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Antagonistic Effect of Extracts of Some Nigerian Higher Fungi Against Selected Pathogenic Microorganisms

[1]*Jonathan Gbolagade,* [2]*Loveth Kigigha and* [2]*Elijah Ohimain*

[1]Department of Botany and Microbiology University of Ibadan, Ibadan, Oyo State, Nigeria
[2]Department of Biological Sciences, Niger Delta University, Wilberforce Island Bayelsa State, Nigeria

Abstract: *In-vitro* studies were carried out to investigate antagonistic effect of crude and purified extracts of some selected Nigerian higher fungi against selected pathogenic microorganisms. Purified and crude extracts of the tested higher fungi showed wide spectrum of antibacterial activity. The highest antibacterial inhibitory activity (24.0 mm) was recorded with the purified extract (PRE) of *Polyporus giganteus* against *E. coli*. The second widest zone of inhibition (22.0 mm) was recorded with the PRE of *Pleurotus florida* against *K. pneumoniae*. Except the extracts of *Pleurotus tuber-regium*, none of the tested macrofungi was able to inhibit the growth of *P. aeruginosa*. Generally, the antifungal activities of these higher fungi were low. Only *P. giganteus* and *T. robustus* inhibited the growth of *C. albicans* with values which are not statistically significant from each other ($p \leq 0.05$). The minimum inhibitory concentration (MIC) of *M. jodocodo* against *E. coli* was 2.75 mg ml^{-1} while that of *T. robustus* against *M. bourlardii* was 15.75 mg ml^{-1}. The implications of these findings were discussed.

Key words: Antagonistic · extracts · higher fungi · pathogenic microorganisms · *in-vitro*

INTRODUCTION

Nigeria is a country with many natural resources and vegetation which support the luxuriant growth of different types of naturally occurring higher fungi [1-4]. Edible macro fungi are usually collected from the wild because farms growing them are very few [2, 5].

In the southern part of Nigeria, people usually use fruitbodies and sclerotia of edible mushrooms as major food condiments which are served at their important family meals [6, 7]. Few higher fungi from Nigeria have also been reported to possess important medicinal ingredients among the traditional doctors [7-10].

Macro fungi that have been implicated of having curative effect against diseases such as high blood pressure, pneumonia, urinary tract infection, intestinal disorder by Nigerian herbalists include *Ganoderma lucidum, Fomes lignosus, Daldinia concentrica, Termitomyces species, Pleurotus* species, *Lycoperdon species Polyporus* species, *Calvatia cynthiaformis* and *Psathyrella atroumbonate* [3, 8, 11].

Information on *in vitro* antimicrobial activities of these Nigerian higher fungi is very scanty or not available in the literatures. Jonathan and Fasidi [7] 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 [3] reported that antibacterial potency of puffballs could be compared to some extent with the commonly used antibiotics. The objectives of this study is to evaluate the antimicrobial potentials of selected Nigerian higher fungi in view of the limited scientific information on their medicinal values.

MATERIALS AND METHODS

Higher Fungi: Eight [8] macro fungi including *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) were used for this study. The fruitbodies of these fungi were collected from Botanical Gardens, University of Ibadan, Ibadan Nigeria. They were identified using

Corresponding Author: Dr. Jonathan Gbolagade, Department of Botany and Microbiology University of Ibadan, Ibadan, Oyo State, Nigeria

the standard descriptions of Zoberi [1] and that of Alexopolous *et al.* [12].

Preparation of the Extracts: The sporophores of collected fungal samples were air dried under a shade for 5 days to avoid inactivation of the bioactive components by ultra violent radiation, then oven dried at 55°C for 48 hrs to a constant weight. The oven dried samples were milled to obtain fine powder. Eighty grammes (80.0 g) portion of the powdered samples were extracted with 320 ml of methyl-alcohol in a soxhlet apparatus for 6 h. The extracts were concentrated using a rotatory evaporator. The semi solid extract, thus obtained was further dried into powder form [3]. To obtain purified extracts, the solid crude extracts were mixed each with 1000 ml of sterile distilled water with stirring at 4°C overnight. The suspension, thus obtained were centrifuged to remove the insoluble matter; the aqueous supernatant was concentrated under reduced pressure to 200 ml. The concentrates were extracted with each 200 ml ethyl acetate and subsequently concentrated using rotatory evaporator to yield a light yellow material known as purified extract [13]. When required, both the crude and purified extracts were mixed with sterile distilled water to desired concentration.

Detection of antibacterial activity: The assay for antibacterial activities in the tested fungal sample was determined by agar well diffusion method described by Stoke and Ridgway [14]. Bacteria used were *Bacillus cereus, Escherichia coli*, *Klebseilla pneumoniae Proteus vulgaris, Pseudomonas aeruginosa* and *Staplylococcus aureus*. The pure culture of each bacterium was inoculated in peptone water for 18 hours, then seeded into nutrient agar plates (one organism per plate). Well (7 mm diameter) was made on each Petri dish using sterile cork borer. About 0.25 ml of the extract was introduced into bore agar wells using sterile dropping pipette. The plates were kept inside the refrigerator at 4°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 0.25 ml of sterilized distilled water into the well in place of the extract in three replicates. The plates were incubated at 37°C for 24 hours. The antibacterial activities of the extracts were expressed as the diameter of the inhibition zones (in mm) appeared on the inculated plants

Detection of antifungal activities: The assay for antifungal potentials of these higher fungi extracts was carried out using *A. niger, A. flavus, C. albicans, M. boulardii* and *T. concentrum* as test organisms seeded on to sterile plates of Saboraud dextrose agar (SDA). Wells were made on the solid agar using 7 mm sterile cork borer. Twenty milligrammes (20.0 mg) of the extract was mixed with 5.0 g of the ointment base. Two grammes (1.5 g) 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°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 tested microorganisms. In this experiment different concentrations (0.5 – 20.0 mg ml^{-1}) of the methyl alcohol extract were prepared by dissolving a known weight of the extract in 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 20.0 mg ml^{-1}) in a Completely Randomized Block Design. Those that were still effective at 8.0 mg ml^{-1} were further diluted until no inhibitory zone was observed. The lowest concentration (dilution) produced was regarded as the minimum inhibitory concentration (MIC) for each extract [15]. Each experiment was carried out in triplicates. The sterile distilled water without any fungal extract served as the control.

Analysis of data: The data obtained were subjected to analysis of Variance (ANOVA) while the Text of significance were carried out using Duncan's multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The crude and purified extracts (CRE and PRE) of all the tested eight higher fungi used for this investigation possessed varying degrees of antibacterial properties against the tested bacteria (Table 1). Pure extract of *Polyporus giganteus* produced the widest zone of inhibition (24.0 mm) against *E. coli* followed by *P. atroumbonata* (18 mm) against the same bacteria ($P \leq 0.05$). Pure extract of both *Fomes lignosus* and *T. microcarpus* produced inhibitory zones of 16.0 mm each against *E. coli*. On the other hand, *M. jodocodo*

Table 1: Antibacterial activities of crude and purified higher fungi extracts

	Test Bacteria					
Higher fungi	*B.cereus*	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*P. aeruginosa*	*S. aureus*
	Zone of Inhibition (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	10	-	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
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) ($p \geq 0.05$)

Table 3: Minimum inhibitory concentration for bacteria and fungi

	Test bacteria and fungi								
Higher fungi	*B. cereus*	*E. coli*	*K. pneumoniae*	*P. vulganis*	*S. aureus*	*A. niger*	*A. flavus*	*C. albicans*	*M. boulardii*
	MIC (mg ml^{-1})								
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) ($p \leq 0.05$)

possessed no antibacterial activities. The strong antibacterial properties possessed by *P. giganteus* and *P. atroubonata* is not a surprise, because these two fungi are important part of medicinal ingredients which are used by the localYoruba people in the south western Nigeria for the treatment of intestinal disorder and some other bacterial infections [3].

All the tested extracts (either pure or crude) except those of *P. giganteus*, inhibited the growth of *S. aureus*. The purified extract (PRE) of both *P. atrombonata* and *T. microcarpus* had the best *in-vitro* antibacterial activities (18.0mm inhibition zone) against *S. aureus* (Table 1). It was interesting to note that *P. aeruginosa* which is resistant to both tetracycline and gentamycin [3, 16] 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 [9, 11] 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 contained potential antibacterial agents against infection from this organism. Oso [8, 9] 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 tested higher fungi were generally poor. Only four of the eight screened mushrooms exhibited weak antifungal properties against 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 [10] for *D. elegans* and *C. occidentalis*. The extracts of *P. giganteus* and *T. robustus* weakly inhibited the growth of *C. albicans* while other tested mushrooms showed no antifungal properties against this fungus. Similar inhibitory effect against *C. albicans* was observed by Jonathan [3] for *C. occidentalis* and *D. concentrica*.

It was generally observed that purified extract (PRE) of the tested macrofungi exhibited more potent 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 8.0 mm respectively. Similar result was obtained for this mushroom against *A. niger* and *A. flavus* (Table 2). Eunjeon *et al* [17] and Kenji *et al* [18] reported similar observation with *Ganoderma lucidum* and *Hericium erinaceum* respectively. Tan and Moore [19] Irinoda *et al*. [20]; and Tochikura *et al* [21] 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 15.75 mg ml^{-1}. The lowest MIC (2.75 mg ml^{-1}) 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 3.50 mg ml^{-1} each against P. *vulgaris* and *K. pneumoniae* respectively. Danielli [22] 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 were generally high. This result confirms the observation made that the higher fungi studied possessed poor antifungal activities.

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