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ANTIBIOTIC ACTIVITIES OF MUSHROOM EXTRACTS AGAINST ANTIBIOTIC - RESISTANT ORGANISMS

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Abstract

Antibiotic resistance accompanied by high rate of chemotherapeutic failure has paved way into the assessment and investigation of medicinal plants in order to assess their antimicrobial potency. This research was aimed at analyzing the antibiotic activities of mushrooms extracts against multi-drug resistant isolates from hospital. The identification of the microorganisms and antibiotic resistant status were investigated using Standard Microbiological, Biochemical and antibiotic sensitivity tests. The Aqueous and Ethanolic extracts of two (2) mushroom species *Agaricus bisporus* and *Omphalotus palmatus* were obtained and analyzed for their quantitative and qualitative phytochemical contents. The results revealed that the resistant index of *Staphylococcus* sp and *Klebsiella* sp was 0.6 and 0.4 respectively. The bioactive compounds contained in the mushroom extracts include different quantities of Alkaloids, Proteins, Flavonoids, reducing sugars, Tannins, Terpenoids, Saponin and Cardiac glycoside. All mushroom extracts were active against test bacteria but at varying degrees with the aqueous extract of the mixed mushroom extracts being the most active. It was also observed that the Minimum Inhibitory Concentration (MIC) of ethanolic extract of *Omphalotus palmatus* and mixed aqueous extract of both mushrooms were at 25mg/ml concentration while that of aqueous extract of *Agaricus bisporus*, aqueous extract of *Omphalotus palmatus* and ethanolic extract of mixed mushroom samples were at 50mg/ml. The antimicrobial metabolites produced by mushrooms and their synergistic actions can be harnessed in the treatment of diseases.

Keywords: Mushroorms, Antibiotics, Multi-drug resistance

Introduction

Antibiotic resistance is on the increase leading to higher morbidity and death, longer hospital stays, and higher hospital expenses (Davey and Marwick, 2008; Piddock, 2012; Thabit *et al*., 2015). According to data, more than seventy percent (70%) of bacteria that cause nosocomial infections are resistant to at least one of the most regularly used antibiotics (Lambert *et al*., 2010; Mertz *et al*., 2015). Antimicrobial resistance is increasing not only in hospital-acquired illnesses, but also in community-acquired infections in most countries partly because it is aided by a number of causes, some of which are related to the agent or host (Odonkor and Addo, 2011; Abella *et al*., 2015; Fistarol *et al*., 2015).

There is an increase in the number of bacteria resistant to broad-spectrum antibiotics and this has become a major public health concern around the world (Khudaibergenova, 2015). The discovery of novel antimicrobial drugs that act on newly adapted microbes has become a priority in light of the rapid growth of multi-drug resistance (Laxminarayan *et al*., 2013; Torjesen, 2013). Gilbert *et al*., (2010) focused on developing antibiotics from products of natural origin that can manage infection without promoting the formation of resistance bacterial strains. This has led to prompt testing of numerous medicinal plants for antimicrobial activity (Colombo and Bolsisio, 1996; Iwu *et al*., 1999). Another factor for seeking alternative treatment is the high cost of developing new therapeutic drugs.

Plants, such as Mushrooms (both edible and non-edible species) have been shown to have medicinal properties and they are readily available around us (Anusiya *et al*., 2021). Jonathan and Fasidi (2003) investigated the effects of a variety of mushrooms on bacteria, including *Agaricus bisporus, Lentinu laedodes, Auricularia auricular*, and *Pleurotus* species, and found inhibitory responses against bacteria such as *Mycobacterium smegnatis* and yeast pathogenic strains. Mushrooms are macrofungi that have a characteristic fruiting body that can be seen with the naked eye. Some mushrooms are eaten for their nutritional value, while others have long been employed in traditional medicine (Stamets, 2000).

Due to the presence of specific molecules of interest in mushrooms, such as terpenes, sesquiterpenes, steroids, anthraquinone and derivatives of benzoic acid, quinolines, and proteins, mushrooms can be regarded a source of natural antibiotics (Alves *et al*., 2012). Many researchers have broadly studied the antibacterial properties of mushroom extracts, but there is still paucity of data on the activity of mushrooms against antibiotic resistant bacteria as well as the identification and quantification of the individual compounds responsible for those properties. The aim of this research is to analyze the antibiotic activities of mushrooms against antibiotic resistant organisms isolated from hospital samples, and phytochemical properties of the mushrooms.

Materials And Method

Sample Collection

Edible mushroom (*Agaricus bisporus*) bought from Eke Awka market and wild mushroom *Omphalotus palmatus* from got from a tree trunk in a nearby forest in Awka were collected in clean polythene bags containing dried ice and transferred to the laboratory within a maximum of 3hours after harvest. The mushrooms collected were identified using the morphological and

microscopic features according to Dickinson and Lucas (1982) and Varma and Kharkwal, (2009). The mushrooms were properly cleaned from dust and contaminants and left at 35^0C in the dark for about 72 hours. The dried mushrooms were subsequently ground to fine powder and stored at -20ºC until analyzed.

Extraction of the phytochemical constituents of Mushroom

The extraction was carried out in accordance with the procedure of Fasidi and Jonathan (1994). Fresh mushrooms were thoroughly washed with clean water, cut into pieces and air-dried. Each of the different air-dried mushroom samples were respectively soaked in ethanol (95%) and cold water. For the ethanol and cold water extraction, 50g of mushroom each sample was soaked in 200 ml of ethanol and cold water respectively and then left for 36 hours at room temperature (28 ± 2ºC) with occasional shaking. Whatman filter paper was used to filter each of the extraction unit, the filtrates were collected in different beakers and were labelled. The filtrates were evaporated to dryness in a steady air-current for about 24 hours in previously weighed evaporation dishes (porcelain dishes). The dishes were re-weighed after evaporation and the differences in weights before and after evaporation were calculated (Trease and Evans 1994). The extracts (residues) were stored (4ºC) in a clean sterile container for further use.

Phytochemical analysis of the Mushroom

Qualitative and quantitative phytochemical analysis of the crude powder of each of the two samples of mushrooms were determined (Harborne, 1973). The phytochemical constituents of crude powders of each of the mushroom extract such as tannins, phenolics and flavonoids were determined spectrophotometrically using the modified methods of Harbonne (1998), Trease and Evans (1983), and as described by Obasi *et al*., (2023)

Preparation of the test isolates

Pure cultures of antibiotic resistant bacteria (*Klebsiella* sp and *Staphylococcus* sp) were obtained from Springboard hospital laboratory Awka, Anambra State, Nigeria. Each of the isolates was subcultured on nutrient agar plates to confirm the purity, the identification and the resistant status (Cheesbrough 2010).

Antibiotic Disc Sensitivity Testing of the test organisms

Antibiotic disc sensitivity testing was conducted by the disk diffusion method. Test isolates were inoculated into physiological saline conforming to 0.5 McFarland turbidity standards. A sterile glass rod was employed each time to evenly spread 0.1ml of the inoculums into prepared plates of Mueller Hinton agar plates and allowed to dry for 5minutes. Gram positive and Gram negative antibiotic disc (Oxoid England) were used for the test isolates respectively. The antimicrobial discs were evenly placed on the surface of the plates using sterile forceps. Plates were incubated at 35°C for 18hours. Zones of inhibition were measured in millimeters and were interpreted as sensitive, intermediate or resistant according to the CLSI (Clinical and Laboratory Standard Institute, 2013) breakpoints for the antibiotics used.

Determination of *In vitro* Antibacterial Activity of the Extracts

The extracts were reconstituted by dissolving 10g of each extract in 10ml of DMSO. These were considered as 1000mg/ml. Cultures of both Gram Positive and Gram negative test bacteria were used for determination of antimicrobial activity using agar well diffusion method.

The isolates (24 hours old) were introduced into sterile physiological saline and were adjusted to 0.5 McFarland standard corresponding to 1.0×10^8CFU/ml were introduced into sterile plates and 19ml of molten Muller Hinton agar at 45^{o}C were added to the plates, and shaken gently to mix the contents. The mixture was allowed to solidify on a flat bench for 30 minutes. A sterile cork borer of 6mm diameter was used to punch the agar plates and 2mls of the various extracts were used to fill the wells using sterilized calibrated loop. Then the setups were allowed to stand for about 15 minutes for proper absorption. The plates were incubated at 28 ± 2°C overnight. Sterile DMSO was used for the negative control experiments. Antibacterial activity was recorded by measuring the zone of growth inhibition around the well in millimeters.

Determination of Minimum Inhibitory Concentration (MIC) of the extracts

Different concentrations (100mg/ml, 50mg/ml, and 25mg/ml) of the extract were prepared by dissolving 100mg of extract in 1.0ml of DMSO, 50mg of extract in 1.0ml of DMSO and 25mg of extract in 1.0ml of DMSO respectively. The mixture was tested against the antibiotic resistant bacteria using Agar Well Diffusion method. The lowest concentration (dilution) at which inhibitory zone was produced is referred to as the Minimum Inhibitory Concentration (MIC) for each extract (Pelczar*et al.*, 1993). Each experiment was carried out in triplicates. Sterile DMSO without any fungal extract was used as the negative control.

Results

The aqueous extract of both edible and wild mushrooms yielded more extract than the ethanolic extract. The aqueous extract of the wild mushroom was of higher quantity (14.5%) than that of edible mushroom (12.5%), while there was no difference in the weights for ethanolic extract (Table 1). The phytochemicals present in the mushroom extracts analysed were presented in Table 2. Alkaloids, Flavonoids, Tannins, saponins, protein, Terpenoids and Cardiac glycosides were detected in all the extracts, Reducing sugar was found in some of the extracts while Resin was absent in all the extracts. The aqueous extracts of both edible and wild mushrooms produced similar phytochemicals but at different levels. Kaempferol, Epihedrine, Anthocyanin, Proanthocyanin, Spartein and Ammodendrine were found in larger quantity in edible mushroom while Sapogenin, Cathechin, Dihydrocystisine, Aphylinidine, Cynogrnic glycoside, Steroid, Narigenin, Tannin, Flavonones, Flavone, Ribalinidine, Oxalate and Phytate were in larger quantity in wild mushroom. There was no Sapogenin found in aqueous extract of edible mushroom. None of the extracts of edible mushrooms contained phytate while there was no Ammodendrine found in aqueous extract of wild mushroom (Table 3).

The biochemical test results identified the test microorganisms as *Klebsiella* sp and *Staphylococcus* sp. (Table 4).

The test *Staphylococcus* sp. was resistant to 6 of the ten (10) antibiotics while susceptible in varying degrees to only four (4)antibiotics and the resistant index was recorded as 0.6 (Table 5).The test *Klebsiella* sp. was resistant to four (4) of the ten (10) antibiotics while susceptible in varying degrees to only 6 of the antibiotics (Table 6).Thus, the resistant index was recorded as 0.4.

All the mushrooms extracts used in this study demonstrated a variety of antagonistic results against the test microorganisms. Aqueous extract of the mixed mushroom samples showed the highest activity against *Klebsiella* sp. and *Staphylococcus* sp. (21mm) while *Klebsiella* sp. was resistant to

the ethanolic extract of edible mushroom. The ethanolic extract of wild mushroom showed the least antimicrobial activity against *Staphylococcus* sp. (16mm) (Table 7).

Table 1: Mushroom Extraction

Sample	Before Extraction (g)	After Extraction	
		Aqueous Extract (g)	Ethanolic Extract (g)
Omphalotus palmatus (Wild Mushroom)	34.1	4.94 (14.5%)	2.89 (8.5%)
Agaricus bisporus (Edible Mushroom)	43.6	5.43 (12.5%)	3.72 (8.5%)

Table 2: Qualitative Analysis of Mushroom Extracts

Test	EEEM	AEEM	EEWM	AEWM
Alkaloid	++	++	++	++
Flavonoid	+	++	++	++
Reducing sugar	- ve	+	-ve	+
Tannin	++	+	++	+
Protein	++	++	++	++
Resin	- ve	-ve	-ve	-ve
Saponin	+	+	++	++
Cardiac glycoside	+	+	++	++
Terpenoids	+	++	+	++

Key: EEEM = Ethanolic Extract of Edible Mushroom; **AEEM =** Aqueous Extract of Edible Mushroom; **EEWM =** Ethanolic Extract of Wild Mushroom; **AEWM =** Aqueous Extract of Wild Mushroom

Table 3: Quantitative Analysis of Mushroom Extracts

Component	EEEM	AEEM	EEWM	AEWM
Sapogenin	3.0558	-	4.0338	2.9756
Kaempferol	7.9326	8.9255	4.2254	2.2342
Epihedrine	5.8101	8.7072	2.7206	2.7042
Catechin	3.6672	4.5703	28.1631	28.9560
Anthocyanin	8.5215	8.4991	7.2409	6.7324
Dihydrocytisine	5.2393	7.8095	16.7936	20.4479
Aphylinidine	6.7623	6.7536	7.0277	6.4247
Cynogenic glycoside	2.9331	2.9395	4.4824	4.5298
Steroid	8.4540	8.6316	10.5084	13.3813
Narigenin	5.8657	8.7274	10.9404	10.0870
Tannin	18.3554	18.3972	25.3877	23.4956
Flavonones	12.5031	12.4944	17.1678	16.9917
Flavone	9.0288	8.9989	9.3973	9.2127
Proanthocyanidin	8.4427	12.6660	8.8967	8.9800
Ribalinidine	3.8262	3.8318	7.5207	7.9498
Spartein	7.6328	11.4196	8.9904	8.9405
Oxalate	17.8919	17.8200	22.8219	22.7144
Ammodendrine	0.0017	5.8400	1.9287	-

Phytate	-	-	7.7220	9.3218

Key: EEEM = Ethanolic Extract of Edible Mushroom; **AEEM =** Aqueous Extract of Edible Mushroom; **EEWM =** Ethanolic Extract of Wild Mushroom; **AEWM =** Aqueous Extract of Wild Mushroom

Table 4: Biochemical Characterization of Microorganisms

Test	***Klebsiella* sp.**	***Staphylococcus* sp.**
Colony Morphology	Large, creamy colonies	Small, creamy and yellowish colonies
Gram Reaction	Gram negative rods	Gram positive cocci in clusters
Motility	-	-
Indole	+	-
Catalase	+	+
Citrate Utilization	+	+
Coagulase	-	+
Methyl Red	-	+
Sugar Fermentation		
Glucose	A + G	A -
Fructose	A + G	A -
Sucrose	A + G	A -
Lactose	A + G	A -

A + G = Acid and Gas production; A - = No Gas production

Table 5: Antibiotic Sensitivity Profile for *Staphylococcus* sp.

Antibiotics	**Zones of Inhibition**
Amoxicillin	0 (R)
Streptomycin	0 (R)
Norfloxacin	0 (R)
Chloramphenicol	0 (R)
Ciprofloxacin	13mm (S)
Erythromycin	15mm (S)
Levofloxacin	19mm (S)
Gentamycin	0 (R)
Ampiclox	0mm (R)
Rifampicin	14mm (S)

Key: R =Resistant; S = Susceptible

Table 6: Antibiotic susceptibility profile for *Klebsiella* sp.

Antibiotics	**Zone of Inhibition(mm)**
Septrin	15mm (S)
Ciprofloxacin	0 (R)
Augmentin	0 (R)
Gentamycin	18mm (S)
Streptomycin	20mm (R)
Pefloxacin	18mm (R)
Taravid	15mm (S)
Sparfloxacin	0 mm (R)
Amoxacillin	0 mm (R)
Chloramphenicol	13mm (S)

Key: R =Resistant; S = Susceptible

Table 7: Antimicrobial Activity of the Extracts(mm)

Extract	*Klebsiella* sp.				*Staphylococcus* sp.			
	100%	50%	25%	Control	100%	50%	25%	Control
EEEM	-	-	-	-	18mm	13mm	-	-
AEEM	18mm	11mm	-	-	19mm	16mm	10mm	-
EEWM	19mm	15mm	13mm	-	16mm	13mm	-	-
AEWM	17mm	16mm	-	-	18mm	14mm	-	-
EEMM	16mm	12mm	-		20mm	12mm	-	-
AEMM	21mm	17mm	15mm		21mm	17mm	12mm	-

Key: EEEM = Ethanolic Extract of Edible Mushroom; **AEEM =** Aqueous Extract of Edible Mushroom; **EEWM =** Ethanolic Extract of Wild Mushroom; **AEWM =** Aqueous Extract of Wild Mushroom; **EEMM =** Ethanolic Extract of Mixed Mushroom; **AEMM =** Aqueous Extract of Mixed Mushroom

Discussion

The crude extracts obtained from each of the mushroom species was relatively low and this could be attributed to the method of extraction. The higher yield of aqueous extracts compared to ethanolic extracts may be explained by higher proportion of water-soluble constituents in mushrooms (Ijeh *et al*., 2005). Obi and Onuoha (2000) obtained a contrary result with the report of ethanol as a better extraction medium for plants than water. Cold water extraction has mostly been reported to produce low quantities of extracts as compared to ethanol extraction (Jha *et al*., 2022).

The phytochemicals present in the mushrooms analyzed were present at varying levels. This varying level could be as a result of the difference in solubility of the constituents of the different mushroom species in the water. However, higher concentration of the constituents in water did not always mean higher activity of aqueous extracts (Bandow *et al*., 2003).

Significantly, there was no phytate(antioxidant and anti-bacterial activity) in both ethanolic and aqueous extracts of the edible mushroom as found in both extracts of the wild mushroom. Also, the aqueous extract of the edible mushroom contained no Sapogenin (possess antimicrobial activity and improves immune function) as seen in all other extracts while the aqueous extract of wild mushroom contained no Ammodendrine (teratogenic activity). It is also noted that the ethanolic extracts of the mushroom samples generally has more constituents than the aqueous extracts quantitatively. The presence of these active compounds could be responsible for the antimicrobial activities of mushroom.

The morphological and biochemical characteristics of *Klebsiella* sp. and *Staphylococcus* sp. obtained in this study are typical of the species obtained in a previous study by Lukas *et al*., (2016). Thiscould be because ofthe standard methods employed in the isolation and identification of the test isolates. Besides, upon purification of the cultures by sub culturing, same results were obtained (Alves *et al*., 2012).

All the mushrooms used in this study were found to exhibit various degrees of synergistic effects against the test microorganisms. This was evidenced by the clear zone of inhibition produced by the bacteria around the test mushroom extracts. The zone of inhibition of the extracts on

Klebsiella pneumonia ranged from 11mm to 21mm with ethanolic extract of the edible mushroom being inactive against the Gram negative isolate and the aqueous extract of the mixed mushroom sample was the most active. It could be said that these extracts can be useful in the treatment of infections caused by *Klebsiella* sp. when they become more resistant to commercial antibiotics. A high concentration of these mushrooms in our diets could be an answer to some urinary tract and blood infections by these bacteria.

Moreover, the zones of inhibition ranged from 16mm to 21mm on *Staphylococcus* sp. with ethanolic extract of the wild mushroom being the least active extract and aqueous extract of the mixed mushroom samplewas the most active against the Gram positive isolate. Multidrug resistant *Staphylococcus aureus* is the most common multidrug resistant organism. These extracts could be an alternative in treatment of infections when the therapeutic effect of antibiotics drastically reduces.

The activity of mushrooms against bacteria in this research increased with higher concentrationof the extract and this has been noted previously that antibiotic activity of Mushrooms are concentration dependent (Ezebialu *et al*., 2021).

According to Tsungai, *et al*. (2016), antimicrobial activities of mushrooms extracts are greatly influenced by their habitat which could have led to the difference in results. The results of the current study show that both edible mushroom and wild mushroom extracts are antagonistic against bacterial pathogens. This is closely related to the study done previously by Thillaimaharan, *et al*. (2016). According to Adams (2014), the spectrum of antimicrobial activities of microorganisms is influenced by the growth curves of the organism and the stage of harvesting the antimicrobial metabolites. Also, similar growth environments may be a factor to the production of the same metabolites (Mishra *et al*., 2017).

The *Staphylococcus* sp. strain was found to be sensitive to Ciprofloxacin (13mm), Erythromycin (15mm), Levofloxacin (19mm) and Rifampicin (14mm) while it was resistant to Amoxicillin, Streptomycin, Norfloxacin, Chloramphenicol, Gentamycin and Ampiclox. On the other hand, *Klebsiella* sp. was susceptible to Septrin (15mm), Gentamycin (18mm), Streptomycin (20mm), Pefloxacin (18mm), Taravid (15mm) and Cholamphenicol (13mm) while being resistant to Ciprofloxacin, Augmentin, Sparfloxacin and Amoxacillin. Thus, the multidrug resistance attributes of these isolates highlighted the increasing need for alternatives to antibiotics.

Conclusion

Mushrooms produce antimicrobial metabolites that can be exploited in treating diseases that affect humans, plants and animals. This study established that the extracted phytochemicals and active compounds in mushrooms inhibited growth in test organisms. The Mushrooms analyzed in this study showed antimicrobial activity against Disease Causing Microorganisms. At the end of the study, we have found that the extracts of *Agaricus bisporus* and *Omphalotus palmatus* demonstrated antimicrobial activities against the test bacteria and hence can be used as treatment to specific health problems.

Recommendations

With the recent rise in Antibiotic resistance, there is need to carry further studies to establish the chemical composition and active compounds of the mushrooms responsible for their antibacterial activities and harness their properties in establishing more effective chemotherapy.

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