
Full Length Research Article

Antimicrobial Activities of Some Selected Nigerian Mushrooms

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Abstract

Methanolic extracts of five Nigerian mushrooms – *Auricularia polytricha*, *Coriolopsis occidentalis*, *Daldinia concentrica*, *Daedalea elegans* and *Tricholoma lobayensis* were investigated for their antimicrobial activities using filter paper disc and hole diffusion methods. Bacteria such as *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Staphylococcus aureus* were well inhibited by these mushroom extracts, while *Pseudomonas aeruginosa* was resistant to all the mushroom samples except *Tricholoma lobayensis*. The study on the antifungal effect of these mushroom extracts revealed that *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Microsporum boulardii* were either weakly inhibited or not inhibited at all. The minimum inhibitory concentration (MIC) ranged between 1.25 and 9.00mg/ml for bacteria and between 10.50 and 17.50mg/ml for fungi. These results are discussed in relation to therapeutic value of the studied mushrooms.

Key Words:

Antimicrobial screening, mushrooms, bacteria, therapeutic value.

INTRODUCTION

The predominating interest of Nigerians in mushrooms is to use them as food. This is because of their good taste, appetizing aroma and nutrient contents (Fasidi and Kadiri, 1993; Jonathan and Fasidi, 2001). Besides, mushrooms have been used extensively in traditional medicine for curing various types of diseases (Oso, 1977; Oso, 1981; Chapela and Lizon, 1993; Tochikura et.al, 1988). For centuries, mushrooms have been prescribed for treatment of diseases such as gastro –intestinal disorder, bleeding, high blood pressure and various bacterial infections (Stamets, 1993; Brodie, 1998).

While some of the medicinal values associated with mushrooms must have arisen from superstitious beliefs and myths, they have provided information for curiosity research studies. Research has shown that some of these claims are not mere myth but are authentic (May et. al., 1998; Jonathan and Fasidi, 2003). Benedict and Brady (1972), tested the activities of some selected mushroom metabolites on some bacteria and reported that the best inhibitory responses were seen against gram positive organisms including acid fast

bacterium (*M. smegnatidis*) and pathogenic strains of yeast (*C. albicans*).

Mushrooms such as *A. bisporus*, *L. edodes*, *A. auricula* and many *Pleurotus* species have been shown to possess antagonistic effects against bacteria, fungi, viruses and cancer (Tochikura et. al., 1988; Stamets, 1993; Jonathan and Fasidi, 2003). It was therefore the aim of this present study to screen *A. polytricha*, *C. occidentalis*, *D. concentrica*, *D. elegans* and *T. lobayensis* for their antimicrobial activities. These will act as possible preventive agents against common bacteria and fungal infections of man.

MATERIALS AND METHODS

Source of the used basidiomycetes

Five mushroom species – *A. polytricha*, *C. occidentalis*, *D. elegans*, *D. concentrica* and *T. lobayensis* used in this study were collected from different areas within the University of Ibadan campus. These areas include University Botanical Gardens, University Teaching and Research Farm and Nursery Section of Botany and Microbiology Department. These basidiomycetes were identified by their spore prints and, by comparing their morphological,

anatomical and physiological characteristics with the standard descriptions of Zoberi (1978), and that of Alexopoulos et. al., (1996).

Microorganisms

The stored culture of *B. cereus*, *E. coli*, *K. pneumoniae*, *P. vulgaris*, *P. aeruginosa* and *S. aureus* were collected from the Medical Microbiology unit of the University College Hospital, Ibadan. Pathogenic fungi strains *C. albicans*, *M. bouliardii* and *T. concentricum* were collected from the Medical Mycology unit of Lagos University Teaching Hospital, Lagos. *A. niger* and *A. flavus* were collected from the Plant pathology unit, Botany department, university of Ibadan.

Preparation of Crude Methanol Extract

The fruit bodies of each of the test mushrooms were cut into bits and dried at 40°C. This dried carpophore was pulverized in a moulinex blender and 50.0g each of the powdered samples were soaked separately in 300ml of 95% methanol in an Erlenmeyer flask. The flasks were covered with aluminum foil and allowed to stand for 7 days for extraction. It was filtered through Whatman filter paper no 1 and the filtrate obtained was concentrated in a rotary evaporator at 40°C. The methanol was recovered and the extract was collected and dried (Jonathan and Fasidi, 2003).

Assay for Antimicrobial Activities and Minimum Inhibitory Concentration of the crude Extract

The standard concentration of the extract used was 20.0mg/ml. Sterile distilled water was used as the diluent. Mushrooms with activities at this concentration were regarded as having antimicrobial properties while other with no activity at this concentration was disregarded (Hirasawa et. al., 1999).

The antimicrobial activities of each of the crude methanol extracts were determined using filter paper disc and agar well diffusion methods of Stoke and Ridgway (1980). The sterile distilled water used in the dilution of solid mushroom extracts was used as the control. The inhibitory zones produced were measured in millimeters. A control experiment was set up by either soaking disc of filter paper with sterile distilled water or by adding drops of this diluent into the agar well on the plates. Negative results were regarded as those in which no zone of inhibition was observed. All the tests were carried out in triplicates and their means recorded. The MIC was determined according to the method of Unaeze (1987).

Assay for Antimicrobial Activities of Purified Extracts

This study was aimed at removing methanol that was used in the extraction of bioactive components from the studied mushrooms. Alcohols have been proved to have antagonistic effects on some microorganisms (Benedict and Brady, 1972).

Twenty milliliters of ethyl acetate were dispensed into conical flasks containing 1g of crude mushroom extract. The mixture was shaken vigorously and 30ml of sodium bicarbonate was added to remove weak acids. It was filtered and the filtrate was evaporated under a reduced pressure at 40°C to obtain pure (methanol free) extract. The process, which took about 15 minutes, was repeated 3 times (Hirasawa et.al., 1999). Antimicrobial activities of the extracts were assayed at 10mg/ml concentration and determined as described in the previous experiment.

Analysis of Data

The results of this study were subjected to the analysis of variance (ANOVA) and Duncan's Multiple Range Tests (DMRT).

RESULTS AND DISCUSSION

All the mushrooms used in this study were found to exhibit various degrees of antagonistic effects against the tested microorganisms. This was evidenced by the clear zone of inhibition produced by the bacteria and fungi around the tested mushroom extracts. The best in-vitro antibacterial activity (22.0mm) was exhibited by *D. elegans* against *E. coli* (Table 1). This was followed in order by *T. lobayensis*, *C. occidentalis* and *A. polytricha* with values 20.0, 19.0 and 18.0mm respectively. This observation was in agreement with the prediction of Brain (1951), that an increasing number of antimicrobial agents from higher fungi are to be expected.

The inclusion of *T. lobayensis* among mushrooms with antimicrobial properties (Table 1) was not a surprise. This is because a member of the same family and genus *T. saponaceum* has been implicated in the production of antibacterial substance (Stamets, 1993; Zoberi, 1978). *A. polytricha* inhibited the growth of *B. cereus*, *E. coli*, *P. vulgaris* and *S. aureus* (Table 1). This observation supported the earlier claim of Lu and Tang (1986), that *A. polytricha* could inhibit the growth of some disease causing bacteria. Also, a member of the same genus *A. auricula* has been found to possess antibacterial property which could be used for the treatment of eye inflammatory disease (Well, 1994).

It was also observed from Table 1 that hole diffusion method was the best for evaluating antimicrobial activities of the mushrooms studied. For *D. concentrica*, the zones of inhibition were 16.0 and 10.00mm against *B. cereus* for hole diffusion and filter paper disc

method respectively. This result indicates that the filter paper disc may act as diffusion barrier between the extracts and the microorganisms. All the mushrooms except *T. lobayensis* were inhibited by *P. aeruginosa*. This supports the suggestion of Madigan et. al., (1997), that *P. aeruginosa* is a gram negative bacteria which is difficult to control by most therapeutic agents.

Antifungal activities of crude mushroom extract were generally low (Table 2). All the tested fungi were inhibited by the extracts of *A. polytricha* and *T. lobayensis*. The dermatophytes (*T. concentrum* and *M. bouldarii*) were either weakly inhibited or not inhibited at all by the crude mushroom extracts (Table 2). This suggests that extracts of the mushrooms studied may not be effective antifungal agents. The least minimum

inhibitory concentration (MIC) value (1.25mg/ml) was obtained in *C. occidentalis* against *E. coli* (Table 3). This was followed by *T. lobayensis* with 2.0mg/ml against the same bacterium. Danielli (1957), suggested that at the lowest MIC, the extract will still be effective because of the presence of bio-active compounds. Therefore, higher concentration, which may consequently poison host cells, may not be required. It was also revealed (Table 3), that MICs of the mushroom extracts against the test fungi were high. This also confirms the earlier suggestion that the studied mushrooms possessed poor antifungal properties. The low MIC values obtained in *A. polytricha*, *C. occidentalis*, *D. elegans* and *T. lobayensis* against the test bacteria indicates that these mushrooms are promising antibacterial agents.

Table 1
Antibacterial Activities of Crude Mushroom Extracts

Organisms	*Zone of Inhibition (mm). Mean of three replicates					
	<i>B. cereus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. vulgaris</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
<i>A. polytricha</i> (HDM)	12.0b	18.0ab		10.0ab		7.0c
<i>A. polytricha</i> (FDM)	7.0b	13.0bc		5.0c		
<i>C. occidentalis</i> (HDM)	10.0b	19.0a	7.0c	13.0a		8.0bc
<i>C. occidentalis</i> (FDM)	8.0b	13.0bc		10.0ab		6.0bc
<i>D. elegans</i> (HDM)	18.0a	22.0a	14.0a	15.0a		12.0b
<i>D. elegans</i> (FDM)	13.0ab	18.0ab	9.0b	12.0ab		8.0bc
<i>D. concentrica</i> (FDM)	16.0a	15.0b	8.0b	9.0b		12.0b
<i>D. concentrica</i> (FDM)	10.0b	12.0c		5.0c		7.0c
<i>T. lobayensis</i> (HDM)	17.0a	20.0a	10.0ab	12.0ab	13.0a	18.0a
<i>T. lobayensis</i> (FDM)	13.0ab	16.0b	5.0c	10.0ab	10.0a	12.0b
Control (Distilled Water)						

*Values followed by the same letters along each vertical column are not significantly different ($P = 0.05$).
HDM = Hole Diffusion Method, FDM = Filter Paper Disc Methods; | = Inactive

Table 2:
Antifungal Activities of Mushrooms Extracts

Mushrooms	* Zone of Inhibition (mm)				
	<i>A. niger</i>	<i>A. flavus</i>	<i>C. albicans</i>	<i>M. bouldarii</i>	<i>T. concentrum</i>
<i>A. polytricha</i>					
<i>C. occidentalis</i>	9.0a	7.0a	7.0a	5.0a	
<i>D. elegans</i>	5.0a	10.0a	10.0a		10.0a
<i>D. concentrica</i>				9.0a	
<i>T. lobayensis</i>					
Control (Distilled Water)					

*Values followed by the same letters along each vertical column are not significantly different ($P = 0.05$). Data represented above are means of 3 replicates.

Table 3:

Minimum Inhibitory Concentration of Crude Mushroom Extracts for Bacteria and Fungi.

Microorganisms	M I C (mg/ml). Mean of three replicates					Distilled water (Control)
	A. <i>polytricha</i>	C. <i>occidentalis</i>	D. <i>elegaus</i>	D. <i>concentrica</i>	T. <i>lobayensis</i>	
<i>B. cereus</i>	3.75c	5.25c	6.50b	7.00bc	5.50b	
<i>E. coli</i>	3.00c	1.25d	2.50c	5.50c	2.00c	
<i>K. pneumoniae</i>		4.50cd	8.50b	9.00b	7.25a	
<i>p.vulgaris</i>	5.50b	3.00d	4.25c	8.00b	5.50b	
<i>P. aeruginosa</i>	N.D	N.D	ND	N.D	N.D	
<i>S. aureus</i>	7.00a	5.75c	5.25bc	6.00c	4.75b	
<i>A.niger</i>		13.50ab				
<i>A. flavus</i>		11.50b	13.75a	17.75a		
<i>C. albicans</i>		14.00a	14.25a	10.75b		
<i>M. bouldarii</i>		15.00a		15.50a		
<i>T. concentrum</i>		N.D	N.D	N.D		

I = Inactive, ND = Not detectable

Values followed by the same letters along each vertical column are not significantly different (P = 0.05).

Table 4:

Antimicrobial Activities of Purified Mushroom Extracts

Microorganisms	Inhibitory Zones. Mean of three replicates					Distilled water (Control)
	A. <i>polytricha</i>	C. <i>occidentalis</i>	D. <i>elegaus</i>	D. <i>concentrica</i>	T. <i>lobayensis</i>	
<i>B. cereus</i>	16.0ab	14.0b	21.0a	19.0a	21.0a	
<i>E. coli</i>	20.0a	21.0a	23.0a	18.0a	22.0a	
<i>K. pneumoniae</i>		11.0bc	16.0bc	10.0c	13.0b	
<i>p.vulgaris</i>	15.0b	15.0b	18.0ab	11.0c	17.0ab	
<i>P. aeruginosa</i>					15.0b	
<i>S. aureus</i>	10.0c	10.0c	14.0cd	15.0b	22.0a	
<i>A .niger</i>		11.0bc	7.0d			
<i>A. flavus</i>		9.0c	11.0cd	10.0c		
<i>C. albicans</i>		10.0c	12.0cd	11.0c		
<i>M. bouldarii</i>		7.0c		10.0c		
<i>T. concentrum</i>			11.0			

I = Inactive

*Values followed by the same letters along each vertical column are not significantly different (P = 0.05).

Table 4 shows that the antimicrobial activities of purified mushroom extracts were generally higher than the crude extracts. For *T. lobayensis*, 18.0mm was obtained for the crude extract against *S. aureus* whereas, the value increased significantly to 22.00mm when purified extract was employed (P< 0.005). Similar trend was obtained for *D. elegans* against *K. pneumoniae*. Hirasawa et. al., (1999) also obtained similar result with the extract of *L. edodes*.

In conclusion, the extracts of all the mushrooms used in this study inhibited some medically important microorganisms. This suggests that they are potential sources of new antimicrobial agents. Work is in progress on the

identification of phytochemicals, which are responsible for the antimicrobial properties.

REFERENCES

- Alexopoulous, C.J., Mims C.W. and Blackwell, M. (1996):** Introductory Mycology. 4th ed. John Wiley and Sons. New York Chichester, Brisbane, Toronto and Singapore.
- Benedict, R.G. and Brady, L.R. (1972):** Antimicrobial Activities of Mushroom Metabolites. *J. Pharm. Sci.* 61, 1820 – 1822.
- Brain, P.W. (1951):** Antibiotics Produced by Fungi: *Bot. Rev.* 17, 357 – 430.
- Brodie, H.J. (1978):** Fungi – Delight of Curiosity. University of Toronto Press, Toronto.
- Chapela, I.H. and Lizon, P. (1993) :** Fungi in Stone Age. *Mycologist* 7, 121 – 122.

- Danielli, I. (1957):** Antimicrobial Activities of *Aframomum* species. *Pharm. Journal.* 16, 470 – 472.
- Fasidi, I.O. and Kadiri, M. (1993):** Effect of sporophore maturity on chemical composition of *volvariella essculenta* (mass) singer, a Nigerian mushroom. *Die Nahrung.* 37 (3) 269 – 273.
- Hirasawa, M. Shoujii, N., Neta, T., Fukushima, K. and Takada. K. (1999):** Three kinds of antibacterial substances from *Lentinus edodes* (Berk.) Sing. (Shitake, and edible mushroom. *Int. J. of Ant. Agents* 11, 151-157.
- Jonathan S.G. and Fasidi I.O. (2003).** Antimicrobial activities of two Nigerian edible macro fungi – *Lycoperdon pusillum* (Bat. Ex) and *Lycoperdon giganteus* (Pers). *African J. Biomed. Research* 6: 85 –90 (2003).
- Jonathan, S.G. and Fasidi, I.O. (2001):** Effect of Carbon, nitrogen and mineral sources on growth of *Psathyrella atroumbonata* (Pegler), a Nigerian edible mushroom. *Food Chem.* 72, 479 – 483.
- Lu, J.Y. and Tang, A.Y., (1986):** Cellulostic enzymes and Antibacterial activities of *Auricularia polytricha*. *J. Food. Sci.* 511 (3), 668 – 669.
- Madigan, M.T.; Martinco, J.M. and Parker, J. (1997):** Brock Biology of Microorganisms. 8th Edition. Prentice Hall Int. Inc.
- Mau, J.L., Wu, K.T., Chan, P.T., Wu, Y.H. and Peng, J.T. (1998):** Flavour Compounds in KingOster mushroom (*Pleurotus eryngii*. *J. Agric. Food Chem.* 46, 4587 – 4589.
- Oso, B.A. (1977):** Mushrooms in Yoruba Mythology and Medicinal Practices. *Eco. Bot.* 31 (31), 367 – 371.
- Oso, B.A. (1981):** Fungi and Mankind. University of Ibadan (Nigeria) Inaugural Lecture pp.40.
- Stamets, P. (1993):** Growing Gourmet and Medicinal Fungi. Ten Speed. Berkeley.CA.
- Stoke, J.E. and Ridgway, G.L. (1980):** Clinical Bacteriology. Edward, Arnold Ltd. London.
- Tochikura, T.S., Nakashima, H., Ohashi, Y. and Yamamoto, (1988):** Inhibition (in vitro) of Replication and of the Cytopathic effect of Human immunodeficiency virus by an extract of the culture medium of *Lentinus edodes* mycelia. *Med. Microbiol. Imm.* 177, 235 – 244.
- Unaeye, N.C. (1987):** The Antimicrobial Activity of Medicinal Plants. The National Conference of Biotechnol. Held in Enugu. *Bull. Of Biotechnol. Soc. Nigeria* 3, 72 – 73.
- Well, K. (1994):** The Jelly Fungi: Then and Now! *Mycologia* 86, 18 –48.
- Zoberi, M.H. (1978):** Some Edible mushrooms from Nigeria. *Nigeria Fields* 38, 81 – 90.

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