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Full Length Research Article

Evaluation of the Inhibitory Potentials of Eight Higher Nigerian Fungi against Pathogenic Microorganisms

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ABSTRACT

Studies were carried out to determine antimicrobial potentials of crude and purified methanol extracts of some selected Nigerian mushrooms against some selected pathogenic microorganisms. The best *in vitro* antibacterial activity (24.0mm) was observed with the purified extract (PRE) of *Polyporus giganteus* against *E. coli*. This was followed in order by PRE of *Pleurotus florida* against *K. pneumoniae* with 22.0mm *(*$P \leq 0.05$). Among the screened higher fungi, only the extracts of *Pleurotus tuber-regium*, were able to inhibit the growth of *P. aeruginosa*. It was also observed that antifungal activities of these mushrooms were generally low. *Candida albicans* were inhibited by the extracts of *P. giganteus* and *T. robustus* only while other fungi produce no zone of inhibition The observed minimum inhibitory concentration (MIC) of *M. jodocodo* against *E. coli* was 2.75mg/ml while that of *T. robustus* against *M. bourlardii* was 15.75mg/ml. The significance of these observations was discussed.

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Key Words; Antimicrobial, extraction, edible fungi, disease, microorganisms

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INTRODUCTION

The vegetation in the tropics supports the growth of different types of naturally occurring macro-fungi Edible mushrooms are usually collected from the wild because farms growing them are very few (Zoberi, 1972; Alofe *et al* 1998; Gbolagade, 2007).

In many parts of Nigeria, indigenous people usually use fruit bodies and sclerotia of edible higher fungi as soup condiments which are served at their important social gatherings and family meals (Fasidi and Ekuere, 1993; Jonathan and Fasidi, 2003) Some Nigerian edible mushrooms have also been reported to possess important medicinal ingredients among the traditional doctors (Oso, 1977a and 1981; Jonathan and Fasidi, 2003 and 2005).

Mushrooms that have been implicated of having therapeutic effect against diseases such as high blood pressure, pneumonia, urinary tract infection, intestinal disorder in Nigerian etnomedical practice include *Ganoderma lucidum, Fomes lignosus, Daldinia concentrica, Termitomyces species, Pleurotus* species, *Lycoperdon species Polyporus* species, *Calvatia cynthiaformis* and *Psathyrella atroumbonate* (Oso,1977a; Jonathan,2002).

Information on *in vitro* antimicrobial activities of these Nigerian mushrooms is very scanty or not available in the literatures as of now. Jonathan and Fasidi (2003) reported that alcoholic extract of *Lycoperdon pusilum* and *L. giganteum* showed significant antimicrobial properties against some disease causing bacteria and fungi when compared with their respective water extracts. Likewise, Jonathan (2002) reported that antibacterial potency of puffballs could be compared with some commonly used antimicrobial agents. It is therefore the objectives of this study to describe the antimicrobial potentials of selected Nigerian edible fungi view of the limited scientific information on their medicinal values.

MATERIALS AND METHODS

Higher Fungi: Eight Higher fungi were used in this study. They include *Fomes lignosus* (Kl Bres), *Marasmius jodocodo* (Henn), *Pleurotus florida* (Mont) Singer, *Pleurotus tuber-regium* (Fries) Singer, *Psathyrella atroumbonata* (Pegler), *Polyporus giganteus* (Fries), *Termitomyces microcarpus* (Berk) and *Termitomyces robustus* (Beeli). The sporophores of these fungi were collected from Botanical Gardens, University of Ibadan, Ibadan Nigeria. They were identified using the standard descriptions of Zoberi (1972) and that of Alexopolous *et al* (1996).

Preparation of methanol Extracts: The fruitbodies of collected fungal samples were air dried under shade for 5 days. They were not exposed to sun-light to prevent the ultra violent radiation from the sun from inactivating bioactive components in these fungal samples. They were oven dried at 55^{o}C for 48hrs to a constant weight. The dried samples were milled to obtain fine powder. Eighty grammes (80.0g) of the powdered sample were extracted with 320ml of methyl-alcohol in a soxhlet apparatus for 6hours. The extract was concentrated using a rotatory evaporator. The semi solid extract thus obtained was further dried into powder (Jonathan, 2002). To obtain purified extract, the solid crude extract was mixed with 1000ml of sterile distilled water with stirring at 4^{o}C overnight. The suspension thus obtained was centrifuged to remove the insoluble matter; the aqueous supernatant was concentrated under reduced pressure to 200ml. The concentrate was extracted with 200ml of ethyl acetate. This was also concentrated using rotatory evaporator to yield a light yellow material known as purified extract (Hirasawa et al, 1999). When required, both the crude and purified extracts were mixed with sterile distilled water to desired concentration.

Detection of Inhibitory Activities against Bacteria

The assay for antibacterial activities in each fungal sample was determined by agar well diffusion method of stoke and Ridgway (1980). Bacteria used were *B. cereus, E. coli, K. pneumoniae P. vulgaris, P. aeruginosa* and *S. aureus*. The pure culture of each bacterium was inoculated to peptone water for 18 hours. These were seeded

into nutrient agar plates (one organism per plate). Well (7mm diameter) was made on each Petri dish using sterile cork borer. About 0.25ml of the extract was introduced into bore agar wells using sterile dropping pipette. The plates were kept inside the refrigerator at 4^{o}C for 12 hours to allow proper diffusion of the extracts into the medium. All the experiments were carried out in triplicates. Control experiments were also set up by adding 0.25ml of distilled water into the well in place of the extract in three replicates. The plates were incubated at 37^{o}C for 24 hours. The zones of inhibition produced were measured in milliliters (mm).

Detection of Inhibitory Activities against Fungi

The assay for antifungal potentials of these higher fungi was carried out using *A. niger, A. flavus, C. albicans*, *M. boulardii* and *T. concentrum*. Sterile plates of Saboraud dextrose agar (SDA) were prepared. Wells were made on the solid agar using 7mm sterile cork borer. Twenty milligrammes (20.0mg) of the extract was mixed with 5.0g of the ointment base. Two grammes (1.5g) of the mixture were introduced into the well on the agar plate. The control experiment was set up with the ointment base alone (without any extract). Each experiment was replicated three times. Each Petri-dish was inoculated with test fungus and incubated at 35^{o}C for 7 days. The plates were observed for any zone of inhibition which was measured in millimeters (mm).

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was aimed at finding out the lowest concentration of the extract that will inhibit the growth of the test microorganisms. Different concentrations (0.5 – 20.0mg/ml) of the methyl alcohol extract were prepared by dissolving a known weight of the extract with a known volume of sterile distilled water. The mixture was tested against microorganisms using hole diffusion method. The test was first carried out by using high concentration of the extract (8.0 to 20.0mg/ml) in a completely randomized block design. Those that were still effective at 8.0mg/ml were further diluted until no inhibitory zone was observed. The lowest concentration (dilution) at which inhibitory zone was produced is regarded as the minimum inhibitory concentration (MIC) for each extract (Pelcza et al, 1993). Each experiment was carried out in triplicates. The sterile distilled water without any fungal extract served as the control.

RESULTS AND DISCUSSION

The crude and purified extracts (CRE and PRE) of all the eight higher fungi used for this investigation possessed varying degrees of antibacterial properties against the test bacteria (Table 1). *Polyporus giganteus* produced the widest zone of inhibition (24.0mm) against *E. coli* followed by *P. atroumbonata* (18mm) against the same bacteria ($P \leq 0.05$). *Fomes lignosus* and *T. microcarpus* produced inhibitory zones of 16.0mm each; while *M. jodocodo* possessed no antibacterial activities against *E. coli*. The strong antibacterial properties possessed by *P. giganteus* and *P. atroubonata* is not a surprise. This is because these two fungi are important part of medicinal ingredients which are used by the Yoruba people in the south western Nigeria for the treatment of intestinal disorder and some other bacterial infections (Jonathan, 2002).

All the tested extracts except *P. giganteus*, inhibited the growth of *S. aureus*. The purified extract (PRE) of both *P. florida* and *T. microcarpus* had the best *in- vitro* antibacterial activities (18.0mm) against this bacteria (Table 1). It was interesting to note that *Pseudomonas aeruginosa* which is resistant to both tetracycline and gentamycin (Jonathan, 2002; Madigan et al, 1997) was found to be sensitive to the methyl-alcohol extract of *P. tuber-regium*. The potent antibacterial activity exhibited by *P. tuber-regium* against most of the tested bacteria supported the earlier report of Oso (1977a and b) that *P.tuber-regium* is a medicinal mushroom.

Klebsiella. pneumoniae was inhibited by all the extracts except *F. lignosus* and *T. microcarpus* (Table 1). This observation suggests that these fungi are potential antibacterial agents against infection from this organism.

Table 1: Antibacterial activities of fungal extracts

	Test Bacteria					
Higher fungi	*B.cereus*	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*P. aeruginosa*	*S. aureus*
Inhibitory zones (mm)						
F. lignosus (CRE)	15.0d	13.0gh	-	13.0fg		16.0bc
F. lignosus (PRE)	17.0b	16.0de	-	12.0g		17.0b
M. Jodocodo (CRE)	4.0j	-	10.0h	8.0i		13.0ef
M. jodocodo (PRE)	8.0i	-	13.0g	10.0h		17.0ab
P. florida (CRE)	-	13.0gh	20.0bc	-		16.0bc
P. florida (PRE)	-	13.0gh	22.0a	-	4.0b	18.0a
P. tuber-regium (CRE)	18.0a	8.0j	17.0de	16.0d	8.0a	12.0fg
P. tuber-regium (PRE)	18.0a	11.0i	19.0c	18.0bc		14.0de
P. atroumbonata (CRE)	12.0g	14.0fg	10.0	10.0		11.0gh
P. atroumbonata (PRE)	15.0d	18.0c	13.0g	14.0ef		15.0cd
P. giganteus (CRE)	13.0f	20.0b	13.0g	-		-
P. giganteus (PRE)	16.0c	24.0a	16.0ef	-		-
T. microcarpus (CRE)	-	13.0gh	-	17.0cd		16.0bc
T. microcarpus (PRE)	-	16.0de	-	20.0a		18.0a
T. robustus (CRE)	10.0h	12.0hi	13.0g	-		6.0i
T. robustus (PRE)	14.0e	15.0ef	15.0ef	-		10.0h
Control (Distilled water)	-	-	-	-		-

Key: CRE = Crude extract PRE = Purified extract

Values followed by the same letter(s) along each column are not significantly different by Duncan's multiple range test (DMRT) ($P \geq 0.05$)

Table 2: Antifungal activities of crude and purified higher fungi extracts

	Test Fungi				
Higher fungi	*A. niger*	*A. flavus*	*C. albicans*	*M. boulardii*	*T. concentrum*
Zone of Inhibition (mm)					
F. lignosus (CRE)	-	-	-	-	-
F. lignosus (PRE)	-	-	-	-	-
M. jodocodo (CRE)	5.0e	7.0e	-	-	-
M. jodocodo (PRE)	9.0cd	8.0de	-	-	-
P. florida (CRE)	-	-	-	-	-
P. florida (PRE)	-	-	-	-	-
P. tuber-regium (CRE)	-	-	-	-	-
P. tuber-regium (PRE)	-	-	-	-	-
P. atroumbonata (CRE)	8.0d	10.0c	-	5.0c	-
P. atroumbonata (PRE)	10.0bc	12.0ab	-	8.0ab	-
P. giganteus (CRE)	10.0bc	12.0ab	9.0a	-	9.0
P. giganteus (PRE)	11.0ab	13.0a	10.0a	-	-
T. microcarpus (CRE)	-	-	-	-	-
T. mirocarpus (PRE)	-	-	-	-	-
T. robustus (CRE)	10.0bc	-	7.0a	5.0c	-
T. robustus (PRE)	12.0a	-	10.0a	9.0a	-
Distilled water (control)	-	-	-	-	-

Key: CRE = Crude extract PRE = Purified extract.

Values followed by the same letter(s) are not significantly different byDuncan's multiple range test (DMRT) ($P \geq 0.05$).

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Table 3:
Minimum inhibitory concentration for bacteria and fungi.

Higher fungi	Test microorganisms								
	B. cereus	*E. coli*	*K. pneumoniae*	*P. vulganis*	*S. aureus*	*A. niger*	*A. flavus*	*C. albicans*	*M. boulardii*
	MIC (mg/ml)								
F. lignosus	5.50de	6.00bc	-	4.75c	4.50e	-	-	-	-
M. jodocodo	7.25a	2.75e	6.00b	8.75a	7.50a	13.75b	15.50a	-	-
P. florida	-	7.25a	7.75a	-	6.00b	-	-	-	-
P. tuber-regium	5.00e	-	6.25b	3.50d	-	-	-	-	-
P. atroumbonata	6.50b	5.00c	6.00b	4.25c	4.00e	14.00ab	15.25a	-	13.50b
P. giganteus	5.75cd	3.75d	3.25c	-	4.75de	10.50c	12.00c	13.25a	-
T. microcarpus	-	7.00a	-	6.00b	5.00cd	-	-	-	-
T. robustus	6.00bc	5.75	3.50c	-	6.25b	14.25a	14.00b	13.75a	15.75a

Values followed by the same letter(s) are not significantly different by Duncan's multiple range test (DMRT) ($P \leq 0.05$).

Oso (1977a and 1981) reported that *T. microcarpus* is a powerful medicinal ingredient for the treatment of gonorrhea among the traditional doctors in the south- western Nigeria. This medicine which is administered orally is prepared by grinding a large quantity of *T. microcarpus* with the pulp of the fruit of *Cucurbita pepo* Linn.; the leaves of *Cassia alata* Linn and some other ingredients From Table 2, it was clearly revealed that the antifungal properties of the test higher fungi were generally poor. Only four of the eight screened mushroomsd exhibited weak antifungal properties on at least two pathogenic fungi. Only crude extract of *P. giganteus* showed inhibitory effect against the dematophyte (*T. concentrum).* Likewise, *P. atroumbonatai and T. robustus* inhibited the growth of *M. boulardii*. This result was similar to that reported by Jonathan and Fasidi (2003) for *D. elegans* and *C. occidentalis*. The extracts of *P. giganteus* and *T. robustus* weakly inhibited the growth of *C. albicans* while other test mushrooms exhibited no antifungal properties against this fungus. Similar inhibitory effect against *C. albicans* was observed by Jonathan (2002) for *C. occidentalis* and *D. concentrica*.

It was generally observed that purified extract (PRE) of the test macrofungi enhanced more antimicrobial activities than crude extracts (CRE). (Tables 1 and 2). The values obtained for CRE and PRE for *M. jodocodo* against *B. cereus* were 4.0 and 8.0mm respectively. Similar result was obtained for this mushroom against *A. niger* and *A. flavus* (Table 2). Eunjeon et al 1997) and Kenji *et al* (1993) reported similar observation with *Ganoderma lucidum* and *Hericium erinaceum* respectively. Tan and Moore (1994) Irinoda *et al* (1992); and Tochikura *et al* (1988) separately observed that purified extracts of edible mushrooms are more effective against microorganisms than crude extracts.

Table 3 shows that the minimum inhibitory concentration (MIC) of the extracts ranged between 2.75 and 15.75mg/ml. The lowest MIC (2.75mg/ml) was found with the extract of *M. jodocodo* against *E. coli*. This was followed by *P. giganteus* extract against *K. pneumoniae*. *Pleurotus tuber-regium* and *T. robustus* had the MIC of 3.50mg/ml each against P. *vulgaris* and *K. pneumoniae* respectively. Danielli (1957) suggested that the lower the MIC, the more sensitive and promising the extract. This implies that most of these higher fungi offer potential therapeutic potency against some of the medically important bacteria. The MIC against fungi was generally high. This result confirms the observation made that the higher fungi studied

possessed poor antifungal activities.

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