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ANTIMICROBIAL ACTIVITIES OF FOUR WILD EDIBLE MUSHROOMS IN NIGERIA

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ABSTRACT
Antimicrobial activities of four wild Nigerian edible mushrooms *(Lentinus squarrosulus, Psathyrella atroumbonata, Volvorella volvacea and Coprinellus micaceus*) were evaluated. The four mushroom extracts showed varying degree of inhibition on the test organisms (*Escherichia coli, Staphylococcus aureus, Aspergillus flavus and Penicillum notatum*). L. *squarrosulus* showed good antimicrobial activities against all the microorganisms tested. The filtrates of *L. squarrosulus* and *P. atroumbonata* showed wider inhibition zones (16.2mm and 12.7mm for *S .aureus* and 18.6 and 14.3 for *E .coli*) in the test bacteria than those of the filtrates of *C. micaceu*s and *V. volvacea (*9.3mm and 10.4mm for *S. aureus* and 8.4mm and 7.7mm for *E. coli*) against the test bacteria. Culture filtrates of *L. squarrosulus* and *V. volvacea* inhibited the two fungi tested while the culture filtrates of *P. atroumbonata* and *C. micaceus* did not show sign of mycelial inhibition of *Penicillum notatum*. *L. squarrosulus* and *P. atroumbonata* were found to be the most effective against the test bacteria while the best antifungal activities was recorded in *L. squarrosulus* and *V. volvacea*. The bioactive contents of the mushrooms are promising natural antimicrobial agents that can be harnessed as potent antibacterial and fungi toxicants.

KEYWORDS: Antimicrobial activities, culture filtrates, growth inhibition, wild edible mushrooms.

INTRODUCTION

The use of mushrooms as food as well as medicine is gaining popularity in recent times. The nutritive and medicinal properties of many mushrooms have been documented. These include protein, fibers and polysaccharides (Bonatti *et.al*.,2004; Agrahar-Murugkar and Subbulakshmi, 2005;Cheung and Cheung, 2005; Lin, 1995; Chihara,1992) . Mushrooms have been found to contain all the essential amino acids (Breene,1990; Chang and Miles, 1987). Edible mushrooms characteristically contain many different bioactive compounds such as polysaccharides, glycolipids, sesquiterpenes etc with diverse biological activities such as anticancer, antibacterial, antifungal and antiviral agent (Waser,2002).

It has been estimated by Chang (1999) that about 50% of the cultivated edible mushrooms contain functional neutraceutical or medicinal properties. They have various degrees of immunomodulatory, lipid-lowering, antitumour and other beneficial and therapeutic health effects (Chang, 1999). According to Lin (1995), *Tremella* has been valued in folk medicine in China as a remedy with nutritive and tonic action in treating debility and exhaustion. It is also used in traditional Chinese medicine to nourish and moisten the lungs, the stomach and promote secretion of saliva. Lentinan extracted from *Lentinus edodes* has been found to be effective therapy for cancer patient because of its antitumour and host defense potentiators' properties (Chihara, 1992).

Stamets (2000),compiled the list of some medicinal mushrooms and their activities in the treatment of different diseases. These activities include anti-arthritic, anti-inflammatory, anti-fatigue, anti-microbial, anti-oxidative, antiviral, chemo-protective and radio-protective activities. These special attributes of edible and medicinal mushrooms might be one of the major reasons why the United States National Cancer Institute has chosen mushrooms as one of the sources of new drugs for cancer (Liu, 1993).

Lentinus squarrosulus, Psathyrella atroumbonata, Volvariella volvacea and *Coprinellus mecaceus* are common edible mushrooms usually collected in the wild for consumption in Nigeria. They are commonly found in both grass lands and forest lands in Nigeria (Kadiri and Fasidi, 1994; Ayodele and Okhuoya, 2007). The nutritive values of these mushrooms have been evaluated and they have been found to be highly nutritious (Breene, 1990; Kadiri and Fasidi, 1994; Ayodele and Okhuoya, 2007). *Lentinus* species have been found to contain medicinal properties such as Lentinan which has been evaluated to be a host defense potentiator (Norrel and Messley,1997). It is on this note that the antimicrobial activities of these four wild edible mushrooms found in Nigeria were studied.

MATERIAL AND METHODS

Collection of Mushrooms Tested

Four wild edible mushrooms (*Psathyrella atroumbonata* Pegler, *Lentinus squarrosulus Mont* , *Volvariella volvacea* Bull. Ex Fr. and *Coprinellus micaceus* Fr. were collected in the grass land area of Kogi State, Nigeria. The longitude and latitude of the collection area is $7^0$11′- 7^0 32′E and 7^0 15′- 7^0 29′N . The pure cultures, preserved specimens and photographs of the mushrooms were taking for identification at the Mushroom Science Unit of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria where there are collections of most Nigerian edible mushrooms. The mushrooms were identified morphologically. To get the

pure cultures of the mushrooms, the freshly collected mushrooms were tissue cultured by cutting the tip of the stipe after removing the pileus and placed on potato dextrose agar (PDA) medium. This was carried out in the Laminar flow under sterile condition. The pure cultures of the mushrooms were maintained on Potato Dextrose Agar (PDA) medium from M- Lab at 25^{o}C for further study.

Test Organisms

Two species of human pathogenic bacteria and two species of fruit rotting fungi were chosen as test organisms for extracts of the four mushrooms. Pure clinical isolates of *Staphylococcus aureus* and *Escherichia coli* (gram positive and gram negative respectively) were obtained from the laboratory of the Grimad Catholic Hospital Anyigba, in Kogi State, Nigeria. The bacterial strains were maintained on nutrient agar (NA) medium at 35^{o}C for further study.

Two rot inducing pathogenic fungi on yam tubers (*Aspergillus flavus* and *Penicillum notatum*) were obtained from the plant pathology unit of the Department of Biological Sciences, Kogi State University, Anyigba, Nigeria and used for the study. The fungi were maintained on Potato Dextrose Agar (PDA) (M-Lab) at 25^{o}C for further study.

Culture Filtrates

The mycelia of the mushrooms were cultured in Potato Dextrose Broth (PDB) and incubated at 25^{o}C in a rotatory shaker (Stuart Scientific) at 150 – 200 rpm for 14 days. To obtain the culture filtrate from the 4 mushrooms species, the liquid cultures containing mycelia were filtered through Whatman No 1 filter paper. The filtrates were used for the study immediately.

Test for antibacterial activities

Two methods (Bacterial growth by optical density method and Modified filter paper disc method) were used to evaluate the antibacterial activities of the culture filtrates. Bacteria for testing the antibacterial activities were grown in Nutrient Agar (NA) (M- Lab.). The antibacterial activity of the mushroom culture filtrates were evaluated by adding 50ml of mushroom filtrates to 50ml of fresh potato dextrose broth (v/v) and then autoclaved at121^{0} C for 15 min (Imtiaj and Lee, 2007) . Cooled liquid medium containing each mushroom filtrate was inoculated with four discs of 2cm agar discs of each bacterium separately in 250ml conical flasks and incubated at 35^{o}C. The bacterial growth was determined by measuring the Optical Density (OD) at 600nm (Imtiaj and Lee, 2007) after every 12hrs and it was done until 72hours. For control experiment, 50ml of fresh PDB (M-lab) was added to 50ml sterile distilled water (without the mushrooms filtrates) and inoculated with each bacterium. The OD was also measured with same method.

A modified filter paper disc method by Norrel and Messley (1997) was also used to determine the antibacterial activity. The culture filtrates were concentrated by a rotary evaporator until a semi-solid state substance was obtained. This was freeze dried at -80^{o}C and diluted to 10% solution (0.1gm1^{-1}) with sterilized distilled water. The sterile paper discs (8mm diameter) were soaked with the solution and placed on bacterial seeded plate (0.1g ml^{1} 10 CFU ml^{-1}) of nutrient agar. The plates were incubated at 35^{o}C for 24h and the inhibition zone was observed and measured. An average inhibition zone was calculated for 4 replicate plates. Sterile paper discs (8mm diameter) were soaked in sterile distilled water and placed on bacterial seeded plate (0.1gml^{1} 10cfu ml^{1}) and this served as control.

Antifungal Activity

Two methods were employed to determine the antifungal activity of the culture filtrates. The methods were Percent inhibition of mycelial growth (PIMG) and the Percent inhibition of mycelial weight (PIMW) (Imtiaj and Lee, 2007) . Culture filtrates of mushrooms were diluted with PDB medium separately to make 50% concentrations (V/V) of each mushroom filtrate and 2% agar was added to make it solid. They were then autoclaved at 121^{o}C for 15 min and poured into sterile Petri dishes (Imtiaj and Lee, 2007). Agar discs taken from 10 days old cultures of two rot inducing fungi (*Aspergillus flavus* and *Penicillum notatum)* were placed in the centre of the Petri Plates respectively. For negative control, agar discs of the same fungi were placed the same way on fresh sterilized PDA plates without culture filtrates. All pairings were carried out in 4 replicates and incubated at 25^{o}C. Inhibitory activity was assessed after 10 days of incubation by measuring the radial growth (mm) along two diameters on the medium containing culture filtrates (R2) and the radial growth on the control (R1) was also measured the same way. The two measurements were transformed into PIMG using the formula PIMG = [(R1 – R2)/R1] x 100 according to Imtiaj and Lee, (2007).

For determination of the percent inhibition of mycelial weight (PIMW), the culture filtrates were diluted as in PIMG but without agar and autoclaved. Incubation of each fungus was made separately with 100ml of PDB media in 250ml of conical flask at 25^{o}C for 10 days on rotatory shaker(Stuart Scientific) . After the incubation period, the fungal mycelium was harvested by filtering through previously dried and weighted Whatman filter paper No 1. It was then dried to constant weight (g). Before weighing, the filter paper were allowed to cool and subsequently weighted in a balance. The weight of the mycelium (g) was calculated by deducting the weight of the filter paper (g) from the final weight. For control, fresh sterilized PDB were used without the culture filtrates. All pairing were carried out in 4 replicates and were incubated at 25^{o}C for 10 days. The inhibition was assessed after the incubation period by measuring the mycelial weight (g) grown in culture filtrates (R2) and the mycelial weight (g) grown in the control (R1). The two readings were transformed into PIMW using the same formula above (Imtiaj and Lee,2007).

Statistical Analysis

All the results were analyzed using simple descriptive statistics such as mean and standard error. Mean was separated using analysis of variance (ANOVA).

TABLE 1: Growth of *E. coli* and *S. aureus* Measured at 600nm (OD) in Liquid Culture Filtrates of Four Edible Mushrooms

Mushroom	*E. coli*	*S. aureus*
Control	1.42 ± 0.02	1.42 ± 0.02
Lentinus squarrosulus	0.44 ± 0.03	0.61 ± 0.05
Psathyrella atroumbonata	0.52 ± 0.01	0.47 ± 0.03
Coprinellus micaceus	0.88 ± 0.03	1.45 ± 0.04
Volvorella volvacea	1.53 ± 0.04	1.01 ± 0.22

Each value is a mean of 4 replicates ± Standard Error: P = 0.005

The Optical Density (OD) value in liquid culture filtrate higher than the OD value of control indicates no inhibition.

TABLE 2: Inhibition Zones of Four Edible Mushrooms Culture Filtrates against *S. aureus* and *E. coli* Growth (mm)

Mushroom	*S. aureus*	*E. coli*
Control		
Lentinus squarrosulus	16.2 ± 0.05	18.6 ± 0.02
Psathyrella atroumbonata	12.7 ± 0.01	14.3 ± 0.04
Coprinellus micaceus	9.3 ± 0.02	8.4 ± 0.01
Volvorella volvacea	10.4 ± 0.05	7.7 ± 0.04

Each value is a mean of 4 replicates ± Standard Error: P = 0.05

TABLE 3: Percentage Inhibitory Effect of Culture Filtrates on the Mycelial Growth(PIMG) of *Aspergillus flavus* and *Penicillum notatum*

Mushroom	*Aspergillus flavus*	*Pencullium notatum*
Lentinus squarrosulus	*32.10 ± 0.01*	*29.92 ± 0.03*
Psathyrella atroumbonata	*28.06 ± 0.05*	-
Coprinellus micaceus	*14.92 ± 0.10*	-
Volvorella volvacea	*17.66 ± 0.05*	*13.82 ± 04*

Each value is a mean of 4 replicates ± Standard Error: P = 0.05

TABLE 4: Percentage Inhibitory Effect of Culture Filtrates on the Mycelial Weight(PIMW) of *Aspergillus flavus* and *Penicillum notatum.*

Mushroom	*Aspergillus flavus*	*Penicillum notatum*
Lentinus squarrosulus	16.82 ± 0.02	17.04 ± 0.01
Psathyrella atroumbonata	10.73 ± 0.04	0.00
Coprinellus micaceus	10.10 ± 0.03	0.00
Volvorella volvacea	12.66 ± 0.03	11.57 ± 0.02

Each value is a mean of 4 replicates ± Standard Error: P = 0.05.

RESULTS

The antimicrobial activity of the four mushrooms extracts are shown in Table 1-4. The mushrooms extracts showed varying degree of inhibition on the test organisms. There was significant difference (P =0.05) in the antimicrobial activity of the mushrooms extracts. The result of optical density showed that the culture filtrates of *L. squarrosulus* and P. atroumbonata were highly effective against *E. coli* and *S. aureus.* The culture filtrates of *V. volvacea* did not inhibit the growth of *E.coli* but showed slight inhibitory effect on S. areus while culture filtrate of *C. micaceus* inhibited the growth of *E. coli* but not *S. aureus* (Table1).

Result of paper disc experiment is presented in Table 2. There was significant deference(P = 0.05) in the zones of inhibition of the culture filtrates. *Lentinus squarrosulus* has the widest of inhibition on E. coli. The filtrates of *L. squarrosulus* and P. atroumbonata showed wider inhibition zone than the filtrates of *C. micaceus* and *V. volvacea*. The gram negative bacterium(E. coli) was more susceptible than the filtrate of gram positive bacterium (*S. aureus*).

The antifungal activity of the mushrooms filtrates are shown in table 3and 4. *Aspergillus flavus* is more susceptible to the culture filtrates of the mushrooms than *P. notatum*. Culture filtrates of *L. squarrosulus* and *V. volvacea* inhibited mycelial growth of *P. notatum* while the culture filtrates of *P. atroumbonata* and *C. micaceus* did not inhibit the mycelial growth of *P. notatum* (Table 3). The highest percentage inhibitory effect on mycelial growth was recorded in *L. squarrosulus* on *A. flavus* (32.10%) while the least was recorded in *C. micaceus* (14.92) on *A. flavus*. The result of mycelial weight analysis of *A. flavus* and *P. notatum* follows a similar pattern with the percentage inhibition of mycelial growth. The highest value was also recorded in *L. squarrosulus* (17.04%) on P. notatum while there was no inhibitory effect (0.00%) of culture filtrates of *P. notatum* and *C. micaceus* on *P. notatum* (Table 4).

DISCUSSION

The filtrates of four wild edible mushrooms under study showed a wide range of antibacterial and antifungal activity. They exhibited moderate to good antibacterial activity against the bacteria pathogens tested. The filtrate of *L. squarrosulus* is very effective against *E. coli* and *S. areus*. This report is similar to the findings of Ishikawa *et al* (2001)' who showed that the mycelial culture filtrate of *Lentinula edodes* inhibited the growth of *B. subtilis*. The observed inhibitory effect of *L. squarrosulus* on both gram negative and gram positive bacteria is in line with the report of Komemushi *et al (*1995, 1996) who worked on the antimicrobial substances in *L. edodes*. Imtiaj and Lee (2007) also worked on the antibacterial and antifungal activities of Korean wild mushrooms and found that several filtrates of wild mushrooms inhibited the growth of many pathogenic bacteria such as *P. aeruginosa* and *S. aureus.*

The result of paper disc method showed that the mushrooms culture filtrates inhibited the test bacteria. The gram positive bacterium was more susceptible than the gram negative bacterium. This observation is in line with the work of Imtiaj and Lee (2007) who reported the antimicrobial activity of some wild mushrooms from

Korea. Jonathan and Fasidi (2003) also worked on two edible Nigerian macro- fungi *Lycoperdon pusilum* and *L. giganteum* using paper disc method and reported that the mushrooms exhibited a strong antibacterial activity against several gram positive and gram negative bacteria such as *S. areus, B. subtilis, E. coli, Micrococcus flavus* and *Proteus mirabilis.*

The mushrooms filtrates also showed antifungal activity against the two rot fungi (Aspergillus *flavus* and *Penicillum notatum*). Mycelial weight of *A. flavus* was more inhibited by the culture filtrates of the four mushrooms. Only the culture filtrates of L. *squarrosulus* and *V. volvacea* inhibited growth of *P. notatum*. The culture filtrates of *P. atroumbonata* and *C. micaceus* did not show sign of mycelial inhibition of *P. notatum.* This observation is similar to the report of Imtiaj and Lee (2007) who reported that the culture filtrates of wild mushrooms from Korea were selective in mycelial inhibition of plant pathogenic fungi tested.

Among the studied mushrooms, L. squarrosulus and *P. atroumbonata* were found to be very effective against the test bacteria while the best antifungal activity was recorded in *L. squarrosulus* and *V. volvacea.* This observation was also similar to the observation of Jonathan and Kadiri (2003) who reported that *Lycoperdon giganteum* ethanolic extract was more active against the fungi tested while *Lycoperdon pusilum* was more active against the test bacteria.

This study has revealed the antimicrobial activity of the four wild edible mushrooms under study and can be suggested that the bioactive contents of the mushrooms are promising natural antimicrobial agents that can be harnessed as potential antibacterial and fungi toxicants. Further extensive studies are recommended for these mushrooms to actually identify the bioactive components responsible for their antimicrobial activities.

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