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Studies on Antioxidant and Antimicrobial Potentials of Some *Pleurotus* sp.

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ABSTRACT
Mushrooms are worldwide honored for their taste and flavor and are taken both in fresh and a refined form. Mushrooms have been not only used as food products with their distinct taste and texture, but also regarded as a major source of chemical substances with biological activity and possible medicinal benefits. In our study, to evaluate the antioxidant and antimicrobial activitiy of the aqueous, methanol and di ethyl extracts of Pleurotus ostreatus, P. florida and P. sajor caju were used. Demonstrated that the various solvents significantly scavenged DPPH, reducing power assay, and hydrogen peroxide assay were analysed. The total antioxidant activity was maximum present in all solvents and were examined by spectroscopic measurements and expressed as percent of inhibition. Antibacterial activities of the extracts against Staphylococcus aureus, Bacillus sp, Streptococcus pneumoniae and Vibriyo sp. were examined by agar well diffusion method and zones of inhibition varied for different organisms but highest in Pleurotus florida and P. sajor caju when compared to P. ostreatus. Maximum zone of inhibition was recorded in Aspergillus niger followed by A.flavus, A.terrus and Pencillium notatum when the extract of P. ostreatus was used. The therapeutic benefits of the mushrooms are greatly influenced by these antioxidants. They can be used as a natural, high-antioxidant food source to help the immune systems protection against osmotic damage. To cure diseases brought by the microorganism, the Pleurotus species are used as an alternate source of treatment. As a result, the prospects of developing antimicrobials from them appears promising. Variations in the quantity of mushrooms antimicrobial activities were also recorded in this research.
Keywords: *Pleurotus sp, antioxidant, antibacterial, anti fungal, zone of inhibition.*

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INTRODUCTION

The *Pleurotus* genus contains several species that are especially notable due to their high nutritional benefits and medicinal significance, including *P. ostreatus, P. florida* and *P. sajor caju* [1]. In contrast with their unique taste and scent, edible mushrooms also include vitamins, proteins, molecules that include antioxidants and natural products that are free of pesticides, all of which contribute to the mushroom's increasing popularity [2]**.** Due to higher mineral content than meat or fish, mushrooms are recognized as a healthy food for vegetarians. They offer therapeutic properties in addition to their nutritional benefits. Both developed and developing nations depend on mushrooms as a major food and economic source [3]. Mushrooms are micro fungi that have characteristic fruiting bodies that are often fleshy, edible, hypogeous or epigeous and can be manually harvested. Mushrooms have been utilised extensively as delicious and healthful foods [4]. About 40 species of the genus *Pleurotus* are referred to as "oyster mushrooms" in popular usage.

Natural antioxidants are becoming major significance as potential protector agents to reduce oxidative damage [5]. Antioxidants are essential compounds that organisms can produce and obtain from a range of environmental sources. They protect their health by restricting free radicals and active oxygen [6]. Various kinds of mushrooms from all over the world have been extensively studied for their antimicrobial properties. Some *Pleurotus* species have the capability to produce antifungal and antibacterial agents that can be utilised to treat bacterial and fungal diseases, respectively [7]. Though many antimicrobial drugs

have lost their effectiveness in treating infectious diseases, almost as a result of the growth of microbial resistance, the globe is currently experiencing significant problems in modern healthcare systems [8].
The requirement for and the necessary impetus for an endless pursuit for a novel antimicrobial agent from various natural sources has been produced by the resistance of the existing antibiotics by the microorganisms and the spread of such drug resistant infections. This study aims to evaluate the antioxidant and antimicrobial activity of certain oyster mushroom species.

MATERIALS AND METHODS

Spawn Collection

The mother spawn of *Pleurotus ostreatus* *Pleurotus florida* and *Pleurotus sajor caju* were collected from Tamil Nadu, Agricultural University, Coimbatore which were cultivated and marketed as edible mushroom from Indian Biotrack Research Institute, Thanjavur.

Spawn Preparation

Grain spawn of three species was prepared using the standard methodology suggested by [9]. Paddy straw waste material was used as substrate for growth of mushroom. The substrate was prepared by soaking in H_2O for 72 hrs and then, it was allowed to extrude extra moisture by spreading on the inclined plane. This substrate was filled in polyethene bags at 1 kg/bag and dry sterilized [10].

Preparation of the Mushroom Extract

The present study was carried out to know the antimicrobial activity of *Pleurotus* sp. (*P.ostreatus*, *P.florida* and *P.sajor caju*) mushrooms cultivated on paddy straw. *Pleurotus* sp. freshly obtained fruiting bodies were shade dried and ground into a fine powder. Mushroom extraction was carried out using aqueous, methanol and di-ethyl ether solvents and used as such for the antioxidant and antimicrobial tested [11].

A) Antioxidant activity

1) Reducing power assay: The reducing power of the mushroom extract was determined by the method [12]. Various concentrations of mushroom extracts (2.5 mL), phosphate buffer (2.5 mL, 0.2 M, pH 6.6), and 1% potassium ferricyanide (2.5 mL) were mixed and incubated at 50°C for 20 min.
Ten percent TCA (2.5 mL) was added to the mixture. The mixture was centrifuged at 3,750×g for 10 min. A portion (2.5 mL) of the supernatant was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride. After 10 min of incubation, the absorbance was measured at 700 nm against a blank.

2) DPPH Radical-Scavenging Activity: The scavenging effect of samples for DPPH radical were monitored according to the method [13]. 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.

3) Hydrogen Peroxide Scavenging: Solution of 0.2 M potassium dihydrogen phosphate and 0.2 M sodium hydroxide solutions were prepared as per the Indian Pharmacopoeia 1996 standards [14]. 50 ml potassium dihydrogen phosphate solution was placed in a 200 ml volumetric flask and 39.1 ml of 0.2M sodium hydroxide solution was added and finally volume was made up to 200ml with distilled water to prepare phosphate buffer (pH-7.4). 50 ml of phosphate buffer solution was added to equal amount of hydrogen peroxide and generate the free radicals and solution was kept aside at room temperature for 5min to complete the reaction. Extracts (1 ml) in distilled water were added to 0.6 ml hydrogen peroxide solution and the absorbance was measured at 230 nm in a spectrophotometer against a blank solution containing phosphate buffer solution without hydrogen peroxide. The percentage of scavenging of H_2O_2 of extract was measured. The ability to scavenge the H_2O_2 radical was calculated using the following equation.

$$H_2O_2 \text{ scavenging activity } (\%) = (A0 - A1) / A0 \times 100$$

Where,
A0 is the absorbance of the control and A1 is the absorbance in the presence of extract sample. A standard of ascorbic acid was run using same concentrations as that of extract.

Antimicrobial activity

Using pathogenic microorganism: In the experiment, four bacterial and fungal strains were used for antimicrobial activity. The preserved strains were obtained from the Indian Biotrack Research Institute, Thanjavur.

Agar well – diffusion method: It was followed for determination of antimicrobial activity [15]. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old-broth culture of respective bacteria and fungi. Agar wells (5mm diameter) were

made in each of these plates using sterile cork borer. About 20, 40, 60 and 80µL of aqueous, methanol and diethyl ether extracts were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions and the plates were incubated in an upright position at 37˚C ± 2˚C for 24 h for bacterial and 28˚C ± 2˚C for fungi. Results were recorded as the presence or absence of inhibition zone. Triplicates were maintained and the average values were recorded for antimicrobial activity.

RESULT AND DISCUSSION

Antioxidant activity

All samples reduction power was concentration-dependent. The reducing power of *Pleurotus ostreatus* extracts from dried samples was often higher than that of extracts from fresh samples, and aqueous extracts typically exhibited this higher reducing power than methanolic extracts. In the case of extracts from dried samples, the aqueous extract produced by boiling the fruiting body displayed the highest value of reducing power. It was found that both under dry and fresh conditions, aqueous extract samples generally had stronger DPPH radical scavenging activity than methanol extract samples [16]. In the present study that the antioxidant properties of reducing power assay in *Pleurotus ostreatus* and using aqueous, methanol and diethyl ether solvents. There five concentrations are used in the property such as 100 µl to 500 µl respectively. For extracts of fresh samples, the 500 µl concentration of aqueous and diethyl ether solvents obtained the highest values for all antioxidant characteristics, and the values of 6.50±0.04% and 6.78±0.06% (Table 1) and DPPH assay, the values of 7.35±0.18% and 7.35±0.18% (Table 2). Similiarly, hydrogen peroxide (H_20_2) were obtained the highest values in 6.84±0.03% and 5.29±0.34% (Table 3). The lowest values in antioxidant properties of *P. ostreatus* in 100 µl concentration of aqueous and diethyl ether solvents respectively. The concentration-dependent reduction power of the *P. florida* acetone, methanol, and hot water extracts increased. The hot water extract had the lowest reducing power inhibition and the acetone extract had the highest reducing power inhibition. The concentration-dependent DPPH radical scavenging abilities of the acetone, methanol, and hot water extracts from *P. florida* fruiting bodies were observed [17]. In the present study that the antioxidant properties of reducing power assay in *Pleurotus florida* and using aqueous, methanol and diethyl ether solvents. The 500 µl concentration of aqueous and methanol solvents obtained the highest values for all antioxidant characteristics, and the values of 7.09±0.03% and 6.11±0.57% (Table 1) and DPPH assay, the values of 7.62±0.11% and 6.81±0.09% (Table 2). Similiarly, hydrogen peroxide were obtained the highest values in 6.71±0.17% and 7.15±0.61% (Table 3). The lowest values in antioxidant properties of *P. florida* in 100 µl concentration of aqueous and diethyl ether solvents respectively. The methanolic extracts of *Pleurotus* sp. have outstanding reducing properties that continuously increased with concentration. A putative scavenger is incubated with H_2O_2 in an experiment for H_2O_2 scavenging activity, and the amount of H_2O_2 lost during the reaction is then measured. The concentration-dependent scavenging activity of the farmed oyster mushroom methanolic extracts in the DPPH assay increased [18]. In the present study that the antioxidant properties of reducing power assay in *Pleurotus sajor caju* and using aqueous, methanol and diethyl ether solvents. The 500 µl concentration of aqueous and methanol solvents obtained the highest values for all antioxidant characteristics, and the values of 7.54±0.15% and 6.79±0.21% (Table 1) and DPPH assay, the values of 6.84±0.13% and 6.81±0.09% (Table 2). Similiarly, hydrogen peroxide were obtained the highest values in 7.69±0.05% and 6.83±0.16% (Table 3). The lowest values in antioxidant properties of *P. sajor caju* in 100 µl concentration of aqueous and diethyl ether solvents respectively. The *Pleurotus* sp. has the majority of all antioxidant qualities, and *Pleurotus florida* has the highest levels of these properties when compared to *P. ostreatus* and *P. sajor caju* respectively.

Antimicrobial activity

All of the mushrooms employed in this investigation had varying degrees of antibacterial properties on the examined microorganisms. For extracts, the zone of inhibition that measured more than 10 millimetres was regarded to be extremely active. Broad-spectrum antibacterial and antifungal action is possessed by *P. ostreatus* [19]. In the present study, the human pathogens of bacteria were used namely *Staphylococcus aureus, Bacillus* sp, *Streptococcus pneumoniae* and *Vibriyo* sp. The *P. ostreatus* of aqueous extract exhibited the maximum zone of inhibition at all concentration against *Streptococcus pneumoniae* and at the diethyl ether extract exhibited the most inhibitory concentration against *Bacillus* sp when compared with other pathogens (Table 4). Similarly, the fungus were used namely *Aspergillus niger, A. flavus, A. terreus* and *Pencillium notatum*. The Pleurotus ostreatus of aqueous extract exhibited the maximum zone of inhibition at all concentration against *Aspergillus terreus* and at the diethyl ether extract exhibit the most inhibitory concentration against *A. niger* when compared with other pathogens (Table 7). The ethanol extract exceeded the others in based on its ability to kill microorganisms. *P. florida*

extracts showed maximum antibacterial activity in methanol and minimum antibacterial activity in chloroform and the most activity was seen from ethanol when tested against three pathogenic fungus, while chloroform extract recorded the lowest activity [20]. In the present study, the *Pleurotus florida* of both aqueous and methanol extract exhibited the maximum zone of inhibition at all concentration against *Bacillus* sp when compared with other pathogens (Table 5). Similarly, the *P. florida* of both aqueous and methanol extract exhibit the most inhibitory concentration against *A. flavus* when compared with other pathogens (Table 8). Maximum inhibition was seen in methanol extract at a concentration level of 80% against all five test pathogens including fungal and bacterial pathogens, and was followed by ethanol and aqueous extracts at the same concentration. When antifungal and antibacterial activity was examined, it was found that all extracts had more antifungal than antibacterial properties [21]. In the present study, the *Pleurotus sajor caju* of aqueous extract exhibited the maximum zone of inhibition at all concentration against *Bacillus* sp and at the diethyl ether extract exhibit the most inhibitory concentration against *Streptococcus pneumoniae* when compared with other pathogens (Table 6). Similarly, the *Pleurotus sajor caju* of aqueous extract exhibited the maximum zone of inhibition at all concentration against *Aspergillus niger* and at the diethyl ether extract exhibit the most inhibitory concentration against *A. terreus* when compared with other pathogens (Table 9).

Table 1: Antioxidant activity of *Pleurotus* species by Reducing power assay

Different concentration (µl)	% of inhibition					
	Pleurotus ostreatus		*Pleurotus florida*		*Pleurotus sajor caju*	
	Aqueous	Diethyl ether	Aqueous	Methanol	Aqueous	Diethyl ether
100	3.61±0.11	4.23±0.05	5.11±0.08	4.16±0.17	5.26±0.18	4.13±0.47
200	4.11±0.24	5.11±0.16	5.70±0.09	4.67±0.19	5.75±0.44	4.91±0.09
300	5.34±0.08	5.84±0.09	6.05±0.45	5.06±0.31	6.16±0.08	5.48±0.16
400	5.71±0.45	6.25±0.11	6.62±0.17	5.81±0.05	6.87±0.32	6.17±0.04
500	6.50±0.04	6.78±0.06	7.09±0.03	6.11±0.57	7.54±0.15	6.79±0.21

The values are expressed in terms of (Mean ± Standard deviation)

Table 2: Antioxidant activity of *Pleurotus* species by DPPH assay

Different concentration (µl)	% of inhibition					
	Pleurotus ostreatus		*Pleurotus florida*		*Pleurotus sajor caju*	
	Aqueous	Diethyl ether	Aqueous	Methanol	Aqueous	Diethyl ether
100	5.12±0.06	4.69±0.11	5.22±0.16	4.49±0.15	4.62±0.05	5.46±0.49
200	5.68±0.19	5.82±0.23	5.86±0.44	5.18±0.51	5.13±0.08	6.12±0.16
300	6.26±0.18	6.46±0.19	6.42±0.21	5.70±0.33	5.86±0.18	6.81±0.05
400	6.89±0.09	6.87±0.07	7.07±0.16	6.27±0.27	6.29±0.07	7.37±0.41
500	7.35±0.18	7.36±0.41	7.62±0.11	6.81±0.09	6.84±0.13	7.95±0.33

The values are expressed in terms of (Mean ± Standard deviation)

Table 3: Antioxidant activity of *Pleurotus* species by Hydrogen peroxide assay

Different concentration (µl)	% of inhibition					
	Pleurotus ostreatus		*Pleurotus florida*		*Pleurotus sajor caju*	
	Aqueous	Diethyl ether	Aqueous	Methanol	Aqueous	Diethyl ether
100	4.12±0.08	3.54±0.00	4.31±0.18	5.11±0.24	5.06±0.28	4.52±0.18
200	4.85±0.31	3.78±0.18	5.01±0.06	5.46±0.12	5.84±0.19	5.01±0.23
300	5.66±0.19	4.16±0.05	5.46±0.48	6.00±0.19	6.31±0.08	5.72±0.16
400	6.05±0.26	4.71±0.12	6.05±0.16	6.79±0.06	7.14±0.61	6.12±0.07
500	6.84±0.03	5.29±0.34	6.71±0.17	7.15±0.61	7.69±0.05	6.83±0.16

The values are expressed in terms of (Mean ± Standard deviation)

Table 4: Antibacterial activity of *Pleurotus ostreatus* against bacteria

Name of the bacteria	Different concentration (µl) and Zone of inhibition (mm)							
	Aqueous extract				Diethyl ether extract			
	20	40	60	80	20	40	60	80
Staphylococcus aureus	11.0±0.02	12.0±0.26	13.0±0.55	15.0±0.01	10.5±0.31	12.5±0.91	14.0±0.08	16.5±0.18
Bacillus sp.	09.5±0.14	13.0±0.09	13.5±0.19	14.5±0.04	10.0±0.17	12.0±0.16	13.5±0.05	15.0±0.09
Streptococcus pneumoniae	11.5±0.64	12.0±0.07	14.0±0.21	15.5±0.18	09.5±0.06	11.0±0.54	12.5±0.04	14.0±0.06
Vibriyo sp.	11.0±0.08	12.5±0.51	14.0±0.04	15.0±0.16	10.0±0.57	12.0±0.01	13.5±0.03	15.5±0.15

The values are expressed in terms of (Mean ± Standard deviation)

Table 5: Antibacterial activity of *Pleurotus florida* against bacteria

Name of the bacteria	Different concentration (µl) and Zone of inhibition (mm)							
	Aqueous extract				Methanol extract			
	20	40	60	80	20	40	60	80
Staphylococcus aureus	12.0±0.16	13.0±0.09	15.0±0.48	16.5±0.07	09.0±0.15	12.0±0.18	13.0±0.23	16.0±0.56
Bacillus sp.	12.5±0.08	13.5±0.19	15.5±0.60	18.0±0.51	14.0±0.09	15.0±0.13	16.0±0.47	16.5±0.61
Streptococcus pneumoniae	11.5±0.13	12.0±0.06	14.0±0.90	16.0±0.42	11.5±0.21	12.0±0.34	14.0±0.71	16.0±0.29
Vibriyo sp.	10.0±0.19	12.0±0.87	13.5±0.04	16.0±0.18	10.0±0.27	12.0±0.69	13.5±0.16	16.0±0.55

The values are expressed in terms of (Mean ± Standard deviation)

Table 6: Antibacterial activity of *Pleurotus sajor caju* against bacteria

Name of the bacteria	Different concentration (µl) and Zone of inhibition (mm)							
	Aqueous extract				Diethyl ether extract			
	20	40	60	80	20	40	60	80
Staphylococcus aureus	20.0±0.34	22.0±0.47	25.0±0.26	26.0±0.19	11.0±0.19	12.0±0.46	13.0±0.42	19.0±0.16
Bacillus sp.	25.0±0.09	30.0±0.11	32.0±0.75	34.0±0.03	12.0±0.75	14.0±0.76	15.0±0.50	17.0±0.37
Streptococcus pneumoniae	17.0±0.08	18.0±0.22	18.5±0.16	20.0±0.56	19.0±0.44	20.0±0.13	22.0±0.72	23.0±0.53
Vibriyo sp.	24.5±0.18	25.5±0.10	26.0±0.04	28.0±0.15	09.0±0.21	10.0±0.67	13.0±0.57	15.0±0.06

The values are expressed in terms of (Mean ± Standard deviation)

Table 7: Antifungal activity of *Pleurotus ostreatus* against fungi

Name of the fungi	Different concentration (µl) and Zone of inhibition (mm)							
	Aqueous extract				Diethyl ether extract			
	20	40	60	80	20	40	60	80
Aspergillus niger	07.5±0.16	08.0±0.06	08.5±0.23	09.5±0.20	06.0±0.31	08.0±0.59	08.5±0.06	09.0±0.11
A.flavus	06.0±0.57	06.5±0.06	08.0±0.34	10.0±0.56	05.0±0.60	05.5±0.78	06.0±0.25	06.5±0.54
A.terreus	13.0±0.26	17.5±0.35	20.0±0.19	23.5±0.34	04.0±0.18	04.5±0.14	05.0±0.71	05.5±0.35
Pencillium notatum	08.0±0.46	15.0±0.06	16.0±0.47	21.0±0.56	05.0±0.11	05.5±0.08	06.0±0.03	07.0±0.49

The values are expressed in terms of (Mean ± Standard deviation)

Table 8: Antifungal activity of *Pleurotus florida* against fungi

Name of the fungi	Different concentration (µl) and Zone of inhibition (mm)							
	Aqueous extract				Methanol extract			
	20	40	60	80	20	40	60	80
Aspergillus niger	05.0±0.11	05.5±0.16	06.0±0.52	06.5±0.03	05.0±0.06	05.5±0.29	06.0±0.21	06.5±0.08
A.flavus	05.5±0.63	06.0±0.57	07.0±0.26	08.0±0.13	05.5±0.14	06.0±0.05	06.5±0.11	07.0±0.09
A.terreus	05.0±0.55	05.5±0.60	06.5±0.74	08.5±0.35	04.5±0.28	05.0±0.16	05.5±0.08	06.5±0.21
Pencillium notatum	04.0±0.26	04.5±0.49	05.0±0.55	05.5±0.08	04.0±0.08	04.5±0.19	05.0±0.56	05.5±0.12

The values are expressed in terms of (Mean ± Standard deviation)

Table 9: Antifungal activity of *Pleurotus sajor caju* against fungi

Name of the fungi	Different concentration (µl) and Zone of inhibition (mm)							
	Aqueous extract				Diethyl ether extract			
	20	40	60	80	20	40	60	80
Aspergillus niger	05.0±0.55	05.5±0.06	06.0±0.18	06.5±0.13	04.5±0.24	05.0±0.67	05.5±0.19	06.0±0.41
A.flavus	04.0±0.17	04.5±0.32	05.0±0.14	05.5±0.07	04.0±0.22	04.5±0.16	05.0±0.81	05.5±0.12
A.terreus	04.5±0.11	05.0±0.03	05.5±0.01	06.5±0.28	05.0±0.89	05.5±0.46	06.0±0.07	06.5±0.13
Pencillium notatum	04.0±0.54	04.5±0.37	05.0±0.19	05.5±0.18	04.0±0.02	04.5±0.11	05.0±0.67	05.5±0.47

The values are expressed in terms of (Mean ± Standard deviation)

CONCLUSION

The data and research made available by this study indicated that the evaluated oyster mushroom extracts have a wide range of potential medicinal qualities. By using analysis results, it is possible to determine that the aqueous, methanol and diethyl ether extracts of these edible mushrooms (*Pleurotus ostreatus, P.florida* and *P.sajor caju*) use to have a broad range of antimicrobial properties, providing the prospect of developing antimicrobials from them rewarding. The results of several antioxidant capacity tests showed that all of the extracts had significant antioxidant characteristics and that the antioxidant activity of the mushroom extracts was concentration controlled. Although more research will be required to establish this new compound's specific mechanism of action, the present findings reveals that it is worthwhile to investigate its potential for treating infectious bacterial and fungal infections. The result suggests that *P.ostreatus, P. florida* and *P.sajor caju* fruiting bodies are a good source of natural antioxidants and antimicrobial agents.

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CONFLICT OF INTEREST

The author's have no conflict of interest

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