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Full Length Research Paper

Antimicrobial activity of some local mushrooms on pathogenic isolates

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The antimicrobial properties of ethanol, hot and cold extracts of some mushroom species (*Russula vesca, Auricularia auricular, Pleurotus squarrosulus, Volvariaella vulvae*) on some Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella typhi*), Gram positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus pneumoniae*) and yeast (*Candida albicans*) were investigated. The Minimum Inhibitory Concentrations (MIC) was evaluated for each of extracts of the mushrooms. Antimicrobial activity was performed by agar disc diffusion. The hot water extracts of *R. vesca* inhibited growth of *E. coli*, *S. typhi*, *P. mirabilis* and *C. albicans*. Ethanolic extract of *A. auricular* showed wide spectrum of antimicrobial effect against test organisms with the exception of *S. typhi* and *P. aeruginosa. P. squarrosulus* showed antimicrobial activity against *K. pneumoniae* (6.14 mm), *S. pneumoniae* (5.12 mm), and *C. albicans* (4.10). *P. aeruginosa* was resistant to almost all extracts of the four species of mushroom except the hot water extract of *P. squarrosulus* which showed zone of inhibition (3.41 mm). *V. vulvae* showed antimicrobial activity against *S. typhi* (4.60 mm). Ethanol and hot water extracts of most of the mushroom species contained more bioactive substance than cold water extract. The significance of antimicrobial activity of mushroom extracts was compared with the standard antibiotics (gentamicin, 5 µg/disc) using chi – square. There were significant difference between the mean zone of inhibition of the ethanol extract of *P. squarrosulus* and the standard antibiotic against test organisms at 5% level. The results obtained in this study suggest that *P. squarrosulus* possessed broad-spectrum of activity against microbial isolates used.

Key words: Mushroom, antimicrobial, phytochemicals, bacteria, yeast.

INTRODUCTION

Antibiotic resistance has become a global concern (Westh et al., 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens (Bandow et al., 2003). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microorganisms has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bolsisio 1996; Iwu et al., 1999). The public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. Worldwide spending on finding new anti-infective agents is increasing. The use of plant extracts as well as other alternative forms of medical treatments is being investigated by researchers.

Mushroom is a macro fungus with a distinctive fruiting body that is large enough to be seen by the naked eyes.

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Abbreviation: MIC, Minimum inhibitory concentrations.

It includes both edible and non edible species. Some mushrooms serve as food because of their nutrient contents while some have been used extensively in traditional medicine (Stamets, 2000; Lindequist et al., 2005). Medicinal values associated with mushrooms have been reported. Mushroom species have been shown to possess antagonistic effects against bacteria, fungi, viruses and cancer (Tochikura et al., 1998; Jonathan and Fasidi, 2003). Jonathan and Fasid (2003) tested the activities of some selected mushrooms such as *Agaricus bisporus*, *Lentinula edodes*, *Auricularia auricular* and *Pleurotus* species on bacteria and reported inhibitory responses against some bacteria including acid fast bacterium (*M. smegnatis*) and pathogenic strains of yeast (*Candida albicans*). *Reishi*, *Polyporus* and *Cordyceps sinensis* are mushrooms of medicinal importance in China (Malthilla et al., 2001; Lakshmi et al., 2004).

This study was designed to evaluate the antimicrobial activity of *Russula vesca* (local name, "Obubunta") *A. auricular* (local name, "Eru nti") *Pleurotus squarrosulus* (local name, "Atakata alu"), *Volvariella vulvae* (local name, "Onyekam etu") mushrooms extracts on bacterial and fungal isolates. Determine their minimum inhibitory concentration (MIC), so as to offer informed recommendation on its use for the treatment of problem of antibiotic resistance. And also determine the phytochemical properties of the mushroom.

MATERIALS AND METHODS

Sources of mushrooms

Some quantities of four different mushroom species were purchased from local markets in Umuahia, Nigeria. The mushroom species (with their local names) were *R. vesca* ("obubunta"), *A. auricular* ("eru nti"), *P. squarrosulus* ("atakata alu"), *V. vulvae* ("onyekam etu").

Extraction of mushroom

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 (96%), hot water and cold water. For the ethanol and cold water extraction, 50 g of mushroom sample was soaked in 200 ml of ethanol and cold water, respectively, and then left for 36 h at room temperature (28 ± 2˚C) with occasional shaking. The hot water extraction involved soaking of the mushroom sample (50.0 g) in 200 ml of hot water (boiled at 100˚C) and then allowed to stand for 4 h with occasional shaking. Each portion was filtered using Whatman filter paper. The filtrates were collected in different beakers and labelled accordingly. The filtrates were evaporated to dryness in a steady air-current for about 24 h in a previously weighed evaporation dishes (porcelain dishes). After evaporation, the dishes were re-weighed and the differences in weights before and after evaporation were calculated (Trease and Evans, 1994). The extracts (residues) were stored (4˚C) in a clean sterile container for further use.

Phytochemical analysis

Qualitative phytochemical analysis of the crude powder of each of the four species of mushrooms was determined. Tannins, alkaloids, saponins, cardiac glycosides, steroids and flavonoids of the mushroom samples were determined (Harborne, 1973).

Sources of microorganisms

Pure culture of *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Escherichia coli* , *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella typhi*, *Bacillus cereus* and *C. albicans* were obtained from bacteriology laboratory of Federal College of Veterinary and Medical Laboratory Technology (FCVMLT), Vom in Plateau State of Nigeria. Each isolate was subcultured on nutrient agar to ensure the purity of the culture and the pure isolate identified according to Cheesbrough (2004) for confirmation.

Antimicrobial assay

The antimicrobial assay was performed by agar disc diffusion methods (Bauer et al., 1966). The surface of Mueller Hinton agar (Oxoid) plate was inoculated with 100 µl (1×10^8 cfu/ml) of the standardized pure culture suspension to obtain a lawn culture. Circular paper discs measuring 7.0 mm were cut from Whatman No. 1 filter paper using a paper perforator and sterilized in an autoclave. The disc (7 mm) was saturated with each of the reconstituted mushroom extracts, allowed to dry and was placed firmly (with the use of sterile forceps) on the surface of the seeded agar plate. The plates were incubated for 24 - 48 h at 37°C. Antimicrobial activities were determined by measuring the diameter (in millimetre) of the zone of inhibition. For each of the bacterial isolates, control was determined by using pure solvents instead of the extract. All experiments were performed in triplicates and the mean value was recorded. The results obtained were compared with the standard antimicrobial agents, gentamicin (5 µg/ml) and nystatin (20 µg/ml). The same method was used for yeast except that the period of incubation was 72 h at room temperature.

Determination of minimum inhibitory concentration (MIC) of the crude extracts

The minimum inhibitory concentration (MIC) was determined by macro-broth dilution techniques as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1998). A two fold serial dilution of the reconstituted extract was prepared in Mueller Hinton Broth. Each dilution was seeded with 100 µl of the standardized suspension of the test organism (1×10^6 cfu/ml) for Gram positive bacteria and (5×10^5 cfu/ml) for Gram negative bacteria and incubated for 24 h at 37°C. MIC was determined as the highest dilution (that is, lowest concentration) of the extract that showed no visible growth.

Determination of Minimal Bactericidal Concentration (MBC) of the crude extracts

MBC was determined by selecting tubes that show no growth during MIC determination and a loopful from each of the tubes was subcultured on the Mueller Hinton Agar. The plates were incubated for 24 h at 37°C. The MBC was determined as the least concentration that showed no visible growth (NCCLS, 1998).

RESULTS

The antimicrobial activities of four species of mushroom extracts were determined by agar disc diffusion method

Table 1. Total yield of crude extract of mushroom species by different solvents.

Solvent	Mushroom	Yield (mg) of crude extract
Ethanol	*Russula vesca*	3.36
	Auricularia auricular	1.60
	Volvariaella vulvae	2.20
	Pleurotus squarrosolus	2.45
Hot water	*Russula vesca*	9.60
	Auricularia auricular	4.60
	Volvariaella vulvae	5.14
	Pleurotus squarrosolus	8.17
Coldwater	*Russula vesca*	3.51
	Auricularia auricular	4.02
	Volvariaella vulvae	3.62
	Pleurotus squarrosolus	1.94

Table 2. Phytochemical characteristics of mushroom species in different solvent extractions.

Extracts	*P. squarrosulus*			*A. auricular*			*V. vulvae*			*R. vesca*		
	Ethanol	Hot water	Cold water	Ethanol	Hot water	Cold water	Ethanol	Hot water	Cold water	Ethanol	Hot water	Cold water
Glycosides	+++	++	-	-	+	++	+	-	-	-	-	++
Tannins	++	+	+	-	+++	++	-	+	++	+	++	+
Saponins	+	+	+	++	+++	+	+	+	-	+++	+	+
Flavonoids	++	+	+	-	++	+	-	+	++	-	-	++
Carbohydrate	++	++	++	+	+++	+	+	+	+++	+	-	+
Protein	+ ++	++	++	+	+++	+	+	+	+	+	++	+
Alkaloid	+	+	-	++	-	-	+	-	+	+	++	+

- = Not present
+ = Present in small amount (concentration)
++ = Moderately present
+++ = Present in large amount

against nine pathogenic isolates. Table 1 shows the total yield of the mushroom's extract using different solvents (ethanol, hot and cold water). The highest total yield of crude extract of 9.60 mg was obtained from *R. vesca* in hot water while A. *auricular* yielded the least amount (1.60 mg) in ethanol extraction. Table 2 showed the qualitative phytochemistry of the four species of mushroom using different solvents (ethanol, aqueous hot and cold water). Ethanol and hot water extract of *P. squarrosulus* contain almost all the phytochemical compounds assayed for, though at varying levels. The mean zone inhibition of crude extract is shown in Table 3. *B. cereus* and *S. pneumoniae* as well as *C. albicans* showed the highest zone of inhibition by various solvents extracts of the mushrooms. *P. aeuroginosa* showed only zone of inhibition (3.41 mm) with hot water extract of *P. squarrosolus*. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of extracts of *P. squarrosolus* by different solvents is shown in Table 4.

DISCUSSION

Antimicrobial activity of extracts of mushrooms species (*P. squarrosulus*, *A. auricular*, *V. vulvae and R. vesca*) as well as the phytochemical characteristics were studied. The total yield of the crude extracts obtained from each of the mushroom species was relatively low and this could probably be due to the extraction methods employed. The gelling of some of these mushrooms components in

Table 3. Mean zone of inhibition (mm) of isolates by different crude extracts of mushroom species.

Extract	Zone of inhibition (mm)								
	B. cereus	*P. aeruginosa*	*S. typhi*	*P. mirabilis*	*K. pneumoniae*	*S. pneumoniae*	*S. aureus*	*E. coli*	*C. albicans*
Russula vesca									
Ethanol	5.70	-	6.27	2.32	0.44	-	2.40	2.93	10.44
Hot water	6.10	-	7.41	7.11	0.96	-	5.16	6.05	2.63
Cold water	3.45	-	2.10	-	1.26	0.19	2.35	-	-
Auricularia auricular									
Ethanol	7.70	-	4.70	3.11	1.77	-	-	1.66	4.89
Hot water	-	-	-	-	-	-	-	-	-
Cold water	4.22	-	-	2.10	1.46	4.70	-	2.62	9.10
Volvariaella vulvae									
Ethanol	1.44	-	-	-	0.16	-	1.75	-	0.69
Hot water	-	-	-	-	-	-	-	-	2.34
Cold water	1.60	-	4.60	-	-	-	-	-	3.95
Pleurotus squarrosolus									
Ethanol	9.76	-	1.04	3.10	6.14	5.12	4.32	2.10	7.10
Hot water	6.01	3.41	6.45	1.88	0.49	6.75	0.86	1.68	-
Cold water	4.44	-	3.47	2.16	2.91	6.14	11.71	-	6.91
Gentamicin (5.0 µg/ml)	18.60	-	17.41	14.61	15.67	19.26	22.08	20.11	-
Nyastatin (20 µg/ml)	-	-	-	-	-	-	-	-	23.6

hot water into thin slime may reduce the total yield as it made filtration through the filter paper some what slower and difficult (Soforowa, 1992). Hot water extract of *R. vesca* yielded the highest value of 9.60 mg while ethanol extract of *A. auricular* yielded the lowest value of 1.60 mg (Table 1). But generally the hot water extracts produced slightly higher yield than the cold water. The higher yield of hot water extracts compared to ethanol extracts may be explained by higher proportion of water-soluble constituents in mushrooms (Ijeh et al., 2005). This result is in agreement with Obi and Onuoha (2000) who reported that ethanol extraction of plant ingredients were better than water extract. Extraction by cold water has generally been reported to produce low amount of extracts compared to ethanol extraction (Ibrahim et al., 2001). However, cold water extraction was adopted because it is usually applied in traditional medicine preparations.

The phytochemical analysis revealed the presence of bioactive compounds as shown in Table 2. The phytochemicals of the mushrooms were present at varying levels. Tannins, saponins, protein and carbohydrate were detected in all the extracts while glycosides, alkaloids and flavonoids were found in some. Some of the hot or cold extracts produced similar phytochemicals though in different levels. This could be explained by the difference in solubility of the constituents in the hot and cold water, respectively. However, higher concentration of the constituents in hot water did not always mean higher activity of hot water extracts (Bandow et al., 2003). In this study, the hot water extract of *A. auricular* contained higher amount of tannins, saponins, carbohydrates and proteins than the cold water extract but had lower antimicrobial activity. The lack of activity in spite of higher concentration of constituents may indicate that the active ingredients are heat-labile (Lillian et al., 2006).

The ethanolic and water extracts of the mushrooms species especially *P. squarrosolus* inhibited the growth of majority of the isolates. Similar antimicrobial activities were reported (Westh et al., 2004; Lacobellies et al., 2005; Iwalokun et al., 2007). This possibly indicated that the extracts possessed substances that can inhibit the growth of some microorganisms (Chika et al., 2007). However, the observed inhibitory activities were more

Table 4. The MIC and MBC of the crude extract of *P. squarrosolus.*

Extract	Test organisms	MIC (mg)	MBC (mg)
	E. coli	50.00	25.00
	P. aeruginosa	0.00	0.00
	S. typhi	12.50	25.00
	P. mirabilis	0.00	0.00
Ethanol	*K. pneumoniae*	50.00	50.00
	S. pneumoniae	0.00	0.00
	S. aureus	50.00	50.00
	B. cereus	0.00	0.00
	C. albicans	50.00	50.00
	E. coli	50.00	0.00
	P. aeruginosa	50.00	0.00
	S. typhi	50.00	0.00
	P. mirabilis	0.00	0.00
Hot water	*K. pneumoniae*	00.00	0.00
	S. pneumoniae	00.00	0.00
	S. aureus	50.00	0.00
	B. cereus	0.00	0.00
	C. albicans	50.00	0.50
	E. coli	00.00	0.00
	P. aeruginosa	0.00	0.00
	S. typhi	50.00	0.00
	P. mirabilis	50.00	0.00
Cold water	*K. pneumoniae*	0.00	0.00
	S. pneumoniae	0.00	0.00
	S. aureus	25.00	0.00
	B. cereus	0.00	0.00
	C. albicans	50.00	50.00

with the ethanolic extracts of the mushrooms species. Extracts of *P. squarrosolus* and *R. vesca* inhibited both Gram positive and negative bacteria as well as *C. albicans* suggesting broad–spectrum antimicrobial potentials. However, the inability of the extracts to inhibit the growth of *P. aeruginosa* could be that the organisms possess a mechanism for detoxifying the active components (Chika et al., 2007). But in this study, only hot water extract of *P. squarrosolus* showed minimal zone of inhibition. The observed antimicrobial properties could be due to the presence of tannins, alkaloids and flavonoids which have been shown to possess antimicrobial properties (Draughon, 2004).

The variations in the antimicrobial activities of mushrooms may be due to the differences in their bioactive compositions or concentrations, methods of extraction and mechanism of action of active ingredients in these edible mushrooms (Iwalokun et al., 2007). Based on the results of this study, it can be concluded that the edible mushrooms possessed a broad-spectrum antimicrobial activities especially *P. squarrosolus*. The potential of developing antimicrobials from plants appears rewarding.

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