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Antimicrobial Activity of *Pleurotus squarrosulus* on Clinical Pathogenic Bacteria and Fungi

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Authors' contributions

This work was carried out in collaboration between both authors. Author AUK designed the study, performed the statistical analysis, managed the analyses of the study and wrote the first draft of the manuscript. Author OCK managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the antimicrobial activities of *Pleurotus squarrosulus* mushroom extracts on bacterial and fungal isolates.
Study Design: *Pleurotus squarrosulus* was obtained from different sources in Umuahia North Local Government, Abia state, Nigeria and identified in the Department of botany, University of Nigeria, Nsukka.
Place and Duration of Study: Antimicrobial activities of *Pleurotus squarrosulus* was carried out in the department of microbiology between January 2016 and August 2016
Methodology: *Pleurotus squarrosulus* was extracted using ethanol, methanol and aqueous. Antimicrobial susceptibility tests were carried out by agar disc diffusion technique using National Committee of Clinical Laboratory Standard. Qualilative phytochemical analysis was carried out using standard methods.
Results: Methanol, ethanol and aqueous extracts of *Pleurotus squarrosulus* were tested against *E. coli, B. cereus, S. aureus, P. aeruginosa, C. albicans* and *C. glabrata.* The different test microorganisms showed varied susceptibility to the test extracts. All the test organisms were

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inhibited by methanol, ethanol and aqueous extract at varied concentrations ranging between 500 mg/ml and 125 mg/ml. Statistically, inhibition of the antibacterial and antifungal control for the test organisms were significantly higher ($P < 0.05$) than that of the extracts. The phytochemical analysis revealed the presence of saponin, carbohydrates, tannins, flavonoids and proteins in all the extracts while glycoside and alkaloids, were found in some.

Conclusion: The finding of this result suggest that *Pleurotus squarrosulus* possess broad-spectrum antimicrobial activity. The potential of developing antimicrobials from plants appear rewarding.

Keywords: Pleurotus squarrosulus; antimicrobial activities; mushroom; phytochemicals; bacteria; yeast.

1. INTRODUCTION

Pleurotus species is one of the choice edible mushrooms which can be cultivated in many countries in the subtropical and temperate zones. Generally *Pleurotus* is referred to as "oyster mushroom" over the world while in China it is known as Abalone mushroom" and „Dhingri" in India. *Pleurotus* species have been used by the people in all over the world for their nutritional value, medicinal properties and other beneficial effects [1].

Oyster mushrooms are easy to grow and process and do not need huge investment. Mushroom farming is being practiced in more than 100 countries and its production is increasing at the rate of 7 per cent per annum. Production of mushroom has already crossed 5 million metric tons annually in the world and is expected to reach around 7 million metric ton in next ten years. India had been known world over for its exotic mushrooms and total mushroom production in India was 48,000.00 tons in 2005. Oyster mushroom cultivation has increased during the last decade [2].

Mushrooms have been used as food supplement from times immemorial not only for their flavor, aroma and nutritive values but also for their medicinal properties [3-5]. Wild mushroom holds a variety of bioactive compounds that have made it possible to be used as an impending source for the improvement of medicine and nutraceuticals [6].

A number of medicinal mushrooms, such as *Aleurodiscus, Coprinus, Clitocybe, Daedalea, Marasmius, Merulius, Pleurotus, Polyporus, Poria, Psathyrella, and Tricholoma* spp., are rich sources of ß-glucan, 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, eryngeolysin etc. [1].

These bioactive compounds have been employed as immune-modulator, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-viral, antioxidant and antimicrobial agents [7]. Besides, mushroom has been used extensively in traditional medicine for curing of various types of diseases [8-10]. For centuries, mushrooms have been prescribed for treatment of diseases such as gastro-intestinal disorder, bleeding, high blood pressure and various bacterial infections [11]. While some of the medicinal values associated with mushroom must have arisen from surperstitious beliefs and myths, they have provided information for curiosity research studies. Research has shown that some of these claims are not mere myth but are authentic [12,13]. Besides medicinal and nutritional use, mushroom can be used as natural dyes for fabrics [14].

2. MATERIALS AND METHODS

2.1 Collection and Identification of Materials

Pleurotus squarrosulus was collected from different sources of Umuahia North Local Government area, Abia state and identified by a botanist in the Department of botany, University of Nigeria, Nsukka.

2.2 Test Organisms Used

Pure cultures of *Escherichia coli* JCM 20135 and *Bacillus cereus* IFO 13804 were obtained from Department of Microbiology, University of Nigeria Nsukka while pure cultures of *Staphylococcus aureus* ATCC 25923*, Candida albicans* ATCC 10231*, Pseudomonas aeruginosa* ATCC 25783 and *Candida glabrata* ATCC 22018 were

obtained from Spectramedics Laboratories No. 2 Adebayo close, Araromi Offiri Ikenne Road, Sagamu, Ogun State.

2.3 Standard Antimicrobials

Tetracycline (5 µg/ml), Amplicillin (5 µg/ml), Oxacillin (5 µg/ml) and Nystatin (20 µg/ml) oxoid discs were used as positive standards.

2.4 Sample Preparation and Extraction

Fresh *Pleurotus squarrosulus* mushrooms were thoroughly washed with distilled water, cut into pieces, air-dried at room temperature and pulverized using manual grinder. Fifty grams of each of the ground samples was soaked in 500 ml ethanol, cold water, and methanol for 24 hours with intermittent shaking. Each sample was filtered using Whatman №1 filter paper. The filtrate was dried with a rotary evaporator in order to obtain the extract which was scooped and poured into well-labeled sample bottles and stored at 4ºC [15].

2.5 Inoculum Preparation

Pure cultures of *Escherichia coli* JCM 20135 and *Bacillus cereus* IFO 13804 were obtained from the Department of Microbiology, University of Nigeria Nsukka while pure cultures of *Staphylococcus aureus* ATCC 25923*, Candida albicans* ATCC 10231*, Pseudomonas aeruginosa* ATCC 25783 and *Candida glabrata* ATCC 22018were obtained from Spectramedics Laboratories, Sagamu, Ogun State, Nigeria. Inoculum was prepared by emulsifying overnight colonies from an agar medium. A 0.5 McFarland standard (equivalent to approximately 10^8 cfu/ml) was used. Media plates were inoculated within 30 minutes of standardizing the inoculum to avoid changes in inoculums density.

2.6 Determination of Antimicrobial Activity of Mushroom Extracts

Antimicrobial activity of mushroom extracts was determined according to the National Committee of Clinical Laboratory Standards [16]. Agar disc diffusion method on SDA and Muller-Hinton agar were used for fungi and bacteria respectively. A micropipette was used to introduce 100 µL of the inoculum onto the agar plate, and spread with glass rod spreader under sterile conditions. The paper discs of 6 mm diameter soaked in 10 µL of different concentrations of the extracts (500, 250, 125, 62.5, 31.25, 15.63 and 7.81 mg/mL) was applied on the agar plate. Similarly, for control plates, paper discs of 6 mm with dilute dimethylsulfoxide were used as negative control and antibiotics discs of tetracycline (10 µg/mL) and ampicillin (10 µg/mL) were used for Gram negative bacteria isolates, oxacillin (5 µg/mL) was used for Gram positive bacteria isolates whereas antifungi disc of nystatin (20 µg/mL) oxoid disc was used as positive control.

This procedure was carried out in triplicate for the entire test organisms and allowed to stand for 30 min on the bench after which they were incubated for 24 h at 37 ± 2ºC for bacteria and 72 at 28 ± 2ºC for yeast. After incubation, the inhibition zone diameters produced by the different concentrations of the crude extracts were measured (in millimeter) using transparent meter rule.

2.7 Determination of Minimum Inhibitory Concentrations (MICs) of the Mushroom Extracts

The MIC of the extracts was determined for the test organisms in triplicates at varying concentrations (250, 125, 62.5, 31.25, 15.62, 7.80 and 3.90 mg/ml). To obtain these concentrations, 1.0 ml of varying concentrations of the extracts with double strength (500, 250, 125, 62.5, 31.25, 15.62 and 7.80 mg/ml) were constituted in different test tubes. About 1.0 ml of Mueller-Hinton broth (for bacteria) and Sabouraud dextrose broth (for fungi) was added and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. Controls of Mueller-Hinton broth and Sabouraud dextrose broth without the mushroom extract were set up. All the bacterial cultures were incubated at 37± 2℃ for 24 hours and yeast culture incubated at 28± 2℃ for 72 hours. After incubation each tube was examined for microbial growth. The lowest concentration of the extract that inhibited the growth of the test organisms as detected by lack of visual turbidity was designated the MIC [16].

2.8 Determination of Minimum Bactericidal Concentrations (MBCs) and Minimum Fungicidal Concentrations (MFCs) of the Mushroom Extracts

MBC was determined by selecting tubes that showed no bacterial growth during the MIC determination. A loopful from each of the tubes

was sub-cultured on the Mueller Hinton Agar and incubated for 24 hours at 37℃ ± 2℃. MFC was determined by selecting tubes that showed no fungal growth during MIC determination. A loopful from each of the test tubes was sub-cultured on Potato Dextrose agar. The plates were incubated for 72 hours at 28 ± 2℃ [16].

2.9 Statistical Analysis

Experimental values were given as means ± standard deviation (SD). Statistical significance of data were analyzed at P ≤ 0.05 (Independent-Samples T Test) using statistical package for social sciences (SPSS, Armonk, NY, USA) version 20.

3. RESULTS AND DISCUSSION

Natural products not only provide valuable components but also an important source of bioactive compounds that provide lead information for developing useful synthetic compounds. Mushrooms contain a large number of biologically active components that impart health benefits and protection against degenerative diseases. They have been traditionally used in all over world for treatment of variety of chronic disease. Antimicrobial activity of the crude extract of *Pluerotus squarrosulus* as well as phytochemical characteristics were studied. Table 1 shows the result of the MIC and MBC of the ethanolic, methanolic and aqueous extracts of *P. squarrosulus* on the test organisms. The MIC of ethanolic extract of *P. squarrosulus* showed that *B. cereus, S. aureus, P. aeruginosa* and *E.coli,* had 15.63, 15.63, 15.63 and 31.25 mg/ml with MBC of 15.63, 31.25, 31.25 and 31.25 mg/ml respectively. The methanolic extract of *P. squarrosulus* showed that the MIC varied between 3.90 and 125 mg/ml with MBC of 7.81 to 125 mg/ml while the MIC of aqueous extract of *P. squarrosulus* varied between 31.25 and 62.50 mg/ml with MBC of 31.25 to 125 mg/ml.

Table 2 shows the result of the average MIC and MFC of the ethanolic, methanolic and aqueous extracts of *P. squarrosulus* on test organisms. The MIC of ethanolic extract of *P. squarrosulus* showed 15.63 mg/ml for *C. albicans* and 125 mg/ml for *C. glabrata* with MFC of 31.25 and 125 mg/ml, respectively, the MIC of methanolic extract of *P. squarrosulus* showed 250 mg/ml for *C. albicans* while *C. glabrata* showed no activity with MFC of 250 mg/ml for *C.albicans* while the MIC of aqueous extract of *P. squarrosulus* showed 7.81 mg/ml for *C. albicans* and 62.5 mg/ml for *C. glabrata* with MFC of 15.25 and 125 mg/ml, respectively.

Table 1. The MIC and MBC of crude extract of *Pleurotus squarrosulus*

Extract	Test organism	MIC (mg/ml)	MBC (mg/ml)
	B. cereus	15.63	15.63
Ethanol	*S.aureus*	15.63	31.25
	P. aeruginosa	15.63	31.25
	E.coli	31.25	31.25
	B. cereus	3.90	7.81
Methanol	*S.aureus*	31.25	62.5
	P. aeruginosa	62.5	62.5
	E.coli	125	125
	B. cereus	62.5	125
Aqueous	*S.aureus*	31.25	31.25
	P. aeruginosa	ND	ND
	E.coli	ND	ND

ND = Not determined

Table 2. The MIC and MFC of the crude extract of *Pleurotus squarrosulus*

Extract	Test organism	MIC (mg/ml)	MFC (mg/ml)
Ethanol	*C.albicans*	15.63	31.25
	C.glabata	125	125
Methanol	*C.albicans*	250	250
	C.glabata	ND	ND
Aqueous	*C.albicans*	7.81	15.25
	C.glabata	62.5	125

ND = Not determined

Table 3 shows the phytochemical analysis that revealed the presence of bioactive compounds which were present at varying levels. Saponins, protein and carbohydrate were detected in all the extracts while glycosides, alkaloids, tannins and flavonoids were found in some.

Fig. 1 shows the antimicrobial activity of *Pleurotus squarrosulus* methanol extract on the test organisms. The mean inhibition zone diameter varied directly with increase in extract concentration. *E.coli* was inhibited at different concentration of 500, 250 and 125 mg/ml, *P. aeruginosa* and *B. cereus* were inhibited at different concentration ranging from 500 mg/ml to 62.5 mg/ml, also *S. aureus* was inhibited at different concentrations ranging from 500 mg/ml to 31.25 mg/ml and *C. albicans* were inhibited at different concentrations of 500 mg/ml to 31.25 mg/ml whereas *C. glabrata* was not inhibited by the extract even at the highest concentration of 500 mg/ml. However, inhibition of the antibacterial and antifungal control for the test organisms were significantly higher ($p < 0.05$) than that of the extract.

Fig. 2 presents the antimicrobial activity of *Pleurotus squarrosulus* ethanol extract on the test organisms. *E. coli, B. cereus, S. aureus* and *C.albicans* were well inhibited at different concentrations ranging from 500 mg/ml to 31.25 mg/ml while *P. aeruginosa* were inhibited at concentrations between 500 mg/ml and 62.5 mg/ml whereas *C. glabrata* that was only

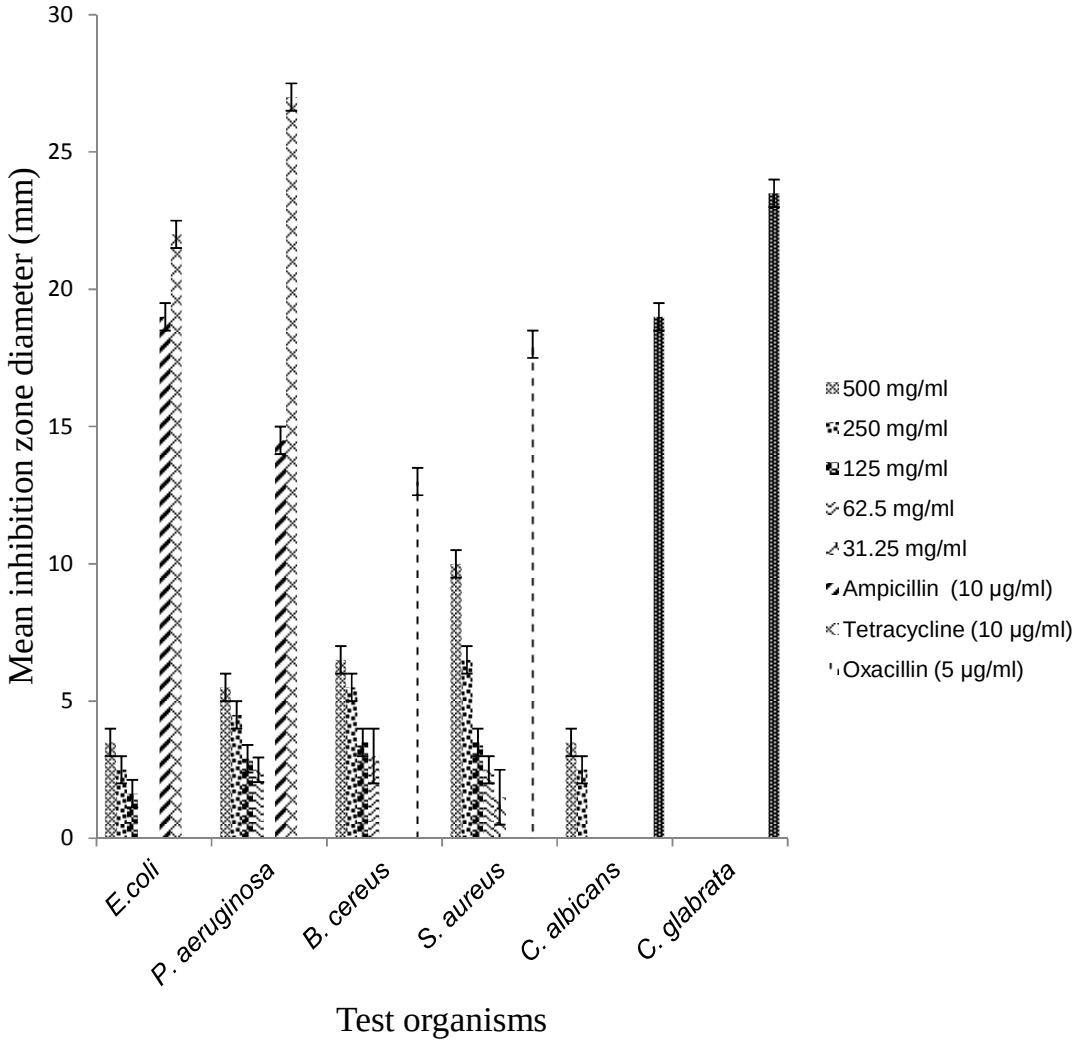

Fig. 1. The antimicrobial activity of *Pleurotus squarrosulus* methanol extract on the test organisms

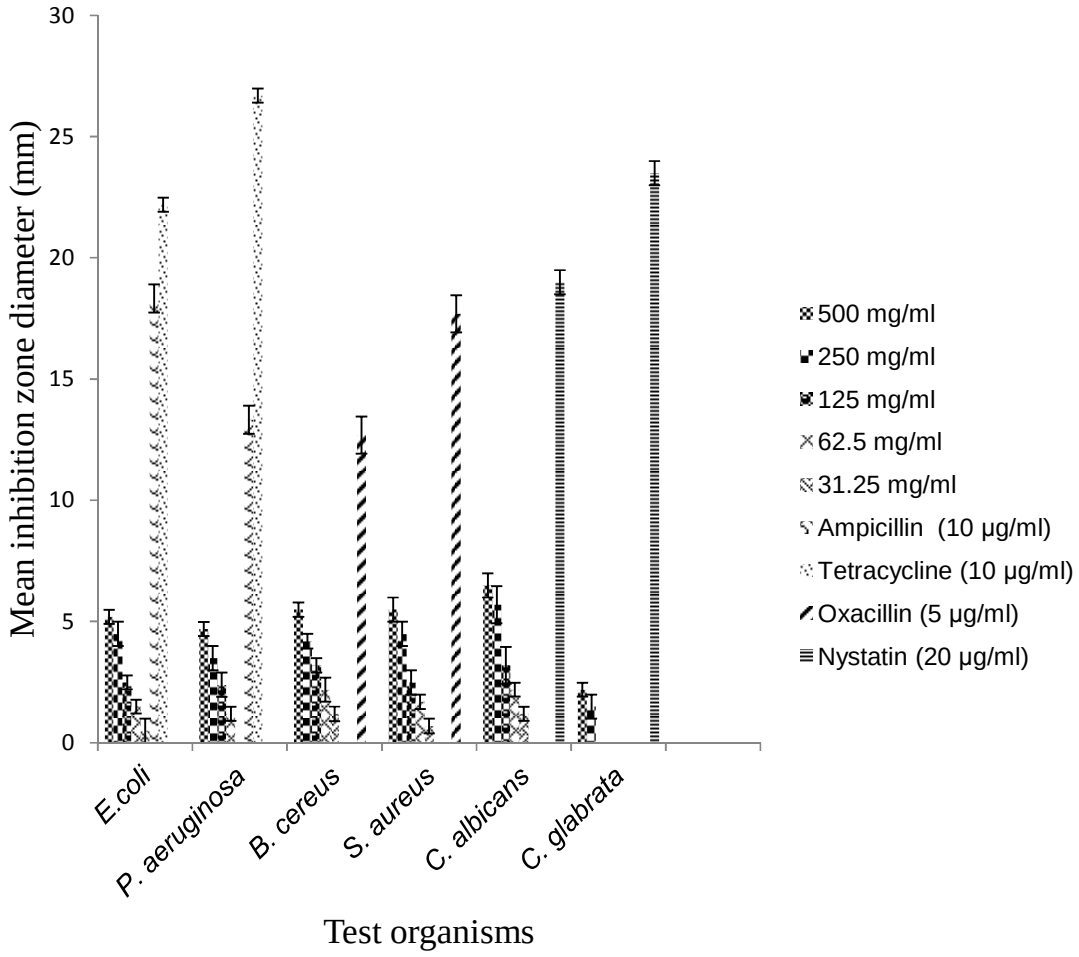

Fig. 2. The antimicrobial activity of *Pleurotus squarrosulus* ethanol extract on the test organisms

Table 3. Phytochemical analysis of *Pleurotus squarrosulus* in different solvent

Solvents	Methanol	Ethanol	Aqueous
Saponin	++	+	+
Tannins	+	++	+
Flavonoid	+	++	+
Alkaloid	+	+	-
Proteins	++	+++	+ +
Glycosides	++	+++	-
Carbohydrates	++	++	++

Legend: - = not present, + = present in low concentration, ++ = moderate, +++ = present in high concentration

inhibited at concentrations of 500 mg/ml and 250 mg/ml. However, inhibition of the antibacterial and antifungal control for the test organisms were significantly higher ($p < 0.05$) than that of the extract.

Fig. 3 shows the result obtained for the antimicrobial activity of *Pleurotus squarrosulus* aqueous extract. *B. cereus, S. aureus, C. albicans* and *C. glabrata* were well inhibited by the extract at concentrations ranging from 500 to 125 mg/ml. *E. coli* and *P. aeruginosa* were not inhibited even at the highest concentration of 500 mg/ml. However, inhibition of the antibacterial and antifungal control for the test organisms were significantly higher ($p < 0.05$) than that of the extract.

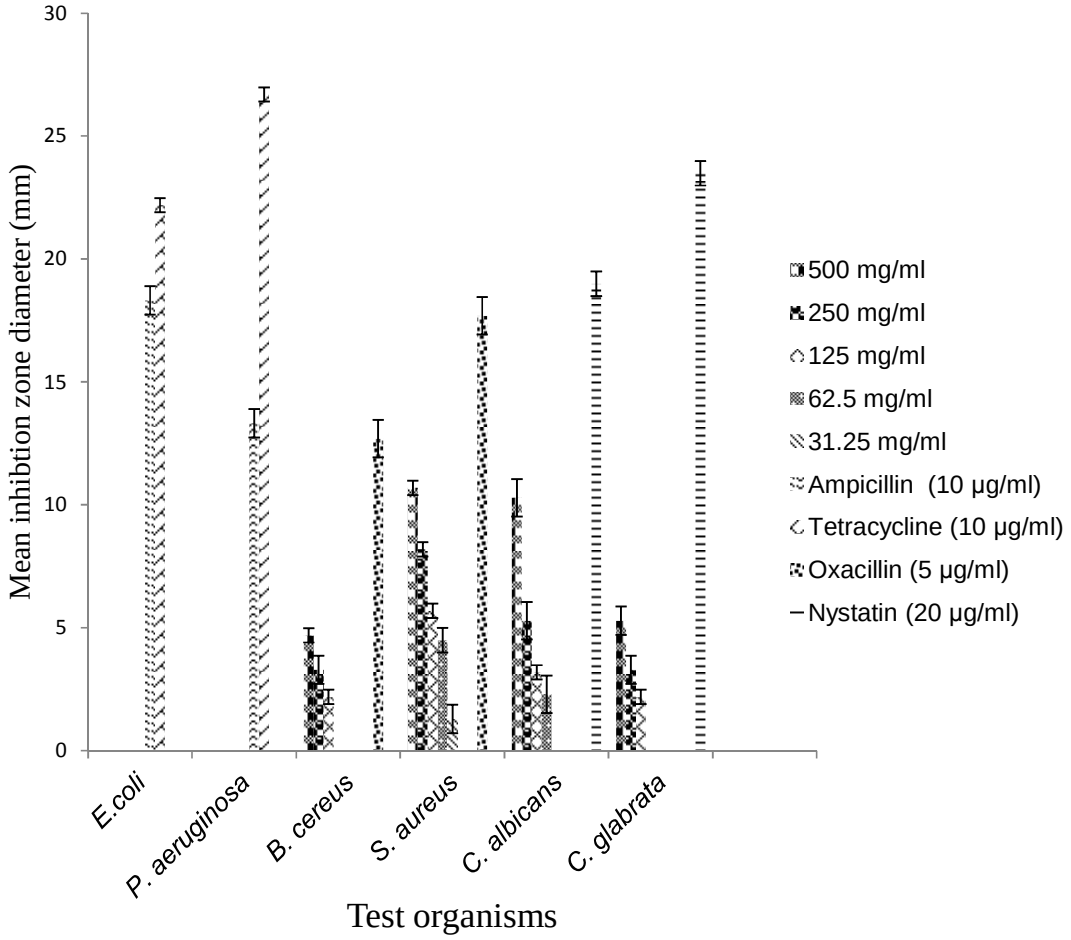

Fig. 3. The antimicrobial activity of *Pleurotus squarrosulus* aqueous extract on the test organisms

The results indicated that extracts from mushroom has similar antimicrobial properties as reported by Nwachukwu and Uzoeto [15]. The sensitivity of isolates to the mushroom extracts implies that intrinsic substance in the extracts is unknown to the microorganisms which made it impossible for them to resist. The variations in the antimicrobial activities of *Pleurotus squarrosulus* extracts may be due to the differences in their bioactive compositions or concentrations, methods of extraction and mechanism of action of active ingredients [17]. The results of the present study strengthened the outcomes of earlier works done by others that showed mushrooms produced a great variety of antimicrobial agents. For instance, it is known that the extract from fruit bodies of several *Lactarius* sp. [18,19]; *Fomitopsis* sp. [20]; *Boletus* sp. [21]; *Cortinarius* sp. [22]; *Ganoderma lucidum*, *Navesporus floccosa* and *Phellinus rimosus* [23]; *Pleurotus tuber-regium* [24]; *Amanita caesarae*, *Armillaria mellea*, *Chroogomphus rutilus*, *Clavariadelphus truncates*, *Clitocybe geotropa*, *Ganoderma* sp., *Ganoderma carnosum*, *Hydnum repandum*, *Hygrophorus agathosmus*, *Lenzites betulina*, *Leucoagaricus pudicus*, *Paxillus involutus*, *Polyporus arcularius*, *Rhizopogon roseo*, *Sarcodon imbricatus*, *Suillus collitinus*, *Trametes versicolor*, *Tricholoma auratum*, *Tricholoma fracticum* [25]; *Lactarius deliciosus*, *Sarcodon imbricatus* and *Tricholoma portentosum* [26]; *Russula delica* [27]; *Pleurotus eryngii* var. *ferulae* [28]; *Infundibulicybe geotropa*, *Lactarius controversus*, *Lactarius delicious* and *Phellinus hartigii* [29]; *Lactarius indigo* [30] and *Stereum*

ostrea [31] contain a wide range of antimicrobial activity.

4. CONCLUSION

This research has further illuminated the medicinal value of *Pleurotus squarrosulus* found in Umuahia North Local Government, Abia State Nigeria. From the present study, the sensitivity of isolates to the mushroom extracts implies that intrinsic substance in the extracts is unknown to the microrganisms, which made it impossible for them to resist.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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