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Antimicrobial activity of whole fruit body of *Auricularia auricular* on clinical pathogenic bacteria and fungi

Akpi Uchenna Kalu[1*], Okobo Uchenna Jude[2], Ebeifenadi Uche Jonathan[3]
[1-3] Department of Microbiology, University of Nigeria, Nsukka, Nigeria

Abstract
The rampant multi-drug resistance among human pathogenic microorganisms has necessitated a continuous search for new and potent antimicrobial substances, especially among plants. In this study, antimicrobial activities of extracts of fruit bodies of *Auricularia auricular* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus cereus*, *Candida albicans* and *Candida glabrata* were investigated. Antimicrobial components from the mushrooms were extracted using ethanol, methanol and water and examined by agar well diffusion method. The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were evaluated for each extract of the mushroom. The aqueous, methanol and ethanol extract of *Auricularia auricular* inhibited the growth of all the tested pathogenic organisms except *P. aeruginosa*. The phytochemical analysis revealed the presence of Flavonoids, saponins, protein and carbohydrate in all the extracts while glycosides, alkaloids and tannins were found in some. The results obtained from this study suggest that *Auricularia auricular* has broad-spectrum of activity against microbial isolates used.

Keywords: Agar well diffusion method, antimicrobial, *Auricularia auricular*, phytochemicals

1. Introduction
Auricularia auricula-judae, known as Eru nti, the Jew'sear, wood ear, jelly ear or by a number of other common names, is a species of edible Auriculariales fungus found worldwide. The fruiting body is distinguished by its noticeably ear-like shape and brown colouration; it grows upon wood, especially elder. The fungus can be found throughout the year in temperate regions worldwide, where it grows upon both dead and living wood [1].
Auricularia auricula-judae has a soft, jelly-like texture. Though edible, it was not held in high culinary regard in the west for many years. It has been likened to "eating an Indian rubber with bones in it" [2] while in 19th century Britain, it was said that "it has never been regarded here as an edible fungus". It has a mild flavour, and is useful for mixed mushroom recipes, but is still considered bland in the west [3]. It can be dried and rehydrated, sometimes swelling to a very large size. Young specimens are best, but the species are not edible when raw, needing to be cooked thoroughly. The whole fruit body can be eaten, but should be thoroughly washed before cooking. The nutritional content of 100 g (3.5 oz) of dried fungus includes 370 kcal, 10.6 g of protein, 0.2 g of fat, 65 g of carbohydrate, 5.8 g ash, and 0.03% mg of carotene. Fresh mushrooms contain about 90% moisture [4]. Dried specimens may be ground up into a powder and used to absorb excess liquid in soups and stews, as it rehydrates into tiny fragments [5].
Mushrooms, also called puffballs are macroscopic fungi that can be found 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 [6]. They are distinctive fruiting bodies which are either hypogeous or epigeous [7]. Mushrooms are differentiated into edible or poisonous, wild or domestic. In many countries of the world, Nigeria inclusive, edible mushrooms are good for food and are used in medicine to protect against free radicals and infections [8]. The use of mushrooms as food as well as medicine is gaining popularity in recent times [9]. Mushrooms have been found to contain all the essential amino acids [9, 10]. Edible mushrooms are attractive because of their flavor, taste and delicacy [11]. Many species of edible mushrooms exist in nature, less than 20 species are used as food and only 8-10 species are regularly cultivated in significant extent [12]. Mushrooms characteristically contain many different bioactive compounds such as polysaccharides, glycosides, sesquiterpenes etc with diverse biological activities such as anticancer, antibacterial, antifungal and antiviral agent [13].
Polysaccharides are the main active components in *A. auricula-judae*, as well as some flavonoids, proteins, etc. In previous studies, it has been found that polysaccharides from *A. auricula-judae* (AAP) have many pharmacological activities such as antidiabetic, antitumor, anticancer, etc. [14]. Therefore, *A. auricula-judae* is a potential health-care food [15, 16]. Currently, the typical method for extraction of AAP is hot water extraction, although this current technology has some drawbacks, such as large energy consumption and low extraction yield. Therefore, it is necessary to optimize the technology of hot water extraction of AAP from the mushroom.
In recent years, it has been found that polysaccharides extracted from some natural products have antimicrobial activity. Until now, there are few studies on the antimicrobial potentials of AAP. Therefore, it is necessary to study their antimicrobial activities. This investigation can provide some fundamental data for further demonstrating the mechanisms of antimicrobial activities of the polysaccharides and other constituents from mushrooms [17].
Recently, some reports demonstrated that some polysaccharides isolated from *A. auricula-judae* had antioxidant activities. A water-soluble purified polysaccharide extracted from *A. auricular-judae*

significantly decreased the level of malondialdehyde and increased superoxide dismutase and glutathione activities in mice [18]. Four purified polysaccharides (APPsA-1, APPsB-1, APPsB-2, and APPsC-1) from the fruiting bodies of *A. polytricha* exhibited different antioxidant abilities in a concentration-dependent manner [19].

Therefore, this study was aimed at evaluating the antimicrobial activity of *Auricularia auricula* mushroom extracts on bacterial and fungal isolates so as to offer informed recommendation on its use for the treatment of antibiotic resistance.

2. Materials and Methods

2.1 Collection and Identification of Materials

Auricularia auricula 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 *Auricularia auricula* 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 [20, 21].

2.5 Inoculum Preparation

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 [1998]. 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, 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 ± 2ºC for 24 hours and yeast culture incubated at 28 ± 2ºC 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 [22].

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 ± 2ºC. 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 hours at 28 ± 2ºC [22].

3. Statistical Analysis

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

4. Results and Discussion

Figure 1 presents the antimicrobial activity of *Auricularia auricular* of methanol extract on the test organisms. *C. albicans* and *S. aureus* were most susceptible to the extract, followed by *C. glabrata* and *B. cereus* while *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.

The antimicrobial activity of *Auricularia auricular* of ethanol extract of the test different test organisms showed varied susceptibility to the extract. *E. coli*, *B. cereus*, *C. albicans* and *S. aureus* were well inhibited. *C. glabrata* was only inhibited at concentrations of 500 mg/ml and 250 mg/ml. whereas *P. aeruginosa* was 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 (Figure 2).

The antimicrobial activity of *Auricularia auricular* of aqueous extract on the different test organisms showed that *C. albicans* was most susceptible followed by *B. cereus*, *C. glabrata* and *E.coli* while *P. aeruginosa* was not even inhibited 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 (Figure 3).

Table 1 shows the result of the average MIC and MBC of the ethanolic, methanolic and aqueous extracts of *A.auricula* on test organisms. The MIC of ethanolic extract of *A. auricular* varied between 31.25 and 62.5 mg/ml with MBC of 31.25 to 125 mg/ml. The MIC of methanolic extract of *A.auricular* varied between 31.25 and 125 mg/ml with MBC of 62.5 to 250 mg/ml while the MIC of aqueous extract of *A. auricular* varied between 125 and 250 mg/ml with MBC of 125 mg/ml for *E. coli* and *B. cereus* while *S. aureus* and *P. aeruginosa* showed no activity.

Table 2 shows the result of the average MIC and MFC of the ethanolic, methanolic and aqueous extracts of *A.auricula* on test organisms. The MIC of ethanolic extract of *A. auricular* showed 15.63 mg/ml with MFC of 31.25 mg/ml for *C. albicans* and no activity on *C.glabrata*, the MIC of methanolic extract of *A.auricular* showed 125 mg/ml for both *C. albicans* and *C. glabrata* with MFC of 250 mg/ml whereas the aqueous extract of *A. auricular* showed MIC of 7.81 and 250 mg/ml for *C. albicans* and *C. glabrata* with MFC of 15.63 and 250 mg/ml, respectively.

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.

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 [21, 23, 24]. They have been traditionally used world-wide for treatment of variety of chronic disease [21]. This study was designed to evaluate the antimicrobial activity of *Auricularia auricular* 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. Mushrooms produce various antiviral, antifungal compounds to survive in the wild against competing or pathogenic agents [25, 26]. The variations in the antimicrobial activities of *Auricularia auricular* extracts may be due to the differences in their bioactive compositions or concentrations, methods of extraction and mechanism of action of active ingredients [21]. The results of the present study strengthened the outcomes of earlier works done by others which showed that mushrooms produced a great variety of antimicrobial agents. For instance, it is known that the extract from fruit bodies of several *Lactarius* sp. [27, 28]; *Fomitopsis* sp. [29]; *Boletus* sp. [30]; *Ganoderma lucidum*, *Navesporus floccosa* and *Phellinus rimosus* [31]; *Pleurotus tuberregium* [32]; *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* [33]; *Lactarius deliciosus*, *Sarcodon imbricatus* and *Tricholoma portentosum* [34]; *Russula delica* [35]; *Pleurotus eryngii* var. *ferulae* [36]; *Infundibulicybe geotropa*, *Lactarius controversus*, *Lactarius delicious* and *Phellinus hartigii* [37]; *Lactarius indigo* [38]; *Trametes hirsuta* [39]; *Stereum ostrea* [40]; *Lycoperdum perlatum* [23]; *Polyporus alveolaris* [24]; *Auricularia auricular* [17] and *Pleurotus squarrosulus* [21] contain a wide range of antimicrobial activity.

5. Conclusion

This research has further illuminated the medicinal value of *Auricularia auricular*. From the present study, it can be concluded that *Auricularia auricular* possesses good quantities of compounds which have potent antimicrobial activity. Therefore, they have lots of potentials for use in the production of novel drugs and medicines, considering the lingering threat of multi-drug resistance. Furthermore, clinical evaluation of *Auricularia auricular* through *in vivo* based research is highly recommended to achieve low cost, less side effect treatment and prevent recurrent infections.

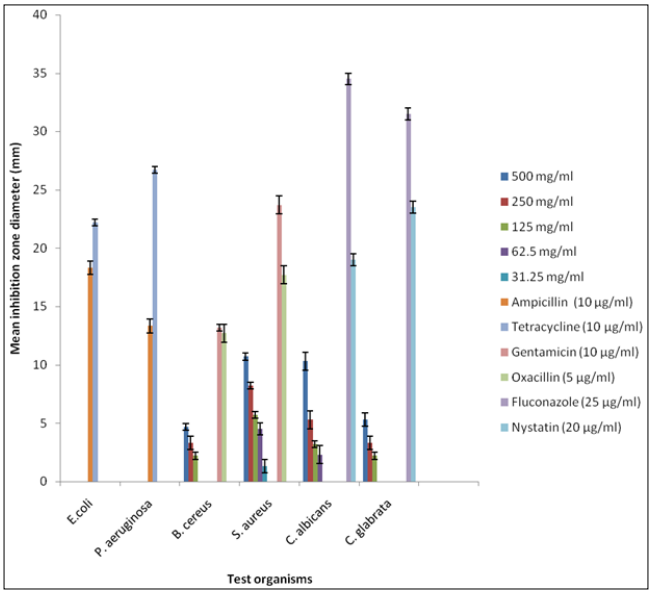

Fig 1: The effect of *Auricularia auricula* methanol extract on the different test organisms

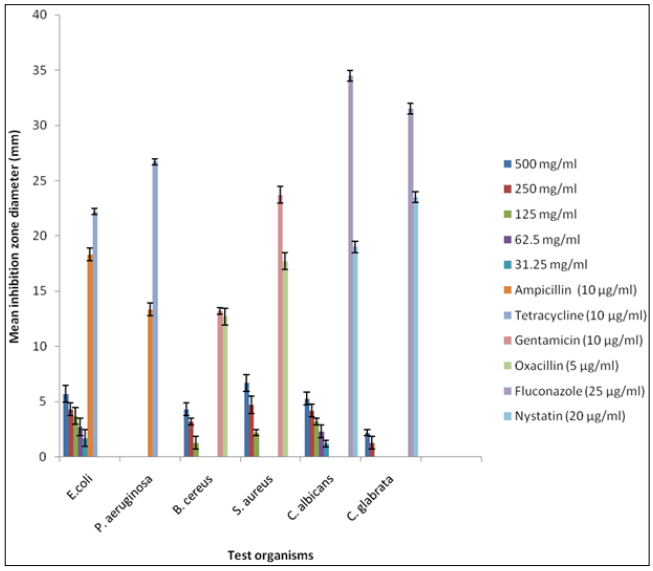

Fig 2: The effect of *Auricularia auricula* ethanol extract on the different test organisms

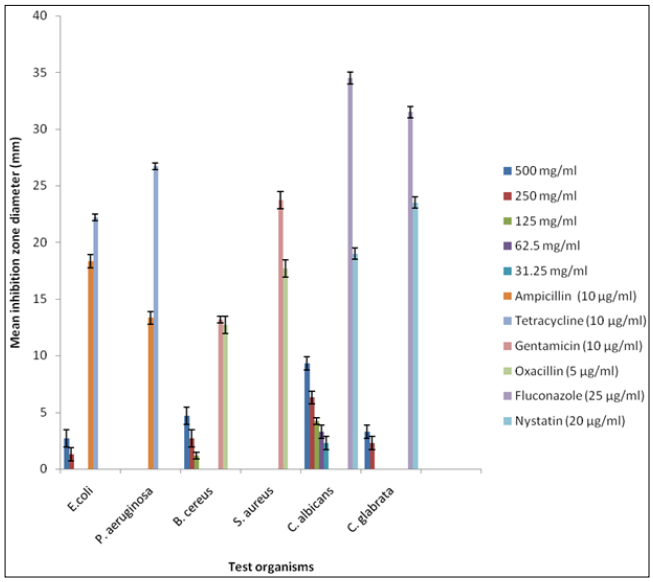

Fig 3: The effect of *Auricularia auricula* aqueous extract on the different test organisms

Table 1: The minimum inhibitory concentration (MIC) and Minimal Bactericidal Concentration (MBC) of the crude extract of *Auricularia auricular.*

Extract	Test organism	MIC (mg/ml)	MBC (mg/ml)
Ethanol	*B. cereus*	62.5	62.5
	S.aureus	62.5	125
	P. aeruginosa	ND	ND
	E.coli	31.25	31.25
Methanol	*B. cereus*	125	250
	S.aureus	125	125
	P. aeruginosa	62.5	62.5
	E.coli	31.25	62.5
Aqueous	*B. cereus*	125	125
	S.aureus	ND	ND
	P. aeruginosa	ND	ND
	E.coli	125	250

ND = not determined

Table 2: The minimum inhibitory concentration (MIC) and Minimal fungicidal Concentration (MFC) of the crude extract of *Auricularia auricular.*

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

ND = not determined

Table 3: Phytochemical Analysis of *Auricularia auricular* in Different Solvent

Mushroom name	Solvents	Gly	Tan	Sap	Fla	Car	Pro	Alk
Auricularia auricular	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.

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