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Antibacterial Activities of *Pleurotus ostreatus* and *Pleurotus djamor* Against Selected Bacterial Pathogens

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ABSTRACT

Mushrooms are the fruit bodies of macrofungal and it is consumed as food by many people across the world. The cultivation of mushrooms is on the increase as it now attracts many consumers because of its medicinal properties apart from their well known nutritional benefits. This study was aimed to determine the antibacterial activities of *Pleurotus ostreatus* and *Pleurotus djamor* against some bacterial pathogens. Mushroom culture of *Pleurotus ostreatus* and *Pleurotus djamor* were collected from Myco-Farms Ltd, Benin City, Edo State, Nigeria while *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* isolates were obtained from Microbiology Department, Federal University of Technology, Owerri. Hot water and Whatman disk filter method was used to get extract from *Pleurotus ostreatus* and *Pleurotus djamor* . Antibacterial assay was determined using Mueller Hinton agar after 24H of incubation at 37^0C. The results revealed that *P. ostreatus* zone of inhibitions measured 1.83±0.15 cm, 1.70±0.10 cm and 0.93±0.15 for *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* respectively at a concentration of 100mg/ml of the extract. While the extract of *Pleurotus djamor* had inhibition zone of 2.33±0.57cm, 1.93±0.15cm and 0.8±0.10cm for *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* respectively. The levels of antibacterial activities of these species of mushrooms showed a significant difference ($P<0.05$) and a possible therapeutic potentials that could be used against common bacterial infections of man. Hence, Pharmacological and research institutions should consider *P. ostreatus* and *P djamor* as a therapeutic source for drug production.

Keywords: Antimicrobial activities, *Pleurotus ostreatus, Pleurotus djamor*.

INTRODUCTION

Mushrooms are the fruit bodies of macrofungal (Nwoko *et al*., 2018) and it's consumed as food by many people across the world. The cultivation of mushrooms is on the increase as it now attracts many consumers. Mushrooms are low in energy and fat content with appreciable amounts of vitamins, minerals, dietary fibre and protein (Cheung and Cheung, 2005) which made it a good meal for many health and diet conscious people. Mushrooms when consumed offer lots of nutritional and health benefits (Kratika, 2018), but mushroom consumption is beyond nutritional purpose. Mushrooms aid healing and the promotion of good health as they are good sources of phytochemicals, nutrients and minerals (Okwulehie and Ogoke, 2013). Mushrooms like many other fungi reproduce when two sexually compatible hyphae got fused to produce many spores. These spores develop into a mushroom when they fall on favourable environment (like damp/moist soil or decaying wood surfaces.

In Asia, about 966 edible mushrooms are recognized in China alone. Out of these, 576 are of therapeutic types and are used to treat different diseases (Dai *et al*., 2009). Some of them have been prominent, sold or have

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been exported to different countries over the world for years.

Despite the huge diversity of antibacterial compounds available, bacterial resistance to first choice antibiotics has been on the increase over many years. Example is *Acinetobacter* spp. with increasing resistance to carbapenems and Colistin (Kempf and Rolain, 2012), and also *Pseudomonas* spp. which have shown and manifested resistance to aminoglycosides, carbapenemics and/or cephalosporins.

In the recent years, high scale usage of synthetic and industrially produced antibiotics has led to the emergence of multi-drug resistance pathogens. These commercial antibiotics have become a threat to the world at large due to the emergence of resistant bacteria. Hence, there is need for natural antimicrobial agents. For this purpose, the antimicrobial properties of many natural compounds from a wide variety of plant species have been assessed (Karuppusamy, 2009).

In this research, the antibacterial activities of *Pleurotus ostreatus* and *Pleurotus djamor* were investigated against *Staphylocucos aureus*, *Escherichia coli* and *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

Substrates Used

Sawdust were obtained from saw mill. The sterilized sawdust was used as substrate to culture the mushroom species.

Spawn/Culture Collection

The mushroom culture of *Pleurotus ostreatus* and *Pleurotus djamor* were collected from Myco-Farms Ltd, Benin City, Edo State, Nigeria and were cultivated as edible mushrooms.

Mother Spawn Preparation

Spawn was prepared by using method of Shazia and Sardar, 2022 with slight modifications. One kg of Sorghum grains was cooked by boiling for 40 minutes. It was thereafter washed in running tap water. The washed grain was drained and 2 g limestone and 8 g gypsum as sources of calcium were added and properly mixed. Then, the mixed grains were filled in poly propylene bags of 1 kg capacity and sterilized in an autoclave at 121^{o}C for 1-1½hours and allowed to cool. After cooling, the Poly propylene bag was inoculated with freshly prepared mycelium culture (from previously prepared PDA plate). This was incubated at 25^{o}C for two weeks in an incubator and mycelia growth by spreading was observed.

Cultivation for fructification

The Sawdust substrate was prepared by addition of powdered limestone and rice brown as supplement. This was packed into the nylon bags and sterilized using the autoclave at 121^{0}C for 1-1½hours. These were allowed to be cooled and the spawn were aseptically inoculated into the sterilized substrates. These were kept in the dark room for two weeks to achieve good colonization of the substrates by the mushrooms. After two weeks (about 14 days) in the dark room, checking for development of mushroom clusters was carried out to check for colonization of the substrate by the mushroom mycelium. These were transferred to shelves in the growth room (production house) where there is proper ventilation and light. As they mature (with the cap tight on the stalk, they were picked gently by holding the basal region of mushroom stalk with fingers, twisting and breaking them carefully from the substrate as recommended by Shazia and Sardar, 2022.

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Preparation of the Mushroom Extract

The extract of *Pleurotus streatus* and *Pleurotus djamor* mushrooms was obtained hot water extraction process. Dried mushroom bodies of *P. ostreatus* and *P djamor* distinctively were reduced to powder and 0.7 grams of powder were soaked in 10 mL of sterile distilled water and boiled to obtain the extract. This was cooled and kept in refrigerator at 4.5° C for 48 hours. The obtained extract was sterilized by filtration using Whatman disk filter of 0.2 µm caliber and was sterilized using the autoclave at 121^0C for 15 minutes. This sterile extract was used for screening of antibacterial activities.

Source of Bacteria for the Antimicrobial Assays

In this experiment, three bacterial strains were used for antimicrobial assay. These bacteria includes: *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa.* The preserved bacterial isolates were obtained from the Federal University of Technology, Owerri, Microbiology Department. These microorganisms were maintained on nutrient agar.

Antibacterial Assay

Agar well diffusion techniques as described by Tendencia 2004 were adopted in this study. Mueller Hinton Agar plates (MHA oxoid) medium were inoculated with 0.1 mL of an over-night broth culture of each bacterial isolates (Equivalent to 3 x 107cfu/ml) aseptically in sterile Petri-dish. These seeded plates were rocked to achieve uniform distribution of isolates across the surface and allowed to set. Wells were made on the plates using standard sterile cork borer of 6 mm diameters and equal volumes of the extract (which is 100µl of 25% solution in water) were transferred into the well with the aid of micropipette. The experiments were carried out in triplicate. The plates were allowed to stand for one hour at room temperature to allow proper diffusion of the extract. The plates were incubated at 37°C for 24 hours until marked decline in the potency of the mushrooms extract to inhibit the growth of the test isolates was observed. Zone of inhibitions were measured in centimeter (cm) and the average values were calculated and recorded accordingly.

RESULTS AND DISCUSSION

Result of the Antibacterial Activities of Mushroom Samples Produced in the Study

The result of the antibacterial activities of *P. ostreatus* is shown on Table 1 below. A good antibacterial activity was observed among the test organisms with *P. aeruginosa* taking the lead, followed by E. *coli*.

Table 1: Antibacterial activities of *P. ostreatus* on bacterial isolates

Bacteria	Antibacterial activities zone of inhibition Mean±Std	Streptomycin (control) Mean±Std
Pseudomonas aeruginosa	1.8333 ± 0.1527^a	2.6333 ± 0.1527^a
Escherichia coli	1.7000 ± 0.1000^a	2.7333 ± 0.2081^a
Staphylococcus aureus	0.9333 ± 0.1527^b	2.8333 ± 0.1154^a

Values are means ± Standard deviation of triplicate determination values with the different superscripts within the column are significant at ($P<0.05$).

The result of the antibacterial activities of *P. djamor* is shown on Table 2 below. A good antibacterial activity was observed among the test organisms with *P. aeruginosa* taking the lead, followed by E. *coli*.

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Table 2: Antibacterial activities of P. *djamor* on bacterial isolates

Bacteria	Antibacterial activities zone of inhibition Mean±Std	Streptomycin (control) Mean±Std
Pseudomonas aeruginosa	2.3333±0.5777[a]	2.600±0.1000[a]
E. coli	1.9333±0.1527[b]	2.800±0.1000[a]
Staphylococcus aureus	0.8000±0.1000[c]	2.7333±0.2083[a]

Values are means ± Standard deviation of triplicate determination values with the different superscripts within the column are significant at (P<0.05).

The graphical presentation of the antibacterial activities of *P. ostreatus* is shown on the Figure 1 below. The zone of inhibition in cm is plotted against the bacterial isolates.

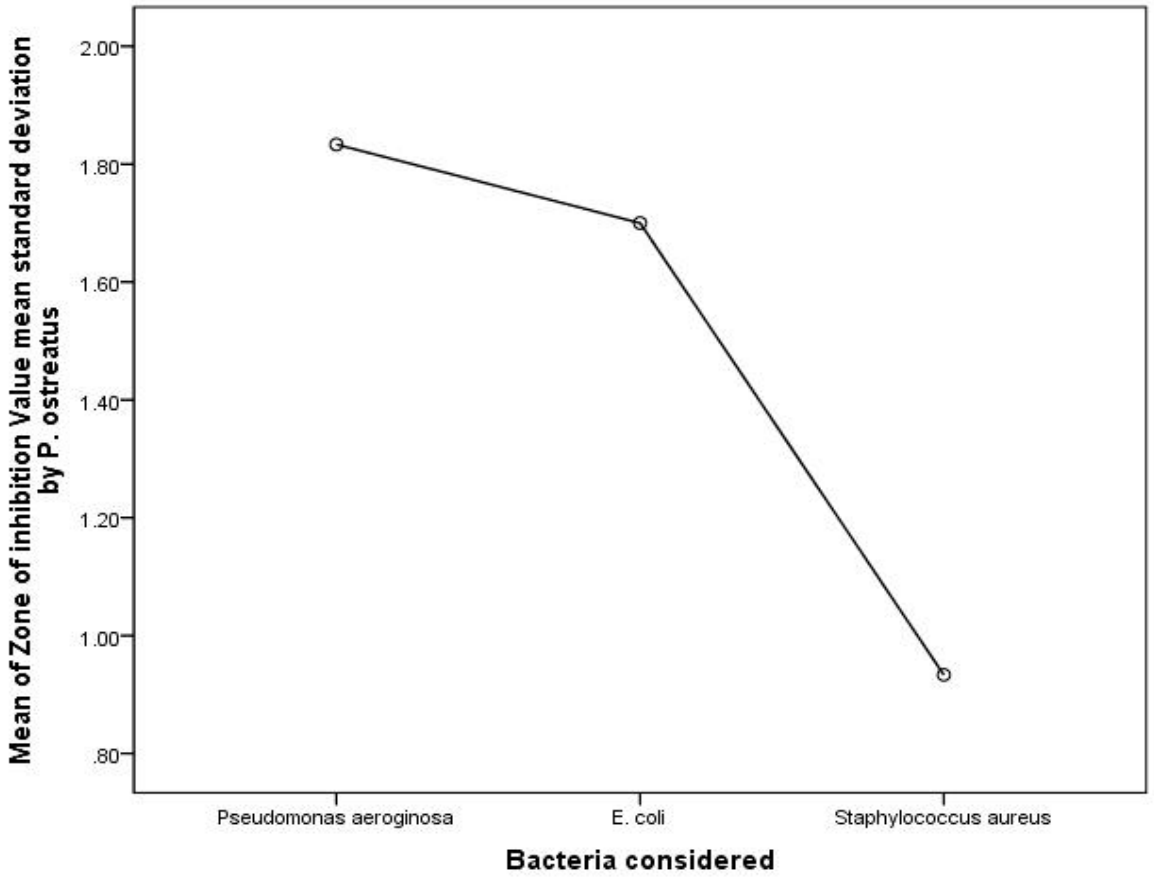

Figure 1: Graphical presentation of zone of inhibition of P. *ostreatus* against *P. aeroginosa, E. coli* and *S. aureus.*

The graphical presentation of the antibacterial activities of *P. djamor* is shown on the Figure 2 below. The zone of inhibition in cm is plotted against the bacterial isolates.

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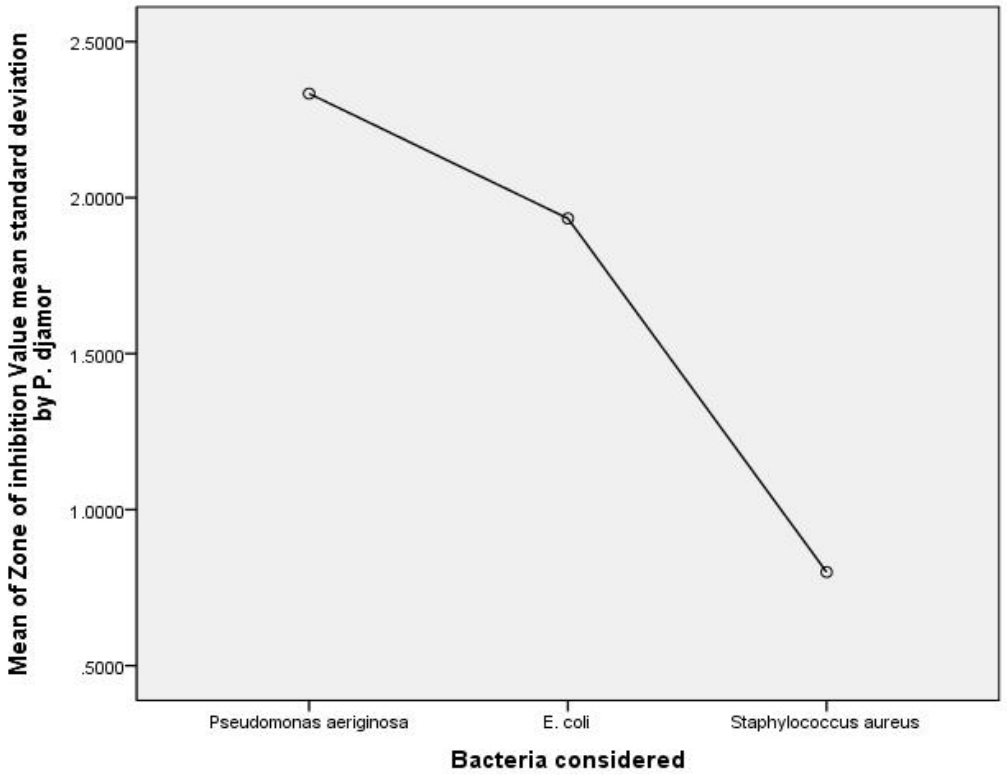

Figure 2: Graphical presentation of Inhibition of P. *djamor* against *P. aeroginosa, E. coli* and *S. aureus*.

The extract of mushroom species, *P. ostreatus* and *P. djamor* used in this study were found to exhibit various degrees of antibacterial effects against the tested microorganisms, *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*. This was evidenced by the clear zone of inhibition produced around the bacterial isolates in response to the mushrooms' extracts. The best *in-vitro* antibacterial activity of 2.33cm was exhibited by *P. djamor* against *P. aeruginosa* and 1.8cm by *P. ostreatus* against *P. aeruginosa*. This was followed by the antibacterial activity of *P. ostreatus* and *P. djamor* against *E. coli* with the zone of inhibition 1.70cm and 1.933cm respectively. These antibacterial activities of the both *P. ostreatus* and *P. djamor* mushrooms are indicators of their therapeutic potentials of the Oyster mushrooms species. Its worthy of note that majority of the Oyster mushroom species are known for their medicinal value, particularly, their antimicrobial properties as reported by Mohamed and Farghaly, (2014). In another development, the wild strain of some species belonging to the genus *Pleurotus* has been found to have pharmacological potential and nutraceutical properties according to Kalaw and Albinto, (2014), this will reflect the importance of these laboratory cultivated mushroom species as a natural antimicrobial agent.

There was no major contamination observed in the process among the *Pleurotus* species possibly due to sterilization of substrates before inoculation and their faster rate of fruition, this might have frustrated competitive microorganisms making it difficult for them to thrive.

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CONCLUSION

Mushrooms are the fruit bodies of macrofungal and it is consumed as food by many people across the world. The cultivation of mushrooms is on the increase as it now attracts many consumers because of its medicinal properties apart from their well known nutritional benefits. The study has revealed the antibacterial activities of *Pleurotus ostreatus* and *Pleurotus djamor against Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* isolates obtained from Microbiology department laboratory at Federal University of Technology, Owerri. The levels of antibacterial activities of these species of mushroom showed possible therapeutic potentials that could be used against common bacterial infections of man. Hence, Pharmacological and research institutions are advised to consider *P. ostreatus* and *P djamor* as a therapeutic source for drug production.

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