Comparative Studies of Antibacterial Properties of Three *Pleurotus* Species (Oyster Mushroom)

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Abstract: *P. sajorcaju, P. florida* and *P. floridanus* three oyster mushroom were taken for studying their antibacterial activities. Fruiting bodies, mycelium and 10 days grown extracellular culture filtrate were taken for these study. All the three species efficiently grown on rice straw. The biological efficiency of *P. sajorcaju* was maximum followed by *P. florida* and *P. floridanus*. Both the 70% alcohol extract and aqueous extract of fruiting body and mycelium of *P. floridanus* showed antibacterial activities. Extracellular culture filtrate of *P. floridanus* showed antibacterial properties only against *S. aureus.* 70% alcohol extract of *P. florida* fruiting bodies showed the antibacterial properties whereas the aqueous fruiting body extract or the mycelial extract (both alcohol or aqueous) did not show any antibacterial property. *P. sajorcaju* on the other hand showed its inefficiency as a source of antibacterial agent in the present experimental conditions.
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1. Introduction

Mushrooms are being used as a food item from the paleolithic time and the medicinal properties of mushrooms have a rich and long history of use not less than 2000 years (Rahaman et al., 2009). Different mushrooms, particularly *Agaricus* spp (button), *Lentinus edodes* (shiitake), *Coriolus versicolor, Ganoderma spp* (reshi)., *Cordyceps sinensis, Grifola frondosa* (maitake), *Polyporus umbellatus, Psilocybe sp., Lactarius sp*., *Tremella fuciformis* (silver-ear), *Poria cocos* (hoelen) etc. have shown number of medicinal properties like antioxidant, antitumor, hypocholesterolaemic, antihypersensitive, antibacterial and antiviral properties (Novaes, 2007; Kuznetsov, 2005; Collins and Ng, 1997; Jonathon and Fasidi, 2003; Chihara, 1993; Turkoglu et al. , 2006; Arora et al., 2008; Acharya et al., 2005).

The oyster mushroom *Pleurotus* spp. are most popular edible mushrooms due to its easy cultivation procedures within a broad range of temperatures(15-30°C) on different varieties of substrates like agro-forest residues, weeds and wastes for the production of food, feed, vitamins, enzymes and a number of pharmaceuticals in addition to their waste degradation and detoxification properties (Gregori et al., 2007; Jonathan et al., 2012). The oyster mushroom positioned second in the world just after the button mushroom *Agaricus* spp. as per its world consumption (Sanchez, 2010). The medicinal properties of *Pleurotus* spp though less but was also reported (Gregori et al., 2007). Though there are about 40 species of *Pleurotus* but according to Ahmed et al. (2009) only 12 species are cultivated in different parts of India. Only 3-4 species of *Pleurotus* are tested for their pharmaceutical importance. Most of the work have been done using fruiting body (Iwalokun et al., 2007). Purification of the medicinally important compound(s) and their therapeutic mechanisms are still largely untouched.

After the serendipitous discovery of penicillin in 1928 and subsequent discoveries of a large number of antibiotics, the treatment of bacterial and fungal diseases seems to be very simple. But repeated and excessive use of antibiotics not only showed harmful side effects to some crucial organs but also disrupted the internal ecology by harming the beneficial bacteria inside our body and disturbs the persons own immunity. Increasing antimicrobial resistance against a large number of antibiotics limited the treatment of invasive diseases caused by different pathogenic bacteria emphasizes the need for alternative therapies. So, search of an alternative drug from medicinal mushroom *Pleurotus spp* not only added another agent (s) to the therapeutic armory to control the bacteriall disease but also minimize the side effects as the source is the nutritious, fleshy, very common edible oyster mushroom (*Pleurotus* spp).

In this context new drug from medicinal mushroom *Pleurotus*, hopefully will be less at risk in confronting the resistance mechanism in different bacteria. The aqueous and 70% alcohol extract of fruiting bodies and mycelia of three *Pleurotus spp* viz *P. florida, P. sajorcaju* and *P floridanus* and their

extracellular culture filtrate from submerged culture were studied against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Bacillus megaterium* and their antimicrobial spectrum were compared with some commonly available antibiotics in agar plates in well method as well as filter paper disc method.

2. Material and Methods

(1) Mushroom species

P. florida (ITCC, 3308) was collected from Society for Rural Industrialization, Ranchi, India. *P. sajorcaju* (MTCC, 1806) and *P. floridanus* (MTCC, 6315) were collected from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. Bacterial cultures of *E. coli*, *Bacillus subtilis*, *B. megaterium* and *Staphylococcus aureus* were collected from dept. of Botany, Presidency University, Kolkata, India.

(2) Materials

Antibiotics and other chemicals used in this study were manufactured by reputed companies and were of analytical grade.

(3) Submerged Fermentation

An inoculum (6mm in diameter) has been taken from the periphery of colonies growing on potato dextrose agar (PDA) for 10 days. The submerged fermenttion has been studied in liquid PD medium in stationary conditions at 25±1˚C.

(4) Spawn preparation

About 1000 gm of wheat grains have been chosen for spawn production. The grains were cooked for half an hour then washed in flowing water. Extra water present was drained off and the substrate was spread on the surface of a clean blotting paper and air dried for 15 min. 5 gm of calcium carbonate and 10 gm of calcium sulphate were mixed with the grains. About 100 gm of spawn (wet wt) has been placed in polythene bag and sterilized in autoclave at 121˚C for 15 min and inoculated with the fungal species and kept at 30°C for 15 days (Das and Mukherjee, 2007; Das et al., 2011).

(5) Substrate preparation

Dried rice straw was collected from a local farm at Barasat, West Bengal, India The rice straw have been chopped into small pieces (5-6 cm), weighed and soaked in water for overnight. Extra water present in the substrate was drained off and the substrate was air dried for 15 min. No heat treatment of the substrate has been done. About 250 gm wet substrate (~ 85% moisture content) was mixed with 10% spawn (wet wt. /wet wt.). The spawned substrate was then put into 30 cm x 42 cm polythene bags. The bags were tightly closed with pin holes on the surfaces and kept in a BOD at 25 ± 1 ºC (Das et al., 2010).

(6) Biological Efficiency

% Biological efficiency (BE) =
(Dry wt. of substrate / Wet wt. of mushroom) x100

(7) Preparation of fruiting body/mycelia extract

10 gm of fresh fruiting body or wet mycelium (extra water was removed by squeezing) was weighed and crushed with either 10 ml of water or 10 ml 70% ethyl alcohol. Centrifuged at 15000 rpm for 20 min. Filtrate was collected, dried and finally 1ml volume was make up with water and maintained as stock solution of aqueous or alcohol extract, respectively.

(8) Antibacterial testing of *Pleurotus* extract

Mycelium/ fruiting body extract or the extracellular medium was tested for antibacterial activity by agar-well diffusion technique (Iwalokun et al., 2007). An overnight culture (approx 10^6 cfu/ml of bacteria) was taken in sterile Petri plate. Molten nutrient agar was poured in the plate with gentle movement in clockwise and anticlockwise direction for uniform mixing of microbes in agar media and confluent growth throughout the plate. Wells have been made on agar plates by using sterile cork borer and filled with 30µl extract/extracellular medium. Standard antibiotics of 30µg/well (30µl of 1mg/ml stock) was used as control. In some experiments paper discs soaked with antibiotics or mushroom extracts/culture filtrate (10 µl unless otherwise mentioned) was placed on agar plates in place of well. Growth inhibition was measured as diameters of inhibitory zones in the nearest 0.1mm after 24 h.

(9) Minimum Inhibitory Concentration

The MICs of the extracts was determined by broth by dilution method. The extract was diluted serially with normal saline and different conc. were given in well or disc in plate as previously mentioned. The minimum conc. of extract that inhibit the bacterial growth as visible as inhibition zone was the MIC.

3. Results

(1) Biological efficiency of fruiting body production

All the three *Pleurotus* spp. i.e. *P. florida*, *P. floridanus* and *P. sajorcaju* efficiently grown in rice straw (Fig. 1). Biological efficiency of three *Pleurotus* species (Table- 1) showed that *P. sajorcaju* produced highest amount of fruiting body followed by *P. florida and P. floridanus.*

Table 1. Biological efficiency (%) of three *Pleurotus* species for fruiting body production

Name of mushroom	1st flush	2nd flush	Total
P. florida	80	50	130
P. sajorcaju	92	46	138
P. floridanus	65	45	110

A **B** **C**

Figure 1. Fruiting bodies of different *Pleurotus* species A) *P. sajorcaju* B) *P. florida* C) *P. floridanus*

(2) Efficiency of alcohol extract of fruiting body of *P. florida, P. sajorcaju* and *P. floridanus* on bacterial growth

70% alcohol extract of *P. florida* and *P. floridanus* fruiting body showed growth inhibition to all the tested bacteria in both well (30µl) and disc (10µl) method but the *P. sajorcaju* extract did not show any inhibition (Table -2).

(3) Efficiency of aqueous extract of fruiting body of *P. florida, P. sajorcaju* and *P. floridanus* on bacterial growth

When 30µl aqueous extract of fruiting body of *P. florida, P. sajorcaju* and *P. floridanus* was applied in different wells to different bacteria, it showed inhibitory effect to all of them by *P. floridanus* extract but not by *P. florida* and *P. sajorcaju* extracts (Table-3).

Table- 2. Zone of inhibition (cm) of alcohol extract of fruiting body of different *Pleurotus* spp. and antibiotics

Sl. No.	*Bacteria*	*P. florida*		*P. sajorcaju*		*P. floridanus*		oxytetracycline	cefotaxime
		well	disc	well	disc	well	disc		
1	*E. coli*	0.9	1.1	-	-	0.8	1.0	1.5	1.5
2	*B. subtilis*	0.5	0.8	-	-	1.1	0.7	1.5	1.1
3	*B. megaterium*	0.6	1.1	-	-	1.2	0.8	1.6	1.0
4	*S. aureus*	0.6	0.9	-	-	0.8	0.8	1.4	1.5

Table- 3. Zone of inhibition (cm) of aqueous extract of fruiting body of different *Pleurotus* spp. and antibiotics

Sl. No.	Name of bacteria	*P. florida*	*P. sajorcaju*	*P. floridanus*	oxytetracycline	penicillin
1	*E. coli*	-	-	0.7	1.5	0.6
2	*B. subtilis*	-	-	1.0	1.5	0.9
3	*B. megaterium*	-	-	0.6	1.6	1.3
4	*S. aureus*	-	-	0.6	1.4	1.0

(4) Zone of inhibition (in cm) of aqueous extract of mycelium and culture filtrate of different *Pleurotus* spp.

When culture filtrate of some oyster mushroom and their aqueous mycelial extracts were compared for antibacterial activities it was found that only *P. floridanus* (6315) mycelial extract showed inhibitory activities against all the bacteria while 6315 culture filtrate showed inhibitory activity against only *Streptococcus* sp. (Table-4).

(5) Zone of inhibition (in cm) of alcohol extract of mycelium of different *Pleurotus* spp.

When alcohol extracts of mycelium of *P. sajorcaju, P. florida* and *P. floridanus* were compared for their antimicrobial activities against different bacteria both in well as well as disc methods it was found that the discs containing *P. floridanus*

extract showed inhibitory effect against all the tested bacteria (Table-5).

Table-4. Zone of inhibition (in cm) of aqueous extract of mycelium and culture filtrate of different *Pleurotus* spp.

Sl. No.	Name of bacteria	*P. florida*		*P. sajorcaju*		*P. floridanus*	
		Mycelium	Culture filtrate	Mycelium	Culture filtrate	Mycelium	Culture filtrate
1	*E. coli*	-	-	-	-	2.5	-
2	*S. aureus*	-	-	-	-	1.2	1.0
3	*B. subtilis*	-	-	-	-	1.1	-
4	*B. megaterium*	-	-	-	-	2.2	-

Table-5. Zone of inhibition of alcohol extract (70%) of mycelium of different *Pleurotus* spp.

Sl. No.	Name of bacteria	*P. florida*		*P. sajorcaju*		*P. floridanus*	
		well	disc	well	disc	well	disc
1	*E. coli*	-	-	-	-	-	0.6
2	*S. aureus*	-	-	-	-	-	0.5
3	*B. subtilis*	-	-	-	-	1.1	0.7
4	*B. megaterium*	-	-	-	-	1.2	0.7

(6) The MIC of alcohol extract of *P. florida* fruiting bodies

The MIC of alcohol extract of *P. florida* fruiting bodies showed as low as 3 µl (equivalent to 30mg fresh mushroom) extract was sufficient to inhibit the growth of all the bacteria in disc method (Table-6) whereas in well method it required 18 µl alcohol extract to inhibit the growth of all the tested bacteria.

(7) MIC of 6315 alcohol extract on different bacteria in well and Disc methods

MIC of alcohol extract of 6315 fruiting body was tested against different bacteria in both well and disc methods (Table-7). It was found that the MIC was 3µl extract which is equivalent of 30mg of fresh fruiting body. Both the well and disc methods are more or less equally effective.

Table-6. Determination of MIC of alcohol extract of fruiting body of *P. florida* on different bacteria.

Sl. No.	Name of bacteria	Well (cm)					Disc (cm)				
		3 µl	12 µl	18 µl	24 µl	30 µl	3 µl	12 µl	18 µl	24 µl	30 µl
1	*B. megaterium*	1.0	1.2	1.3	1.5	1.8	0.5	0.6	0.8	1.0	1.3
2	*B. subtilis*	0.9	1.2	1.0	1.2	1.3	0.5	0.6	0.8	0.8	1.0
3	*E. coli*	-	-	0.5	1.0	1.0	0.5	0.6	0.6	0.6	0.7
4	*S. aureus*	-	-	0.6	1.2	1.2	0.5	0.5	0.5	0.6	0.7

Table 7. MIC of *P. floridanus* fruiting body extract against some bacteria Table 7. MIC of *P. floridanus* fruiting body extract against some bacteria

Sl. No.	Name of bacteria	Well (cm)				Disc (cm)			
		3 µl	12 µl	18 µl	30 µl	3 µl	12 µl	18 µl	30 µl
1	*B. megaterium*	0.6	0.6	0.8	1.2	0.5	0.6	0.8	0.8
2	*B. subtilis*	0.6	0.6	0.7	1.1	0.6	0.7	0.8	0.7
3	*E.coli*	0.7	0.6	0.8	1.0	0.6	0.7	0.8	0.8
4	*S. aureus*	0.5	0.5	0.6	0.8	0.6	0.5	0.7	0.8

(4) Discussion

In the present investigation the therapeutic significance of the edible fruiting bodies has been carried out in addition to submerged fermentation product (mycelium/extracellular culture filtrate) of different *Pleurotus* species against some bacteria in *in vitro* experiments. *P. sajorcaju* produced highest amount of fruiting bodies in rice straw followed by *P. florida* and *P.floridanus* (Table-1). There are a number of report about the cultivation of *P. florida* and *P. sajorcaju* whereas the *P. floridanus* cultivation is not very common (Sanchez, 2010, Gregory, 2007).

The resistance of bacteria against antibiotics is now-a-days a world-wide problem (D'Costa et al., 2006). To come over the antibiotic resistance, sometimes more than one antibiotic are used

simultaneously which have more chance of antibiotic related adverse effect (Kim *et al.*, 2001). According to Iftikhar et al. (2011) as mushroom has taken as food item , they have devoid of dose related adverse effect as found with antibiotics whereas the mushroom along with antibiotics might lessen the undesirable effect (Kim et al, 2001).

In the present investigation fruiting bodies, mycelium and extracellular culture filtrate after 10 days growth in PD medium of three oyster mushroom viz. *P. florida, P. sajorcaju and P. floridanus* were studied for their antibacterial activities. It was observed that the 70% ethanolic extracts of fruiting bodies of *P. florida* and *P. floridanus* showed the antibacterial activities (Table-2). *P. ostreatus* , *P.eryngii* and *P. sajorcaju* was also reported for their antimicrobial activities (Gregory, 2007; Akyuz and Kirbag, 2009). Iwalokun *et al*. (2007) reported that both petroleum ether and acetone extract of *P. ostreatus* fruiting body also inhibited the fungal growth. In the present experimental condition antifungal activity was not done in any of the extracts or culture filtrate of the tested mushrooms. The aqueous extract of fruiting body of *P. floridanus* showed the antibacterial activity but the other two extracts are not efficient (Table-3). In addition to fruiting body, mycelial extract of *P. florida, P sajorcaju and P. floridanus* were tested for antibacterial activities. It was found that *P. floridanus* showed antibacterial activities in both aqueous or alcoholic condition (Table 4 and 5). *P. floridanus* mycelium showed better inhibition zone in aqueous condition than the alcohol extract (data not shown). Again both well and disc methods are used for the inhibitory experiment. The disc method showed better result than the well method (Table-5). The extracellular PD culture filtrate of *P. floridanus* also showed the antibacterial activity against a gram positive bacterium *Staphylococcus aureus* (Table-4). The antibacterial activities of mushroom extract showed that they have broad spectrum activities. Similar results were shown by Iwalokun, 2007; Iftekhar et al 2011, Rahaman et al, 2009. The MIC of alcohol extract of *P. florida* and *P. floridanus* was also studied (Table 6 and 7). The MIC of alcohol extract of fruiting body of *P. florida* and *P. floridanus* was as low as 3µl (=30mg fresh Fruiting body).

The alcoholic extract of fruiting body of *P. florida* showed the inhibitory activity for bacterial growth. The result agree with the experiments of Fagade and Oyelade (2009) and Iftekhar (2011). According to Iwalokun *et al.*, (2007) the difference in response of mushroom extracts to test organisms might be due to a variety of factors like the nature of environment, growth media, mushroom species, solvent system for extraction, and obviously the genetic structure of mushroom species. Gao et al (2005) commented that the sensitivity pattern of microorganisms undergo alteration to chemotherapeutic agents depending on their strains and susceptibility or resistance to antibiotics.

In the present investigation *P. floridanus* is the most efficient mushroom so far its antibacterial properties are concerned. The aqueous and alcohol extract of mycelium, fruiting body, even extracellular culture filtrate showed bactericidal activity to some extent. The nature of inhibition indicate possible presence of different antibactericidal agents in alcohol and aqueous extract.

Ramesh and Patter (2010) commented that antibacterial activities of mushroom extract have been attributed to presence of biologically active components that have immunomodulatory activities. The antibacterial activities of mushroom extract was recognized in presence of terpene, polysaccharide, lectins etc. Wasser and Weis (1999) suggested that polysaccharides from mushroom do not directly act but altered the immune response of host by increasing the macrophage activity which then kill the pathogens i.e. bacteria, viruses etc.

(5) Conclusion

P. sajorcaju produced highest amount of fruiting bodies in rice straw followed by *P. florida* and *P. floridanus.*70% alcohol extract of fruiting body of *P. florida* showed antibacterial activities against all the tested bacteria but the extracellular culture filtrate did not showed antibacterial activities. Both alcohol and aqueous extract of mycelium and fruiting body of *P. floridanus* showed antibacterial activities against all the tested bacteria. The extracellular culture filtrate of *P. floridanus* showed antibacterial activity against only *S. aureus*. The MIC of alcohol extract of fruiting body of *P. florida and P. floridanus* was as low as 3µl (=30mg fruiting body). Both the alcohol and aqueous extract of fruiting body of *P. sajorcaju* showed no antibactericidal activity. So, in this investigation *P. floridanus* is the most efficient oyster mushroom as per its antibacterial activities are concerned .

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