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Antibacterial Effect of Crude Polysaccharide Extracts from Sclerotium and Fruitbody (Sporophore) of *Pleurotus tuber-regium* (Fried) Singer on Some Clinical Isolates

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Abstract: The antibacterial effect of crude polysaccharide extracts of the sclerotial mushroom *Pleurotus tuber-regium* on some bacterial pathogens were investigated using the agar cup diffusion and disc diffusion methods. Ethanolic polysaccharide extracts of *P tuber-regium* fruitbody showed mean inhibition zones of 19.33, 20.67, 23.00 and 26.67 mm on *Enterococcus feacalis, Escherichia coli, Staphylococcus aureus* and *Salmonella* typhi respectively. The ethanolic extracts of the sclerotium and aqueous extracts of both the sclerotium and fruitbody did not produce any observable zones of inhibition. None of the extracts showed visible zones of inhibition in the disc diffusion technique. The Minimum Inhibitory Concentrations (MIC) determined by the agar cup diffusion techniques for the ethanolic extracts from the fruit body were 6.25 mg mL^{-1}, 12.5 mg mL^{-1} and 12.5 mg $mL^{-1}$and 25 mg mL^{-1} for *Staphylococcus aureus Escherichia coli Salmonella* typhi and *Enterococcusfeacalis* respectively.

Key words: Antibacteria, *Pleurotus tuber-regium* fruit body, sclerotium, crude polysaccharide, pathogen

INTRODUCTION

Pleurotus tuber-regium (Fries) singer is an edible sclerotial Basidiomycete that belongs to the family Pleuroteceae. It occurs in both tropical and subtropical regions of the world[1,2]. It is a common mushroom in the southwest province of Cameroon and in the eastern and western parts of Nigeria. It forms large spherical to oval subterranean sclerotia which sometimes measure up to 30 cm in diameter[3] and which is dark brown on the outside and white on the inside. Oso,[4] The fungus infects dead wood where it produces sclerotia usually buried within the wood tissues but also found between the wood and the bark or buried in the soil[5]. When cropped in warm and humid atmosphere sclerotia produce fruit-bodies. Both the sclerotia and the fruit-bodies are consumed as food or used as condiment to add flavor in food[1,2,4]. It is an essential food thickener amongst the Igbo tribe of Eastern Nigeria.

Edible and medicinal (macro) fungi not only convert huge cellulose biomass waste into human food but also most remarkably can produce notable myco-pharmaceuticals and myco-nutriceuticals[6]. Pharmaceutical substances with potent and unique health enhancing properties have been isolated from mushrooms[7,8]. Some of the pharmaceuticals contain several anti-bacterial and anti-tumor polysaccharrides [9]. Mushroom have been found to possess anti-viral, anti-tumor, anti-diabetic, anti-allergic, anti-inflammatory, anti-oxidant, anti-bacterial, anti-parasitic, diuretic adaptogenic properties and can be used as a cardio tonic, laxative, sedative immunostimulant immunoregulator and hepatic tonic[10].

P. tuber-regium is not only consumed in Nigeria for its flavor and nutritive value but also for its medicinal effects. This mushrooms has been widely used by traditional medicine (native doctors) in the treatment of many ailments[4].

Resistance of microorganisms to antibiotics has created an immense clinical problem in the treatment of infectious diseases[11] and has complicated the treatment of infectious diseases in immuno-compromised patients and nosocomial infections[12]. This has rekindled the search for novel antibiotics.

This study is designed in pursuance of the efforts to search for drugs from mushrooms and the verification of the scientific basis for its traditional medicinal uses. We report here on the antibacterial activities of the crude polysaccharide extracts from the mushroom *P. tuber- regium.*

MATERIALS AND METHODS

Collection, identification and cultivation of mushrooms: Fresh sclerotia of *Pleurotus tuber-regium* were purchased from a local market at Ndoro, Ikwuano LGA, Abia State of

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Nigeria and identified in the Department of Biological Sciences Michael Okpara University of Agriculture, Umudike by a mycologist. The cultivation of *Pleurotus tuber-regium* was carried out according the methods of and Fasidi and[13-15]. The Sclerotia weighing 602.5 g and 730.5 g were each kept on moist cotton wool in separate transparent plastic buckets with covers inside a room at temperatures 30 + 2°C to create a conducive humid atmosphere for the sporophores to sprout. The sclerotia were observed daily to make sure that it remained moist Fig. 1 (A and B).

Preparation of Mushroom samples and Extraction of Crude Polysaccharides: The resulting fruit-bodies were harvested after two weeks of cultivation and dried to a constant mass in the oven at 35°C. Uniform powders of fresh sclerotium (520 g) and dried fruit-bodies were obtained by pulverizing each sample of *P. tuber-regium* and stored in airtight containers for further use. The crude ethanolic and aqueous polysaccharide extracts were obtained from the powered samples according to the methods of [9] . The extracts were tested for sterility by exposure to ultraviolet light for 24 h and streaking out on nutrient agar plates and incubating for 24 h. The percentage extracts obtained were calculated using the formula.

$$\text{Percentage (\%) yoeld of extracts} = \frac{\text{Weight of residue extract}}{\text{weight of mushroom powder}} \times \frac{100}{1}$$

Antibacterial screening tests: Clinical isolates of *Escherichia coli, Salmonella typi, Staphylococcus aureus and Enterococcus faecalis* were collected from the Microbiology laboratory of Federal Medical Centre, Umuahia, Abia State, Nigeria. The identities of the isolates were confirmed by their morphological cultural, physiology and molecular characteristics.

Agar diffusion method[16,17] and the disc diffusion technique reported by[18,19] were used. Solutions of extracts were prepared by dissolving 0.5 g of the crude extracts of fruit-body and sclerotium in 3mL of normal saline in test tubes and the resultant solution diluted to concentration of 100 mg mL^{-1} by addition of 2mL of normal saline[9] Agar plates were seeded with 0.2mL of standardized broth cultures of the test bacteria. The surfaces of the seeded media were allowed to dry in the incubator for 30 min. Wells of 6.00 mm diameter and 8.00 mm deep were aseptically made in the seed agar using sterile cork borers[20]. This was done in triplicates for each extract. The antibiotic Gentamycin and normal saline were used as positive and negative controls respectively. The whole set-up was incubated as 37°C for 18 h[17] after which diameter of zones of inhibition were measured. The agar disc diffusion techniques involved placing sterile paper discs (Whatman No. 1 filter paper) of 5 mm diameter impregnated with different crude extracts and dried in a hot air oven at 60°C on agar plates seeded with the test organism. Discs use also prepared for Gentamycin and Normal saline as controls.

Determination of minimum inhibitory concentration (MIC): Analysis of the Minimum Inhibitory Concentration (MIC) was carried out according to the standards recommended by the National Committee for Clinical laboratory Standards[21]. This test was carried out for only the crude ethanol extract of the fruit-body of *P. tuber-regium*. A two-fold dilution of the crude ethanol polysaccharide extract of the fruit-body in the normal saline was prepared. The minimum inhibitory concentration was then determined.

Statistical analysis: Tests of significance were calculated using analysis of variance and mean were separated using T – statistics.

RESULTS

The results obtained showed that the fruit-body of *P tuber-regium* gave a higher quantity of crude polysaccharide extract than the sclerotium (Table I). The differences in yield was however not statistically significant. The ethanol extraction gave higher quantity of crude extract than aqueous extraction (Table I). There was no significant difference in the mean yield for both methods of extraction ($p<0.05$). None of the organism was inhibited in the disc diffusion technique by any of the extracts. Antibacterial screening of the stock concentration of the different crude polysaccharide

Table 1: Percentage extracts obtained from *P. tuber-regium*

Mushroom	Method of extraction	Colour of extract	Weight of powder g	Weight of extract g	Percentage yield of extract
Sclerotium	Ethanol	Cream	50.00	1.01	2.02
	Aqueous	Cream	50.00	0.82	1.64
Fruit-body	Ethanol	Dark brown	50.00	4.30	8.60
	Aqueous	Brown	50.00	2.21	4.42

Table 2: Sensitivity of test Bacteria to Crude Polysaccharide extract (100 mg mL^{-1}) from *P. tuber-regium* (Fried) singer.

	Sclerotium		Fruit-body		Controls	
Bacterial Pathogen	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract	Gentamycin	Normal saline
E. faecalis	-	-	+	-	+	-
E. coli	-	-	+	-	+	-
S. aureus	-	-	+	-	+	-
S. typhi	-	-	+	-	+	-

\+ = Sensitive
\- = Resistant

Table 3: Mean Inhibition Zone diameter (MM) of the crude polysaccharide extracts of *P. tuber-regium* against test bacterial pathogen

Crude Polysaccharide extract and controls	Concentration mg mL^{-1}	Mean Growth Inhibition zone *E. faecalis*	*E. coli*	*S. aureus*	*S. typhi*	Proportion of strain susceptible (%)
Ethanol extract of fruit-body	100	19.33+0.73	20.67+0.16	23.00+0.16	25.67+0.68	(100)
Aqueous extract fruit-body	100	-	-	-	-	000
Ethanol extract sclerotium	100	-	-	-	-	000
Aqueous extract Sclerotium	100	-	-	-	-	000
Gentamycin	000.50	20.73+0.41	22.33+0.24	25.00+0.57	26.67+0.62	100
Normal Saline	008.50	-	-	-	-	000

- = No Inhibition, mm = millimeter, % = percentage. Figures are Mean + SD

Table 4: Minimum Inhibitory Concentration test of the crude ethanol polysaccharide extract of the fruit body of *P tuber-regium*

Concentration (mg mL^{-1})*	Dilution	*Enterococcus faecalis*	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*
100	2^{0}	19.8 ± 0.87	21.30± 0.34	24.60 ± 0.62	25.3 ± 0.47
50	2^{-1}	12.7 ± 0.63	19.00± 0.00	19.30 ± 0.05	19.0 ± 0.4
25	2^{-2}	8.67 ± 0.47	9.300± 0.85	13.30 ± 0.46	12.7 ± 0.47
12.5	2^{-3}	-	2.000± 1.47	9.17 ± 0.48	3.400± 0.16
6.25	2^{-4}	-	-	4.33 ± 0.33	-
3.125	2^{-5}				
Crude extract		25	12.5	6.25	12.5
Gentamycin (Control)		1.95×10^{-9}	4.88×10^{-9}	6.1×10^{-9}	9.77×10^{-9}

MIC – Lowest concentration that exhibited observable zones of inhibition (mm ±. SD)

–No zones of inhibition

* The test was conducted in triplicates SD-Standard Deviation

extracts of *P. tuber-regium* showed observable zones of growth inhibition with the Agar cup diffusion method (Table 2).

Ethanolic extracts (100 mg mL^{-1}) from the fruit body exhibited antibacterial activity on the four tests bacteria. The highest zone of inhibition diameter with the ethanolic extracts of the fruit-body of *P. tuber-regium* was observed on *Salmonella typhi* (25.67 + o.47 min) while the lowest (19.33 + 0.77 mm) was observed for *Enterococcus faecalis* (Table 3).

The Minimum Inhibitory Concentration (MIC) of the crude polysaccharide ethanol extracts from the fruit-body of *P. tuber-regium* determined by the agar cup diffusion method Fig. 2 (A and B) were 6.25 mg mL^{-1}, 12.50 mg mL^{-1}, 12.50mg mL^{-1} and 25mg mL^{-1} on *S. aureus, E. coli, S. typhi and E. faecalis* respectively (Table 4).

DISCUSSION

Results obtained from this study show that the yield of extracts differed with the mushroom sample and method of extraction. The ethanol extraction gave higher yield than aqueous extraction on both sclerotium and fruit body of *P. tuber-regium* (fres) Singer. Ethanol is a well-known organic solvent and dissolves phytochemical metabolites better than water. The ethanol extracts of the fruit body of *P. tuber-regium* inhibited all four bacterial test organism used for this study. This finding shows that the mushroom contained active principles which justify its use by traditional medical practitioners (folk-doctors) to treat ailments and diseases associated with bacterial infections as reported by various authors[4-15].

The fruit-body of *P. tuber-regium* produced observable zones of inhibition while the sclerotium did not. This may be due to the differences in concentration of the active principles present in the two mushroom samples. Other workers[14-22] also reported higher concentrations of the active principles – polysaccharides, terpernoids, alkaloids, tannins, phenols and other nutrients in the fruit-body than the sclerotium of *P. tuber-regium.*

The use of ethanol for extraction seem to impact positively on the potency of the active principles since the ethanolic extract from the fruit-body produced appreciable zones of inhibitions while the aqueous extract from the same fruit-body did not. The better extractability of ethanol when compared to water is however well known. The results show that the active principles against the organism was only extracted by alcohol or that the aqueous extract did not yield enough concentration of the active principles that can inhibit these organisms. These results agree with the report of Stowe[23]. The inability of even the ethanol extract from the fruit body to inhibit the test organism using the agar disc diffusion technique may be due to easy evaporation of components of the mushroom on paper disc or that the quantity of extracts absorbed by the paper was too small. Even in the agar cup technique the concentration was an important factor, since higher concentrations gave higher zones of inhibition. It is noteworthy that the spectrum of antibacterial activity of the crude ethanol polysaccharide extracts of the fruit-body of *P. tuber-regium* is comparable to that of gentamycin. The recent study on carbohydrates of submerged mycelium by Shcherba and babitsskaya [24]showed that the qualitative composition of xylographic

basidiomycetes were mainly structural polysaccharides which were hetero – and homo glycans with β and α - glycoside bonds. The need for more research to determine the best methods of extraction and the particular active ingredient in this mushroom, *P. tuber-regium,* is here emphasized.

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