**RESEARCH PAPER**

# Antimicrobial and Antioxidant Property of *Pleurotus Florida* on *Butea Monosperma* Flower Extract along with other Extracts

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**Abstract**

The present study has been undertaken to evaluate antimicrobial and antioxidant activity of methanolic BMF (*Butea monosperma* flower powder treated fruiting body), methanolic, chloroform and aqueous extracts of *Pleurotus florida.* In the DPPH (2,2-diphenylpicrylhydrazyl) radical scavenging activity aqueous, chloroform and methanolic extract all these three showed more or less similar DPPH scavenging effects with increasing concentration while methanolic BMF extract exhibited the greatest potency when compared to the ascorbic acid (AC) standard drug. Antimicrobial activity of methanolic BMF, methanolic, chloroform and aqueous extracts of *Pleurotus florida* was evaluate at the concentration 0, 312.5, 625, 1250, 2500 and 5000 µg/disk. In the present studies, BMF methanolic extract showed significant activity against *E. coli*, aqueous extract showed significant activity against *B. subtilis*, chloroform extract showed significant activity against *S. aureus*, methanolic BMF extract showed significantly higher activity against *B. subtilis*. In case of fungi methanolic BMF extract showed significantly higher activity against *C. albicans* and *A. niger.* The new findings of our study is that *Butea monosperma* flower methanolic BMF extract showed maximum inhibition zone against bacteria and fungi.

**Keywords:** Antimicrobial, antioxidant, potency, scavenging

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**Introduction:** Among edible mushrooms, *Pleurotus* (oyster mushroom) species have been utilized by human everywhere on the world for their dietary benefit, medical effects and other beneficial impacts. They inhibit tumour growth, modulate the inflammation and immune system, have antithrombotic actions and hypoglycemic, prevent hypertension and atherosclerosis, lower blood lipid concentrations and other actions. *Pleurotus florida* also known as white oyster mushroom is an comestible mushroom that becoming popular due to its cheap production cost, high nutritional values, and easy to cultivate. Fungal polysaccharides, derived from *Pleurotus* have shown multiple beneficial therapeutic action, including immunomodulation, anticancer, antimicrobial, hypocholesterolemic, and hypoglycemic actions (Hassan *et al*., 2022).

Antimicrobial drugs have long been used for prophylactic and therapeutic purpose. Unfortunately the recent increase in the occurrences of drug resistant bacterial strains is creating serious problems. Consequently, the antimicrobial activities of various antitumor polysaccharides from medicinal mushrooms are being re-evaluated in terms of their clinical efficacy. Such compounds would be expected to function by mobilizing the body's humoral immunity toward off viral, bacterial, fungal and protozoal infections resistant to current antibiotics. Different substances occurring in higher Basidiomycetes are effective against various kinds of viral, bacterial and parasitic infections, including AIDS (Hobbs, 1995; Wasser & Weis, 1999; Wang *et al*., 2004).

Antioxidants are substances that are available at low concentrations and remarkably prevent or delays oxidation of compounds. Antioxidants are effective due to they can donate their own electrons to reactive oxygen species (ROS) and neutralize the adverse effects of a latter. Antioxidant from our diet are important because its helps in engulfing ROS and neutralize the oxidative stress. The nutrient antioxidant deficiency is one of the causes of many degenerative and chronic diseases. When an antioxidant destroys a free radical, this antioxidant becomes oxidized. Thus, the antioxidant resources must be regularly replaced in the body (Sheriff *et al*., 2022). Phenolic compounds widely distributed in mushrooms which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. (Miller, 1996). The antioxidative and free radical scavenging properties of the phenolic content of mushroom methanolic extracts have been reported, suggesting possible protective roles of these compounds, due to their ability to capture metals, inhibit lipoxygenase and scavenge free radicals (Mau, Chang, Huang, & Chen, 2002).

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**Material and Methods**

**Preparation of mushroom extracts:** The fruiting body of *Pleurotus florida* were collected from mushroom house (Department of Botany, Dr. Harisingh Gour University Sagar). In order to facilitate drying in shade and prevent the loss of active phytoconstituents as well as any microbial growth, the fruiting body was periodically turned over. The dry materials were crushed into a coarse powder with a mechanical grinder and kept in an airtight container with labels for future research. On the basis of their polarity, different solvents (chloroform, methanol, and water) were consecutively extracted from the powdered mushroom material using the Soxhlet equipment. After the extraction process was finished, the mushroom material was treated with each subsequent solvent while the concentrated extracts were dried in a water bath (40–45°C). The dried crude extracts' weight and yield percentage were determined, transferred to airtight containers, and stored at 4°C in the refrigerator.

**Antibacterial activity**

The Zone Inhibition Method was used to assess the antibacterial activity (Kirby-Bauer method). The MHA plates were inoculated by spreading 100 µl of bacterial culture (adjusted to 0.5 McFarland Unit) and then 10 µl of various concentrations (ranging from 0 to 500 mg/ml) into the wells. Each plate had a single well that was filled with solvent alone as a vehicle control and Ciprofloxacin (10 µg) as a positive control. Bacterial plates were incubated at 37 °C for 24 hours. Measurements and records were made of the clear zone formed around the well.

**Antifungal activity**

Zone Inhibition Method was used to test the antifungal activity (Kirby-Bauer method). The SDA plates were inoculated with 100 µl of fungal culture (adjusted to 0.5 McFarland Unit) before the discs containing 10 µl of various concentrations (0 to 500 mg/ml) were added. As a vehicle control, one disc in each plate was loaded only with solvent, and one disc (250 g) was used as a positive control. Fungal plates were incubated at 37 °C for 24 hours. Measurements and records were made of the clear zones formed around the disc.

**In-vitro antioxidant activity:** Methanolic, Aqueous, chloroform and methanolic BMF (*Butea monosperma* floral powder) extracts of *Pleurotus florida* were evaluated for in-vitro antioxidant activity using following methods:

**Total Phenolic content:** Phenols and phosphomolybdic acid in the Folin-Ciocalteu reagent react to produce a blue complex in an alkaline media that can be measured at 725 nm on a microplate reader. The modified Folin-Ciocatleu technique was used to determine the amount of total phenolics in the extracts (Barros et al., 2007; Barros et al., 2007a). Pipette out an aliquot of each sample in sequence, and then add distilled water to the volume until it reaches 500 µl. Folin-Ciocalteu reagent in the volume of 250 µl was added, properly mixed, and incubated for 5 minutes at room temperature. The mixture was mixed with 1250 µl of 20% sodium carbonate solution, incubated for 1 hour in the dark, and then the absorbance was measured at 725 nm.

**DPPH (1,1-Diphenyl-2-Picryl Hydrazyl) free radical scavenging activity:** When an antioxidant component that may donate hydrogen combines with the DPPH radical, the radical is reduced. When an antioxidant reacts with DPPH, diphenyl picryl hydrazine is synthesized. The transformation of purple to light yellow can be used to identify this. 500 µl of methanol were combined with various concentrations of the extract and the standard drug ascorbic acid (AC) (20, 40, 60, 80, and 100 µg/ml), and 100 µl DPPH was added (prepared in methanol). 30 minutes were given for the test solutions to stand at room temperature. The sample's absorbance was determined at 517 nm. As a control, reagent solution without test sample was used. The formula was used to calculate the radical scavenging activity.

$$\% \text{ radical scavenging activity} = \frac{(\text{Abs of control} - \text{Abs of sample})}{(\text{Abs of control})} \times 100$$

**Nitric oxide radical scavenging activity:** At physiological pH, sodium nitroprusside in aqueous solution spontaneously produces nitric oxide, which reacts with oxygen to form nitrite ions with an estimated wavelength of 546 nm. With 0.1 M phosphate buffer, different extract concentrations (20, 40, 60, 80, and 100 g/ml) were prepared up to 3 ml (pH 7.2). Each tube has 1 ml of sodium nitroprusside (5 mM, formulated in buffered saline pH 7.2). At room temperature, the reaction mixture was incubated for 30 minutes. After 30 min, 1.5 ml of the aforementioned solution and 1.5 ml of Griess reagent were combined (Kumar et al., 2008).

$$\% \text{ radical scavenging activity} = \frac{(\text{Abs of control} - \text{Abs of sample})}{(\text{Abs of control})} \times 100$$

**Result and Discussion:**

**Antimicrobial activity**: The present study has been undertaken to evaluate antimicrobial activity of methanolic BMF (*Butea monosperma* flower powder treated fruiting body), methanolic, chloroform and aqueous extracts of *Pleurotus florida* at the concentration 0, 312.5, 625, 1250, 2500 and 5000 µg/disk against some pathogenic bacteria *E coli, S. aureus, B. subtilis* as well as some fungal strains such as *C. albicans, A. niger*. Results of the antimicrobial examination of various extract concentrations have been compiled in tables. In the present studies, methanolic BMF extract showed significant activity

against *E. coli* 6.5±0.41 and 8.5±0.41 mm at 2500 and 5000 µg/disk respectively and 8.5±0.41 mm at 5000µg/disk with methanolic extract while no activity was observed with aqueous and chloroform extract (Table 1). In case of *S. aureus* chloroform extract showed significant activity 9 ±0.00, 9 ±0.00, 8.5±0.41, 9.5±0.41 and 10 ±0.00 mm at 312.5, 625, 1250, 2500 and 5000 µg/disk respectively (Table 2). In case of *B. subtilis* all the four extracts aqueous, methanolic BMF, chloroform and methanolic extracts showed antimicrobial activity. But significantly high zone of inhibition was observed 8±0.00 mm at 5000 µg/disk with aqueous extract, 9±0.00 mm at 5000 µg/disk with methanolic BMF extract, 7±0.00 mm at 5000 µg/disk with chloroform extract, 6±0.00 mm at 5000 µg/disk with methanolic extract (Table 3).

Results of anti-fungal activity of *Pleurotus florida* are compared in table 4 and 5. All the four extracts aqueous, methanolic BMF, chloroform and methanolic extracts showed anti-fungal activity against *C. albicans*. But significantly high zone of inhibition was observed in 7±0.00 mm at 5000 µg/disk with aqueous extract, 7.5±0.41 mm at 5000 µg/disk with methanolic BMF extract, 6.5±0.41 mm at 5000 µg/disk with chloroform extract, 6.5 ±0.41 mm at 5000 µg/disk with methanolic extract. In case of *A niger* extracts aqueous, methanolic BMF, chloroform extracts showed similar zone of inhibition 9±0.00 mm at 312.5, 625, 1250, 2500 µg/disk. Methanolic extract showed 8.5 ±0.00 mm at 312.5, 625, 1250 µg/disk zone of inhibition. Significantly high zone of inhibition was observed with methanolic BMF extract 10±0.00 mm at 5000 µg/disk. The new findings of our study are that *Butea monosperma* flower methanolic BMF extract of *Pleurotus florida* showed significantly good results then other extracts.

Mushrooms need antibacterial and antifungal compounds to survive in their natural environment. Therefore, antimicrobial compounds could be isolated from many mushroom species and could be of benefit for humans (Zjawiony, 2004). Macrofungi such as mushrooms contain unique antimicrobial compounds that include; flavonoids, alkaloids, tannins, peptides, proteins, steroids, terpenoids, and anthraquinones (Alves *et al*., 2012). Several studies have found that most mushroom extracts antimicrobial potentials are largely dependent on the mushroom strains, vegetative forms, cultivation conditions, methods of extract preparation, evaluation and results interpretation (Canli *et al*., 2015). *Pleurotus* species have different type of phytochemical compounds and this explains the variation noticed in their antimicrobial activity. A previous study of Menaga *et al*., (2012) on the antimicrobial activity of the ethanolic extract of *Pleurotus florida* exhibited the highest activity against *Pseudomonas* sp. and *Campylobacter* sp., whereas the methanol extract showed higher activity against the *E. coli, Salmonella typhi*, *Staphylococcus aureus*, *Camphylobacter* sp., and *Vibrio* sp. In aqueous extract, also revealing a high zone formation against *Vibrio* sp. of 24 mm. It should be pointed out that the majority of the extracts present an antimicrobial activity against the tested species, which is in agreement with the results previously reported by research group concerning *E. coli, K. pneumonia*, and *P. aeruginosa* (Barros *et al*., 2008). Nevertheless, a recent study describes methanolic extracts obtained from mushroom as having high antibacterial activity against *E. coli*, assessed by the disc diffusion method (Ozturk et al., 2011).

**Table 1: Antibacterial activity of *Pleurotus florida* extract against *E. coli***

| Amount (µg/disk) | Aqueous extract | BMF methanolic extract | Chloroform extract | Methanolic extract |
|---|---|---|---|---|
| | Zone of inhibition (mm) | | | |
| PC | 39.5±0.41 | 37±0.82 | 37.5±1.22 | 37.5±0.41 |
| 0 | NI | NI | NI | NI |
| 312.5 | NI | NI | NI | NI |
| 625 | NI | NI | NI | NI |
| 1250 | NI | NI | NI | NI |
| 2500 | NI | 6.5±0.41 | NI | NI |
| 5000 | NI | 8.5±0.41 | NI | 8.5±0.41 |

Values are mean of two replicates ± SE.
NI=No inhibition

**Table 2: Antibacterial activity of *Pleurotus florida* extract against *S. aureus***

| Amount (µg/disk) | Aqueous extract | BMF methanolic extract | Chloroform extract | Methanolic extract |
|---|---|---|---|---|
| | Zone of inhibition (mm) | | | |
| PC | 36±0.82 | 34.5±1.22 | 35±0.00 | 33.5±0.41 |
| 0 | NI | NI | NI | NI |
| 312.5 | NI | NI | 9±0.00 | NI |
| 625 | NI | NI | 9±0.00 | NI |
| 1250 | NI | NI | 8.5±0.41 | NI |

| | | | | |
|---|---|---|---|---|
| 2500 | NI | NI | 9.5±0.41 | NI |
| 5000 | NI | NI | 10±0.00 | NI |

Values are mean of two replicates ± SE.
NI=No inhibition

**Table 3: Antibacterial activity of *Pleurotus florida* extract against *B. subtilis***

| Amount (µg/disk) | Aqueous extract | BMF methanolic extract | Chloroform extract | Methanolic extract |
|---|---|---|---|---|
| | Zone of inhibition (mm) | | | |
| PC | 30±0.82 | 29.5±0.41 | 29.5±0.41 | 28.5±0.41 |
| 0 | NI | NI | NI | NI |
| 312.5 | 7±0.00 | 8±0.00 | 6±0.00 | NI |
| 625 | 7±0.00 | 8.5±0.41 | 6.5±0.41 | 6.5±0.41 |
| 1250 | 7±0.00 | 8.5±0.41 | 6.5±0.41 | 6.5±0.41 |
| 2500 | 7.5±0.41 | 8.5±0.41 | 6.5±0.41 | 6.5±0.41 |
| 5000 | 8±0.00 | 9±0.00 | 7±0.00 | 6±0.00 |

Values are mean of two replicates ± SE.
NI=No inhibition

**Table 4: Antifungal activity of *Pleurotus florida* extract against *C. albicans***

| Amount (µg/disk) | Aqueous extract | BMF methanolic extract | Chloroform extract | Methanolic extract |
|---|---|---|---|---|
| | Zone of inhibition (mm) | | | |
| PC | 16.5±0.41 | 15±0.82 | 15±0.00 | 14±0.00 |
| 0 | NI | NI | NI | NI |
| 312.5 | NI | NI | 3±2.45 | NI |
| 625 | NI | 6.5±0.41 | 6±0.00 | 6±0.00 |
| 1250 | 6.5±0.41 | 7±0.00 | 6.5±0.41 | 6.5±0.41 |
| 2500 | 7±0.00 | 6.5±0.41 | 6.5±0.41 | 6.5±0.41 |
| 5000 | 7±0.00 | 7.5±0.41 | 6.5±0.41 | 6.5±0.41 |

Values are mean of two replicates ± SE.
NI=No inhibition

**Table 5: Antifungal activity of *Pleurotus florida* extract against *A. niger***

| Amount (µg/disk) | Aqueous extract | BMF methanolic extract | Chloroform extract | Methanolic extract |
|---|---|---|---|---|
| | Zone of inhibition (mm) | | | |
| PC | 10±0.00 | 10±0.00 | 10±0.00 | 10±0.00 |
| 0 | NI | NI | NI | NI |
| 312.5 | 9±0.00 | 9±0.00 | 9±0.00 | 8.5±0.41 |
| 625 | 9±0.00 | 9±0.00 | 9±0.00 | 8.5±0.41 |
| 1250 | 9±0.00 | 9±0.00 | 9±0.00 | 8.5±0.41 |
| 2500 | 9±0.00 | 9±0.00 | 9±0.00 | 9±0.00 |
| 5000 | 9±0.00 | 10±0.00 | 9±0.00 | 9.5±0.41 |

Values are mean of two replicates ± SE.
NI=No inhibition

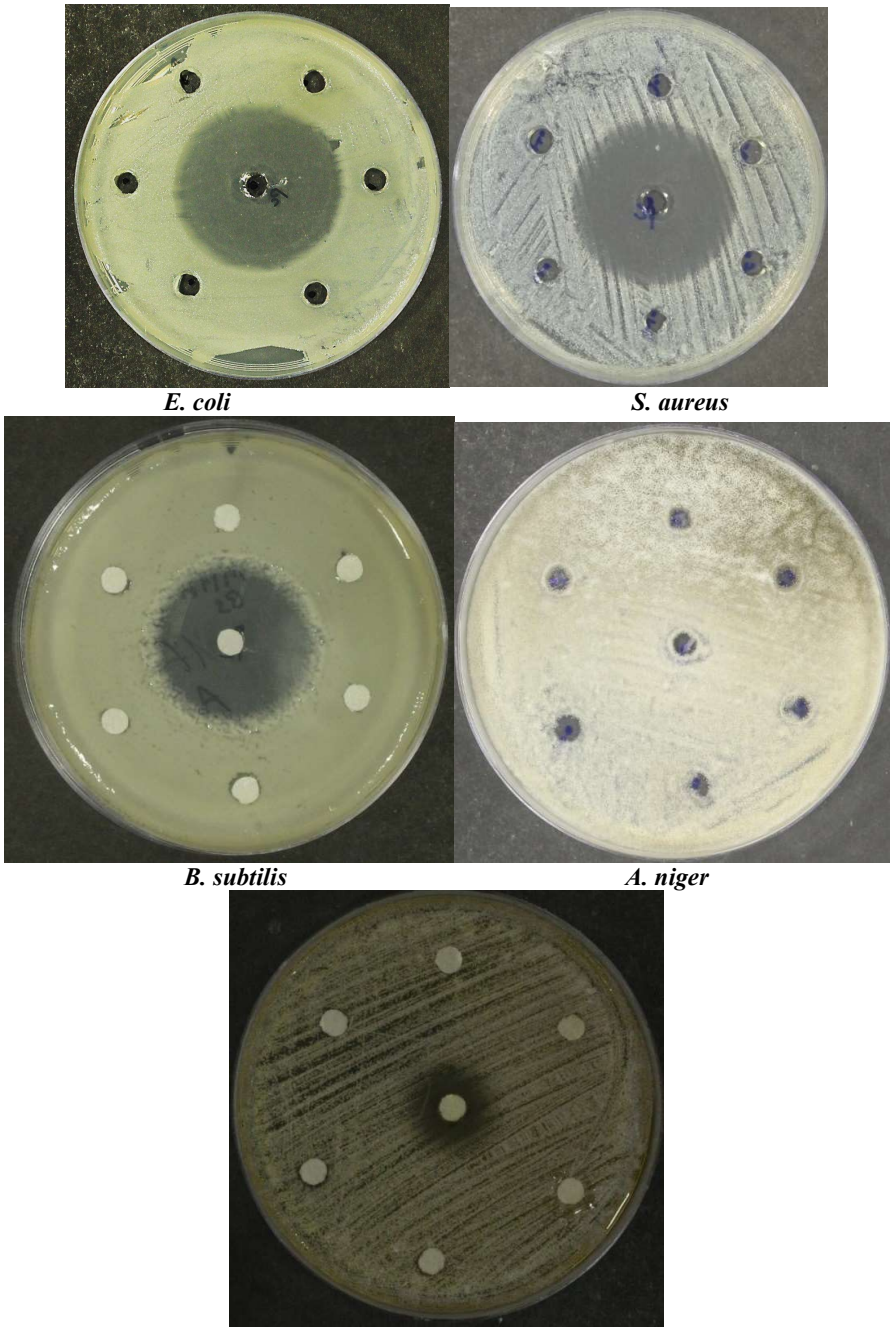
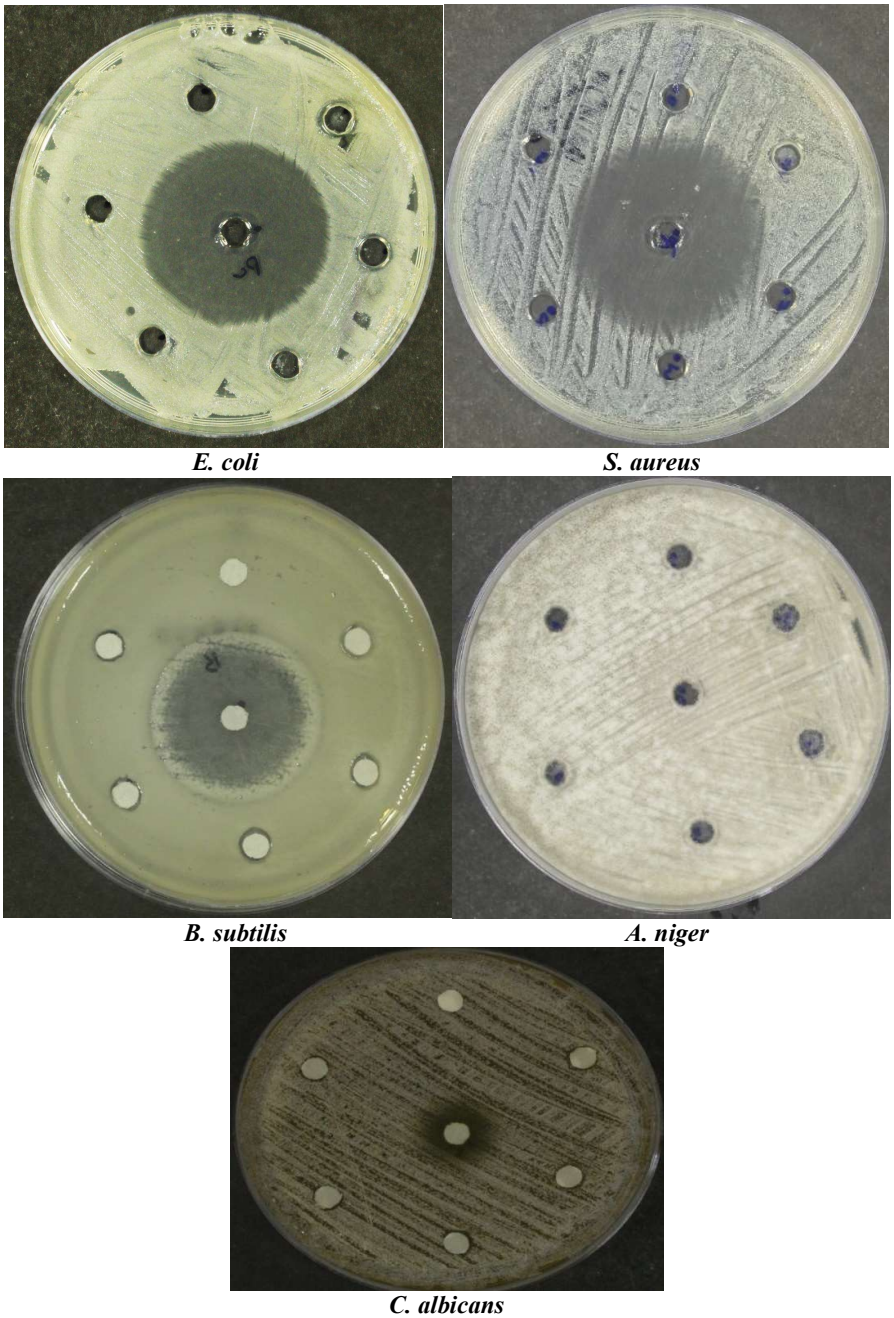



**Figure 1: Photograph showing antimicrobial activity of aqueous extract of *Pleurotus florida***

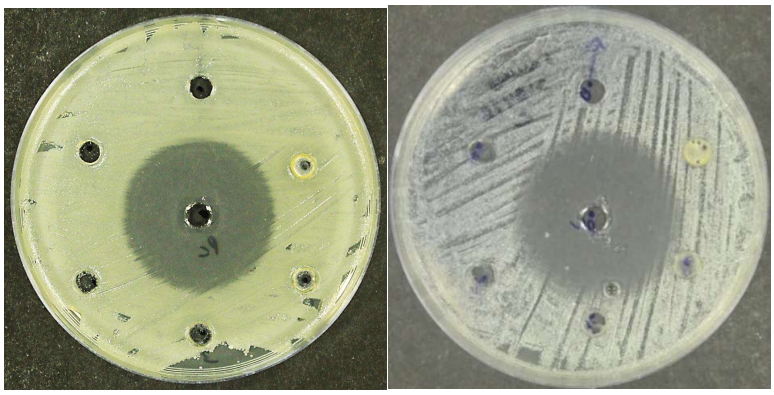


***E. coli*** ***S. aureus***

***B. subtilis*** ***A. niger***

***C. albicans***

**Figure 2: Photograph showing antimicrobial activity of methanolic BMF extract of *Pleurotus***



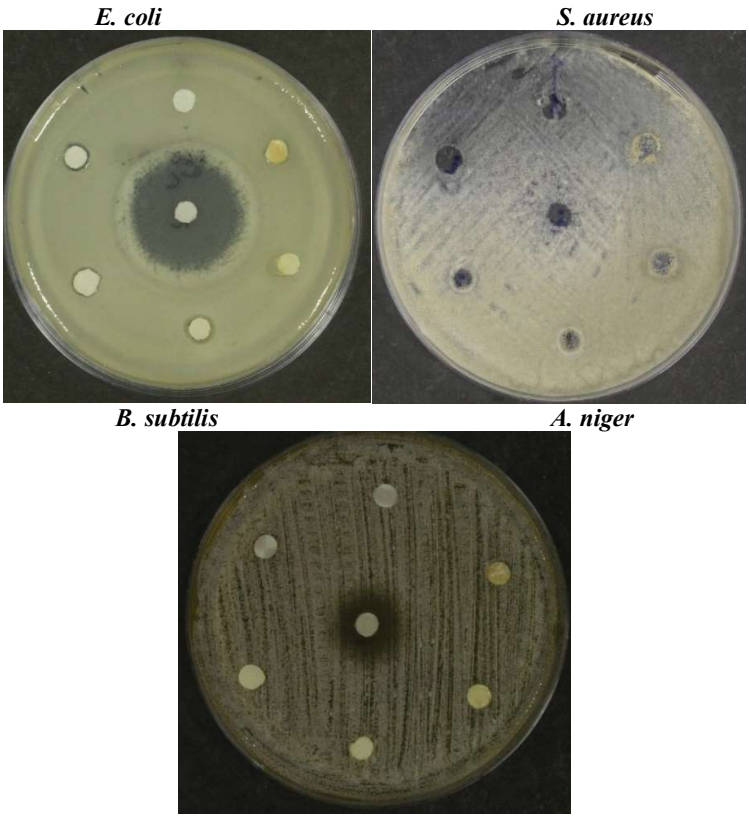
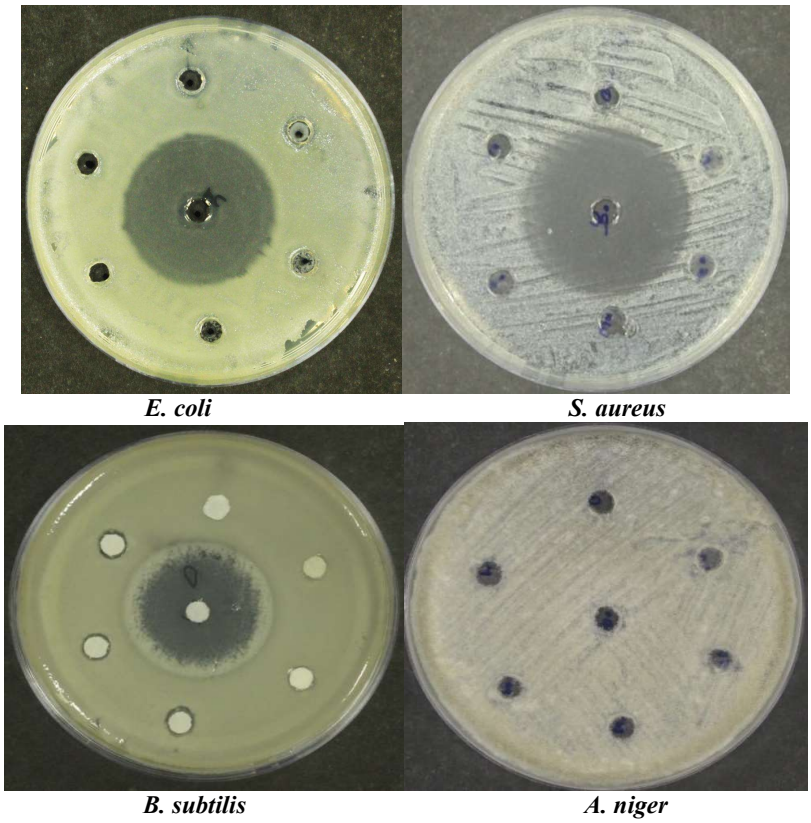



**Figure 3: Photograph showing antimicrobial activity of chloroform extract of *Pleurotus florida***



***C. albicans***

**Figure 4: Photograph showing antimicrobial activity of methanolic extract of *Pleurotus florida***

**In-vitro Antioxidant activity**

The objective of this research was to evaluate the antioxidant activity of chloroform, methanolic, aqueous and methanolic BMF (*Butea monosperma* flower treated fruiting body) extracts of *Pleurotus florida* using total phenolic content, DPPH radical scavenging, and nitric oxide radical scavenging methods.

**Total Phenolic content**

The total phenolic content (TPC) of chloroform, methanolic, aqueous and methanolic BMF extract of *Pleurotus florida* was found to be 7.30±1.14, 30.50±0.62, 8.99±1.20 and 60.63±1.13 µg/mg of gallic acid equivalent respectively when measured by Folin-Ciocalteau reagent. The highest concentration of phenolic contents was measured in methanolic BMF and methanolic extract (without treated) 60.63 µg/mg and 30.50 µg/mg whereas lowest found to be in chloroform extract 7.30 µg/mg.

The findings of the study are intended to demonstrate the phytochemical composition of *Pleurotus* species. The *Pleurotus* species are therefore used as a complementary source of medicine to treat disorders caused on by microorganisms. The levels of phytochemicals in the various *Pleurotus* species are also useful. The current research generally supports their inclusion in human diets as a source of possible phytochemicals.

Because of their ability to scavenge free radicals due to their hydroxyl groups, phenols are crucial components of plants. Consequently, the phenolic content of mushrooms may directly contribute to their antioxidant function (Tosun *et al*., 2009). Several polarity solvents were used to extract phytochemical compounds with various chemical structures. Many studies on the qualitative composition of plant extracts have shown that the extracts produced using polar solvents contain significant amounts of phenols. The maximum antioxidant activity is seen in extracts with the highest phenol concentrations (Čanadanović-Brunet *et al*., 2008). Similar trends have been documented by Azieana *et al*., (2017), who found that wild edible mushrooms had the highest phenolic and flavonoid content.

High concentrations of phenolic compounds, which can act as hydrogen donors or electron donors and have the ability to chelate metal ions, are present in mushroom extracts. These compounds are composed primarily of one or more aromatic rings bearing one or more hydroxyl groups. The phenolics may have stronger antioxidant action because they contain more hydroxyl groups (Rangkadilok *et al*., 2007).

In the present study, total phenolics of *Pleurotus florida* varied from 7.30 to 60.63 µg gallic acid /mg (Table 6) when compared to the reported quantities in other mushrooms like *Coriolus versicolor* (23.28 mg/g), *Ganoderma lucidum* (47.25 mg/g), *Ganoderma tsugae* (51.28 mg/g), and *G. lucidum* (antler) (55.96 mg/g), (Mau, Lin, & Chen, 2002) and *P. ostreatus* (1.8 mg/g) (Palacios *et al*., 2011).

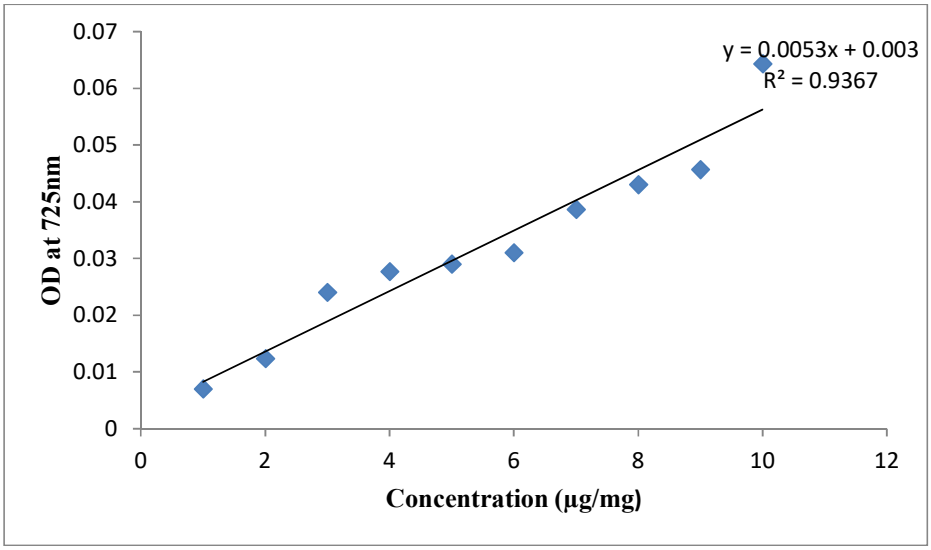



**Figure 5: Standard Gallic acid calibration curve**

**Table 6: Total phenolic content in different extracts of *Pleurotus florida***

| **Extract** | **Gallic acid equivalent phenolic (µg/mg) of extract** |
|---|---|
| Chloroform | 7.30±1.14 |
| Methanolic | 30.50±0.62 |
| Aqueous | 8.99±1.20 |
| Methanolic (BMF) | 60.63±1.13 |

BMF- *Butea monosperma* flower powder

**Inhibition of DPPH radical scavenging assay**

In the DPPH radical scavenging activity aqueous extract, chloroform extract and methanolic extract all these three showed more or less similar DPPH scavenging effects with increasing concentration while methanolic BMF extract exhibited the greatest potency when compared to the ascorbic acid (AC) standard drug. The aqueous extract showed antioxidant activity (35.53, 38.87, 44.77, 69.39 and 72.21 %) inhibition while chloroform extract showed (34.92, 47.49, 55.67, 68.43 and 76.34 %) inhibition and methanolic extract showed (52.59, 57.08, 65.70, 74.76 and 76.69 %) inhibition. However, methanolic BMF extract showed most potent antioxidant activity (53.21, 60.42, 65.96, 75.73 and 82.32 %) inhibition at the concentration of 20, 40, 60, 80 and 100 µg/ml respectively. $IC_{50}$ values for aqueous, chloroform, methanolic, methanolic BMF extract and Ascorbic acid were found 67.34, 222.22, 59.48, 190.21 and 120.57 µg/ml respectively (Table 7; Figure 6).

Moreover, polysaccharides found in both edible and therapeutic mushrooms have the highest capacity to scavenge hydroxyl radicals and inhibit the growth of tumour cells (Chen *et al*., 2015, Zeng *et al*., 2018). The ability of mushroom polysaccharides to neutralise ROS and reduce their detrimental effects on human bodies is one of its useful features. Furthermore, the functional group -COOH may be responsible for the substantial antioxidant activity found in mushroom polysaccharides with high uronic acid levels (Li *et al*., 2016). The antioxidant activity of some polysaccharides is linked to functional groups that function as effective electron or hydrogen donors. Many food compositions may utilize this functional property as an ingredient with increased antioxidants (Khan *et al*., 2015). The polysaccharide produced from oyster mushrooms, however, demonstrated potent DPPH radical and superoxide anion radical scavenging action, according to numerous investigations (Piska *et al*., 2017). The antioxidant properties of fruits, vegetables, and mushrooms are thought to be greatly influenced by polyphenols (Ferreira *et al*., 2007).

According to Sreeyan and Rao (1996), the antioxidant combines with the stable free radical DPPH and converts it into the compound 1, 1- diphenyl-2-picryl hydrazine. The reducing ability of an extract may be a significant indicator of its likely antioxidant capacity, and the reducing properties of antioxidants are typically linked to the presence of reductones, such as ascorbic acid (Liu, Ooi and Chang, 1997).

**Table 7: Effect of *Pleurotus florida* extract(µg/ml) in DPPH radical scavenging assay**

| **Concentration (µg/ml)** | **Extract** | | | | |
|---|---|---|---|---|---|
| | **AC** | **Aqueous** | **Methanolic BMF** | **Chloroform** | **Methanol** |
| | **% Inhibition** | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 20 | 57.78±4.03 | 35.53±1.3 | 53.21±3.13 | 34.92±3.83 | 52.59±5.01 |
| 40 | 61.65±0.63 | 38.87±2.16 | 60.42±0.76 | 47.49±4.12 | 57.08±6.17 |
| 60 | 67.02±2.75 | 44.77±4.23 | 65.96±5.24 | 55.67±5.59 | 65.70±3.05 |
| 80 | 77.57±2.68 | 69.39±0.3 | 75.73±2.75 | 68.43±1.78 | 74.76±6.82 |
| 100 | 87.86±0.85 | 72.21±1.53 | 82.32±2.6 | 76.34±3.03 | 76.69±4.86 |
| $IC_{50}$ | 120.57 | 67.34 | 190.21 | 222.22 | 59.48 |

**AC: Ascorbic acid**

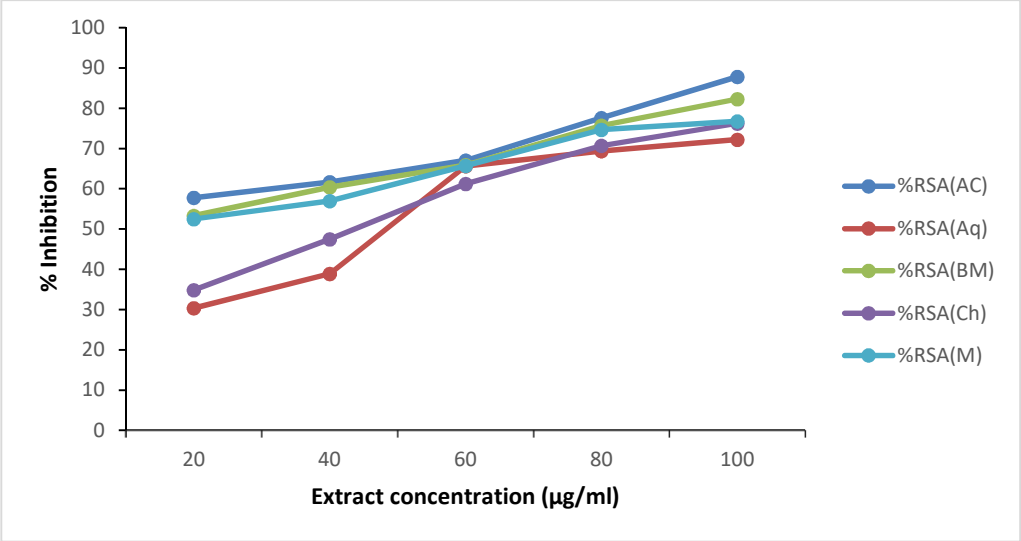



**Figure 6: Effect of *Pleurotus florida* extract (µg/ml) in DPPH radical scavenging assay**

**Nitrous oxide radical scavenging assay**

The results of the current investigation demonstrated that all of the extracts significantly reduced the production of free radicals in a dose-dependent manner. Methanolic BMF extract showed most potent activity (14.12, 35.23, 40.47, 42.94 and 88.79 %) inhibition as compared to aqueous extract (16.3, 17.47, 32.75, 36.54 and 71.18 %) inhibition, chloroform extract (10.04, 14.85, 48.76, 50.8 and 60.55 %) inhibition and methanolic extract (15.87, 21.25, 38.14, 50.8 and 69.29 %) inhibition, while ascorbic acid (standard drug) showed (17.03, 42.65, 46.72, 56.77 and 92.29 %) inhibition at the concentration of 20, 40, 60, 80 and 100 µg/ml respectively. $IC_{50}$ values for aqueous, chloroform, methanolic, methanolic BMF extract and Ascorbic acid were found 234.23, 50.44, 120.15, 230.09 and 430.80 µg/ml respectively (Table 8; figure 7).

Nitric oxide is an unstable free radical that is involved in a broad range of biological functions and is associated with a variety of diseases. Nitric oxide can be harmful in high concentrations, thus preventing its overproduction is a key objective. Nitric oxide interacts with oxygen to form the stable products nitrate and nitrite through intermediaries (Wang *et al*., 2005). *P. florida* mushroom displayed a dose-dependent percentage of inhibition in the scavenging experiments, irrespective of the dosage range employed. The methanolic BMF extract therefore demonstrated strong antioxidant capacity, which may indicate its therapeutic potential for a variety of diseases. The current findings demonstrated that the methanolic BMF extract of the *P. florida* mushroom had a comparatively high reduction power and performed well in the DPPH radical scavenging assay.

When compared to other mushrooms like *Agaricus bisporus*, *Volvariella volvaceae*, *Calocybe indica*, and *Hybsizus ulmarius*, the radical scavenging activity of *P. ostreatus* is observed to be higher (6mg/ml) (Ramkumar *et al*., 2010). The methanolic extract of *P. florida* has stronger chelating activity against ferrous ion as compared to *P. ostreatus* mushroom (Mohamed Imran *et al*., 2011). As compared to other commercial and therapeutic mushrooms, the methanolic extract of *P. florida* appears to have superior reducing power. The fruit bodies of *Pleurotus* mushrooms have been found to have a significant amount of antioxidant elements, including phenolic compounds, flavonoids, carotenoids, and vitamins C and E. (Jayakumar *et al*., 2011).

**Table 8: Effect of *Pleurotus florida* extract (µg/ml) in Nitrous oxide scavenging assay**

| **Concentration (µg/ml)** | **Extract** | | | | |
|---|---|---|---|---|---|
| | **AC** | **Aqueous** | **Methanolic BMF** | **Chloroform** | **Methanol** |
| | **% Inhibition** | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 20 | 17.03±2.52 | 16.3±4.05 | 14.12±1.05 | 10.04±4.97 | 15.87±1.91 |
| 40 | 42.65±2.27 | 17.47±1.97 | 35.23±2.4 | 14.85±4.21 | 21.25±2.49 |
| 60 | 46.72±0.67 | 32.75±0.91 | 40.47±4.15 | 48.76±1.29 | 38.14±2.67 |
| 80 | 56.77±0.25 | 36.54±5.93 | 42.94±3.48 | 50.8±1.72 | 50.8±2.17 |
| 100 | 92.29±0.81 | 71.18±1.9 | 88.79±0.63 | 60.55±4.6 | 69.29±2.53 |
| $IC_{50}$ | 430.80 | 234.23 | 230.09 | 50.44 | 120.15 |

**AC: Ascorbic acid**

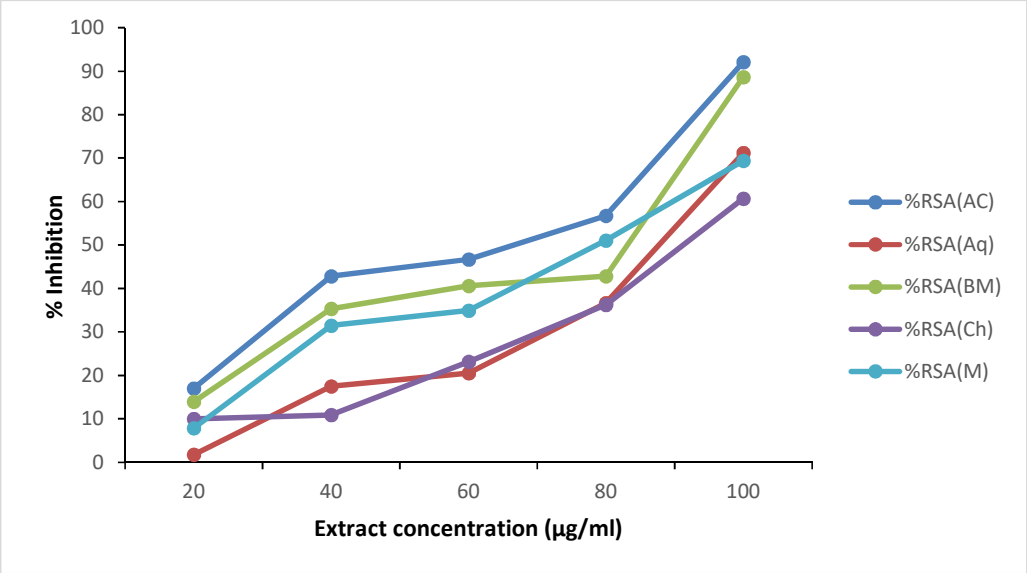



**Figure 7: Effect of *Pleurotus florida* extract (µg/ml) in Nitrous oxide scavenging assay**

**Statistical analysis**

Mean values and standard error of the mean were used to express the results. The IC50 value was determined using a linear regression analysis.

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