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# Studies on Phytochemical Characteristics and Antimicrobial Activity of *Pleurotus spp.* Cultivated on Different Agro Wastes

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**A B S T R A C T**

In the present study Mushrooms *pleurotus ostreatus* and *pleurotus florida* were cultivated on different agro wastes namely as gram ($S_1$), Pea straw ($S_2$) and pearl millet cuttings ($S_3$) for the screening of phytochemical characteristics and antimicrobial activity. Qualitative analysis revealed the phytochemicals alkaloids, saponins, flavonoids and tannins were present in methanolic and aqueous extracts of both *Pleurotus* spp. while anthraquinones and phobetanins were found absent. Antimicrobial activity was carried out against human pathogenic microorganism *Escherichia coli*, *Stphylococcus aureus*, *Protius mirabilis* and *Candida albicans*. *P.ostreatus* cultivated on substrate $S_1$ was recorded for highest antibacterial activity against *S.aureus* (18 mm), *P.mirabilis* (13.8 mm) and *E.coli* (16.2 mm).However methanolic extract of *P.florida* gave strong antifungal activity against *C.albicans* (12.5 mm) when compared to *P.ostreatus* .Therefore results suggested that *P.ostreatus* and *P.florida* cultivated on Substrate $S_1$ were found with highest antimicrobial activity in comparison to other substrates. The results supported the methanolic extracts of *P.ostreatus* and *P. florida* might indeed be potential sources of phytochemicals and antimicrobial agents.

## Introduction

Mushroom are the most conspicuous structures of the fungi world. Mushroom is a fleshy, spore bearing fruiting body of fungi, typically produced above ground on soil or on its food source. Mushroom belongs to phylum Basidiomycota and some of them in the Ascomycota are known as the higher fungi (Moradali *et al.*, 2007; Sicoli *et al.*, 2005).

The oyster mushroom *Pleurotus* spp. is widely cultivated on a wide range of substrates which are composed of lignin and cellulose. Cultivation of *Pleurotus* spp supports a broad range of temperatures (15-30°C) on different range of substrates like agro waste residues, weeds and wastes after 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).

Mushroom is being used as a valuable food source and traditional medicine around the world since ancient times especially in China and Japan. Mushrooms are rich sources of bio active compounds as ß-glucan, proteoglucan, 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, AHCC, maitake D-fraction, ribonucleas, eryngeolysin. Pharmacological and nutritional aspects make the mushroom as an important tool for ailment of severe diseases like antimicrobial and antiviral infections, anticancer, antitumor, antiinflamatory, cardiovascular diseases, immunomodulating diseases (Benedict and Brady, 1972; Conchran, 1978; Karacsonyi and Kuniak, 1994; Gunde-Cimerman, 1995; Wang and Ng, 2007; Iwalokun *et al.*, 2007)

The bioactive compounds present in *Pleurotus* spp. makes it a medicinally important mushroom (Gregori *et al.*, 2007). Ahmed *et al.*, (2009) pointed out 12 species among 40 species of *Pleurotus* were being cultivated in different parts of India. Only 3-4 species of *Pleurotus* are tested for their pharmaceutical importance.

*Pleurotus* spp. is promising as medicinal mushrooms, exhibiting hematological, antiviral, antitumor, antibiotic, antibacterial, hypocholesterolic and immunomodulation activities (Cohen *et al.*, 2004). The oyster mushroom may also be considered as medicinal mushroom for its hypocholesterolic property, because it contains statins such as lovastatin which reduces cholesterol (Gunde-Cimerman, 1995).

In recent years, high scale usage of synthetic antibiotic leads the emergence of multi drug resistance pathogens, is now posing a threat to the world. Therefore, a search for natural plant based antimicrobial agents is in need. This development is the consequence of the limited effectiveness of synthetic products to fight against newer and drug resistant bacteria. For this purpose, the antimicrobial properties of many natural compounds from a wide variety of plant species have been assessed (Karuppusamy, 2009).Therefore this study was done to evaluate the effect of different substrate on the phytochemicals and antimicrobial activity of *P.ostreatus* and *P.florida*.

## Materials and Methods

This study was carried out to evaluate the antimicrobial activity of mushroom after cultivation on different substrate.

### Spawn collection

The mother spawn of *Pleurotus florida* and *Pleurotus ostreatus* were purchased from Directorate of Mushroom Research, Chambaghat, (Himachal Pradesh) India.

### Spawn preparation

Spawn was prepared by using method of Bano and Shrivastava (1962) with slight modifications. Take one kg of wheat grain and cooked for 40 min after than washed in tap-water. Grain was drained and supplemented with 2 g lime and 8 g gypsum and mixed manually. Then grain was filled in poly propylene (PP) bags of 1 Kg capacity and sterilized in autoclave at 121ºC for 15 min. After cooling, PP bag was inoculated with freshly prepared mycelium (previously prepared PDA plate) and incubated at 25ºC for two weeks in an incubator.

## Cultivation

Cultivation of mushroom *Pleurotus spp.* were carried out at College of life sciences and CHRI, Gwalior, Madhya Pradesh (India). Three different agricultural wastes were selected for cultivation as Gram straw ($S_1$), Pea Straw ($S_2$) and Pearl Millet cuttings ($S_3$) were collected from rural areas of Gwalior region.

These agro wastes were used as substrate material in present study. *Pleorotus* spp. namely as *Pleurotus ostreatus*, and *Pleurotus florida* are agricultural lignocellulose utilizing species. One kg of each substrate was filled in jute bags and sterilized chemically (Formaldehyde and carben) the substrate materials.

Each substrate was mixed with freshly prepared spawn and then filled in pre sterilized poly propylene (pp) bags which were incubated at 27±2 °C in the dark cultivation room for 2 to 3 weeks or until the mycelium completely colonized the substrate material. *P. ostreatus*, and *P. florida* need different incubation temperature. Humidity (80 - 85%) of culture room was maintained by spraying water on pp bags once or twice day. Fruiting bodies were harvested after the maturation.

## Preparation of the mushroom extract

Freshly harvested fruiting bodies from *P.ostreatus* and *P.florida* were shade dried and finely powdered. Twenty grams of the powder were extracted with 200 ml of 95% solvent methanol, and aqueous separately using soxhlet apparatus. The remaining extract was filtered and evaporated by vacuum distillation; the filtrate thus obtained was used as mushroom extract (Jayakumar *et al.*, 2009).

## Preliminary phytochemical characteristics

Preliminary phytochemicals were qualitativly analysed by using methods of Trease and Evans (1994) and Harborne (1973) for alkaloids, tannins, saponins, anthrquinone, phlobatanenes flavonoids, steroids and glycosides.

## Antimicrobial activity

Antimicrobial activity of the different extracts of *P.ostreatus* and *P. florida* mushroom were evaluated against microorganisms namely *Escherichia coli* (MTCC 1610), *Staphylococcus aureus* (MTCC 3160), *Protius mirabilis* (MTCC 425), *and Candida albicans* (MTCC 854) using agar well diffusion technique (Akpata and Akinrimisi, 1977). An overnight culture of each microbial isolate was mixed with nutrient broth to a turbidity of 0.5 McFarland (108 cfu/ml). 100 µl of each standard inoculum was then streaked on Mueller-Hinton and PDA (Potato Dextrose agar medium). Each mushroom extract and standard (streptomycin and fluconazole) was dissolve in DMSO (di methyl sulphoxide) in a concentration of 10 mg / mL and stored at $4^oC$. Five Wells of 6 mm were made on the agar plate using a sterile cork borer and filled with 20 µL, 40 µL, 60 µL, 80 µL and100µL of mushroom extracts. After incubation for 24 hours at 30°C, a clear zone around a well was formed that considered as antibacterial activity. Diameter of the zones of inhibition was measured in millimeters. Solvent was used as a negative control.

## Result and Discussion

The present study was carried out to know the phytochemical potentiality and antimicrobial activity of *Pleurotus* spp. (*P.ostreatus and P.florida*) mushrooms cultivated on different agro wastes namely Gram straw ($S_1$), Pea straw ($S_2$) and Pearl millet cuttings ($S_3$).

Findings of qualitative analysis of phytochemicals shown in table-1. Phytochemical analysis of *P.ostreatus* showed that the methanolic and aqueous extracts contain alkaloids, tannins, saponins, flavonoids, steroids, and glycosides as reported by Okwulehie and Ogoke (2013). Phytochemicals present in methanolic and aqueous extracts of *.P florida* was supported by studies of Menaga *et al., (*2012).

## Antimicrobial activity

Antimicrobial activity of *Pleurotus* spp (*P.ostreatus* and *P.florida*) was carried out against pathogenic microorganisms namely *E. coli, S. aureus, P. mirabilis, and C. albicans*. Maximum activity was recorded against *S.aureus* was 18 mm showed by methanolic extract of *P.ostreatus* when cultivated on substrate $S_1$ and minimum was 7.2 mm by aqueous extract as shown in table 2 and 3 (graph 1).The obtained results showed similarity with the findings of Menaga *et al.*, (2012) .In the present study, methanolic extracts of *Pleurotus spp* showed the activity against *S.aureus* (7.6 mm-18.0 mm), *E.coli* (10.8 mm-16.2 mm), *P.mirabilis* (9.5 mm-13.8 mm), *C.albicans* (7.1 mm-13.2 mm) as shown in table 2,4,6and 8(graph 1,2,3 and 4) .On the other hand Aqueous extracts showed the antimicrobial activity against *S.aureus* (7.2 mm – 17.3 mm), *E.coli* (10.5 mm-15.1 mm),*P.mirabilis* (8.4 mm-12.9 mm), *C.albicans*(6.5mm-12.6 mm) as given in table 3,5,7 ,9 and Graph 1,2 and 3 .Mushroom obtained from Substrate $S_1$ found with excellent antimicrobial activity whereas the mushrooms obtained from substrate $S_3$ were recorded with poor antimicrobial activity because of low production of bioactive compounds. Methanolic extracts of *P.ostreatus* from substrate $S_1$ gave best results against *E.coli* (16.2mm) *S.aureus* (18 mm) and *P.mirabilis* (13.8 mm).However methanolic extract of *P.florida* was also recorded with high antifungal activity against *C.candida* (13.2mm). The results of Akyuz *et al.*, (2009) on antimicrobial activity of methanolic extract of *Pleurotus* spp. against *B. megaterium, E. coli, K. pneumonia, S. aureus, C. albicans, C. glabrata Epidermophyton spp. Trichophyton spp*. explained that petroleum ether and acetone extracts of *P. ostreatus* were found effective against *Staphylococcus spp*. (7.0-7.6 mm), *Bacillus spp*. (7.1-7.8 mm), *S. thyphi* (7.0-7.5 mm), *E. coli* (7.0-8.2 mm), *K. pneumoniae* (7.0-7.1 mm) and *Candida spp.* (8.0-8.3 mm). *P.ostreatus* showed high activity against *C.glabrata* was 15.5 mm. Jagadish *et al.* (2008) reported the ethanol extract of *P. florida* and *P. aureovillosus* did not exhibit antimicrobial effect against *K. pneumoniae, P. vulgaris, P. aeruginosa* and *C. albicans*, but showed activity against *S. aureus* (16.0 mm and 20.0 mm), *S. mutans* (14.0 and 17.0 mm), *M. luteus* (16.0 and 19.0 mm), *B. subtilis* (9.0 and 14.0 mm) and *E. coli* (12.0 and 14.0 mm), respectively.

Iwalokun (2007) also reported the similar results. Mondal *et al.*, (2013) found the inhibition zone ranged from 3.5mm-17mm was formed by extract of *P.ostreatus* during an antimicrobial study. Its methanolic extracts gave best results against *E.coli* (15.2 mm) and *S.aureus* (16.6 mm) was very close to present study. Surekha *et al.*, (2011) reported the antimicrobial activity of *P.ostreatus* against pathogenic bacteria *E.coli* (15 mm), *S.aureus* (24mm) and *P.vulgaris* (18mm).

**Table.1** Qualitative analysis of phytochemicals

| Mushroom Species | Substrate | Extract | Phytochemicals | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Alkaloids** | **Anthraquinones** | **Saponins** | **Flavonoids** | **steroids** | **phlobatannins** | **tannins** | **glycosides** |
| *P.ostreatus* | $S_1$ | Methanolic | + | - | + | + | + | - | + | + |
| | | Aqueous | + | - | + | + | - | - | + | - |
| | $S_2$ | Methanolic | + | - | + | + | + | - | + | + |
| | | Aqueous | + | - | + | + | - | - | + | - |
| | $S_3$ | Methanolic | + | - | + | + | + | - | + | + |
| | | Aqueous | + | - | + | + | - | - | + | - |
| *P.florida* | $S_1$ | Methanolic | + | - | + | + | + | - | + | + |
| | | Aqueous | - | - | + | + | - | - | + | - |
| | $S_2$ | Methanolic | + | - | + | + | + | - | + | + |
| | | Aqueous | - | - | + | + | - | - | + | - |
| | $S_3$ | Methanolic | + | - | + | + | + | - | + | + |
| | | Aqueous | - | - | + | + | - | - | + | - |

**Table.2** Antibacterial activity of methanolic extract of *Pleurotus* spp. cultivated on different substrates

| Bacteria used | Volume of extract per well (µL) | zone of inhibition in( mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ***P.ostreatus*** | | | ***P.florida*** | | | **Control** |
| | | **Substrate $S_1$** | **Substrate $S_2$** | **Substrate $S_3$** | **Substrate $S_1$** | **Substrate $S_2$** | **Substrate $S_3$** | **Streptomycin** |
| ***S. aureus*** | **20** | **13.9** | **13.4** | **7.6** | **13.5** | **13.3** | **9.5** | **24.5** |
| | **40** | **14.8** | **13.7** | **8.4** | **14.6** | **14.2** | **10.2** | **25.8** |
| | **60** | **16** | **15.4** | **9.1** | **15.4** | **15.1** | **10.8** | **27** |
| | **80** | **17.1** | **16.1** | **9.5** | **16.3** | **15.8** | **11.8** | **28.5** |
| | **100** | **18** | **17.4** | **10.5** | **17.1** | **16.3** | **13.5** | **30** |

Where, Substrate $S_1$ = Gram straw, $S_2$ = Peas straw, $S_3$ = Pearl millet cuttings; Concentration of extract (10mg/mL)

**Table.3** Antibacterial activity of aqueous extract of *Pleurotus* spp. cultivated on different substrates

| Bacteria used | Volume of extract per well (µL) | zone of inhibition in( mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *P.ostreatus* | | | *P.florida* | | | Control |
| | | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Streptomycin |
| *S.aureus* | 20 | 13.5 | 13.1 | 7.2 | 12.9 | 12.7 | 9.1 | 18 |
| | 40 | 14.2 | 13.9 | 7.9 | 14 | 13.5 | 9.9 | 20.5 |
| | 60 | 14.9 | 14.8 | 8.5 | 14.8 | 14.3 | 10.5 | 21 |
| | 80 | 16.5 | 16 | 9.1 | 15.2 | 14.9 | 11 | 22.8 |
| | 100 | 17.3 | 16.9 | 10.2 | 16 | 15.8 | 12.7 | 25 |

Where, Substrate $S_1$ = Gram straw, $S_2$ = Pea straw, $S_3$ = Pearl millet cuttings; Concentration of extract (10mg/mL)

**Table.4** Antibacterial activity of methanolic extract of *Pleurotus* spp. cultivated on different substrates

| Bacteria used | Volume of extract per well (µL) | zone of inhibition in( mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *P.ostreatus* | | | *P.florida* | | | Control |
| | | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Streptomycin |
| *P.mirabilis* | 20 | 11 | - | - | 10.8 | - | - | 18 |
| | 40 | 11.8 | 11.5 | - | 11.2 | 10.7 | - | 20 |
| | 60 | 12.2 | 12 | 9.9 | 11.9 | 11.5 | 9.5 | 21.5 |
| | 80 | 12.9 | 12.8 | 10.6 | 12.3 | 12.1 | 10.3 | 22.8 |
| | 100 | 13.8 | 13.4 | 11.1 | 13.1 | 12.9 | 11.9 | 23.1 |

Where, Substrate $S_1$ = Gram straw, $S_2$ = Pea straw, $S_3$ = Pearl millet cuttings; Concentration of extract (10mg/mL)

**Table.5** Antibacterial activity of aqueous extract of *Pleurotus* spp. cultivated on different substrates

| Bacteria used | Volume of extract per well (µL) | zone of inhibition in( mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *P.ostreatus* | | | *P.florida* | | | Control |
| | | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Streptomycin |
| *P.mirabilis* | 20 | 9.9 | 9.5 | - | 9.5 | 9.2 | - | 11 |
| | 40 | 10.5 | 10.4 | 8.4 | 10 | 10.9 | - | 12.9 |
| | 60 | 11.4 | 11.5 | 9.2 | 11.4 | 11.1 | 9.3 | 13.1 |
| | 80 | 12.1 | 11.9 | 10.1 | 11.9 | 11.8 | 10.2 | 16.9 |
| | 100 | 12.9 | 12.2 | 10.9 | 12.3 | 12.1 | 11.1 | 18.5 |

Where, Substrate $S_1$ = Gram straw, $S_2$ = Pea straw, $S_3$ = Pearl millet cuttings; Concentration of extract (10mg/mL)

**Table.6** Antibacterial activity of methanolic extract of *Pleurotus* spp. cultivated on different substrates

| Bacteria used | Volume of extract per well (µL) | zone of inhibition in( mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *P.ostreatus* | | | *P.florida* | | | Control |
| | | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Streptomycin |
| *E.coli* | 20 | 11.5 | 11.1 | 10.3 | 11.1 | 10.8 | - | 16 |
| | 40 | 12.9 | 11.9 | 11.1 | 11.8 | 11.5 | 11 | 20 |
| | 60 | 14.8 | 13.5 | 12.9 | 13.5 | 12.9 | 12.1 | 22 |
| | 80 | 15.6 | 14.2 | 13.8 | 14.1 | 13.8 | 12.9 | 23 |
| | 100 | 16.2 | 15 | 14.5 | 15.9 | 14.7 | 14.7 | 25 |

Where, Substrate $S_1$ = Gram straw, $S_2$ = Pea straw, $S_3$ = Pearl millet cuttings; Concentration of extract (10mg/mL)

**Table.7** Antibacterial activity of aqueous extract of *Pleurotus* spp. cultivated on different substrates

| Bacteria used | Volume of extract per well (µL) | zone of inhibition in( mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *P.ostreatus* | | | *P.florida* | | | Control |
| | | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Streptomycin |
| *E.coli* | 20 | 10.9 | 10.8 | - | 11 | 10.5 | - | 15 |
| | 40 | 12 | 11.7 | - | 11.9 | 11.7 | - | 17 |
| | 60 | 13.5 | 13.2 | 10.5 | 12.5 | 12.2 | 10.9 | 20 |
| | 80 | 14.6 | 14.1 | 11.2 | 13.4 | 13.1 | 11.5 | 21 |
| | 100 | 15.1 | 14.9 | 11.9 | 14.2 | 13.9 | 12.1 | 23 |

Where, Substrate $S_1$ = Gram straw, $S_2$ = Pea straw, $S_3$ = Pearl millet cuttings; Concentration of extract (10mg/mL)

**Table.8** Antifungal activity of methanolic extract of *Pleurotus* spp. cultivated on different substrates

| Fungi used | Volume of extract per well (µL) | zone of inhibition in( mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *P.ostreatus* | | | *P.florida* | | | Control |
| | | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Fluconazole |
| *C.albicans* | 20 | 7.5 | 7.1 | 7.3 | 9.6 | 9.1 | 8.5 | 15.1 |
| | 40 | 9.2 | 7.9 | 8 | 9.9 | 9.7 | 9.3 | 15.9 |
| | 60 | 10.1 | 8.8 | 8.9 | 11.2 | 10.6 | 10.9 | 16.8 |
| | 80 | 11 | 9.5 | 10.1 | 12.5 | 11.5 | 11.3 | 17.2 |
| | 100 | 12.5 | 10.6 | 11.2 | 13.2 | 12.9 | 11.8 | 18 |

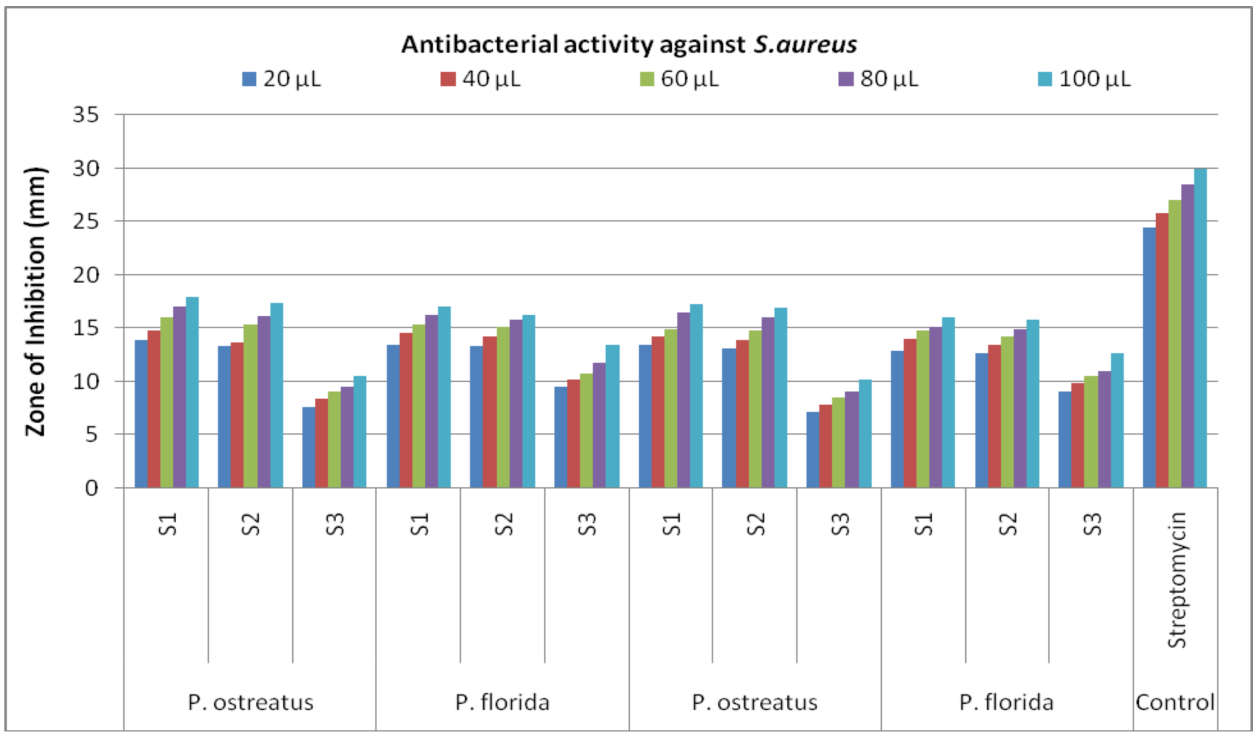
Where, Substrate $S_1$ = Gram straw, $S_2$ = Pea straw, $S_3$ = Pearl millet cuttings; Concentration of extract (10mg/mL)

**Table.9** Antifungal activity of aqueous extract of *Pleurotus* spp. cultivated on different substrates

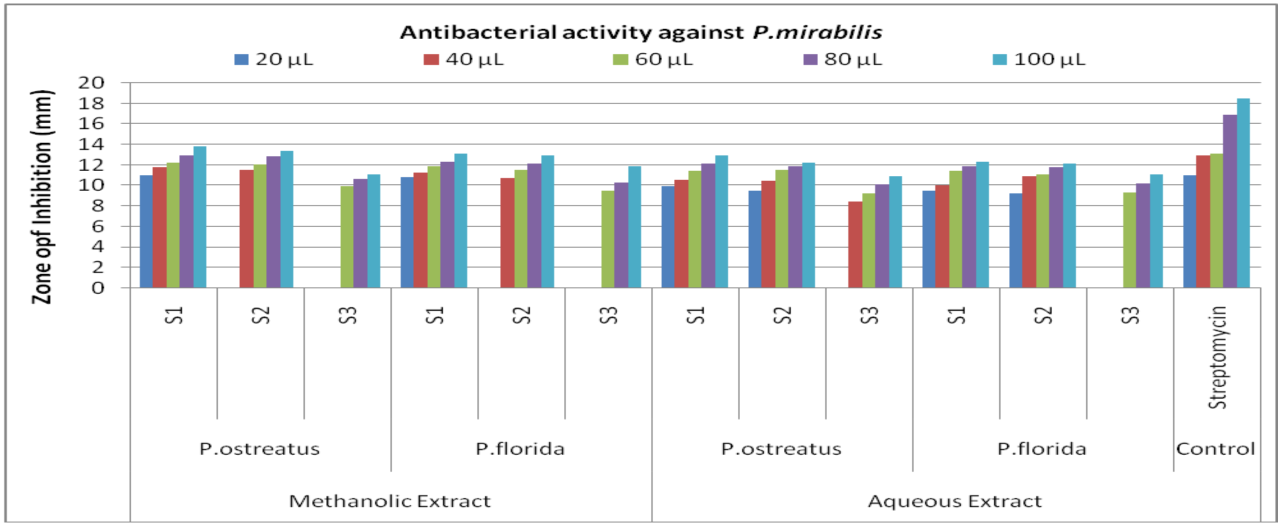
| Fungi used | Volume of extract per well (µL) | zone of inhibition in( mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *P.ostreatus* | | | *P.florida* | | | Control |
| | | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Substrate $S_1$ | Substrate $S_2$ | Substrate $S_3$ | Fluconazole |
| *C.albicans* | 20 | 7.1 | 6.5 | 6.9 | 7.8 | 7.0 | 7.2 | 15.1 |
| | 40 | 8.9 | 7.2 | 7.8 | 9.4 | 7.9 | 8.5 | 15.9 |
| | 60 | 9.5 | 7.8 | 8.5 | 10.9 | 8.5 | 9 | 16.8 |
| | 80 | 10.4 | 8.7 | 9.6 | 11.5 | 9.7 | 10.2 | 17.2 |
| | 100 | 11.2 | 10 | 10.5 | 12.6 | 10.4 | 10.8 | 18 |

Where, Substrate $S_1$ = Gram straw, $S_2$ = Pea straw, $S_3$ = Pearl millet cuttings; Concentration of extract (10mg/mL)

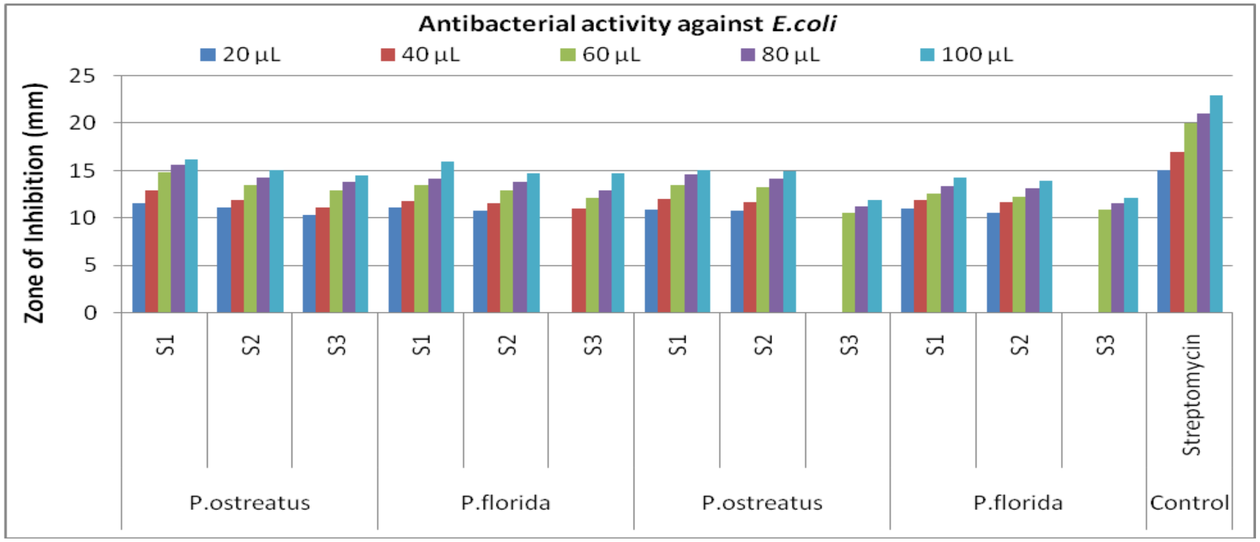
**Graph.1** Antibacterial activities of *Pleurotus* spp cultivated on different substrates



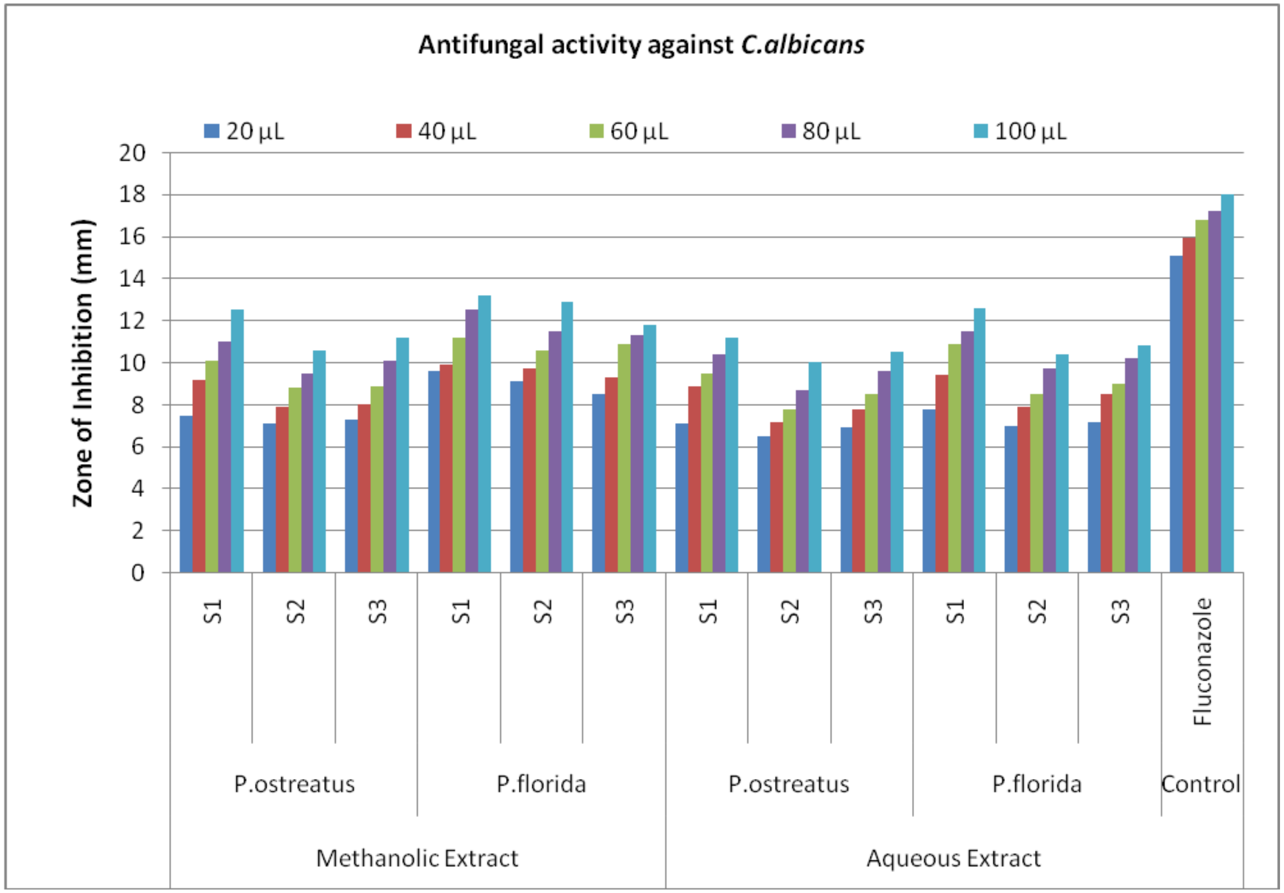
**Graph.2** Antibacterial activities of *Pleurotus* spp cultivated on different substrates




**Graph.3** Antibacterial activities of *Pleurotus* spp cultivated on different substrates



**Graph.4** Antibacterial activities of *Pleurotus* spp. cultivated on different substrates



Thillaimaharani *et al.*, (2013) reported antibacterial activity of different extracts of *P. florida* were tested against 8 human bacterial pathogens *E. coli*, *S. typhi*, *K. pneumoniae*, *V. parahaemolyticus*, *K. oxytoca*, *P. murabilus*, *V. cholarae* and *Streptococcus spp*. antibacterial activity of ethanol extract of *P. florida* was found Maximum (23 mm) against *Streptococcus spp* . and minimum 4 mm against *V. parahaemolyticus.* Antimicrobial activity against *E. coli* was found 11 mm. Akyuz and Kirbag (2009) reported the same results for 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).

Previous study of Menaga *et al.*, (2012) on antimicrobial activity of ethanolic extract of *P.florida* exhibited highest activity against *Pseudomonas spp*. and *Campylobacter spp.* whereas methanol extract showed higher activity against *E.coli, Salmonella typhi, Staphylococcus aureus*, *Camphylobacter sp.,* and *Vibrio sp.* aqueous extract also revealed high zone formation against *Vibrio sp* was 24±1.5 mm. Ethyl acetate and hexane extract showed highest antibacterial potency against *Staphylococcus aureus* and *Pseudomonas spp.*, respectively. Menaga *et al.*, (2012) concluded that methanol extract showed activity against *E.coli* (21 ± 0.9 mm), *Salmonella typhi* (20 ± 0.5 mm), *Staphylococcus aureus* (20 ± 0.4 mm), *Camphylobacter spp.* (19 ± 0.8 mm), *Bacillus spp.* (14 ± 0.5 mm), *Pseudomonas spp.* (8 ± 0.5 mm), *Klebsiella spp.* (12 ± 0.6 mm) and *Vibrio spp.* (20 ± 0.9 mm). In a previous study, Jonathan (2007) reported that the sporophore methanolic extract of *Pleurotus florida* showed activity in *E.coli* (13 mm), *Klebsiella spp.* (20 mm) and no activity against *Bacillus spp.*, *Pseudomonas* and *Proteus spp.* 30. Menaga *et al.*, (2012) reported the zone formation in *Pseudomonas spp.*, (20 ± 0.6 mm), *Salmonella spp.*, (20 ± 0.5 mm) and *Klebsiella pneumonae* (13 ± 0.8 mm) whereas ethanolic extract of mycelium showed zone formation in *Staphylococcus aureus* (16 mm), *Streptococcus mutans* (14 mm), *Escherichia coli* (12 mm), *Micrococcus luteus* (16 mm), *Bacillus subtilis* (9 mm) and no zone formation against *Pseudomonas aeruginosa*, *Salmonella abony*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Candida albicans*.

In conclusion, in present study mushroom obtained from substrate (Gram straw, Pea straw and Pearl millet cuttings) showed strong antimicrobial activity against Pathogenic microorganism. Moreover, mushroom species can be used as easily available source of natural antimicrobial agent for gram negative, gram positive bacteria and pathogenic fungi. Results showed that methanol is a good solvent for extraction. Used substrate can be used for commercial cultivation of mushroom.

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