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The Antimicrobial Activity of Sclerotia of *Pleurotus tuberregium* (Osu) on Some Clinical Isolates

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Authors' contributions

This work was carried out in collaboration between all authors. Author DBO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors HOS and CJU managed the analyses of the study. Author DBO managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

This study was carried out to determine the medicinal potential of the sclerotia of *Pleurotus tuberregium* on some clinical isolates. The method employed for this test was the Kirby-Bauer disc-diffusion method. The ethanol extract showed some level of potency with zones of inhibition: *Klebsiella sp* (21 mm±0.2); *Bacillus sp* (15 mm±0.4); *Staphylococcus aureus* (13 mm±0.2); *Candida albicans* (7 mm± 0.1). *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus* sp were not susceptible to the ethanol extract. There was no observed level of susceptibility when hot and cold water extracts of sclerotia of *Pleurotus tuberregium* were used on the selected test organisms. Ethanol extract of sclerotia of *P. tuberregium* showed a good therapeutic outcome with its inhibitive effect on clinical isolates.

Keywords: Sclerotia; Pleurotus tuberregium; clinical isolates; extracts.

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1. INTRODUCTION

The sclerotium of *Pleurotus tuberregium* is a stage in the life cycle of some fungi and is a compact mass of hardened fungal mycelium containing food reserves which can survive extreme environmental conditions [1]. Sclerotia of *P. tuberregium* which is rich in fibre may grow to a considerable size (up to 25cm in diameter), and they are also very dense and quite resistant to long periods of desiccation, and have been shown to support successive fruiting over consecutive seasons.

Over the years, scientists have been able to structure the information concerning mushroom and its medicinal properties [1]. Several mushroom species with antimicrobial properties have also been reported [2-4]. Bioactive compounds in mushroom are effective against pathogenic bacteria such as *Salmonella* and *Pseudomonas, Staphylococcus aureus* and *Escherichia* coli [5-7]. The aim of this study was to assess the potency of ethanol, hot water and cold water extracts of sclerotium of *Pleurotus tuberregium* on some clinical isolates of public health importance.

2. MATERIALS AND METHODS

Sclerotium of *Pleurotus tuberregium* used for this study was obtained from the Omalelu Market in Ikwerre Local Government Area of Rivers State, Nigeria and identified at Botany Department, University of Port Harcourt.

2.1 Preparation of Extracts of the Sclerotia of *P. tuberregium*

Preparation of extracts of the Sclerotia of *P. tuberregium* was done according to the method of Stanley *et al*. [4]. The sclerotia of *P. tuberregium* was shade dried and blended into smaller pieces. A 500.50 g weight of the powder was measured and transferred into one litre (1000 ml) each of distilled cold water, hot water (heated up to 100°C) and 70% ethanol respectively and stirred vigorously. The cold and hot water preparations were allowed to stand for 30 minutes to discourage fermentation, while the ethanol preparation was allowed to stand for 1hour before extraction process was commenced Rotary evaporator machine was used to concentrate the extract at the rate of 102.3 rps at 60°C, for 60 seconds to recover the ethanol before drying in Gallekamp hot air oven for 91 hours. The mass of extract was determined with the aid of Gallekamp electric weighing balance, expressed as percentage yield.

2.2 Collection and Confirmation of Test Organisms

The test organisms: *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus* sp., *Bacillus* sp., *Escherichia coli, Klebsiella* sp., and *Candida albicans* were obtained from the University of Port Harcourt teaching hospital, Nigeria. The isolates were passed through standard microbiological procedures to confirm their identities.

2.3 Preparation of Whatmann No. 1 Disc

Whatmann No. 1 filter paper was cut into a disc with the aid of a perforator, to a diameter of approximately 6 mm. The discs were then placed in a glass Petri-dish and wrapped in a foil to avoid moisture penetrating to soak the disc. They were then sterilized in a hot air oven at 121°C for 15 minutes after which they were allowed to cool to room temperature and kept until required [4].

2.4 McFarland Standardization of the Test Inocula

A 0.6 ml volume of 1% Barium Chloride solution ($Bacl_2.\ 2H_2O$) was added to 33.4 ml of 1% Sulphuric acid solution (H_2SO_4) and was mixed thoroughly. The preparation was then dispensed into sterile and clear bijou bottle, tightly sealed and placed in a dark at room temperature until when required. The media preparation was based on the manufacture's guidelines.

2.5 Antimicrobial Sensitivity Testing

Pure cultures of the isolates were prepared, and turbidity was adjusted to 0.5 McFarland standards to maintain an even distribution on inoculation. A10 ml volume of sterile nutrient agar was poured into sterile petri dishes and seeded with a 0.1 ml volume of the various inoculums and allowed to set. Sterile Whatmann number 1 filter papers of approximately 6 mm diameter were impregnated with 1000 ppm and 600 ppm concentration of the mushroom extracts prepared with distilled water and gently placed on the surface of the seeded agar. The plates were allowed to stand for about fifteen minutes and then incubated at 37°C for 24 hours [4].

Table 1. Percentage yield of extract from Sclerotia of *P. tuberregium*

Mushroom	Method of extraction	Colour of extract	Sample (g)	Weight of Extract (g)	% Yield of Extract
Sclerotium	Cold water	Creamy	500.50	0.15	7.31
	Hot water	Light brown	500.50	0.18	9.06
	Ethanol	Creamy	500.50	0.10	4.98

Table 2. Antimicrobial activity of the Sclerotia of *P. tuberregium* by disc diffusion method

Bacterial and fungal Pathogen	Ethanol extract	Cold water extract	Hot water extract	Control Sterile medium
Bacillus sp.	15 mm±0.4	NA	NA	NG
E. coli	NA	NA	NA	NG
Klebsiella sp.	21 mm±0.2	NA	NA	NG
Pseudomonas aeruginosa	NA	NA	NA	NG
Staphylococcus aureus	13 mm±0.2	NA	NA	NG
Streptococcus sp.	NA	NA	NA	NG
Candida albicans	7 mm±0.1	NA	NA	NG

NG= No growth; NA= Not active

3. RESULTS AND DISCUSSION

Result for percentage yield of extract from sclerotia of *P. tuberregium* using different methods of extraction is as presented in Table 1. Table 2 shows results for an antimicrobial sensitivity of the test bacterial and fungus to crude extracts of sclerotia of *P. tuberregium*.

The aqueous extracts sclerotia of *P. tuberregium* had the highest yield but did not demonstrate activity against tested isolates: *Bacillus* sp, *E. coli, Klebsiella* sp*, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus sp* and *Candida albicans*. This is in line with the study conducted by Patel et al. [5] where he concluded that the antimicrobial activity of mushroom depends on the nature of the solvent of extraction and substrate used in the growing of that fungus. Maness et al. [8] reported bacterial load reduction with cold water extracts, which can be used as a chemo-preventive supplement. The study carried out by Ezeronye et al. [3] corroborate our finding, which the crude cold water extract did not exert any antimicrobial activity on clinical isolates including *E. coli* and *Staphylococcus aureus*.

The results obtained for the ethanol extract of the Sclerotia of *P. tuberregium* showed that among the selected isolates, *Klebsiella* sp. was more susceptible while *Candida albicans* was the least susceptible. Hence, the extract was more potent against the bacterial isolates than the fungal. *E. coli*, *Streptococcus* sp. and *Pseudomonas aeruginosa* were not susceptible. In comparison with the study carried out by Ezeronye et al. [3], the *Staphylococcus aureus* which showed a noticeable level of susceptibility in this study was reported to be resistant, but the result of *E. coli* been resistant corresponds to theirs.

4. CONCLUSION

The sclerotia of *P. tuberregium* is evidently a source of bioactive compounds potent against some clinical isolates. A good utilization of the sclerotia of this indigenous mushroom would make a great addition to pharmaceutical products of natural origin. More work still needs to be done to further refine and identify the specific antimicrobial compound(s) in the extract to harness their full potential.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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