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Antimicrobial properties of some *Nigerian edible* mushrooms

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ABSTRACT

In recent years, scientists have become more aware of the problems associated with drug resistance. This investigation was carried out to determine the proximate analysis and antimicrobial properties of three species of *Pleurotus sajor* caju, *Pleurotus ostreatus* and *Pleurotus florida*. Phytochemical compounds of the mushrooms were extracted using either ethyl acetate or distilled water as solvent, and the extracts were tested against 9 clinical isolates. The result of the proximate analysis showed that ash content ranged from 2.1 to 2.3, moisture (8.1-9.3%), dry matter (90.7 - 91.9%), crude protein (27.5 - 29.0%), fat (0.30 - 0.92%), crude fiber (17.3 – 19.2%), and carbohydrate (30.88 – 42.66 %). Qualitative phytochemical screening indicated that different *Pleurotus* species have various type of bioactive compounds, and antimicrobial activity study revealed that *Candida albicans* was inhibited by ethyl acetate extract (20.0 mm). Highest *in-vitro* antibacterial activity (23.0 mm) was exhibited by *P. florida* (ethyl acetate extract) against *Enterobacter aerogenes*. This was followed by the same extract against *Staphylococcus aureus* (22.0 mm) and *P. ostreatus* (ethyl acetate extract) against *Klebsiella oxytoca* (22.0). A significant zone of inhibition was observed in ethyl acetate extract of *P. florida* against all the test organisms except *Escherichia coli*. In conclusion, different mushrooms produced specific phytochemical compounds, and ethyl acetate extracts were more effective than aqueous extracts. Finally, further studies are needed to underpin and test these extracts against some other human pathogenic microbes coupled with purification and characterization of the bioactive compounds.

Keywords: Antimicrobial properties, Mushrooms, Proximate analysis, Phytochemical compounds, Test organisms

INTRODUCTION

One of the major challenges facing the healthcare service delivery all over the world is the fact that some of the common active antimicrobial agents have lost their combativeness in curing diseases mainly because of the ineffectiveness of most drugs currently available for treatment [1]. Microbial products are major constituents of new drug molecules [1] and researchers are beginning to search for novel antimicrobial agents from biological sources that include fungi [2,3,4]. The used of antimicrobial agents in the disease treatment involves using stains of microorganisms (test organisms) with high survival rate, even at high doses [5]. This will lead to growth inhibition of the target organism(s) when the appropriate drug is applied [6].

Mushrooms are any rapid-growing, woodcolonizing fleshy fungi, belonging to Basidiomycota phylum. Basidiospores formation is a major characteristic of this group of fungi. Some mushrooms live as saprophytes when they are alone or symbiotic association when they are found with plants. A lot of these macrofungi are edible and possess beneficial medicinal characteristics while some are also poisonous (toadstools). Mushroom is consumed among rural people than urban people however, older consumer's value mushrooms more than the young do [7]. A study conducted in 2017 over a period of 10 years revealed that about 28.9% of Nigerians consumed mushrooms and 13.3% had never tasted mushrooms [8].

Mushrooms are known as functional foods and as a source of drugs, confectionery and convenience foods [9,10,11]. Mushrooms do adapt their metabolism in their habitat for survival. That is why their metabolites have antimicrobial, antioxidation, and anti-inflammation activities [3]. Mushrooms bioactive compounds such as terpenoids, favonoids, tannins, alkaloids, and polysaccharides [12,13]. The basidiocarps and mycelia of mushrooms promote health benefit such as antioxidative, antibacterial and immunostimulatory activities [14]. The combination of these properties usually increases therapeutic values of the mushroom of interest. An investigation by Alves *et al.* [3] revealed that some mushroom extracts had

antimicrobial activity against antibiotic resistant strains of test organisms. *Russula delica* and *Fistulina hepatica* inhibited the growth of *E. coli*, *Morganella morganni* and *Pasteurella multocida* (Gram-negative bacteria), *Listeria monocytogenes*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus agalactiae* and *S. pyogenes* (Gram-positive bacteria).

Water, methanol, ethanol and ethyl acetate are among the solvents of choice for extracting antimicrobial compounds from mushrooms. For most biochemical reactions, water is commonly used because of its strong polarity, which facilitate easy extraction of active ingredients from most biological materials. Santoyo *et al.* [15] reported that water extracts of *L. edodes*, *Boletus edulis*, and *Pleurotus ostreatus* had stronger antibacterial activities as compared to other extracts. It has been suggested that water extraction may be affected by temperature. It should be noted that higher temperature may increase the ease of extraction, it may also lead to degradation of antimicrobials that are heat-sensitive. As more biological materials are being explored for possible solution to drug-resistance, a given mushroom could be an alternative or additional source for a new and novel antimicrobial agent. Therefore, the study sought to complement existing literature on the antimicrobial effect of mushrooms on some clinical isolates. This study aimed to investigate the antimicrobial effect of three common *Pleurotus* species.

MATERIALS AND METHODS

Sample Collection

The three (3) species of mushrooms (*Pleurotus florida, Pleurotus osteratus* and *Pleurotus sajor caju*) used for this present study were collected from Forestry Research Institute of Nigeria, Ibadan, Oyo State, Nigeria.

Proximate analysis

The proximate analysis of all mushrooms were performed according to the methods described by Oyeleke [16] and Sunday *et al.* [17].

Extracts preparation

Freshly harvested samples of each mushroom were cut into slices, air dried in a drying oven (Selecta, Madrid, Spain) at 50ºC to constant weight. A fine powder (20 mesh) was obtained with a hammer stirrer (IKA, Staufen, Germany). A 5 g sample of each dried mushroom was extracted by stirring with 50 mL of either distilled water or ethyl acetate in a beaker and was continuously shaken for 96 hours to dissolve the constituents' phytochemicals such as tannins, flavonoids, alkaloids, terpenoids and others. The aqueous solution was filtered using Whatman's No 1 filter paper.

Qualitative determination mushrooms' phytochemical compounds

The phytochemicals compounds were determined according to the methods described by Adeoyo *et al.* [18].

Test organisms

Pure cultures of the test organisms (*E. coli, Staphylococcus aureus, Klebsiella oxytoca, Salmonella pullorum, Moraxella* sp, *Enterobacter aerogenes, Burkhoderia pseudomallei, Pseudomonas aeruginosa* and *Candida albicans*) were obtained from the stock culture of previously identified isolates of the Microbiology Laboratory of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The microbes were maintained on agar slants at 4˚C to avoid contamination.

Preparation of inoculum

The test organisms were subcultured on appropriate media and incubated at 37˚C for 24 hours. Colonies of microorganisms were inoculated into sterile distilled water. The bacterial suspension was mixed evenly and then diluted to meet the turbidity of 0.5 McFarland standards which was approximately 1.5×10^8 cfu/mL.

Antimicrobial studies

Antimicrobial test was carried out using the agar well diffusion method. In the agar well diffusion method, a 24-hour old broth culture was swabbed onto a sterile Mueller Hinton agar in Petri dishes using sterile cotton swab. The inoculated plates were allowed to dry after which three wells were punched on the agar at equidistant positions using a sterile standard 6 mm Cork borer. Each hole was filled with 0.1 mL of extract. A positive control was set up, a hole was filled with 0.1 mL chloramphenicol (50 µg/mL) while a negative control holes contained the extraction solvent. The plates were allowed to stand on the bench for 40 min for proper diffusion of the extract to occur and then incubated at 37ºC for 24 hours. The resulting zone diameter of inhibition around each test well was measured using a transparent ruler calibrated in millimeters.

RESULTS

Proximate analysis of mushrooms

The result of the proximate analysis include ash (ranged from 2.1 to 2.3), moisture content (8.1-9.3%), dry matter (90.7-91.9%), crude protein (27.5 - 29.0%), fat (0.3 – 0.92%), crude fibre (17.3 – 19.20%) and carbohydrate (30.88 – 42.66 %) (Table 1).

Table 1: Proximate analysis of mushroom samples

Organism	Ash (%)	M (%)	D.M (%)	C.P (%)	Fat (%)	C.F (%)	CHO (%)
P. sajor caju	2.1	8.1	91.9	29.0	0.30	18.3	41.70
P. ostreatus	2.3	9.3	90.7	27.5	0.74	17.3	42.66
P. florida	2.2	8.5	91.5	28.3	0.92	19.2	30.88

Key: M = Moisture, D.M = Dry matter, C.P = Crude protein, C.F = Crude fibre, CHO = Carbohydrate

Phytochemical compound in mushrooms

The major phytochemical compounds present in each mushroom sample were determined. The results showed that all samples were able to produce alkaloids, phenols, and cardiac glycosides (Table 2).

Antimicrobial properties of mushrooms

Ethyl acetate extract of the three *Pleurotus* species used for this study had higher degrees of antagonistic effects against most of the tested microorganisms compared to the water extracts. This was noted by the clear zones of inhibition found on bacterial and fungal isolates. The best *in-vitro* antibacterial activity (23.0 mm) was revealed by *P. florida* ethyl acetate extract against *Eenterobacter aerogens* (Table 1). This was followed by the same plant extract against *Staphylococcoccus aureus* (22.0 mm) and *P. ostreatus* ethyl acetate extract against *Klebsiella oxytoca* (22.0). Generally, significant zone of inhibition was observed in ethyl acetate extract of *P. florida* against all the test organisms except *E. coli* (4 mm). Also, *P. ostreatus* ethyl acetate

extract exhibit significant zone of inhibition against all the test organisms except *E. coli* (0 mm), *Burkhoderia pseudomallei* (0 mm), *Salmonella pullorum* (7 mm) and *Moraxella* spp. (10 mm). However, all the test organisms inhibited were by the ethyl acetate extract of *P. sajor caju* (Figure 1 to 3).

Table 2: Qualitative determination mushroom extracts for phytochemical compounds

Phytochemical compound	*Pleurotus florida*	*Pleurotus ostreatus*	*Pleurotus sajor caju*
Alkaloids	+	+	+
Phenols	+	+	+
Flavonoids	+	-	+
Saponins	+	+	-
Steroids	-	Nd	-
Cardiac glycosides	+	+	+
Tannins	-	+	-
Terpenoids	+	Nd	+

Key: + = positive, - = negative, Nd = not determined

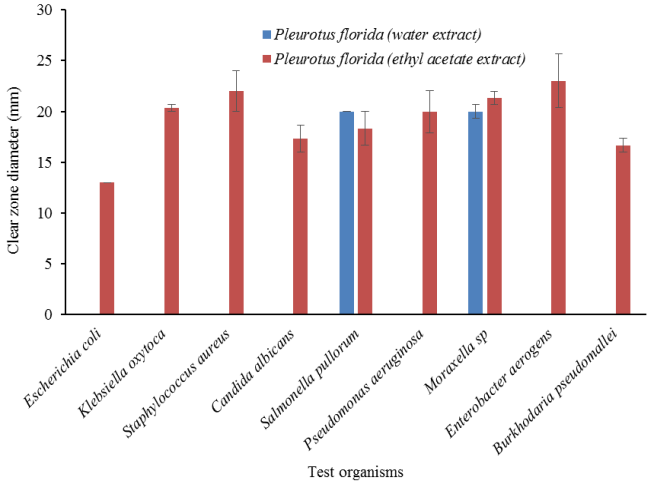

Figure 1: Zone of inhibition (mm) of *P. florida* against some test organisms. Error bars represented the standard errors of the means (±SEM).

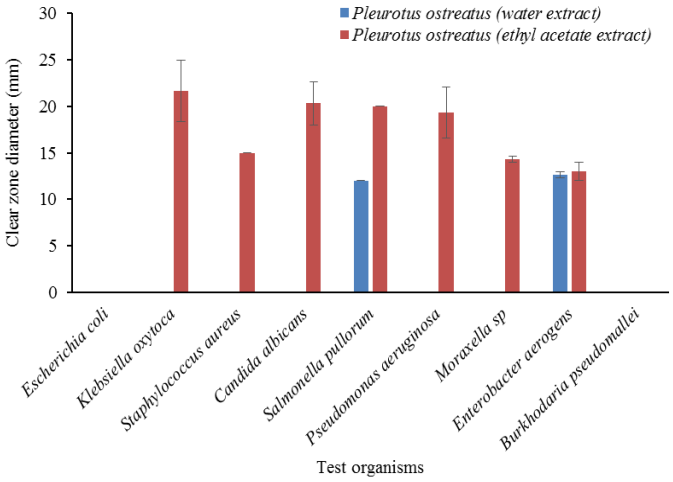

Figure 2: Zone of inhibition (mm) of *P. ostreatus* against some test organisms. Error bars represented the standard errors of the means (±SEM).

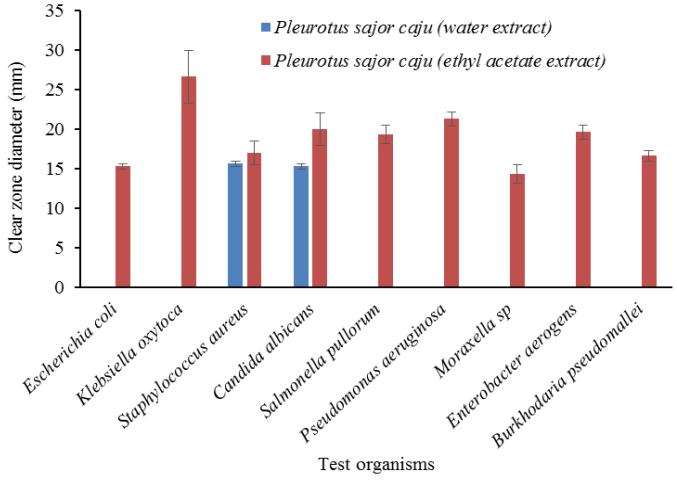

Figure 3: Zone of inhibition (mm) of *P. sajor caju* against some test organisms. Error bars represented the standard errors of the means (±SEM).

DISCUSSION

The crude protein content of the mushroom ranged from 27.5 to 29.0%. This showed that most mushrooms have appreciable quantities of protein from nutritional perception, thus these *Pleurotus* species are rich sources of protein. The obtained values compare favourably with the reported protein values in oyster mushroom (16.35%) by Afiukwa *et al.* [19]. Ash content for the mushrooms were relatively low (2.1% - 2.3%). This is a little more than the 1.5% ash content by Sunday *et al.* [17]. A low ash content (1.41%) value has also been reported by Zahid *et al.* [20]. The crude fibre contents of the mushrooms were between 17.3 – 19.2%, which is low when compared to the values obtained by Afiukwa *et al.* [19] who recorded 29% of crude fibre and higher than 2.05% reported by Sunday *et al.* [17]. High fibre contents in mushrooms were obtained, which suggests that some mushrooms have the capacity to improve the health of humans through quickening the removal of unwanted substances from the body.

The results of the moisture contents of the mushrooms (8.1 - 9.3%) indicated that they are among the perishable foods. When the moisture content of a food material is high, it encourages growth of unwanted microbes and increased activity of enzymes [21]. The result showed that the lower moisture contents observed in the mushroom would make them have longer shelf life. The high values of carbohydrate recorded revealed that these mushrooms are good energy food sources. The carbohydrate contents of the mushrooms in the *Pleurotus* species were 41.7, 42.66 and 30.88% for *P. sajor caju, P. ostreatus* and *P. florida*, respectively. The results compared well with the amount reported for some mushrooms [19]. The amounts of fat in the mushrooms spanned from 0.3 to 0.92%. The fat content reported is very low when compared to values obtained for carbohydrate and protein in the mushroom samples. Similar observations were made by Wani *et al.* [22].

Macrofungi such as mushrooms contain unique antimicrobial compounds that include; flavonoids, alkaloids, tannins, peptides, proteins, steroids, terpenoids, and anthraquinones [3]. Several studies have found that most mushroom extracts antimicrobial potentials are largely dependent on the mushrooms' strains, vegetative forms, cultivation conditions, methods of extract preparation, evaluation and results interpretation [23]. *Pleurotus* species have different type of phytochemical compounds (Table 2), and this explains the variation noticed in their antimicrobial activity.

The study also revealed that the antimicrobial activities of ethyl acetate extracts were better than those of water extracts against some selected clinical isolates. A study by Awala and Oyetayo [24] indicated that antimicrobials from mushrooms vary with solvents used for extraction. The ethyl acetate extract of the mushrooms were found to be effective against all the isolates except *E. coli.* In contract to our findings, water extracts of some mushrooms were observed to have higher activities against *B. subtilis, P. aeruginosa*, *E. coli*, and *C. albicans* isolates [25]. The lower activities obtained with water extracts can be explained based on the method of extraction used, solvent's extraction capacity, bioactive compounds solubility and period of extraction [26].

Despite the effectiveness of the ethyl acetate extracts against the test isolates, the ethyl acetate extracts of *P. osteratus* resulted in weak inhibitory effects towards *Burkhoderia pseudomallei, Moraxella* sp, and *Salmonella pullorum*. However, significant inhibitory effect was observed towards other test organisms tested. Akyuz *et al.* [27] also reported similar outcome. In their study, ethyl acetate extract of *P. ostreatus* showed activity against *S. aureus* (10.0 mm) and *P. aeruginosa* (11.3 mm). However, their study also reported activity against *E. coli* (8.7 mm), which is contract to the findings of this study. This could be due to the genetic characteristics of this oyster mushroom specie that lead to alterations in chemical composition [28].

Antimicrobial agents have long been used for therapeutic purposes and prophylactic; however, the drug-resistant bacterial strains have been creating serious treatment problems. This situation has necessitated the search of new antimicrobial substances effective against pathogenic micro-organisms resistant to conventional treatments. Evolutionary processes are often implicated in the emergence of disease resistance to antibacterial drugs [29]. Natural resources, such as those herein studied, could be an alternative, which when included in diet take advantage on the synergistic effects of all the bioactive compounds present, help to improve the health of consumers.

CONCLUSION

In conclusion, ethyl acetate extracts of *P. sajor caju, P. florida,* and *P. ostreatus* have shown varying degrees of antimicrobial activities against the tested organisms. Further investigations are needed to underpin the strength of these extracts against a wider range of human pathogenic microorganisms. In addition, purification, identification, and determination of bioactive compounds responsible for the antimicrobial activities are important in the development of antibiotics. Finally, the use of *P. florida, P. ostreatus* and *P. sajor caju* as sources of antimicrobial agents should be encouraged.

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Conflict of interest

Authors declare no conflict of interest

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