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Full Length Research Article

Antibacterial and Antifungal Activity of *Pleurotus florida* using different Types of Solvents by Agar well Diffusion Method

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Abstract

In the present investigation suggested that the oyster mushrooms are edible for human being. The effect of antimicrobial properties was rich in mushrooms because of rich amount of bioactive molecules were represented. The antibacterial activity of *Pleurotus florida* against *Staphylococcus aureus*, *Bacillus* sp, *Vibrio* sp and *Streptococcus aureus* using different types of solvents of aqueous, chloroform, ethanol and methanol were performed. The organic solvent aqueous was maximum inhibitory activity showed then the other solvents. Similarly act against some human pathogenic fungi namely *Aspergillus flavus, A. terreus, A. niger* and *Penicillium* sp were determined. The maximum inhibitory of antifungal activity of *P. florida* with aqueous solvent against *Aspergillus flavus* and *A. niger* were analyzed when compared to other fungi and also other solvents. The minimum zone of inhibition observed in *Aspergillus terreus* and *Pencillium* sp. Hence, the edible mushroom *P. florida* was excellent candidature for insight of biological activities to the society.

***Key words*:** Oyster mushroom, *Pleurotus florida*, Antibacterial activity, Antifungal activity

A definition of food as medicine is the concept that underlies the use of mushrooms as a medicinal agent. In the past, local communities, especially in China, Japan, and Korea, used them as food and remedies. There are many of bioactive macromolecules in oyster mushrooms. Because of its nutritional benefits and frequent use as therapeutic agents, mushrooms are still used in the medical industry [1-2]. Due to their abundance of nutraceutical ingredients, mushrooms are well-known as functional foods. These have a well-known nutritional value because to factors including their high protein, low fat, and low energy contents. The best nutritional supplements, with exceptional medical benefits, are believed to be mushrooms. Some edible mushrooms have the ability to fight against numerous human diseases and are antimicrobial. These were discovered to have antifungal and antibacterial properties against tough pathogens [3-4].

Mushrooms are now getting significant importance due to their nutritional and medicinal value and its cultivation is being done in about 100 countries. Mushrooms are a reliable and rich source of unconventional food protein to the health concern population of today's world. The FAO data show that the world's mushroom cultivation reached almost 9 million tonnes in 2018, making it one of the horticulture's fastest-growing subfields. The harvest of fruiting bodies has doubled over the past ten years, and species diversity has also increased [5-6].

The use of antibiotics and fungicides to combat infectious diseases has greatly contributed to a number of medical advances. On the down side, these strategies have recently led to a large increase in the number of multi-drug tolerant species. The effectiveness of modern antibiotics is in jeopardy due to the increased use of commercialized antimicrobial drugs in the treatment of infectious diseases and bacteria's genetic capacity to propagate and develop treatment resistance [7].

MATERIALS AND METHODS

Determination of antimicrobial activity [8]

Sample collection

The *Pleurotus florida* sample has been collected from our own cultivation process. The fruit bodies are dried under controlled condition further used for the process.

Extraction preparation

Ten gram of dried finely crushed mushrooms was extracted for 24 hours in 100 ml of aqueous, chloroform, hexane and methanol at room temperature under dark condition. The extraction was twice repeated, the extracts were filtered through glass funnel and Whatman No.1 filter paper.

Test microorganisms

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The following bacterial and fungal strains were used for the screening of antimicrobial activity. The test organisms have been collected from Indian Biotrack Research Institution, Thanjavur. The bacterial organisms such as *Bacillus* sp, *Staphylococcus aureus*, *Streptococcus aureus*, *Vibrio* sp. The fungal organisms namely *Aspergillus flavus, A. niger, A. terreus* and *Pencillium* sp.

Media used

Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were used for testing the antibacterial and antifungal activity.

Composition of nutrient agar (g/l)

Ingredients	g / Litre
Peptone	5.0 g
Beef extract	3.0 g
Sodium chloride	5.0g
Agar	15.0 g
Distilled water	1000 ml
Final pH	7.0 ± 0.2

Composition of potato dextrose agar (g/l)

Ingredients	g / Litre
Potato Infusion	200 g
Dextrose	20.0 g
Agar	20.0g
Distilled water	1000 ml
Final pH	5.5 ± 0.5

Agar well diffusion method

Agar well diffusion method was followed to determine the antimicrobial activity. 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 100µl of different solvent *P. florida* extracts were added using sterilized dropping pipettes into the wells and plates were left for one 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 the plates were incubated in an upright position at 37 °C ± 2 °C for 24 h for bacterial pathogens and 28 °C ± 2 °C for fungi. The organic solvents alone were acted as a negative control. Results were recorded, as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of tested organism and it was reported as positive and absence of zone is negative. The diameters of the zones were measured using diameter measurement scale. The effect of *P.florida* extract was compared with standard antibiotics

Statistical analysis

The antibacterial and antimicrobial values were studied with triplicate and the results were expressed with error bars. Both the mean and standard deviation were determined where appropriate, using the statistical package in Microsoft Excel version 2007.

RESULTS AND DISCUSSION

Antibacterial activity of P. florida

The antibacterial activity of *P.florida* evaluated by using some of the following pathogens namely *Staphylococcus aureus*, *Bacillus* sp, *Vibrio* sp and *Streptococcus aureus*. The results were recorded in (Table 1-4).

Effect of Staphylococcus aureus

The *Staphylococcus aureus* organisms evaluate antibacterial activity using different solvents such as aqueous, chloroform, hexane and methanol. The maximum zone of inhibition was measured 6.00±0.80 in aqueous solvent, followed by 3.11± 0.07 in chloroform, 3.00± 0.55 in hexane and 5.10 ± 0.16 methanol. The minimum zone of inhibition occurred in methanol solvent 1.21±0.00, 2.03±0.07 in hexane and chloroform, 4.05±0.88 in aqueous.

Effect of Bacillus sp

The antibacterial activity of *Bacillus* sp has been observed maximum zone of inhibition occurred in aqueous 3.11 ± 0.07 followed by chloroform 3.30 ± 0.18, hexane 2.03 ± 0.15 and methanol 2.86 ± 0.08. The minimum zone of inhibition showed in aqueous 1.40 ± 0.10, chloroform 1.00 ± 0.01, hexane 1.03 ± 0.14 and methanol 1.03 ± 0.40.

Effect of Vibrio sp

Effect of *Vibrio* sp shows the following maximum zone of the inhibition occurs in *P. florida* sample using four different types of solvents 3.00±0.55 in aqueous, 2.60±0.06 in chloroform, 2.56±0.01 in hexane and 2.51±0.37 in methanol. The minimum zone inhibition shows 1.66± 0.50 in aqueous, 1.15±0.07 in chloroform, 1.11±0.07 in hexane and 1.51±0.17 in methanol.

Effect of Streptococcus aureus

In antibacterial activity *P. florida* of against to *Streptococcus aureus* shows the maximum zone of inhibition occur in aqueous 5.10±0.16, 2.80±0.19 in chloroform, 2.86±0.18 in hexane and 3.10±0.96 in methanol. The minimum zone of inhibition occurs in aqueous solvent 2.15±0.07, 1.05±0.50 in chloroform, 1.10±0.50 in hexane and 1.15±0.27 in methanol. The results were recorded in (Table 1-4).

Antifungal activity of P. florida

In antifungal screening of *Pleurotus florida* was carried by the pathogen namely *Aspergillus flavus, A. terreus, A. niger, Penicillium* sp using the solvent such as aqueous, chloroform, hexane and methanol. The results were recorded in (Table 5-8).

Effect of Aspergillus flavus

P. florida antifungal shows maximum zone of inhibition occurs in aqueous 12.6± 0.90, in chloroform 15.0± 0.94, in hexane 13.0± 0.88 and 13.0± 0.57 in methanol. The minimum zone of inhibition activity shows 2.33± 0.57 in aqueous, 5.03± 0.57 in chloroform, 3.33± 0.20 in hexane and3.01± 0.57 in methanol.

Effect of A. terreus

Antifungal activity of *P. florida* in *A. terreus* maximum zone of inhibition shows aqueous 3.01± 0.57, in chloroform 4.30± 0.88, in hexane 3.73±0.15 and 4.04±0.88 in methanol. The minimum zone of inhibition occurs in aqueous 1.40±0.15, in chloroform 1.80± 0.51, in hexane 1.33± 0.14 and 1.63± 0.40 in methanol solvent.

Effect of A. niger

The *A.niger* shows maximum zone of inhibition 14.0± 0.59 in aqueous, 11.0±0.96 in chloroform, 13.6± 0.91 in hexane and in methanol 16.0± 0.57. Respectively 3.66± 0.57 in aqueous, 2.05± 0.57 in chloroform, 2.01± 0.57 in hexane and 4.01± 0.57 minimum zone of inhibition showed.

Effect of Penicillium sp

The antifungal activity of *P. florida* to against *Penicillium* sp shows maximum inhibition formed in aqueous 11.05±0.96, in chloroform 10.0±0.89, in hexane 9.66± 0.88 and 11.02±0.96 in methanol. The minimum zone of inhibition formed in aqueous 2.05± 0.57, 2.02± 0.37in chloroform, 2.00±0.57in hexane and 2.01± 0.54 in methanol solvent.

Table 1 Antibacterial activity of *Pleurotus florida* in aqueous solvent

S. No	Name of the organisms	Zone of inhibition (mm) *Pleurotus florida*			
		20µl	40 µl	60 µl	80 µl
1.	*Staphylococcus aureus*	2.00 ± 0.07	4.05 ± 0.88	6.00 ± 0.80	5.66 ± 0.00
2.	*Bacillus* sp	1.40 ± 0.10	2.10 ± 0.16	2.48 ± 0.17	3.11 ± 0.07
3.	*Vibrio* sp	1.66 ± 0.50	2.00 ± 0.07	2.18 ± 0.17	3.00 ± 0.55
4.	*Streptococcus aureus*	2.15 ± 0.07	3.33 ± 0.18	4.00 ± 0.80	5.10 ± 0.16

Table 2 Antibacterial activity of *Pleurotus florida* in chloroform solvent

S. No	Name of the organisms	Zone of inhibition (mm) *Pleurotus florida*			
		20µl	40 µl	60 µl	80 µl
1.	*Staphylococcus aureus*	2.03 ± 0.07	3.66 ± 0.88	4.10 ± 0.18	4.50 ± 0.94
2.	*Bacillus* sp	1.00 ± 0.01	1.50 ± 0.52	2.43 ± 0.04	3.30 ± 0.18
3.	*Vibrio* sp	1.15 ± 0.07	1.53 ± 0.18	2.00 ± 0.08	2.60 ± 0.06
4.	*Streptococcus aureus*	1.05 ± 0.50	1.53 ± 0.88	2.20 ± 0.08	2.80 ± 0.19

Table 3 Antibacterial activity of *Pleurotus florida* in hexane solvent

S. No	Name of the organisms	Zone of inhibition (mm) *Pleurotus florida*			
		20µl	40 µl	60 µl	80 µl
1.	*Staphylococcus aureus*	2.03 ± 0.00	2.43 ± 0.57	3.00 ± 0.80	4.01 ± 0.08
2.	*Bacillus* sp	1.03 ± 0.14	1.53 ± 0.14	1.81 ± 0.15	2.03 ± 0.15
3.	*Vibrio* sp	1.11 ± 0.07	1.66 ± 0.80	2.06 ± 0.08	2.56 ± 0.01
4.	*Streptococcus aureus*	1.10 ± 0.50	1.51 ± 0.51	2.05 ± 0.07	2.86 ± 0.18

Table 4 Antibacterial activity of *Pleurotus florida* in Methanol solvent

S. No	Name of the organisms	Zone of inhibition (mm) *Pleurotus florida*			
		20µl	40 µl	60 µl	80 µl
1.	*Staphylococcus aureus*	1.21 ± 0.00	1.85 ± 0.10	2.20 ± 0.50	3.00 ± 0.40
2.	*Bacillus* sp	1.03 ± 0.40	1.70 ± 0.40	2.06 ± 0.12	2.86 ± 0.08
3.	*Vibrio* sp	1.51 ± 0.17	1.90 ± 0.07	2.00 ± 0.27	2.51 ± 0.37
4.	*Streptococcus aureus*	1.15 ± 0.27	2.33 ± 0.28	2.90 ± 0.88	3.10 ± 0.96

Table 5 Antifungal activity on *Pleurotus florida* in aqueous solvent

S. No	Name of the organisms	Zone of inhibition (mm) *Pleurotus florida*			
		20µl	40 µl	60 µl	80 µl
1.	*Aspergillus. flavus*	2.33± 0.57	6.05 ± 0.88	9.00 ± 0.88	12.6 ± 0.90
2.	*A. terreus*	1.40± 0.15	2.01 ± 0.15	2.41 ± 0.57	3.01 ± 0.57
3.	*A. niger*	3.66± 0.57	8.00 ± 0.57	11.0 ± 0.57	14.0 ± 0.59
4.	*Penicillium* sp	2.05± 0.57	6.33 ± 0.88	9.00 ± 0.88	11.05 ± 0.96

Table 6 Antifungal activity on *Pleurotus florida* in chloroform solvent

S. No	Name of the organisms	Zone of inhibition (mm) *Pleurotus florida*			
		20µl	40 µl	60 µl	80 µl
1.	*Aspergillus. flavus*	5.03± 0.57	8.66 ± 0.88	12.0 ± 0.88	15.0 ± 0.94
2.	*A.terreus*	1.80± 0.51	2.63 ± 0.52	3.43 ± 0.54	4.30 ± 0.88
3.	*A.niger*	2.05± 0.57	6.33 ± 0.88	9.00 ± 0.88	11.0 ± 0.96
4.	*Penicillium* sp	2.02± 0.37	6.35 ± 0.88	9.05 ± 0.88	10.0 ± 0.89

Protein extracts showed antimicrobial effects against studied pathogens except for *T. mentagrophytes*, *M. gypseum*, and *M. ferugineum*. The Tris buffer protein extract showed the strongest effect against *E. coli* with DIZ of 6.00±0.00 mm, followed by *P. aeruginosa* (4.00±0.00 mm), and *S. aureus* (3.66±0.53 mm). The warm aqueous extract exhibited the most effectiveness against *E. coli* and *S. aureus* with the diameter of the inhibition zone of 3.66±0.53 mm [9]. In present study reported *Streptococcus aureus* shows the maximum zone of inhibition 5.10±0.16 mm found in aqueous solvent.

The antimicrobial activity evaluation was carried out against human pathogenic microorganisms, namely, *Escherichia coli*, *Bacillus subtilis*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, and *Salmonella typhi* by using the disc diffusion method. Methanolic extracts of *P. ostreatus* cultivated on a S*orghum* grain residue substrate were recorded for the highest antibacterial activity against *E. coli* (19.8 mm) and *P. aeruginosa* (16.4 mm), and methanolic extracts of *P. florida* cultivated on a wheat grain substrate were recorded for the highest antibacterial activity against *E. coli* (18.6 mm) and *S. faecalis* (14.8 mm) [10]. The current research finding observed antibacterial activity *P.florida* against to *Bacillus* sp maximum zone of inhibition found 3.30±0.18 mm in chloroform solvent.

Table 7 Antifungal activity on *Pleurotus florida* in hexane solvent

S. No	Name of the organisms	Zone of inhibition (mm) *Pleurotus florida*			
		20µl	40 µl	60 µl	80 µl
1.	*Aspergillus. flavus*	3.33 ± 0.20	6.33 ± 0.57	10.0 ± 0.88	13.0 ± 0.88
2.	*A. terreus*	1.33 ± 0.14	2.23 ± 0.14	3.01 ± 0.15	3.73 ± 0.15
3.	*A. niger*	2.01 ± 0.57	9.66 ± 0.88	5.66 ± 0.88	13.6 ± 0.91
4.	*Penicillium* sp	2.01 ± 0.54	3.01 ± 0.57	5.05 ± 0.57	9.66 ± 0.88

Table 8 Antifungal activity on *Pleurotus florida* in methanol solvent

S. No	Name of the organisms	Zone of inhibition (mm) *Pleurotus florida*			
		20µl	40 µl	60 µl	80 µl
1.	*Aspergillus. flavus*	3.01± 0.57	6.05 ± 0.57	10.0 ± 0.57	13.0 ± 0.57
2.	*A.terreus*	1.63± 0.40	2.40 ± 0.49	2.56 ± 0.52	4.4 ± 0.88
3.	*A.niger*	4.01± 0.57	10.0 ± 0.57	13.0 ± 0.57	16.0 ± 0.57
4.	*Penicillium* sp	2.30± 0.58	6.38 ± 0.88	10.00 ± 0.88	11.02 ± 0.96

In recently [11] *Agaricus bisporus* greater antimicrobial effect against *Staphylococcus aureus* (14.3 ± 0.24 mm) and *Candida albicans* (14.2 ± 0.12 mm) was observed compared to *Streptococcus pyogenes* (10.8 ± 0.12 mm), *Shigella flexneri* (10.8 ± 0.21 mm), *Klebsiella pneumoniae* (11.1 ± 0.33 mm) and *Aspergillus fumigatus* (11.2 ± 0.33 mm). In this research observed antifungal activity *P. florida* using different solvent against *Aspergillus flavus, A. terreus, A. niger* and *Penicillium* sp.

Ethanolic extract of *Pleurotus florida* showed highest inhibition activity against *Fusarium oxysporu, and S. commune* showed maximum zone of inhibition (ZOI) against *A. niger*, while, *P. pulmonarius* showed maximum zone of inhibition (ZOI) against *A. solani* was reported by [12]. In the present research work observed the antifungal activity *P. florida* using different solvent against *Aspergillus flavus, A. terreus, A. niger*. In the three different *Aspergillus* species the maximum zone of inhibition occurs in 16.0± 0.57 mm methanol solvent in 80 µl concentration.

CONCLUSION

In this study analyzed antibacterial activity *Pleurotus florida* act against to *Staphylococcus aureus*, *Bacillus* sp, *Vibrio* sp and *Streptococcus aureus* using different types of solvent, similarly act against also some fungi namely *Aspergillus flavus, A. terreus, A. niger* and *Penicillium* sp. In the present study concluded *Pleurotus florida* mushroom highly response for act against some common human pathogens. It contains the antibacterial and antifungal activity.

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