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ANTIMICROBIAL ACTIVITIES AND PHYTOCHEMICAL CONSTITUENTS OF EDIBLE MUSHROOMS *PLEUROTUS OSTREATUS* AND *AGARICUS BISPORUS* SOLD IN ENUGU METROPOLIS

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Abstract

The continuous rise in antimicrobial resistance has led to an unending search for newer, cheaper, and safer sources of antimicrobials. Mushrooms are known to possess bioactive metabolites, which can serve as pharmaceutical agents. The antimicrobial activity of aqueous and methanol extracts from edible mushrooms *Pleurotus ostreatus* and *Agaricus bisporus* against four pathogenic bacterial strains; *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa,* and the yeast *Candida albicans* were evaluated using the agar well diffusion method. Preliminary phytochemical screening of the mushroom *P. ostreatus* and *A. bisporus* extracts revealed the presence of some essential phytochemicals which include; alkaloids, glycoside and saponins. The best antibacterial activity was displayed by the methanol extract of *P. ostreatus* against *B. subtilis* (IZD=8.00 ± 1.40 mm) and *S. aureus* (6.0 ± 0.11 mm) at a concentration of 50mg/ml, whereas aqueous extract showed comparatively lesser activity. None of the extracts displayed any antifungal activity in this study. The methanol extracts of *P. ostreatus* may contain bioactive compounds which also serve as potential antibacterial agents for the treatment of infections caused by *B. subtilis.*

Keywords: *Pleurotus ostreatus*, *Agaricus bisporus* antimicrobial, phytochemicals, mushroom

Introduction

Growing concerns about global antibiotic resistance have plunged into new depths as the World Health Organization (WHO), is now warning that the world is "running out of antibiotics" (WHO, 2020). The incessant emergence of drug-resistant bacteria has posed a critical challenge to the treatment of clinical diseases (Zhu and Lu, 2019), resulting in a gradual increase in the frequency of adverse community and nosocomial infections. The search and exploration for bioactive compounds effective in treating pathogenic microorganisms resistant to present-day drugs have become very intense. Presently, there is a growing interest in searching for new antimicrobial agents from natural sources such as bacteria, fungi, and plants (Gebreyohannes *et al*., 2019). Natural products especially microbial and plant products, constitute the major sources of new drug molecules (Nwobodo *et al*., 2020).

Mushrooms are fungi fruiting bodies bearing spores that grow above the soil or on their substrates, and forms a major group of smaller plant kingdom. Mushrooms are large enough to be seen by the naked eyes, they have distinctive fruiting body, because of the nutritive contents, some mushrooms are edible while some are utilized extensively in traditional medicines (Karaman *et al*., 2012). People have harvested wild mushrooms for medicine and foods for thousands of years. There are about 1200 known species of mushroom utilized in 85 dissimilar countries for their medicinal properties (Wasser, 2014).

Mushrooms have medicinal properties especially due to richness in biologically effective compounds that have antioxidant, antimicrobial properties strengthening the immune system and assuring against carcinogens (Akpi *et* al., 2017; Golak-Siwulska *et al*., 2018; Gebreyohannes *et al*., 2019).

The use of synthetic drugs remains a great concern to human health, in that it has numerous side effects which could probably lead to other diseases and death. Other report has it that some of these synthetic antibiotic drugs could lead to induced hepatoxicity, which would be more severe in patients that are immunocompromised (Sharma *et al*., 2014). In advancement, scientists now prefer the use of medicinal plants, which includes mushrooms that possess phytochemicals with antimicrobial activities. Mushrooms such as *Pleurotus ostreatus* (Oyster) and *Agaricus bisporus* have been reported to have numerous health benefits like anticarcinogenic, anti-inflammatory, antitumor activities (Sathishkumar *et al.*, 2016; Fakoya *et al*., 2020).

Mushroom species are known to produce and release various bioactive compounds which include; alkaloids, terpenoids, flavonoids, tannins, and polysaccharides (Fakoya *et al*., 2020). Despite the wealth of bioactive compounds possessed by mushrooms, they remain underexplored for useful natural compounds. The bioactive compounds are found in various cellular components and secondary metabolites, which have been isolated and identified from the fruiting bodies. The fruiting bodies and mycelium of mushrooms exhibit health promoting values such as immunostimulatory antibacterial, and antioxidative properties (Gebreyohannes *et al*., 2019). Whereas there is a lesser shortage of agents active against Staphylococci, the prevalence of infections with methicillin-resistant *Staphylococcus aureus* (MRSA) remains extremely high in many countries. The use of natural sources such as mushroom has proven to be imperative in a way that is less detrimental, unlike chemically synthetized drugs that have numerous side effects. Generally, antimicrobial compounds produced by algae and fungi (mushrooms) against pathogens have received considerable attention as a new source of unique antimicrobial substances (Ramesh and Pattar 2010; Turkoglu *et al*., 2016). This study is therefore aimed at evaluating the antimicrobial activities of two edible mushrooms sold in Enugu metropolis.

Materials and Methods

Collection and Processing of Mushroom Samples.

Fruiting bodies of mushroom samples were purchased from Shoprite mall Enugu, Enugu State, Nigeria, and authenticated at taxonomy department of the Federal Institute of Industrial Research (FIIRO) Oshodi, Lagos, Nigeria. About one kilograms of procured mushrooms was cut into small pieces with the help of a knife, sun dried for 10 days. These dried pieces of mushrooms were then pulverized into a coarse powder with the help of a grinding machine (FFC-15, China) and stored in an airtight container for further use.

Extraction of Mushroom Crude Extracts

Methanol Extracts.

Fifty grams of each sample was weighed and successively extracted with 500 mL of 70% v/v methanol. The mixture was allowed to stand at room temperature for three days with frequent agitation. The supernatant was filtered using Whatman filter paper (number 1) (Sigma-Aldrich, Michigan, USA). The filtrates were concentrated in a rotary evaporator at 40°C under reduced pressure and lyophilized. The extracts were properly kept in air tight containers, labelled, and stored until required for use.

Warm aqueous extraction

Fifty grams of each sample was weighed and transferred into 500 mL of heated warm water (50 °C) in a beaker. The beaker was placed on a hot plate at 70 °C and allowed to stand for 4 hours with intermittent stirring. The samples were filtered using sieve cloth and centrifuged at 4500 rpm, for 30 minutes. The supernatants were conventionally heated to a final volume of 5 ml.

Qualitative Phytochemical Analysis

Preliminary phytochemical analysis was carried out for the detection of the following bioactive compounds; tannins, saponins, flavonoids, steroids, terpenoids, alkaloids, glycosides, in both the crude aqueous and methanolic extracts using standard procedures described by Fakoya *et al*. (2020).

Antimicrobial Assay

Microbial Test Isolates

A total of five (5) clinical microbial isolates comprising of Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and a yeast (*Candida albicans*), were used in this study. Each of the isolates were appropriately identified and confirmed using the standard protocols.

Preparation of Test Inoculums

All bacterial test isolates were cultured on MHB and incubated at 37 °C for 18-24 hours, while fungal isolate was grown in MEB at 27°C for 48 hours. The inoculum size of each test organism was adjusted to a concentration of 1.5×10^8 CFU/mL by comparing with 0.5 McFarland standards.

Determination of Inhibition Zone Diameter (IZD) of the Mushroom Crude Extracts The inhibition zone diameter was determined by the agar well diffusion method as described by Nwobodo *et al*. (2020), with little modifications. 20 ml of sterile MHA and MEA stabilized at 45°C for 15 minutes were seeded with 100 μL of 1.5×10^8 CFU/mL of test bacteria and fungus, respectively, and aseptically poured into the respective sterile Petri dishes and allowed to set. Five wells (6 mm) equidistant from each other were created with the aid of a sterile cork borer. The wells were filled with 100 μL of 50 mg/mL of the respective mushroom extracts. The plates were allowed to stand on the bench for 45 minutes to allow diffusion of the extract. The zones of growth inhibition were measured after 24 h of incubation at 37°C for bacteria and after 72 h at 28°C for the fungus. Ciprofloxacin (10 µg/ml) and ketoconazole (50 µg/ml) served as reference antimicrobial agents against test bacteria and fungus, respectively. The procedures were performed in triplicates and the mean zones of growth inhibition determined.

STATISTICAL ANALYSIS

Experimental values are represented as means ± standard deviation (SD). Statistical significance was determined by one-way variance analysis (ANOVA), with significant difference considered at $P<0.05$. The Microsoft Excel (2016) and SPSS version 20 software were used for analysis. All experiments were conducted in duplicates.

RESULT

Qualitative Phytochemical Analysis

Table 1 shows the result of the phytochemical analysis carried out on the various extracts of *P. ostreatus* and *A. bisporus*. Both the methanol and aqueous extracts of *P. ostreatus* and *A. bisporus* were all found to contain alkaloids and Glycoside. However, only the methanol and aqueous extracts of *P. ostreatus* were found to contain saponins. Tannins and flavonoids were absent in all four extracts examined. Alkaloids and glycoside were found to be present in all the

tested extracts, while saponins were present only in the extracts of *P. ostreatus*.

Table 1: Phytochemical analysis of the extracts of *Pleurotus ostreatus* and *Agaricus bisporus*

Phytochemical	Mushroom extracts			
	P. ostreatus **(methanol)**	***P. ostreatus*** **(aqueous)**	**A. *bisporus*** **(methanol)**	**A. *bisporus*** **(aqueous)**
Alkaloids	+	+	+	+
Tannins	-	-	-	-
Flavonoids	-	-	-	-
Saponins	+	+	-	-
Glycoside	+	+	+	+

Key: +: presence of secondary metabolite; −: absence of secondary metabolite.

Antimicrobial Activity

The results of antimicrobial activities of the extracts of *P. ostreatus* and *A. bisporus* are represented in Table 2. All test organisms used in this study displayed a high level of resistance towards 75% of the various mushroom extracts studied, with only the methanol extract of *P. ostreatus* displaying any inhibition against *B. subtilis* (Plate 2) and *S. aureus*. The methanol extract of *P. ostreatus* at a concentration of 50mg/mL showed mean zone of growth inhibition of 8.00 ± 1.40 mm and 6.0±0.11 against the Gram-positive Bacteria *B. subtilis* and *S. aureus* respectively. At a value of $P < 0.05$, there is a strong significant difference between the mean IZD of the oyster methanol extract against *B. subtilis* when compared with the control drug ciprofloxacin. The P value was found to be 0.005731. Similarly, there is a significant difference in the mean IZD value obtain against *S. aureus* when compared with ciprofloxacin, with $P < 0.05$.

Table 2: Antimicrobial Inhibition Zone Diameters of Various Extracts of *Pleurotus ostreatus* and *Agaricus bisporus*

Mushroom Extract. (50 mg/ml)	IZD of Test Organisims (mm)				
	S. a	***B.s***	***P. a***	***E. c***	***C.a***
P. ostreatus Methanol Extract	**6.0±0.11[a]**	**8.0±1.40[c]**	0.0±0.00	0.0±0.00	0.0±0.00
P. ostreatus Aqueous Extract	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00

A. bisporus Methanol Extract	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
A. bisporus Aqueous Extract	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
DMSO	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Ciprofloxacin (10µg/ml)	24±1.10^{b}	21±0.05	23±0.75^{b}	20±1.0	NA
Ketoconazole (50µg/ml)	NA	NA	NA	NA	17±0.52

Keys: *S.a = Staphylococcus aureus, E.c = Escherichia coli, Ps.a = Pseudomonas aeruginosa, B.s = Bacillus subtilis, C.a = Candida albicans.* NA = Not Applicable.
Values are means of triplicates ±SD, Samples carrying the same superscripts in the same column are not significantly different at ($p<0.05$)

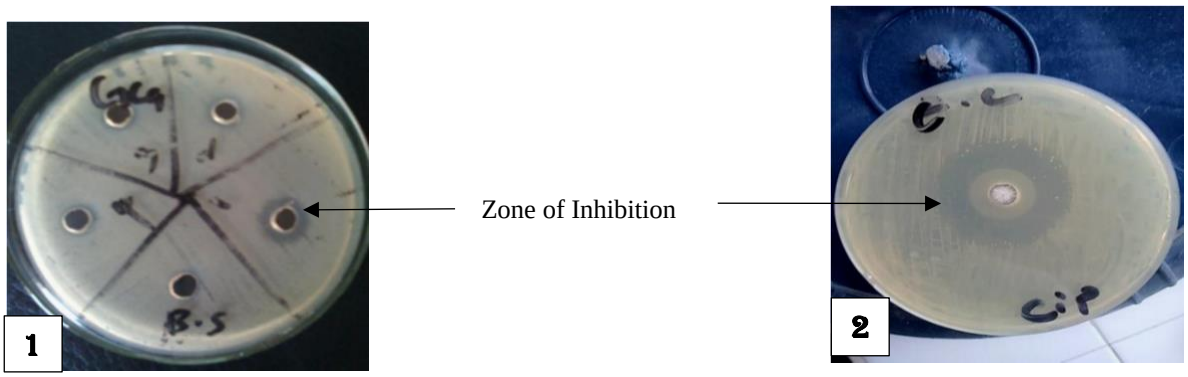

Plate 1. Inhibition zone as exhibited by the positive control ciprofloxacin against *Eschericha coli*
Plate 2. Inhibition zone as exhibited by methanol extract of *Pleurotus ostreatus* against *Bacillus subtilis*

DISSCUSSION

Many edible mushrooms are believed to contain bioactive compounds, which have increased their applications in ethnobotanical medicine for the treatment of specific health problems. The preliminary phytochemical screening in this study reveals the presence of various active constituents. These active metabolites are well known for their curative activities against several human problems such as diuretic choleretic, spasmodic, chronic eczema, diarrhoea, dysentery and menstrual disorders (Sathishkumar *et al.*, 2016).

In this study, both the methanol and aqueous extracts of *P. ostreatus* and *A. bisporus* were all found to contain alkaloids and glycoside. However, only the methanol and aqueous extracts of *P. ostreatus* were found to contain saponins. Tannins and flavonoids were absent in all four extracts examined. This is similar to the findings of Fakoya *et al*. (2020), who recently reported the presence of alkaloids saponins and glycoside in the aqueous and ethanol extract of oyster mushroom. The absence of Tannins and flavonoids may be due to the differences in

substrates from which the various mushroom samples were cultivated (Kimenju *et al*., 2009). Mushrooms are regarded as non-sources of flavonoids. The absence of flavonoids in mushrooms may be of biological advantage in their various ecological niches, since these bioactive compounds inhibit activities involved in their pigmentation, growth and development (Mattila *et al.,* 2001). Fungal species have been proven to be outstanding potential sources of bioactive compounds of high therapeutic value. They are also the richest sources of secondary metabolites (Nwobodo *et al*., 2020). The two mushrooms genera addressed in this study have previously been shown to possess promising antimicrobial activities against the tested organisms (Sathishkumar *et al.,* 2016; Fakoya *et al*., 2020).

All test organisms used in this study displayed a high level of resistance towards 75% of the various mushroom extracts studied, with only the methanol extract of *P. ostreatus* inhibiting *B. subtilis* (Plate 2) and *S. aureus* (Table 2). The methanol extract of *P. ostreatus* at a concentration of 50mg/mL showed mean zone of growth inhibition of 8.00 ± 1.40 mm and 6.0±0.11 against the Gram-positive Bacteria *B. subtilis* and *S. aureus* respectively. However, there is a strong significant difference ($P < 0.05$) between the mean IZD of the *P. ostreatus* extract against *B. subtilis* when compared with the control drug ciprofloxacin (Table 2). About 75% of the mushroom metabolites extracted in this study were not effective in inhibiting growth among Gram positive bacteria. This is in agreement with the findings of similar study carried out elsewhere by Thillaimaharan *et al*. (2016), who also reported the same result. According to Tsungai *et al.* (2016), antimicrobial activities of mushrooms extracts are greatly influenced by their habitat which could lead to differences in the type of secondary metabolites produced and bioactive outcomes. This is because the habitats and culture substrates, which are important factors in the development and production of bioactive metabolites of the studied mushrooms, are unknown. Other researchers have previously reported the resistance of *P. ostreatus* extracts by *E. coli,* and *S. aureus* (Owaid *et al*., 2015; Akyuz *et al*., 2010). The fungi isolate (*C. albicans*) used in this study displayed complete resistance to both methanol and aqueous extracts of *P. ostreatus* and *A. bisporus*. Similarly, Akyuz *et al*. (2010), reported no antifungal activity of the extracts of both *P. ostreatus* and *A. bisporus* against *Candida albicans*. There are several other reports stating the resistance exhibited by fungi pathogens towards mushroom extracts, irrespective of the extraction methods and solvents used (Gbolagade and Fasidi, 2005)

It is worthy to note that the test microorganisms used in this study are sensitive strains, as evidenced by the inhibition zones observed when subjected to the positive control ciprofloxacin (Table 2 and Plate 1). The resistance exhibited by the test isolates used in this study towards the mushroom extracts might as well be as a result of either the development or acquisition of resistance genes, since they were all laboratory isolates. On the other hand, the absence of flavonoid which has been reported to be responsible for antimicrobial activity (Wang *et al*., 2013) could as well be responsible. Based on reviews, it seems that the antimicrobial activity of *P. ostreatus* and *A. bisporus* are changeable as reported by other researchers (Iwalokun *et al.,* 2007), which may arise from the genetic structure of mushroom species, physical, biochemical constituents, chemical differences of mushroom extracts, solvents and test microorganisms that other research shows clearly when it's compared to the other mushroom species (Wang and Ng,

2004; Cohen *et al*., 2002). This study indicated that there are differences in the antimicrobial effects of mushroom groups, due to phytochemical differences among species. Mushroom species possess different constituents and in different concentration, which account for the differential antimicrobial effect. The antibacterial activities against Gram positive bacteria (*S. aureus* and *B. subtilis*) exhibited by the methanol extract of *P. ostreatus* denotes narrow spectrum activity, and may be attributed to the presence of other bioactive metabolites of various chemical types in mushrooms compounds.

CONCLUSION

The extracts of *P. ostreatus* and *A. bisporus* prepared with methanol revealed potential antimicrobial activities against Gram positive bacteria (*S. aureus* and *B. subtilis*), with the best activity against *B. subtilis*. However, 75% of the extracts studied had no antagonistic effect against any of the test bacteria or yeast used in this study. This study justifies the antibacterial claims and usage of mushrooms in herbal treatment. Nevertheless, there is need to further fractionate, identify and characterize the bioactive compounds embedded in the mushrooms, to maximize their pharmaceutical potentials and applications.

DECLARATION OF COMPETING INTERESTS

The authors declare that they have no conflict of interest

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