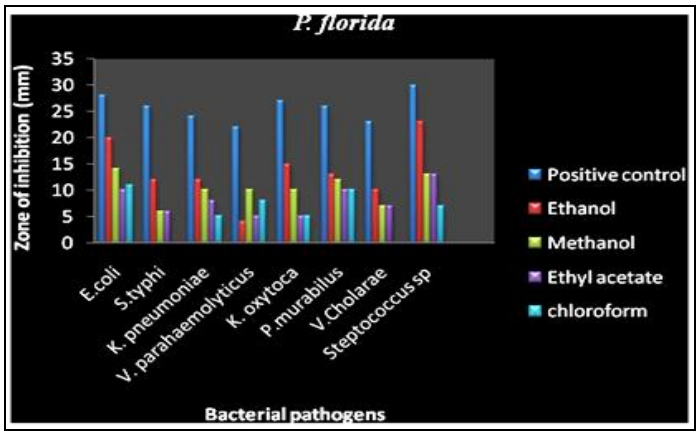

FIG. 2: ANTIBACTERIAL ACTIVITY OF VARIOUS *P. FLORIDA* EXTRACT AGAINST 8 BACTERIAL PATHOGENS

Antifungal Activity of Four Different Extract of *P. florida*: The antifungal activity of 4 different solvent extracts of *P. florida* against 3 dermatophytic fungi such as *T. rubrum*, *E. floccosum* and *M. gypseum* were observed in this study (**Fig. 3**.). Among these four solvent tested ethanol exhibited highest activity against 3 pathogenic fungi and lowest activity was observed in chloroform extract. This showed maximum zone (20mm) of inhibition against *E. floccosum* and minimum (17mm) against *M. gypseum*. Akyuz and Kirbag [19] reported that ethanol extract of *P. eryngii* showed maximum antifungal activity against *C. albicans* (7.7 mm), *C. albicans* (7.7 mm), *C. glabrata* (7.7-9.3 mm), *Epidermopyton* sp. (7.7-8 mm) and *Trichophyton* spp. (7.7-8.7 mm).

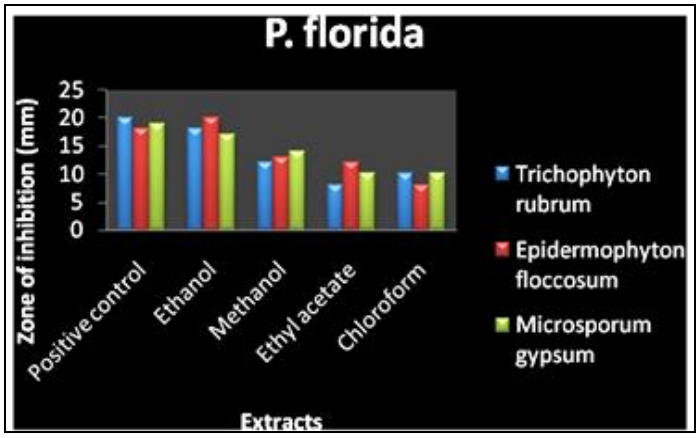

FIG. 3: ANTIFUNGAL ACTIVITY OF VARIOUS *P. FLORIDA* EXTRACT AGAINST 3 DERMATOPHYTIC FUNGI

Similar antimicrobial activities were reported by Iwalokun *et al.*, [20] ; Lacobellies *et al.*, [21]; Nwachukwu and Uzoeto [22]; Westh *et al.*, [23]. This possibly indicated that the extracts possessed substances that can inhibit the growth of some microorganisms [24].

The extracts of various mushrooms inhibited the growth of some microorganisms at different ratios. Different mushroom species posses different constituents and in different concentration, which account for the differential antimicrobial effect as suggested.

Minimum inhibitory concentration (MIC) of ethanol extract of *P. florida* against bacteria and fungi: Maximum antimicrobial activity showed extract was selected for minimum inhibitory concentration. MIC of ethanolic extract of *P. florida* against 4 bacterial pathogens and one fungal pathogen was observed in this study (**Table 1**). Ethanolic extract of mushroom *P. florida* produced minimum inhibitory concentration at 25mg/ml against *E. coli* followed by *K. oxytoca* (25mg/ ml), *P. murabilis* (75mg/ml) and *Sterptococcus* sp. (50mg/ml). In case of fungi, ethanolic extract of *P. florida* formed minimum inhibitory concentration 50 mg/ml against *E. floccosum*.

TABLE 1: MINIMUM INHIBITORY CONCENTRATION OF *P. FLORIDA* AGAINST PATHOGENIC BACTERIA AND FUNGI

Pathogens	Concentrations mg/ml			
	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
E. coli	30.45	39.23	43.94	44.47
K. oxytoca	32.69	71.48	72.65	75.34
P. murabilis	59.85	52.15	34.44	55.14
***Sterptococcus* sp.**	11.59	11.54	23.79	79.38
E. floccosum	36.64	27.43	42.54	65.91

This is correlated to the results of Gbolagade and Fasidi [25] observed that least minimum inhibitory value (1.25mg/ml) was obtained in *C. occidentalis* against *E. coli*. This was followed by *T. lobayensis* with 2.0mg/ml against the same bacterium. It was also revealed that MIC of the mushroom extract against the test fungi such as *A. niger, A. flavus, C. albicans, M. boulardii* and *T. concentrum* were high.

Danielli [26] suggested that at the lowest MIC, the extract will still effective because of the presence of bioactive compounds. Therefore higher concentration, which may consequently poison host cell, may not be required.

Total phenolic content: The phenolic content of four different solvent extract (Ethanol, Methanol, Chloroform and Ethyl acetate) of *P. florida* was evaluated using Folin ciocalteu method (**Fig. 4**.).

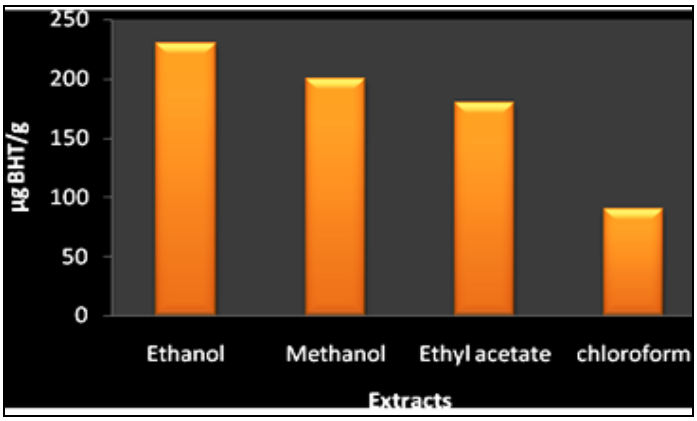

FIG. 4: TOTAL PHENOLIC CONTENT OF *P. FLORIA* USING DIFFERENT SOLVENTS

Among the solvent tested, ethanol extract of *P. florida* showed high phenolic content (6.25 mg gallic acid/g of dry extract) followed by methanol (4.17 mg gallic acid/g of dry extract), ethyl acetate (3.15 mg gallic acid/g of dry extract) and chloroform (0.85 mg gallic acid/g of dry extract).

This was more or less similar with the result of Imran *et al.*, [27] estimated the total phenolic content of *P. florida* and *P. eous* as 3.125 mg GAE/gm of dry extract and 2.725 mg GAE/gm of dry extract. The content of total phenols was higher for *P. florida* than that for *P. eous* extract. Higher extraction yields of phenolics were noted with increased polarity. Low level of phenolics noticed in the present study might be due the solvent. It is reported that the polarity of the extraction solvent affect the level of phenolics [28, 29].

Total antioxidant activity: Fig.5 indicated that the good antioxidant activity was observed at ethanolic extract of *P. florida* (230 µg equivalent of BHT/g). Methanol also showed good antioxidant activity (200 µg equivalent of BHT/g) next to ethanol and chloroform exhibited least antioxidant activity (90 µg equivalent of BHT/g). This was agreed with the result of Vamanu *et al.*, [30] reported that ethanol was the most appropriate solvent. If ethanol was used, *P. ostreatus* strain had an antioxidant activity of 94.54%, it was higher than that of methanolic extracts. Imran *et al.*, [26] reported the total antioxidant activity of the methanol extracts were found to be 220 µg equivalent of BHT/gm of *P.florida* and 540 µg equivalent of BHT/gm of *P. eous* in phosphomolybdenum assay.

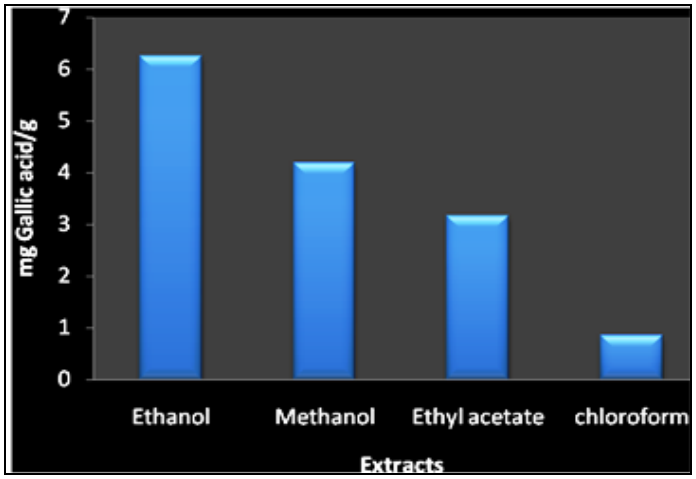

FIG. 5: TOTAL ANTIOXIDANT ACTIVITY OF *P.FLORIDA* USING DIFFERENT SOLVENT

DPPH: Free radical scavenging capacities of the four different extracts of *P. florida* was shown in fig.6. The strongest reducing power inhibition was identified as ethanol (79.24%) and methanol (71.29%) extract of *P. florida*. Weakest inhibition was observed at ethyl acetate (69.26%) and chloroform (63.18%) extracts of P. *florida*.

According to Gezer *et al.*, [31] studied free radical scavenging activity. The 50% of inhibition value for *Ramaria flava* ethanol extract seem to be fairly significant when compared to commonly used synthetic antioxidants BHA and alpha tocophenols.(IC50=276µg/ml ethanolic extract was necessary to obtain 50% of DPPH degradation).

Shahidi and Wanasundra [32] indicated that the acetone extract possessed good activity, whereas the methanol and hot water extracts showed moderate and poor activity, respectively, at the concentration tested. The variation may be attributed to differences in the concentrations of the antioxidant compounds because of the solvent used for the extraction.

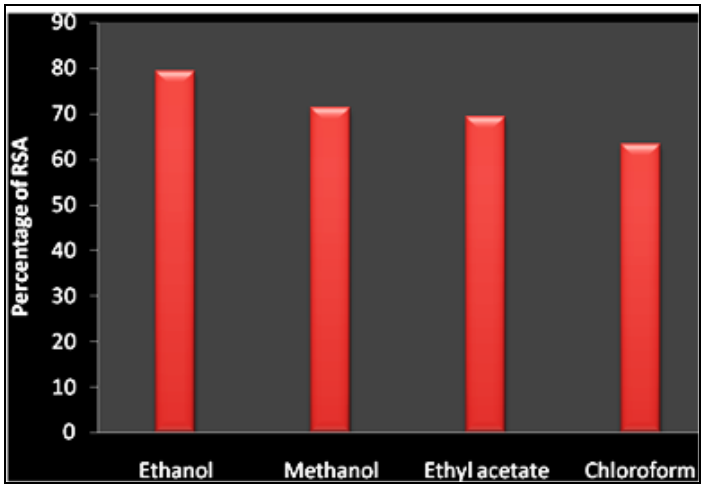

FIG. 6: DPPH RADICAL SCAVENGING ACTIVITY OF *P. FLORIDA* USING DIFFERENT SOLVENTS

CONCLUSION: The extract of *P. florida* used in this study, which was inhibited some medicinally important microorganisms and contain significant amount of antioxidant activity. Present finding encourages their use in human diets, which in turn might serve as protective agents to reduce oxidative damage and microbial diseases.

Further researches are needed to be conducted in order to analyze the active substances in details and chemical characteristics of the antioxidative components.

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