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In Vitro Antimicrobial Activity of *Polyporus Alveolaris* on Clinical Pathogens

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Background: Time immemorial, mushrooms have been used as a part of regular diet due to their nutritional values. They have been found to contain minerals, vitamins and nutritive compounds, proteins, polysachharides and a low fat content.

Objectives: The objective of this study is to evaluate the antimicrobial activities of *Polyporus alveolaris* mushroom extracts on bacterial and fungal isolates.

Method: *Polyporus alveolaris* was obtained from different sources in Umuahia North Local Government, Abia state, Nigeria and identified in the Department of Botany, University of Nigeria, Nsukka. *Polyporus alveolaris* was extracted in ethanol, methanol and aqueous solution. Antimicrobial susceptibility tests were carried out by agar well diffusion technique using National Committee of Clinical Laboratory Standard. Qualilative phytochemical analysis was carried out using standard methods.

Result: The Methanol, ethanol and aqueous extracts of *Polyporus alveolaris* 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 well inhibited by ethanol and aqueous extract while methanol extract only inhibited *E.coli*, *P.aeruginosa* and *C. albicans* at varied concentrations ranging between 500 mg/ml and 62.5 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 and proteins in all the extracts while glycoside, alkaloids, tannins and flavonoids were found in some.

Conclusion: The findings of this result suggest that *Polyporus alveolaris* possess broad-specrum antimicrobial activity. The potential of developing antimicrobials from plants appear rewarding.

Keywords*: Polyporus alveolaris, antimicrobial activities, phytochemicals, bacteria, yeast*

Highlights

- *Polyporus alveolaris* is a promising antibacterial agent.
- *Polyporus alveolaris* is a promising antifungal agent.
- *Polyporus alveolaris* possess bioactive constituents.

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Introduction

Mushrooms, also called puffballs are macroscopic fungi that can be seen in various places such as wet environments, decayed plants and animal sites, termites nest, palm wastes, leaf litters, under shades, to mention but a few.[1] They are distinctive fruiting bodies which are either hypogeous or epigeous.[2] Mushrooms are differentiated into edible or poisonous, wild or domestic. The application of mushrooms as food as well as medicine has agained popularity in recent times.[3] The nutritive nature and properties of many mushrooms have been documented.[4,5] Mushrooms have been found to possess all the essential amino acids.[3] Mushrooms naturally contain different bioactive compounds such as polysaccharides, glycosides, sesquiterpenes e.t.c. with various biological activities such as anticancer, antibacterial, antifungal and antiviral agents.[6]

Mushroom has been used widely in traditional medicine for curing of various types of diseases.[7] For centuries, mushrooms have been prescribed for treatment of diseases such as gastro-intestinal disorder, bleeding, high blood pressure and various bacterial infections.[8] As some of the medicinal values associated with mushroom must have arisen from superstitious beliefs and myths, they have provided information for curiosity based-research studies. Research has revealed that some of these claims are not mere myth but are real.[9] Besides medicinal and nutritional use, mushrooms can be used as natural dyes for fabrics.[10]

The fruit bodies of *P. alveolaris* are 1–10 cm in diameter, rounded to kidney or fan-shaped. Fruit bodies sometimes have stems, but they are also seen attached directly to the growing surface. The cap surface is dry, covered with silk-like fibrils, and is an orange-yellow or reddish-orange color, which weathers to cream to white. The context is thin (2 mm), tough, and white. The pores are large compared to other species in this genus typically 0.5 – 3 mm wide, angular (diamond-shaped) or hexagonal. The pore surface is a white to buff color. The stripe, if present, is 0.5 – 2 cm long by 1.5 – 5 mm thick, placed either laterally or centrally, and has a white to tan color. The pores extend decurrently on the stripe. The spore deposit is white.[11]

Polyporus alveolaris can be seen growing singly or grouped together on branches and twigs of hardwoods, commonly on shagbark hickory in the spring and early summer.[11] It has been reported growing on the dead hardwoods of genera *Acer*, *Castanea*, *Cornus*, *Corylus*, *Cratageus*, *Erica*, *Fagus*, *Fraxinus*, *Juglans*, *Magnolia*, *Morus*, *Populus*, *Pyrus*, *Robinia*, *Quercus*, *Syringa*, *Tilia*, and *Ulmus*.[12]

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.

This study was designed to evaluate the antimicrobial activity of *Polyporus alveolaris* mushroom extracts on bacterial and fungal isolates. The study aimed to determine their minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) and the phytochemical properties of the mushroom so as to offer informed recommendation on its use for the treatment of problem of antibiotic resistance.

Methods

Collection and identification of materials

Polyporus alveolaris was collected from different sources of Umuahia North Local Government area, Abia state and identified in the Department of Botany, University of Nigeria, Nsukka.

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.

Standard antimicrobials

Tetracycline (5 µg/mL), Gentamycin (5 µg/mL), Ampicillin (5 µg/mL), Oxacillin (5 µg/mL), Fluconazole (5 µg/mL) and Nystatin (20 µg/mL) oxoid discs were used as positive standards.

Sample preparation and extraction

The extraction was carried out in accordance with the procedure of Nwachukwu and Uzoeto[13] with slight modification. Fresh *Polyporus alveolaris* was thoroughly washed with clean water, cut into

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pieces, air-dried at room temperature and pulverized into powder using manual grinder. Fifty grammes of each of the ground samples was, respectively, soaked in 500 mL ethanol, cold water and methanol for 24 h with intermittent shaking. Each sample was filtered using Whatman No.1 filter paper. The filtrate was dried with a rotary evaporator in order to obtain the extract. The extract was scooped and put into well-labeled sample bottles and stored in the refrigerator at 4ºC.

Inoculum preparation

The inoculum was prepared by emulsifying overnight colonies from an agar medium. A 0.5 McFarland standard was used for visual comparison to adjust the suspension to a density equivalent to approximately 10^8 CFU/mL. Media plates were inoculated within 30 min of standardizing the inoculum, to avoid changes in inoculum density.

Determination of antimicrobial activity of mushroom extracts

Antimicrobial activity of mushroom extracts was determined according to the National Committee of Clinical Laboratory Standards.[14] 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) and gentamicin (10 µg/mL) were used for Gram positive bacteria isolates whereas antifungal discs of fluconazole (25 µg/mL) and nystatin (20 µg/mL) oxoid disc were 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. Antimicrobial activities were expressed in terms of the mean inhibition zone diameter (mm) produced by the mushroom extracts in triplicate experiments.

Determination of minimum inhibitory concentrations (MICS) of the mushroom extracts

The minimum inhibitory concentration (MIC) was determined by macro-broth dilution techniques as specified by National Committee for Clinical Laboratory Standards [14]. A two fold serial dilution of the reconstituted extract was prepared in Mueller Hinton Broth and Sabouraud Dextrose Broth. Each dilution was seeded with 100 µl of the standardized suspension of the test organism for bacteria and fungi respectively, and then incubated for 24 h at 37 ± 2°C for bacteria and fungi incubated for 28 ± 2º C for 72 h. MIC was determined as the highest dilution (that is, lowest concentration) of the extract that showed no visible growth.

Determination of minimum bactericidal concentrations (MBCS) 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 subcultured on the Mueller Hinton Agar. The plates were incubated for 24 h at 37 ± 2º C. The MBC was determined as the least concentration that showed no visible growth on the plate.[14]

Determination of minimum fungicidal concentrations (MFCS) of the mushroom extracts

MFC was determined by selecting tubes that showed no fungal growth during MIC determination. A loopful from each of the test tubes was subcultured on potato dextrose agar. The plates were incubated for 72 h at 28 ± 2º C. The MFC was determined as the least concentration that showed no visible growth on the plate.[14]

Statistical analysis

Experimental values are given as means ± standard deviation (SD). Statistical significance was determined by one way variance analysis (ANOVA). Differences at $p < 0.05$ were considered to be significant.

Results

The antimicrobial activity of *Polyporus alveolaris* was determined by agar well diffusion method against six pathogenic isolates. Table 1 shows the result of the average MIC and MBC of the ethanolic, methanolic and aqueous extracts of *P. alveolaris* on test organisms. The MIC of ethanolic extract varied between 62.5 and 125 mg/mL with MBC of 62.5 to 125 mg/mL, MIC of the methanolic extract varied between 7.81 and 31.25

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mg/mL with MBC of 15.63 to 62.5 mg/mL while the MIC of aqueous extract varied between 31.25 and 125 mg/mL with MBC of 62.5 to 125 mg/mL.

Table 1: The MIC and MBC of crude extract of *P. alveolaris*

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

ND = Not determined

Table 2: The MIC and MFC of the crude extract of *P. alveolaris*

Extract	Test organism	MIC (mg/mL)	MFC (mg/mL)
Ethanol	*C.albicans*	125	125
	C.glabrata	125	125
Methanol	*C.albicans*	ND	ND
	C.glabrata	62.5	62.5
Aqueous	*C.albicans*	62.5	62.5
	C.glabrata	62.5	125

ND = Not determined

Table 2 shows the result of average MIC and MFC of the ethanolic, methanolic and aqueous extracts of *P. alveolaris* on test organisms. Ethanolic extract of *P. alveolaris* showed MIC of 125 mg/mL with MFC 125 mg/mL for *C. albicans* and *C. glabrata*. The MIC of methanolic extract of *P. alveolaris* showed 62.5 mg/mL with MFC of 62.5 mg/mL for *C. glabrata* with no activity on *C. albicans*. The MIC of the aqueous extract showed 62.5 mg/mL with MFC of 62.5 mg/mL for *C. albicans* and MIC of 62.5 mg/mL with MFC of 125 mg/mL for *C. glabrata*.

Table 3 shows the qualitative phytochemistry of *Polyporus alveolaris* using different solvents (ethanol, methanol and aqueous). The phytochemical analysis 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.

Figure 1 shows the antimicrobial activity of *Polyporus alveolaris* methanol extract on the test organisms. The different test microorganisms showed varied susceptibility to the extract. *E. coli* and *P. aeruginosa* were well inhibited by the extract. *C. glabrata* was only inhibited at concentrations of 500 mg/mL, 250 mg/mL and 125 mg/mL while *B. cereus, S. aureus* and *C. albicans* were not inhibited even at the highest tested concentration of 500 mg/mL. Statistically, inhibition of the antibacterial and antifungal control for the test organisms were significantly higher ($p < 0.05$) than that of the extract.

Figure 2 presents the antimicrobial activity of *Polyporus alveolaris* ethanol extract on the test organisms. This extract exhibited a broad spectrum of activity, inhibiting all the tested organisms including *P.aeruginosa* that was resistant to other crude extracts. However, the mean inhibition zone diameter of *P.aeruginosa* was significantly ($p < 0.05$) lower than that of other inhibited organisms.

Figure 3 shows the antimicrobial activity of *Polyporus alveolaris* aqueous extract on the test organisms. This extract exhibited a broad spectrum of activity, inhibiting all the tested organisms except *P. aeruginosa* that was not inhibited even at the highest tested concentration of 500 mg/mL. Statistically, inhibition of the antibacterial and antifungal control for the test organisms were significantly higher ($p < 0.05$) than that of the extract.

Discussion

Herbal plants are among the most commonly used antimicrobial agents in food and have been used traditionally for thousands of years in the control of various health complications including infectious diseases [15]. Antimicrobial activity of the crude extract of *Polyporus alveolaris* as well as phytochemical characteristics were studied. The results indicated that extracts from mushroom have antimicrobial properties as reported by Nwachukwu and Uzoeto[13]. It is interesting to note that the pathogenic microorganism, *Pseudomonas aeruginosa*, which is resistant to conventional synthetic antibiotic like gentamicin was found to show susceptibility to methanol and ethanol extracts of *Polyporus alveolaris*. Mushrooms produce various antiviral, antifungal compounds to survive in the wild against competing or pathogenic agents[16,17]. Also observed in this study is that there

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were variations in the degree of antimicrobial activities of mushrooms. This result is in agreement with the reports of Akyuz *et al*.[18] in Turkey and that of Jaggadish *et al*.[19]

The broad spectrum activity of mushrooms was also brought to light as the extracts of mushrooms showed inhibitory effects on clinical isolates used for this investigation. This suggests that the bioactive products which are contained in mushrooms are in concentrations which exude varying degrees of antimicrobial activity. It is interesting to note from the results of this study that clinical isolates both Gram positive and Gram negative bacteria were sensitive to the extracts. This is in collaboration with the findings of Onyeagba *et al*. [20] and Desouza *et al*. [21]. 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 *Polyporus alveolaris* extracts may be due to the differences in their bioactive compositions or concentrations, methods of extraction and mechanism of action of active ingredients [22]. Based on the results of this study, it can be concluded that *Polyporus alveolaris* possessed a broad-spectrum antimicrobial activity. The potential of developing antimicrobials from plants appears rewarding.

Table 3: Phytochemical Analysis Of *Polyporus Aveolaris* In Different Solvents

Mushroom Name	Solvents	Gly	Tan	Sap	Fla	Car	Pro	Alk
Polyporus alveolaris	Ethanol	++	+	++	+	+	++	+
	Methanol	+	-	+	-	++	+	+
	Aqueous	++	-	++	-	+++	+	+

- = not present, + = present in low concentration, ++ = moderate, +++ = present in high concentration, Gly = Glycoside, Tan = Tannins, Sap = Saponins, Fla = Flavonoids, Car = Carbohydrates, Pro = Proteins, Alk = Alkaloids

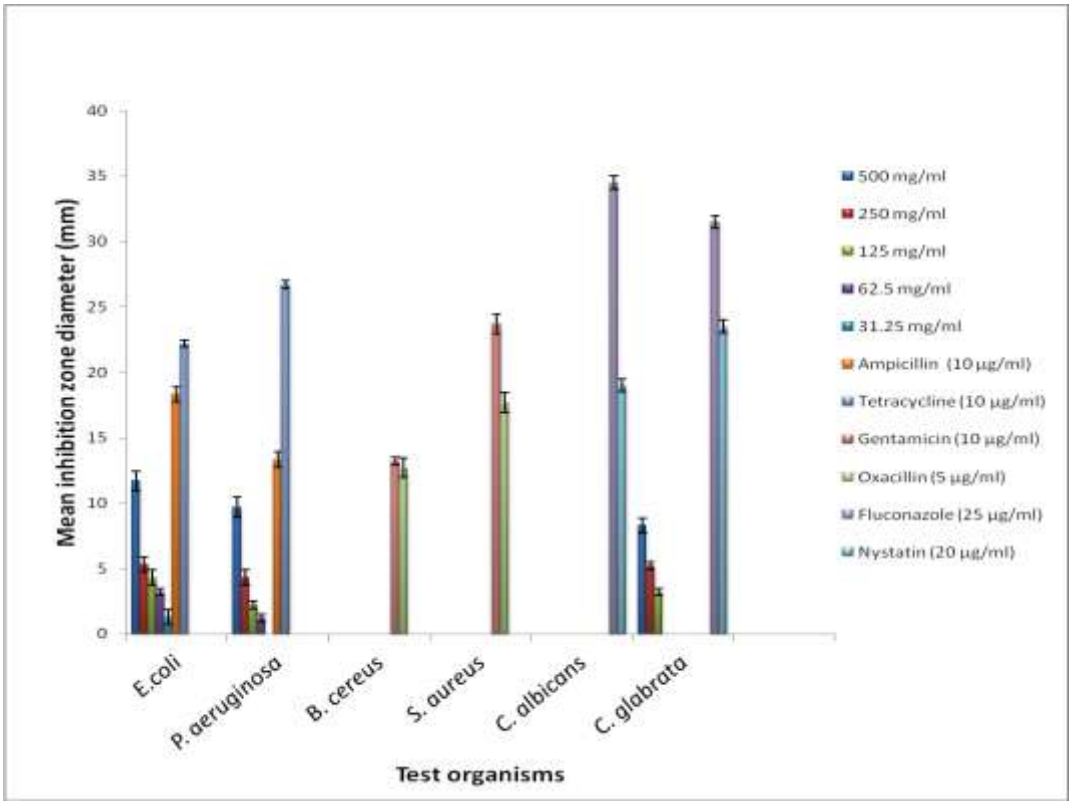


Figure 1: The antimicrobial activity of *Polyporus alveolaris* methanol extract on the test organisms

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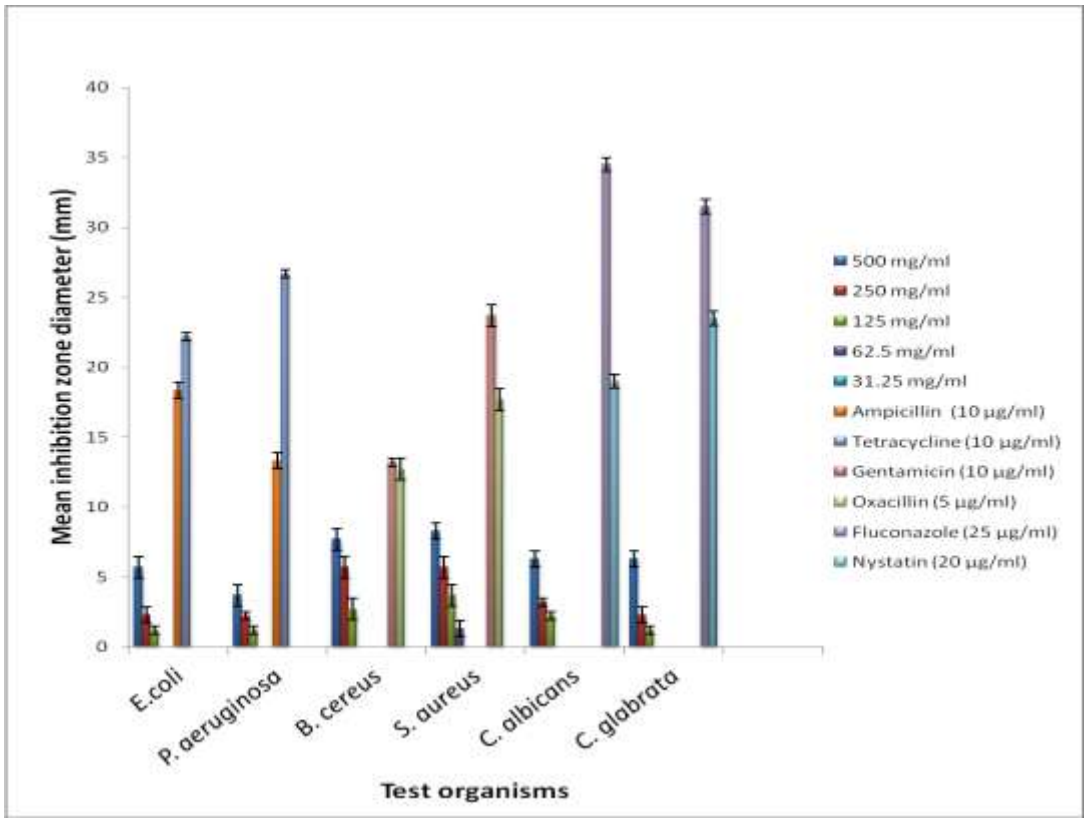


Figure 2: The antimicrobial activity of *Polyporus alveolaris* ethanol extract on the test organisms

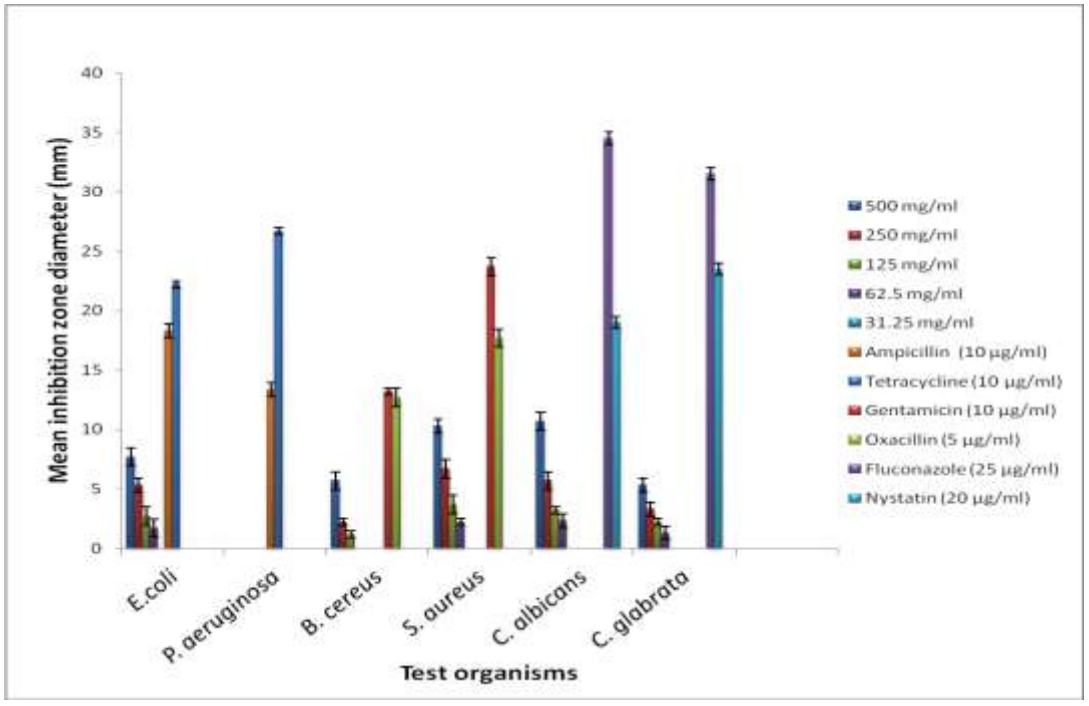


Figure 3: The antimicrobial activity of *Polyporus alveolaris* aqueous extract on the test organisms

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