PERSPECTIVE APPROACHES OF BIOLOGICAL ACTIVITIES IN *Pleurotus* sp.

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Abstract:

Edible mushroom produces enormous bioactive compounds that are known to have potential antibacterial, antioxidant and anti-inflammatory properties. Natural antioxidant can protect against free radicals without side effects. The present study was evaluated the biological activities of *Pleurotus* sp. and fusant strain of *Pleurotus* sp. The fusant strain was produced by using protoplast fushion technique from the wild strains namely *P. florida* and *P. eous* collected from Thanjavur MM spawn lab, GSP mushroom farm, 1154, Pookulam, Karanthai, Thanjavur, India. The antibacterial activities of the extracts of *P. florida* and fusant strain of *Pleurotus* was tested against *Escherichia coli, Enterococcus* sp., *K. pneumoniae, Proteus* sp., *Pseudomonas* sp. and *Staphylococcus aureus*. The higher concentration (100 µL) of the extract was *Plerotus fusant* showed excellent antibacterial activity. The efficiency of antioxidant properties of different concentration of 100, 300, 500, 700 and 900µg/mL were performed by various methods like DPPH, ABTS, superoxide, nitric oxide and hydroxyl radical assay were determined. Statistical significance of the antioxidant properties was performed. The effect of anti-inflammatory properties of the *P. florida* and *Pleurotus* fusant with different concentration (0.5, 1.0, 1.5% and Diclofenicol sodium as a standard) of the extracts were treated. *P. florida* and *Pleurotus* fusant were showed promising biological activities such as antibacterial, antioxidant and anti-inflammatory properties, were represented as an alternative regular drugs available in the market.

Key words: *Pleurotus* sp., fusant strain, properties of antibacterial, antioxidant, anti-inflammatory.

Introduction

Mushrooms are nutritionally functional foods that have been part of our diet over 2000 years ago. They have not just been consumed for their cooking essential because of their unique taste and flavour (Kalac, 2013), but also due to their important therapeutic properties which are observed as effective to treat and prevent varieties of aliments (Lim *et al.,* 2007; Moro *et al*., 2012 and Silveira *et al*., 2014).

The world most cultivated mushroom namly *Pleurotus* genus is very simple to cultivate and the members of this genus have more nutritional value. They are also well known to degrade lignocelluloses, low-value wastes, primarily produced through the activities of the agricultural and food-processing industries, can be converted into new resources to produce value added food items. However, since the substrates play the important role of maintenance the growth, development and fruiting of mushrooms, *Pleurotus* spp. have been shown to be a useful tool to upgrade and degradation of lignin solid-state fermentation, in fact, many agricultural wastes (cereal straw and husk) results in better animal feed. The strong oxidative activity of the *Pleurotus* spp. ligninolytic enzymes has also explained a low-cost bioremediation process. In some agri-food industry residues, in fact, *Pleurotus* spp. are able to reduce toxicity of agro-wastes similar to the presence of toxic compounds (phenols, tannins and caffeine, etc.). Furthermore, *Pleurotus* spp. are higher sources of natural antibiotics, where the cell wall glucans are well known for their immunomodulatory properties and secondary metabolites against bacteria, fungi and viruses also have been used in traditional medicine for curing various types of diseases. These oyster mushrooms are familiar for their antimicrobial, antioxidant, antiviral, anticancer, antitumor, anti-inflammatory, cardiovascular diseases (CVD), immunomodulating, central activities etc. (Chellal and Lukasova, 1995; Iwalokun *et al*., 2007 and Jagadish *et al*., 2009.,).

Mushrooms need antibacterial and antifungal compounds to survive in their natural environment to fight against their competitors. Therefore, antimicrobial compounds could be isolated from many mushroom species and could be of benefit for the treatment of various human illnesses as well as defects'. Not much literature is available with regards to their antimicrobial, antiinflammatory and antioxidant activities of wild and fusant of *Pleurotus florida* and *P. eous*. The aim of the present

study is to screen the fusant strain of *Pleurotus* sp. for their antibacterial, anti-inflammatory and antioxidant activities.

Materials and methods

Collection of *Pleurotus* sp. spawn

The mother spawn of oyster mushroom species namely *Pleurotus eous* and *P. florida* was collected from Department of Plant Pathology, Tamilnadu Agricultural University (TNAU), Coimbatore.

Protoplast fushion and isolation

Protoplasts of *P. eous* and *P. florida* were fused and the fusant strain of *Pleurotus* was prepared and isolated by the standard methods (Hashiba, 1992; Porselvi and Vijayakumar, 2020**)**, and used for further pharmaceutical studies including antibacterial, antioxidant and anti-inflammatory activities.

Antibacterial activities

Test bacteria

The test bacteria namely *Escherichia coli* (ATCC 35218), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas* sp. (ATCC 15442), *Proteus* sp. (ATCC 7829), *Staphylococcus aureus* (ATCC 6633) and *Enterococcus* sp. (ATCC 10042) were obtained from American Type Culture Collection (ATCC) and used for the evaluation of the antibacterial activities of the mushroom species.

Agar well diffusion method

The antibacterial assay was performed by agar well diffusion method. The surface of the nutrient agar was separately swabbed with all test bacteria. Four wells

were made in each nutrient agar plates by using sterile cork borer (6.0 mm in dia). The different concentrations (25, 50, 75 and 100 µL) of the mushroom fusant and *P. florida* extracts were added separately into each well, and the plates were incubated for 24-48h at 37°C. The antibacterial activities were determined by measuring the diameter of zone of inhibition.

Antioxidant activity

DPPH Radicals

The antioxidant activity of the mushroom species was evaluated by the standard methods of Blois (1958). Different concentrations (100, 300, 500, 700 and 900 µg/mL) of *P. florida* and fusant strain extracts were taken and mixed with 2 mL of acetate buffer followed by 1.9 mL of absolute ethanol and 1 mL DPPH solution. The mixture was shaken immediately after adding DPPH, and allowed to stand in dark condition at room temperature for 30 min. The absorbance was measured at 517 nm using a UV-spectrophotometer. BHT was used as positive control and the sample solution without DPPH was used as blank. The radical scavenging activity was measured as a decrease in absorbance of DPPH.

Superoxide radical scavenging activity

Different concentrations (100, 300, 500, 700 and 900 µg/mL) of *P. florida* and fusant extracts were taken and dissolved in water and dispensed into each well of a 96-well microtitre plate. $K_2HPO_4^{-}/KH_2PO_4$ buffer (0.05 mol/L) was served as the control. Pyrogallol (8 µL, 6.25 x 10^{-4}mol/L), luminal (50 µL, 1 mM) and sodium

carbonate buffer (100 μL 0.05 mol/L) were then added to each well and the intensity of luminescence was determined at 0.6 intervals for a total of 30 using a Clarity Microplate Luminometer (Bio-Tek, USA) (Guo *et al.,* 2007).

ABTS radical cation scavenging activity

The ABTS radicals were produced by the reaction held between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. Prior to use, the solution was diluted with ethanol to get an absorbance of 0.700±0.025 at 734 nm. Free radical scavenging activity was assessed by mixing 10 μL of test sample with 1.0 mL of ABTS working standard in a micro-cuvette. The decrease in absorbance was measured exactly after 6 min. The percentage inhibition was calculated according to the formula: ((A0-A1)/A0) x 100 (Re *et al*., 1999), where, A0 was the absorbance of the control, and A1 was the absorbance of the sample.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of the mushroom was studied by the standard procedure described by Qin *et al*. (2000). Aliquots of H_2O_2 (10 μL) were first dispensed into each well of a 96-well micro titre plate. Test samples (10 μL) were dissolved in 70% ethanol to various concentrations (25, 50, 75 and 100 μg/mL), luminol and sodium carbonate buffer solution (150 μL) were then added to each well. Double distilled water was served as the control.

Nitric oxide radical scavenging assay

Nitric oxide radical scavenging activity was measured by spectrophotometric method (Govindharajan *et al*., 2003). When sodium nitroprusside was mixed with aqueous solution at physiological pH, suddenly nitric oxide was generated, which reacts with oxygen and produced nitrite ions that was estimated using Greiss reagent. Nitric oxide scavengers compete with oxygen leading to reduce the production of nitrite ions. About 1 mL of sodium nitroprusside (5 mM) in phosphate buffer (pH 7.4, 0.1 M) was mixed with different concentrations of the *P. florida* and fusant aqueous extract (100, 300, 700 and 900 μg/mL) in phosphate buffer (pH 7.4, 0.1 M). The tubes were then incubated at 25°C for 2 h. After incubation, 1.5 mL of reaction mixture was removed and diluted with 1.5 ml of Greiss reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% of N-(1- naphthyl) ethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-(1- naphthyl) ethylene diamine dihydrochloride) was measured spectrophotometrically at 546 nm.

Anti-inflammatory studies

This HRBC suspension was used for the estimation of anti-inflammatory property. Different concentrations of extract, standard drug and control were separately mixed with 1 mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of HRBC instead of hyposaline 2 mL of distilled water were also used in the control. All the assay mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm. The supernatant liquid was decanted, and the hemoglobin content was estimated by

using spectrophotometer at 560 nm (Azeem *et al*., 2010). The percentage of hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

$$\%\ \text{Percentage Protection} = \frac{100 - \text{Optical Density of sample}}{\text{Optical Density of Control suspension}} \times 100$$

Result and Discussion

Strain improvement is an effective breeding technique in mushrooms which is commonly carried out by protoplast fusion technique. Generally, the fusant strains have the greater pharamaceutical valus than the wild strains of the mushroom. In the present study the antibacterial activities of *Pleroteus* sp. and fusant strain were investigated against six different bacteria namely *E.coli, Enterococcus* sp., *K.pneumoniae, Proteus* sp., *Pseudomonas* sp. and *Staphylococcus aureus*. Maximum (16.4±1.8 mm) antibacterial activity was found against *S. aureus* at higher concentration (100 µL), followed by *E. coli* (16.3±1.4 mm), *K. pneumoniae* (15.2±1.1 mm), *Proteus* sp. (14.5±0.8 mm), *Pseudomonas* sp. (13.9±0.6 mm) and *Enterococcus* sp. (13.3±0.6 mm). Whereas, the maximum efficiency of *P. florida* was exhibited against *E. coli* (16.0±0.5 mm), followed by *S. aureus* (15.7±2.3 mm) *Pseudomonas* sp. (14.9±1.0 mm), *K. pneumoniae* (14.5±0.8 mm) and *Enterococcus* sp (14.0 ±3.2 mm) at 100 µL No activities were found against *Proteus* sp. in all the concentration of *P. florida* extract (Table 1). In accordance with the present study, the phytochemistry, antioxidant and antimicrobial potencies of petroleum ether and acetone extracts of *Pleurotus ostreatus* was elucidated by Iwalokun *et al*. (2007). 79 (89.8%) of the 88 isolates tested were showed sensitivity to acetone and petroleum ether extracts of *P. ostreatus.* The petroleum ether (PE) extract of *P. ostreatus* showed stronger inhibition

activity against test bacteria namely *E. coli, Salmonella typhi, Pseusomonas aeruginosa, Haemophillus influenza*, *Bacillus licheniformis, B. subtilis, Staphylococcus aureus* and *Lactobacillus acidophilus,* and test fungi like *Candida albicans* and *Saccharomyces cerevisiae* when compared to the acetone extract (AE).

Free radical scavenging is a mechanism which inhibits lipid oxidation and is used to measure the antioxidant activity. The radical scavenging activities of mushroom extracts were tested against the DPPH activity, β-carotene-linoleic acid assay and hydrogen peroxide reducing power activity (Jina *et al.,* 2018). DPPH is a stable free radical with good absorption at 517 nm, it is used to study radical scavenging activity of test samples. When antioxidant donate proton to these radicals then absorption of samples decreases. Radical scavenging activity is measured by decrease in absorption of samples. Samples with more phenolic content exhibited in antioxidant activity (Vishwakarma, *et al*., 2017). In the present study, various methods like DPPH, ABTS, superoxide, nitric-oxide and hydroxyl radical assay were employed to measure the antioxidative properties of mushroom extracts for various levels of antioxidant activities. Greater percentage of antioxidant activities (94.10±1.02%, 92.95±1.02%, 89.96±1.03%, 78.84±1.02% and 58.93±1.02%) was observed in higher concentration at 900, 700, 500, 300 and 100 µg/mL respectively in ABTS method. Whereas, minimum percentage of inhibition effects of *P. florida* was observed in superoxide method. The other three methods were exhibited moderate level of antioxidant activities. Here, the significant results were observed by P-values and F-values determined (Table 2).

Similarly, theextraordinary antioxidant activities of *Pleurotus* fusant strain was observed and recorded as 92.15±1.42%, 93.45±1.42%, 89.96±1.03%,

83.84±1.42% and 78.74±1.73% at 900, 700, 500, 300 and 100 µg/mL respectively. But, minimum antioxidant properties were observed in *Pleurotus* fusant strain when employed superoxide method. The fusant strain of *P.leurotus* had much antioxidant properties than that of the native strain of *P. florida* (Table 3). The variation may be attributed to difference in the concentration of the antioxidant compounds and solvents used for the extraction. In a similar manner, the antioxidant activity of the *Pleurotus* sp. extracts was studied by DPPH and ABTS methods revealed a disparate VCEACs of 3.6 – 3.8 mM for PE and 4.1 – 4.4 mM for AE compared to 6.2 – 6.4 mM in the green tea infusion (Iwalokun *et al*., 2007).

In the present study, the anti-inflammatory properties of fusant mushroom strain and *P. florida* was studied at different concentration of extract like control (without extract), 0.5%, 1.0%, 1.5% and with standard drug (Diclofenicol sodium salt 1.2 mg), and found that the percentage of anti-inflammatory activity of fusant strain was recorded as 22.54±0.07, 45.6±1.0, 53.7±1.4, 64.8±1.8 and 42.12±0.33% respectively. The anti-inflammatory efficiency of *P. florida* was found as 22.54±0.07, 32.61±0.08, 49.52±0.05 and 48.12±0.06% at control, 0.5%, 1.0% and 1.5% respectively. On the other hand, the anti-inflammatory activity of the extract was also compared with standard drug, and reported that the mushroom extract had better activity than the standard drug. Low concentration (0.5%) of the mushroom extract was also possessed high percentage of activity (Table 4). Similarly, *In vitro* and *in vivo* anti-inflammatory activities of methanolic extract of *P. florida* exhibited significant results for its inhibition of protein denaturation (69.30±4.98% at 1.0 mg/mL); inhibition of proteinase activity (27.89±0.14% at 1.0 mg/mL) in a dose-dependent manner and also anti-inflammatory activity in Carrageenan induced acute

inflammatory animal model (50.17% at 200mg/kg b.w) (Prabu and Kumthakalavalli, 2014). Our study showed superior activity than that of previous reports. Presence of phytochemicals namely phenols, flavonoids, saponins and tannins may be responsible for such anti-inflammatory activity (Javed *et al.*, 2019 and Banukie *et al*., 2020).

Conclusively, the high reducing power of mushroom extract might have been due to the high level of bioactive compounds that break the free radical chain by donating an electron to stabilize and terminate radical chain reactions. Also, it has been suggested that the mushroom with biological activities could be an effective alternative drug to the commercially available anti-oxidant and anti-inflammatory drugs.

Table 1. Effect of antibacterial activity of *Pleurotus* sp. (Fusant strain) and *P. florida* against bacterial pathogens

S. No	Name of the bacteria	Zone of inhibition (mm)							
		Fusant (µl)				*P. florida* (µl)			
		25	50	75	100	25	50	75	100
1.	*Escharchia coli*	11.7±0.3	13.1±0.6	15.1±0.5	16.3±1.4	11.0±0.6	13.1±0.5	15.1±1.1	6.0±0.5
2.	*Enterococcus* sp.	-	-	10.1±0.6	13.3±0.6	11.7±0.9	12.3±1.8	13.0±2.3	14.0±3.2
3.	*Klebsiella. pneumoniae*	10.0±0.1	10.7±0.3	12.8±0.3	15.2±1.1	-	11.5±0.8	13.5±0.8	14.5±0.8
4.	*Proteus* sp.	10.1±0.6	11.0±0.6	13.2±0.6	14.5±0.8	-	-	-	-
5.	*Pseudomonas* sp.	9.9±0.1	11.2±0.4	13.1±0.5	13.9±0.6	-	10.4±0.3	13.1±0.9	14.9±1.1
6.	*Staphylococcus aureus*	12.1±1.0	14.2±0.4	17.6±0.4	16.4±1.8	9.8±1.3	11.6±1.7	15.0±1.5	15.7±2.3

Values are expressed in mean±S.D

Table 2. Determination of antioxidant activity of *P. florida*

S. No.	Concentration (µg/ml)	Free radical scavenging inhibition (%)				
		DPPH	ABTS	Superoxide	Nitric oxide	Hydroxyl
1	100	74.67 ± 1.01	58.93 ±1.02	56.54 ± 1.03	68.25 ± 1.03	63.11 ± 1.05
2	300	76.15 ± 1.03	78.84 ±1.02	60.75 ± 1.02	73.15 ± 1.02	66.73 ± 1.02
3	500	78.66 ± 1.02	89.96 ± 1.03	66.67 ± 1.02	75.75 ± 1.03	68.82 ± 1.01
4	700	80.34 ± 1.03	92.95 ± 1.02	70.14 ± 1.03	76.82 ± 1.01	70.13 ± 1.02
5	900	82.61 ±1.02	96.15 ± 1.02	74.65 ± 1.02	80.29 ± 1.02	72.82 ± 4.01
P-Value		0.000	0.000	0.000	0.000	0.000
F-Value		4.742444	1.109666	1.945555	7.641444	1.358666

Values are expressed in mean±S.D

Table 3 Determination of antioxidant activity of *Pleurotus* sp. fusant by *in vitro* method

S. No	Concentration (µg/mL)	Free radical scavenging inhibition (%)				
		DPPH	ABTS	Superoxide	Nitric oxide	Hydroxyl
1	100	82.81± 1.24	78.74± 1.73	66.57 ± 1.13	71.25 ± 1.54	69.21±1.05
2	300	83.85 ± 1.23	83.84 ± 1.42	68.55 ± 1.72	76.35 ± 1.32	70.64±2.02
3	500	85.26 ± 1.12	89.96 ± 1.03	70.67 ± 1.42	78.75 ±1.23	72.82±1.11
4	700	88.34 ± 1.33	93.45 ± 1.42	72.14 ± 1.43	81.82 ± 1.41	75.13±2.02
5	900	85.61 ± 1.42	92.15 ± 1.42	71.65 ± 1.52	80.29 ± 1.32	71.82±1.71
P-Value		0.000	0.000	0.000	0.000	0.000
F-Value		6.64557	1.158776	2.156666	8.751555	2.758776

Values are expressed in mean±S.D

Table 4. Efficacy of anti-inflammatory activity of edible mushroom by *in vitro* method

S. No.	Different concentration (%)	Percentage of activity (%)	
		Fusant strain	***P. florida***
1.	Control	22.54 ±0.07	22.54 ±0.07
2.	0.5	45.6±1.00	32.61 ±0.08
3.	1.0	53.7±1.40	49.52 ±0.05
4.	1.5	64.8±1.80	48.12 ±0.06
5.	Standard drug (1.2 mg) Diclofenicol sodium salt	42.12 ±0.33	42.12 ±0.33

Values are expressed in mean±S.D.

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