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Medicinal mushrooms with methicillin resistant *Staphylococcus aureus* (MRSA) inhibitory activity

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Abstract
Although significant progress has been made in the last few decades in the field of antimicrobial chemotherapy, indiscriminate antibiotics use and the growing trend of bacterial resistance have made it necessary to search for newer antimicrobial agents. Various mushrooms have been used in the Nigerian traditional medicine to treat several infectious diseases. The aim of this work is to investigate the antimicrobial activity of five medicinal mushroom species *viz. Ganoderma lucidum*, *Lycoperdon umbrinum*, *Trametes versicolor*, *Ganoderma applanatum* and *Tricholoma* spp against seven clinical strains of methicillin-resistant *Staphylococcus aureus* (MRSA). The methanol extracts of the five mushrooms were subjected to agar diffusion and agar dilution assays to determine the antibacterial and minimum inhibitory concentration (MIC), respectively, against seven strains of MRSA. The extracts displayed varying growth inhibitory activity on the clinical strains of MRSA, with the extracts of *Lycoperdon umbrinum* and *Trametes versicolor* eliciting the highest growth inhibitory effects across the MRSA under investigation. In particular, *L. umbrinum* (3.125 mg/mL) had diameter of zone of inhibition (ZOI) ranging from 7 – 13 mm with MIC value as low as 1.47 mg/mL against the MRSA strains, while *T. versicolor* displayed ZOI between 3 mm and 12 mm. Meanwhile, the extract of the three other species displayed little antibacterial activity against the MRSA strains. The antimicrobial activity of *Trametes versicolor* and *Lycoperdon umbrinum* displayed in this study indicate they could be potential source of novel antimicrobial agents and research is needed to identify the bioactive molecules responsible for their biological activity.

Keywords: Mushroom; Methicillin-Resistant *Staphylococcus aureus; Trametes versicolor; Lycoperdon umbrinum*

INTRODUCTION

Mushrooms are group of fleshy, aerial, umbrella shaped, achlorophyllous macro-fungi that derive their nutritional requirements from the metabolism of non-living organic matters, such as putrefying leaves and tree trunks. Mushrooms are extremely abundant and present in several nations of the world. Recent studies revealed that approximately 150, 000 mushroom species are currently found on the earth with only about 10% of them fully characterised and the nearly 5% being investigated for the biological properties [1]. Mushrooms have been used as nutritional foods for several decades in Nigeria mainly because of their pleasant taste, appetizing aroma and nutritional contents. In the Nigerian traditional medicine, mushrooms have been utilized for the treatment of several ailments including gastrointestinal disorder, high blood pressure, viral infections, cancer and diabetes [2-4]. While some believe that

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the medicinal values attributed with mushrooms stem from superstitious beliefs and myths, several researches have supported the ethnomedicinal use of mushrooms. As an illustration, several studies have reported the antineoplastic, antidiabetic, antiviral, antiparasitic and immunomodulatory properties of mushrooms [5]. Mushrooms are vast and yet largely untapped source of drug molecules as they contain several polysaccharide and polysaccharide-protein complexes with remarkable biological properties. Several secondary metabolites derived from mushrooms are currently in various clinical trials for diverse pharmacological investigations. Krestin, a polysaccharide peptide obtained from *Trametes versicolor* inhibited tumor growth in animal studies [6]. Schizophyllan isolated from *Schizophylla commune* displayed potent antitumor activity in several animal models. Other notable secondary metabolites with immunomodulatory, antimicrobial and antitumor properties include lentinane, copsin and befungin, respectively [7].

Infectious diseases are leading cause of mortality worldwide accounting for more than 25% of the global annual mortality [8]. *Staphylococcus aureus* is an important opportunistic pathogen that have been indicated in many diseases including furuncles, abscess, sepsis, pneumonia and toxic shock syndrome. Historically, Penicillin and its structural analogues were the agent of choice for the treatment of *S. aureus* infections. However, the development of resistance by *S. aureus* to multiple antibiotics led to the development of methicillin in 1959 as the agent of choice. Unfortunately, *S. aureus* developed resistance to methicillin leading to the emergence of methicillin resistant *Staphylococcus aureus* (MRSA) in 1961 [9-10]. Although significant progress has been made in the last few decades in the field of antimicrobial chemotherapy, the global microbial spread has been worsened as a result of poor sanitary conditions especially in developing countries, increase in worldwide travel, indiscriminate and excessive use of antibiotics and the emergence of new pathogenic organisms [11-13]. In view of this, it is imperative to search for new anti-infective agents for the prevention and treatment of infectious diseases.

In this study, we evaluated the antimicrobial activities, particularly anti-MRSA property, of five medicinal mushroom species *viz. Ganoderma lucidum, Lycoperdon umbrinum, Trametes versicolor, Ganoderma applanatum* and *Tricholoma* spp collected from Nigeria.

EXPERIMENTAL

Mushroom collection. The fruiting bodies of five mushroom species including *Ganoderma lucidum, Lycoperdon umbrinum, Trametes versicolor, Ganoderma applanatum* and *Tricholoma* spp were collected from the botanical and zoological gardens of the University of Ibadan, Nigeria. The mushrooms were identified at the Department of Botany, University of Ibadan, by comparison of their spore prints, morphological, anatomical and physiological features with standards as described previously.

Preparation and extraction of mushroom. The fresh fruiting bodies of each mushroom were cut into bits, air-dried at room temperature (25-29 °C) and pulverised. 50 g of each powdered sample was macerated in 95% methanol (500 mL) at room temperature for 72 h. At the expiration of the extraction procedure, the filterate obtained using a Whatman filter paper no 1 was concentrated *in vacuo* at 40 °C and the dried crude extract was stored at 4°C prior to antimicrobial investigation.

Bacterial strains. The test organisms used in this study were seven clinical isolates of MRSA obtained from the Department of

Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan. The phenotypic identification of the isolates had been carried out previously using resistance to cefoxitin [14]. The bacterial strains were cultured and maintained on nutrient agar slants.

Agar diffusion assay. The antimicrobial activities of each of the mushroom extract were evaluated using the agar well diffusion methods as described previously by Stoke and Ridgway [15]. Pure culture colony of each bacterial strain was transferred using a sterile inoculating loop into a nutrient broth and cultured at 37 °C for 24 h. Thereafter, the MRSA cell suspensions adjusted to 0.5 McFarland standards (10^4 CFU/mL) were prepared and 100 µL of the prepared bacterial culture was seeded into 15 mL of molten and cooled Muller Hinton agar (MHA) (Oxoid, UK), mixed thoroughly, and poured into sterile Petri dishes before allowing the agar to solidify. A sterilized cork-borer of 6 mm in diameter was used to bore wells equidistance from each other on the solidified agar. The mushroom extracts were serially diluted to obtain working concentrations ranging from 200 – 3.125 mg/mL. 100 µL of the different working concentrations of the mushroom extract was placed in each of the wells of the solidified inoculated agar and allowed to diffuse for 2 h. Thereafter, the plates were incubated at 37 C for 24 h after which the resulting zones of inhibition were measured in millimetres. The negative control for this experiment utilized sterile distilled water, while disc, containing ciprofloxacin (50 µg) was used as the positive control for all bacterial strains. The experiment was conducted on three different occasions and the antimicrobial activity was expressed as the mean zone of inhibition produced by the mushroom extracts.

Determination of the minimum inhibitory concentration (MIC). The MIC was determined using the agar dilution method according to a previously described method [16]. Briefly, serial dilutions of the mushroom extracts were made to obtain working concentration between 200 and 3.125 mg/mL. Two mL of the extract from each working concentration was mixed with 18 mL of molten agar and poured into sterile petri dishes to allow the agar to set. Thereafter, the surface of the agar was allowed to dry before streaking with overnight culture of susceptible organisms (10^8 CFU/mL). The plates were incubated in inverted position for 24 h at 37 °C and examined for the presence or absence of growth. The lowest concentration preventing visible growth of the organisms was taken as the MIC of the extracts.

RESULTS AND DISCUSSION

Although the discovery and development of antibiotics for the treatment of several infectious diseases as been considered one of the most important scientific achievements of the past seven decades, the growing trend of *Staphylococcus aureus* resistance to the available antimicrobial agents is worrisome. In addition, the inadequate availability of effective anti-infectives against new emerging bacterial species has limited the success story associated with antimicrobial chemotherapy. It was reported that the antimicrobial activities of mushroom extracts are attributable to polysaccharides, proteins, peptides and several secondary metabolites including sesquiterpenes, sterols and anthraquinones [17]. In this study, the anti-MRSA activity of five mushroom (basidiomycetes) extracts used in the Nigerian traditional medical practice was evaluated. The result of the analysis of the antimicrobial activity according to the agar diffusion method is presented in Table 1. The extracts displayed varying growth inhibitory activity on the clinical strains of MRSA, with the extracts of *Lycoperdon umbrinum* and *Trametes versicolor* eliciting the highest inhibitory effects across the MRSA under

investigation. In particular, *L. umbrinum* had the most profound effect at the least tested concentration (3.125 mg/mL) on *S. aureus* C and *S. aureus* E with diameter of zone of inhibition (ZOI) as 13 mm and 7 mm, respectively. *T. versicolor* exerted the highest growth inhibitory effect against *S. aureus* A and *S. aureus* B with ZOI of 7 mm and 5 mm, respectively. Meanwhile, the extract of *L. umbrinum* and *T. versicolor* displayed similar inhibitory activity against *S. aureus* D and *S. aureus* F with ZOI values of 11 mm and 2 mm, respectively. All the MRSA isolates were sensitive to *Ganoderma lucidum* at the highest tested concentration (200 mg/mL), while only isolates B, D and F were sensitive to the extract of *Ganoderma lucidum.* Based on the ZOI results of the investigated mushroom, the minimum inhibitory concentration (MIC) of the extracts of *L. umbrinum* and *T. versicolor* was determined using the agar dilution method. From the MIC evaluation, *L. umbrinum* displayed the lowest MIC value against isolate D with a value of 1.47 mg/mL, while it exerted the highest MIC value against isolates A and B with MIC value of 5 mg/mL. However, both *S. aureus* D and *S. aureus* E were sensitive to the extract of *T. versicolor* with MIC values of 3.17 mg/mL and 4.25 mg/mL, respectively (Table 2).

Trametes versicolor (Syn: *Coriolus* versicolor; *Polyporus versicolor*) is a mushroom that grows on dead trunk of trees and has the top surface of its cap in concentric zones of different colours [18]. Although previous studies reported that the extract of *T. versicolor* displayed no antibacterial activity against *S. aureus, E. faecalis* and *E. coli* with a moderate antimicrobial activity against *P. aeruginosa* [19], the discovery of coriolin antibiotics in other species of *Trametes* that elicited potent antibacterial activity against various bacterial strains indicate that this genus may possess other useful bioactive molecules with promising antimicrobial potentials [20]. Meanwhile, protein bound polysaccharide (PBP) isolated from the mycelium of *T. versicolor* elicited *in vitro* activities against several human cancer cell lines as well as displayed significant antitumor activity in *in vivo* studies [6,21]. Human pilot studies reported that PBP obtained from *T.* versicolor may reduce cancer reoccurrence as well as be beneficial as an adjuvant in the treatment of several human tumors including gastric, colorectal, breast and lung cancers [22].

Lycoperdon umbrinum, commonly referred to as umber-brown puffball is indigenous to China, Europe and Africa. Like other member of the *Lycoperdon* genus, it lacks an open cap with spore-bearing gills but possess spores produced internally in a spheroidal fruiting body. The mushroom had been used by traditional healers in south-western Nigeria for various traditional remedies including wound dressing, diabetes, diarrhoea and inflammatory diseases [23]. Previous phytochemical investigation revealed that *L. umbrinum* is rich in flavonoids and phenolic compounds [24]. There is dearth of scientific information available on the biological properties of *L. umbrinum.* A recent study revealed that the *L. umbrinum* extract (12.5 mg/mL) exerted potent antimicrobial activity towards several gram-positive and gram-negative bacteria including *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus* with ZOI values ranging from 13 mm – 18 mm as well as displayed significant antifungal activity against *Alternaria alternate, Alternaria tomentosa* and *Colletotrichum dematium* [25].

The anti-MRSA activity of *T. versicolor* and *L. umbrinum* documented in this work is the first to be reported in literature. Further studies are needed to identify compounds responsible for their antimicrobial effects.

Table 1. Anti-Methicillin Resistant *Staphylococcus aureus* activity of five mushroom extracts

Extract	Concn.	Mean diameter zone of inhibition ± S.D of clinical isolates (mm)						
	(mg/mL)	A	B	C	D	E	F	G
Ganoderma	200	10 ± 0.00	15 ± 0.00	23 ± 1.86	10 ± 0.00	12 ± 0.54	13 ± 1.86	9 ± 0.00
lucidum	100	8 ± 0.54	12 ± 0.54	15 ± 0.54	14 ± 1.86	10 ± 0.54	9 ± 0.54	5 ± 0.54
	50	-	11 ± 0.00	12 ± 1.86	10 ± 0.54	7 ± 0.00	8 ± 0.00	-
	25	-	7 ± 0.54	12 ± 0.54	9 ± 1.86	-	7 ± 0.00	-
	12.5	-	7 ± 0.00	10 ± 0.00	10 ± 0.00	-	5 ± 0.54	-
	6.25	-	-	10 ± 0.00	9 ± 0.00	-	13 ± 0.00	-
	3.125	-	-	8 ± 0.00	8 ± 0.54	-	10 ± 0.54	-
Ganoderma	200	-	14 ± 0.54	-	25 ± 0.54	-	26 ± 1.86	5 ± 0.00
applanatum	100	-	12 ± 1.86	-	22 ± 0.54	-	22 ± 0.00	-
	50	-	11 ± 0.54	-	15 ± 0.54	-	20 ± 1.86	-
	25	-	9 ± 1.86	-	13 ± 0.00	-	18 ± 0.00	-
	12.5	-	7 ± 1.86	-	11 ± 0.00	-	15 ± 0.00	-
	6.25	-	-	-	9 ± 0.00	-	13 ± 1.86	-
	3.125	-	-	-	9 ± 0.00	-	10 ± 0.00	-
Lycoperdon	200	10 ± 0.00	14 ± 0.00	22 ± 1.86	24 ± 0.00	20 ± 0.54	15 ± 1.86	6 ± 0.00
umbrinum	100	7 ± 0.00	12 ± 1.86	20 ± 0.54	21 ± 1.86	18 ± 0.00	13 ± 0.54	-
	50	5 ± 0.00	11 ± 0.54	18 ± 0.54	20 ± 0.00	15 ± 0.00	11 ± 1.86	-
	25	5 ± 0.00	10 ± 0.00	21 ± 1.86	18 ± 0.00	13 ± 0.00	9 ± 0.54	-
	12.5	3 ± 0.00	9 ± 0.54	20 ± 0.00	15 ± 1.86	11 ± 0.54	7 ± 0.00	-
	6.25	3 ± 1.86	6 ± 1.86	15 ± 0.00	13 ± 0.54	9 ± 0.54	5 ± 0.00	-
	3.125	-	3 ± 0.00	13 ± 1.86	11 ± 0.54	7 ± 0.00	2 ± 0.00	-
Trametes	200	21 ± 1.86	10 ± 0.00	28 ± 0.00	26 ± 0.00	12 ± 0.00	15 ± 0.54	7 ± 0.00
versicolor	100	18 ± 1.86	10 ± 1.86	24 ± 0.00	25 ± 0.54	10 ± 0.54	13 ± 1.86	5 ± 0.54
	50	16 ± 0.54	9 ± 0.00	22 ± 0.54	23 ± 0.54	10 ± 0.54	11 ± 0.00	3 ± 0.54
	25	14 ± 0.00	10 ± 0.00	21 ± 0.54	21 ± 0.00	9 ± 1.86	9 ± 0.00	-
	12.5	12 ± 0.00	8 ± 1.86	18 ± 0.00	20 ± 0.54	7 ± 0.00	7 ± 1.86	-
	6.25	11 ± 1.86	7 ± 0.54	15 ± 0.00	15 ± 0.00	5 ± 0.00	5 ± 1.86	-
	3.125	7 ± 1.86	5 ± 0.00	12 ± 0.00	11 ± 1.86	3 ± 1.86	2 ± 0.00	-
Tricholoma	200	-	-	6 ± 0.00	20 ± 0.54	15 ± 1.86	18 ± 0.54	-
spp	100	-	-	-	18 ± 0.54	12 ± 0.54	15 ± 0.54	-
	50	-	-	-	15 ± 0.00	10 ± 1.86	12 ± 1.86	-
	25	-	-	-	13 ± 0.00	9 ± 0.54	11 ± 1.86	-
	12.5	-	-	-	10 ± 0.54	7 ± 0.00	9 ± 0.00	-
	6.25	-	-	-	9 ± 0.00	5 ± 0.54	7 ± 0.00	-
	3.125	-	-	-	5 ± 1.86	-	5 ± 0.54	-
Ciprofloxacin	50 µg/mL	27 ± 0.00	29 ± 0.00	26 ± 0.00	29 ± 0.00	26 ± 0.00	25 ± 0.00	27 ± 0.00

- = not active

Table 2: Minimum inhibitory concentration (MIC) values of mushroom methanol extract on test organisms

Extract	Minimum inhibitory concentration (mg/mL)						
	A	B	C	D	E	F	G
Lycoperdon umbrinum	5.00	5.00	2.54	1.47	1.72	1.50	ND
Trametes versicolor	ND	ND	ND	3.17	4.25	ND	ND

ND = not determined

Conclusion. To summarize our findings, we report on the antimicrobial activity of five mushroom extracts, used traditionally in the treatment of infectious diseases, against seven clinical strains of methicillin-resistant *Staphylococcus aureus* (MRSA). Amongst the investigated mushrooms, *Trametes versicolor* and *Lycoperdon umbrinum* displayed the best antibacterial activity against the pathogenic organisms and work in ongoing in our

laboratory to isolate and identify the bioactive molecules responsible for their biological activity.

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