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Studies on Phytochemical Constituents and Bio-pesticide Attributes of Methanol Extract of *Ganoderma lucidum* (Curtis) on Selected Pathogenic Fungi and Bacteria in Humid Tropical Environment


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1 ABSTRACT

Ganoderma lucidum **(Reishi) is a medicinal mushroom used for the treatment of various health problems because of its abundant bioactive compounds, which have pharmacological effects. This underscores why the efficacy of methanol extract of *G. lucidum* against selected fungal and bacterial pathogens was evaluated to ascertain if the extract can be used as an antimicrobial agent/bio-pesticide to handle some fungal and bacterial pathogens especially in cases of frequent drug/pesticide resistance among pathogenic microorganisms, emergence of new infectious diseases, high cost of synthetic drugs/pesticides and their adverse side effects often encountered in both plant and animal pathology. Thus, this research was carried out to examine the phytochemical constituents and *in-vitro* bio-pesticide/antimicrobial activities of the methanol extract of *G. lucidum* on selected pathogenic fungi and bacteria. The phytochemical contents were determined quantitatively using standard spectrophotometric analytical procedures while the antimicrobial actions were determined using Agar Cup Diffusion Technique. The Minimum Inhibitory Concentrations (MICs) of the extract were determined on various test organisms. *G. lucidum* extract contains varying levels of alkaloids (8.0%), flavonoids (82.0%), phenol (22.7%), cyanogenic glycoside (0.325%) and tannins (0.012%). *G. lucidum* extract had high inhibition zone diameters on *Salmonella typhi* (1-5.6mm), *Streptococcus pneumoniae* (2-6mm), *Staphylococcus aureus* (3-7mm), *Pseudomonas aeruginosa* (6-15mm), *Aspergillus flavus* (0.2-0.45mm), *Penicillium notatum* (2-7mm), *Fusarium oxysporum* (2.5-6mm) and *Candida albicans* (0.5-10mm). Although the MICs of the extract were higher than the standard drugs tested because of its crude nature, the extract showed both antibacterial and antifungal activities. This showed that methanol extract of *G. lucidum* could be used to produce broad-spectrum biopesticides/antimicrobial agents when properly harnessed.**

2 INTRODUCTION

The use of plant medicinal product has a long history in the treatment of both plant and animal diseases. In the past few decades, numerous reports on the efficacy of plant extracts especially as regards their antimicrobial properties have been documented (Eze and Ogonnaya, 2010). Davicins *et al.* (2007) reported that all parts of medicinal plants such as the leaf, flower and root could possess inhibitory properties against bacteria, fungi and insects. Quinine and penicillin drugs are good examples of medicinal products from plants that have been used successfully in the treatment of human and animal infections. Besides, Eze *et al.* (2009) observed that plant extracts have broad spectrum of activity against both bacterial and fungal pathogens and may lower risk of resistance. Thus, there is a need to investigate plants and other forest products with abundant curative phytochemical constituents. This is because both timber and non-timber forest products continued to provide superb substances from their metabolic activities for the treatment of plants and animal diseases caused by pathogens. Mushrooms are good examples of non-timber forest products with plentiful therapeutic phytochemicals that are often found as saprophytes in many places such as soil, open fields, farmlands, woods and roadsides (Stanley *et al.*, 2011). They possess compounds of medicinal value such as terpenoids, steroids, phenol, nucleotides, glycoprotein (amino acid, lysine and leucine) and polysaccharides (Sandodiya *et al.*, 2009). These compounds have antimicrobial effects and can help to strengthen the immune system with minimum side effect (Tsao, 2010). *Ganoderma lucidum* is a type of mushroom from the genus polypores in the family of Ganodermataceae that possess scores of desirable phytochemicals of medicinal importance (Eze and Chinekwu, 2022). It is one of the species of *Ganoderma* that its medicinal properties has been discovered and used in China for thousands of years ago (Wachtel-Galor *et al.,* 2004). It belongs to the family of Ganodermataceae and is commonly called *Reishi* by Chinese because of its inherent safe nature (Kirk *et al.*, 2008). *G. lucidum* like many other mushrooms grow wide on dead decaying log of woods and can be cultivated artificially using substrates like sawdust, grains and wood log (Boh *et al*, 2007). It is mainly identified by the colour, spore size and shape, chlamydospore production and enzymatic constituents (Mandalvo, 2000). Wang *et al.* (2020) reported *G. lucidum* to have specified pharmacological effects such as analgesic, hepato-protective role, antioxidant activity, immune-stimulation, anti-tumor and stable inhibitory activity against HIV-1 protease (Wang *et al.*, 2020). For instance, Aviva *et al.* (2010) observed that *G. lucidum* is one of the primary herbs of choice in immune deficiency disease because it possesses a broad spectrum of immuno-stimulating activities. Despite the numerous medicinal potential of *G. lucidum,* the knowledge of its bio-pesticide/antimicrobial activities is still scarce. This work was therefore carried out to study the bio-pesticide/antimicrobial properties of methanol extract of *G. lucidum* against some selected pathogenic fungi and bacteria.

3 MATERIALS AND METHODS

The conduct of this Experiment started from the Department of Crop Science Research Field, Faculty of Agriculture, University of Nigeria, Nsukka and ended in the Department of Plant Science and Biotechnology Laboratories, Faculty of Biological Science, University of Nigeria, Nsukka. Nsukka is located in the derived savannah ecological zone on the latitude 06° 52' North, longitude 07° East and altitude 447.26 m above sea level.

3.1 Materials: All the reagents used for the study were of Analar grade. Methanol, ethanol, dimethyl sulphoxide (DMSO), *G. lucidum* (see Fig 9), Sabouraud Dextrose agar (SDA) and Sabouraud Dextrose broth, autoclave, cotton wool, laminar flow chamber, oven (Gallenkamp,

England) and potato dextrose agar (PDA) were used for the study.

3.2 Sources of Materials: The *G. lucidum* mushroom used for this study was obtained from logs of wood in the Department of Crop Science Pathology/Biotechnology Laboratory, The *G. lucidum* was identified in the Department of Plant Science and Biotechnology Laboratories, The test microorganisms were obtained from the Department of Crop Science Pathology Laboratory Unit, all in the Faculty of Agriculture, University of Nigeria, Nsukka.

3.3 Method of Extraction: The extraction of the *G. lucidum* mushroom was carried out as described by Eze *et al.* (2010). Sixty-three grams of the homogenized *G. lucidum* mushrooms were weighed out using Mettler sensitive balance and poured into 500 ml flat bottom flask andthese were soaked in 200 ml of absolute methanol to get 300.2 mg/ml. This was stirred with magnetic stirrer for18 hours and left to stand for 24 hours before it was filtered using a clean muslin cloth and concentrated at 60°C in an oven (Gallenkamp, England).

3.4 Susceptibility of the Pathogens Used: The susceptibility of the pathogens to both the *G. lucidum* extract and standard drugs was carried out using agar cup diffusion technique as described by (Agboke *et al.*, 2005).

3.5 Determination of Inhibition Zone Diameter (IZD) of *G. lucidum* Extract on the test

3.5.1 Pathogens: Sabourand dextrose agar (SDA) and nutrient agar used for fungal and bacterial pathogens, respectively were prepared, sterilized and allowed to cool to 45^{0}C. About 0.5 ml of the suspension of the test pathogen was pipetted into a sterile Petri dish and then placed on a sterile chamber. Twenty millilitre of the prepared SDA and nutrient agar were poured into different plates for fungal and bacterial pathogens, respectively and swirled three times in clockwise and in anticlockwise directions to ensure an even distribution of the test organism. It was then allowed to set or gel. Four millilitre of the *G. lucidum* extract was dissolved in 2.5 ml of DMSO and 2-fold serial dilution of the dissolved extract was done to get a concentration of the extract in the range of 31.3, 62.5, 125 and 250 mg/ml and introduce into three other sterile test tubes labelled 1, 2, 3 and 4, respectively. These different concentrations were prepared for the sensitivity testing. The agar plate was divided into four sections using a marker and labelled 1, 2, 3, and 4 representing the different concentrations (31.3, 62.5, 125 and 250 mg/ml) got from the serial dilution above. Using a cork borer of diameter 8 mm, cups were made at the centre of each of the four sections. Then, 0.05ml each of the dilution of *G. lucidum* extract was aseptically introduced into the cups starting from the lowest concentration to the highest. The plate was labelled and incubated at 35 °C for 24 hours and the zones of inhibition (IZD) were measured using a metre rule. The result was tabulated and a graph of IZD square against the logarithm of concentration was plotted. The MIC was determined from the graph**.** This was repeated for the two standard drugs, nistatin and ciprofloxacin used for fungal and bacterial pathogens, respectively and their MICs determined.

4 RESULTS

The study revealed a high level of flavoniods in *G. lucidum* extract (Table 1). This was followed by total phenols and alkaloids whose values were 22.7% and 8.0%, respectively. The phytochemical contents of the *G. lucidum* extract showed that tannin was 0.012% while cyanide was 0.325 %. The inhibition zone diameter (IZD) of both the extract and all the control drugs increased with increased concentrations of the agents in fungal pathogens (Table 2 - 9). The values for the inhibition zone diameter of the test organisms increased as follows; *Aspergillus flavus* (0.45mm-0.00mm), *Candida albicans* (10.00mm- 0.50mm), *Penicillum notatum* (7.00mm-2.00mm), *Fusarium oxysporium* (6.00mm-2.50mm), *Staphylococcus aureus* (7.00mm-3.00mm), *Streptococus pneumonia* (6.00mm-0.00mm*), Pseudomonas aeruginosa* (15.00-6.00) and *Salmonella typhi* (5.60mm-1.00mm). In Table 2, the concentration of the extract (31.3

mg/ml), failed to produce any zone of inhibition on *Aspergillus flavus* and thus, allowed the mycelial growth. The inhibition zone diameter (IZD) of both the extract and control drugs also increased with increased concentrations of the agents in bacterial pathogens (Table 10 - 17). Thus, the values of the inhibition zone diameter of the test bacterial isolates increased as follows; *Salmonella typhi* (5.60mm-1.00mm), *Streptococus pneumonia* (6.00mm-0.00mm), *Staphylococcus aureus* (7.00mm-3.00mm) and *Pseudomonas aeruginosa* (15.00-6.00). In Table 17, the concentration of the ciprofloxacin ((15.63mg/ml), failed to produce any zone of inhibition on against *Pseudomonas aeruginosa* and *Salmonella typhi.* The minimum inhibition concentration (MIC) values of the extract were significantly higher than that of the control drugs in the fungal pathogens tested (Table 17 and 18). There was increase in the minimum inhibition concentration (MIC) value of the extract on *Staphylococcus aureus* and *Streptococcus pneumonia* compared with that of the control drug. It was observed that the MIC value (19.95mg/ml) of *Pseudomonas aeruginosa* were the same for both the extract and control drug. The MIC value of the extract decreased significantly compared with that of the control drug in *Salmonella typhi* (Table 19 and 20). There was increase in the minimum inhibition concentration (MIC) value of the extract on *Staphylococcus aureus* and *Streptococcus pneumonia* compared with that of the control drug. It was observed that the MIC value of *Pseudomonas aeruginosa* were the same for both the extract and control drug. The MIC value of the extract decreased significantly compared with that of the control drug in *Salmonella typhi* (Table 19 and 20).

Table 1: Concentration of phytochemical constituents in *G. lucidum* extract

Phytochemical	%
Alkaloids	8.00
Flavonoids	82.0
Tannins	0.012
Cyanide	0.325
Total phenols	22.7

Values are mean of three replicates analysis

Table 2: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the *G. lucidum* against *Aspergillus flavus*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	0.45	0.20	250	2.398
2	0.40	0.16	125	2.097
3	0.20	0.04	62.5	1.796
4	-	-	31.3	1.495

Values are mean of three replicates analysis

Table 3: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc.) and log concentration (log conc) of the *G. lucidum* against *Candida albicans*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	10.00	100.00	250	2.398
2	8.10	65.60	125	2.097
3	4.60	21.20	62.5	1.796
4	0.50	0.30	31.3	1.495

Values are mean of three replicates analysis

Table 4: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the *G. lucidum* against *Penicillum notatum*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	7.0	49.0	250	2.398
2	5.0	25.0	125	2.097
3	4.0	16.0	62.5	1.796
4	2.0	4.0	31.3	1.495

Values are mean of three replicates analysis

Table 5: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the *G. lucidum* against *Fusarium oxysporum*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	6.00	36.00	250	2.398
2	5.29	28.00	125	2.097
3	4.00	16.00	62.5	1.796
4	2.50	6.25	31.3	1.495

Values are mean of three replicates analysis

Table 6: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the control drug (nistatin) against *Aspergillus flavus*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	13.00	169.00	125.00	2.097
2	12.00	144.00	62.50	1.796
3	10.95	120.00	31.25	1.495
4	10.00	100.00	15.63	1.194

Values are mean of three replicates analysis

Table 7: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the control drug (nistatin) against *Candida albicans*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	16.00	256.00	125.00	2.097
2	13.46	181.25	62.50	1.796
3	11.45	131.25	31.25	1.495
4	11.00	121.00	15.63	1.194

Values are mean of three replicates analysis

Table 8: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the control drug (nistatin) against *Penicillum notatum*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	13.00	169.00	125.00	2.097
2	12.00	144.00	62.50	1.796
3	11.14	124.00	31.25	1.495
4	10.00	100.00	15.63	1.194

Values are mean of three replicates analysis

Table 9: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the control drug (nistatin) against *Fusarium oxysporum*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	15.00	225.00	125.00	2.097
2	12.00	144.00	62.50	1.796
3	10.00	100.00	31.25	1.495
4	7.07	50.00	15.63	1.194

Values are mean of three replicates analysis

Table 10: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the *G. lucidum* against *Staphylococcus aureus*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	7.00	49.00	250.00	2.398
2	6.16	38.00	125.00	2.097
3	5.00	25.00	62.50	1.796
4	3.00	9.00	31.25	1.495

Values are mean of three replicates analysis

Table 11: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log Conc.) of the *G. lucidum* against *Streptoccocus pneumoniae*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	6.00	36.00	250.00	2.398
2	4.79	23.00	125.00	2.097
3	2.00	4.00	62.50	1.796
4	-	-	31.25	1.495

Values are mean of three replicates analysis

Table 12: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the *G. lucidum* against *Pseudomonas aeruginosa*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	15.00	225.00	250.00	2.398
2	12.00	144.00	125.00	2.097
3	9.00	81.00	62.50	1.796
4	6.00	36.00	31.25	1.495

Values are mean of three replicates analysis

Table 13: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the *G. lucidum* against *Salmonella typhi*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	5.60	31.40	250.00	2.398
2	5.00	25.00	125.00	2.097
3	3.31	11.00	62.50	1.796
4	1.00	1.00	31.25	1.495

Values are mean of three replicates analysis

Table 14: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the control drug (ciprofloxacin) against *Staphylococcus aureus*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	12.50	156.30	125.00	2.097
2	10.00	100.00	62.50	1.796
3	7.50	56.30	31.25	1.495
4	5.00	25.00	15.63	1.194

Values are mean of three replicates analysis

Table 15: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the control drug (ciprofloxacin) against *Streptococcus pneumoniae*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	25.00	625.00	125.00	2.097
2	23.00	529.00	62.50	1.796
3	20.00	400.00	31.25	1.495
4	17.30	300.00	15.63	1.194

Values are mean of three replicates analysis

Table 16: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (Conc) and log concentration (log conc) of the control drug (ciprofloxacin) against *Pseudomonas aeruginosa*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	20.00	400.00	125.00	2.097
2	15.00	225.00	62.50	1.796
3	7.00	49.00	31.25	1.495
4	-	-	15.63	1.194

Values are mean of three replicates analysis

Table 17: Inhibition zone diameter (IZD), Inhibition zone diameter square (IZD^2), concentration (conc) and log concentration (log conc) of the control drug (ciprofloxacin) against *Salmonella typhi*

S/N	IZD (mm)	IZD^2 (mm^2)	Conc (mg/ml)	Log conc
1	6.00	36.00	125.00	2.097
2	4.00	16.00	62.50	1.796
3	2.00	4.00	31.25	1.495
4	-	-	15.63	1.194

Values are mean of three replicates analysis

Table 18: Minimum inhibitory concentration (MIC) of the *G. lucidum* against *Aspergillus flavus, Candida albicans, Penicillum notatum and Fusarium oxysporum*

S/N	Fungi	MIC (mg/ml)
1	*Aspergillus flavus*	15.85mg/ml
2	*Candida albicans*	25.12mg/ml
3	*Penicillum notatum*	19.95mg/ml
4	*Fusarium oxysporium*	17.78mg/ml

Values are mean of three replicates analysis

Table 19: Minimum inhibitory concentration (MIC) of the control drug (nistatin) against *Aspergillus flavus, Candida albicans, Penicillum notatum and Fusarium oxysporum*

S/N	Fungi	MIC (mg/ml)
1	*Aspergillus flavus*	0.63mg/ml
2	*Candida albicans*	3.16mg/ml
3	*Penicillum notatum*	0.63mg/ml
4	*Fusarium oxysporium*	7.20mg/ml

Values are mean of three replicates analysis

Table 20: Minimum inhibitory concentration (MIC) of the *G. lucidum* against *Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi*

S/N	Fungi	MIC (mg/ml)
1	*Staphylococcus aureus*	14.13mg/ml
2	*Streptococcus pneumonia*	39.81mg/ml
3	*Pseudomonas aeruginosa*	19.95mg/ml
4	*Salmonella typhi*	3.16mg/ml

Values are mean of three replicates analysis

Table 20: Minimum inhibitory concentration (MIC) of the control drug (ciprofloxacin) against *Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi*

S/N	Fungi	MIC (mg/ml)
1	*Staphylococcus aureus*	10.00mg/ml
2	*Streptococcus pneumoniae*	1.58mg/ml
3	*Pseudomonas aeruginosa*	19.95mg/ml
4	*Salmonella typhi*	19.95mg/ml

Values are mean of three replicates analysis

Table 17: Minimum inhibitory concentration (MIC) of *G. lucidum* against *Aspergillus flavus, Candida albicans, Penicillum notatum and Fusarium oxysporum*

S/N	Fungi	MIC (mg/ml)
1	*Aspergillus flavus*	15.85mg/ml
2	*Candida albicans*	25.12mg/ml
3	*Penicillum notatum*	19.95mg/ml
4	*Fusarium oxysporium*	17.78mg/ml

Values are mean of three replicates analysis

Table 18: Minimum inhibitory concentration (MIC) of control against *Aspergillus flavus, Candida albicans, Penicillum notatum* and *Fusarium oxysporum*

S/N	Fungi	MIC (mg/ml)
1	*Aspergillus flavus*	0.63mg/ml
2	*Candida albicans*	3.16mg/ml
3	*Penicillum notatum*	0.63mg/ml
4	*Fusarium oxysporium*	7.20mg/ml

Values are mean of three replicates analysis

Table 19: Minimum inhibitory concentration (MIC) of the *G. lucidum* against *Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi*

S/N	Fungi	MIC (mg/ml)
1	*Staphylococcus aureus*	14.13mg/ml
2	*Streptococcus pneumonia*	39.81mg/ml
3	*Pseudomonas aeruginosa*	19.95mg/ml
4	*Salmonella typhi*	3.16mg/ml

Values are mean of three replicates analysis

Table 20: Minimum inhibitory concentration (MIC) of control against *Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa and Salmonella typhi*

S/N	Fungi	MIC (mg/ml)
1	*Staphylococcus aureus*	10.00mg/ml
2	*Streptococcus pneumonia*	1.58mg/ml
3	*Pseudomonas aeruginosa*	19.95mg/ml
4	*Salmonella typhi*	19.95mg/ml

Values are mean of three replicates analysis

4 DISCUSSION

The presence of phytochemicals of medicinal importance such as alkaloids, flavonoids, phenol, glycoside (0.325%) and tannins (0.012%) in the *G. lucidum* extract showed that the mushroom has therapeutic potentials that can be exploited for use in pharmaceutical sectors for drugs and bio-pesticide production. Subbulakshmi and Kannam (2016) observed that mushrooms possess many secondary metabolites known as phytochemicals that have curative properties. For instance, the alkaloid level seen in the *G. lucidum* extract (8.0%) can be successfully used to produce sedative and other medicinal products with analgesic effects applied as pain relieving drugs (Eze and Orjioke, 2010). The high level of flavonoid (82.0%) in the *G. lucidum* extract has a correlation with broad spectrum of antimicrobial activities against plant and animal bacterial, fungal and viral pathogens (Friedman, 2007;Schuier *et al.,* 2005).Cazarolli *et al.,* (2008) also reported flavonoids to have anti-inflammatory, antioxidant and anti-cancer effects. This showed that *G. lucidum* could be used to treat inflammatory diseases and to boost general body defences against toxic substances in the case of immuno-compromised diseases, which other synthetic drugs/pesticides cannot handle. The high level of phenol (22.7%) in the *G. lucidum* extract also supported the fact that the extract has a good antimicrobial property. Agbo*et al.* (2012) reported the presence of phenol in plant to be correlated with high antimicrobial action. Besides, Barros *et al.* (2009) also observed that some phenols found in plants are highly curative in action. Thus, the choice of methanol extract was because both flavonoids and phenols, which were phytochemical of main, interest and were only highly soluble and extractable with the solvent. Eze *et al.* (2010) had reported methanol extract of *Gongronema latifolia* to possess a reliable therapeutic potential against bacterial isolate in clinical setting. The presence of 0.012% tannin in the extract can also be of therapeutic importance. Although tannins may reduce protein absorption by decreasing digestibility, and palatability, they also have good antimicrobial properties (Eze and Orjioke, 2010). The sensitivity of both the fungal and bacterial pathogens to *G. lucidum* extract and the control drugs can be seen from the varying inhibition zone diameters (IZDs) which increased with increased concentrations of the extract and the control drugs. This agrees with the findings of Eze and Eze (2010) on the sensitivity of some fungal pathogens of white yam tubers to *Cassia alata* leaf extract. The

inhibition zone diameter of the extract that increased as the concentration increased resulted in the reduction of the mycelial growth of the fungal pathogens (Fig 1-4) as well as the decrease in the numbers of colonies in bacterial pathogens (Fig 5-8). Besides, the mean effects/values of both the extract and control drugs showed no significant difference since they all decreased with the concentration of the antimicrobial agents used. However, it was observed that the extract was more sensitive to *Candida albicans* and *Pseudomonas aeruginosa* than the rest of the organisms tested. This agrees with the finding of Eze *et al.* (2011) as well as Eze and Ogonnaya (2010) on the effect of *Physcia grisea* on *Candida albicans* and *Pseudomonas aeruginosa,* respectively.

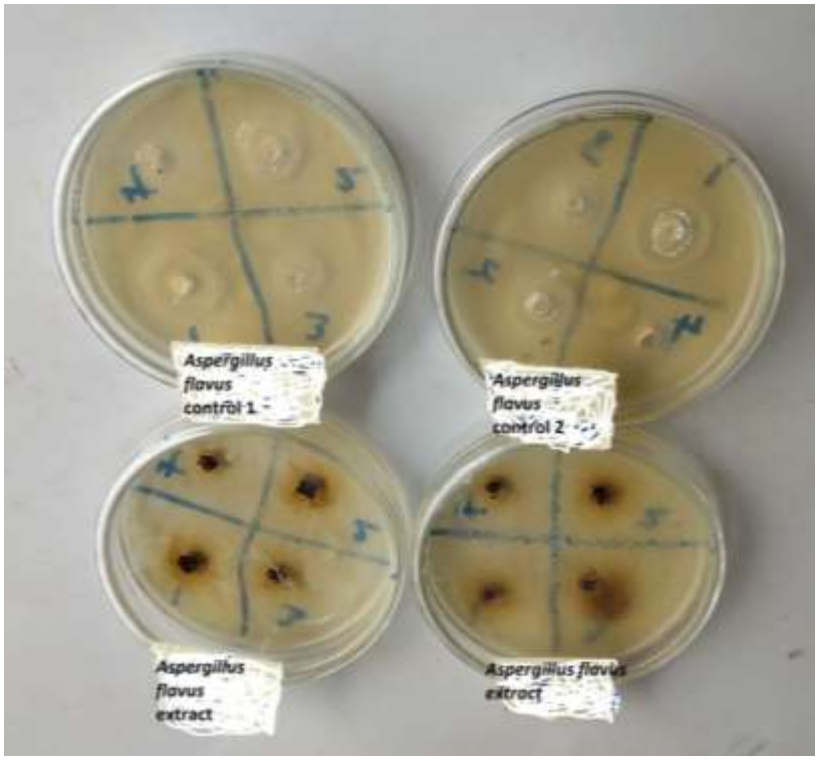

Fig 1: *Aspergillius flavus* extract and control

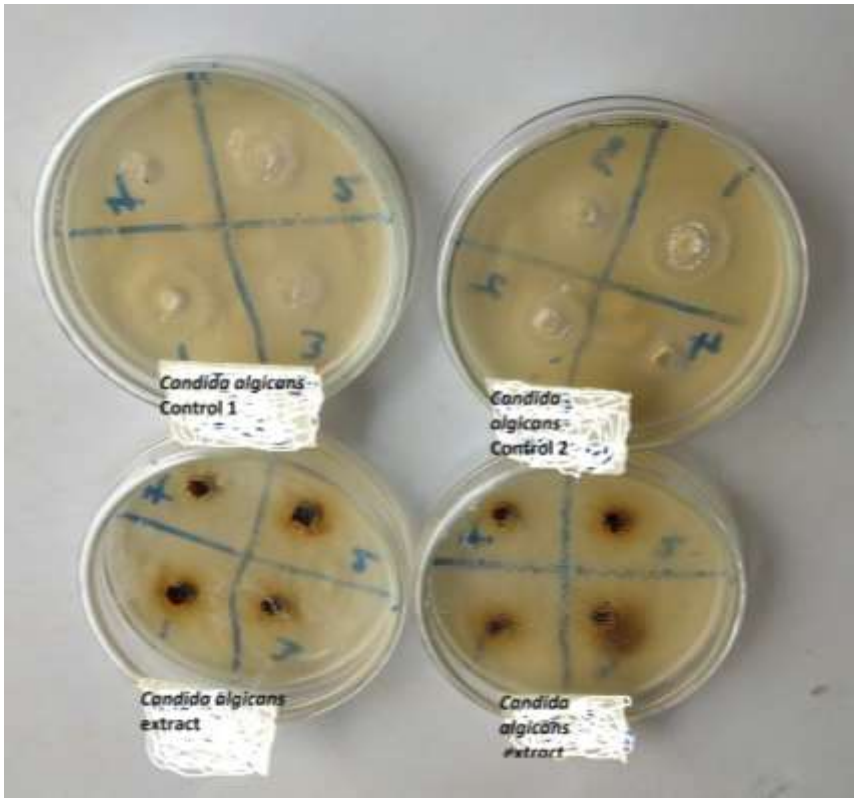

Fig 2: *Candida albicans* extract and control

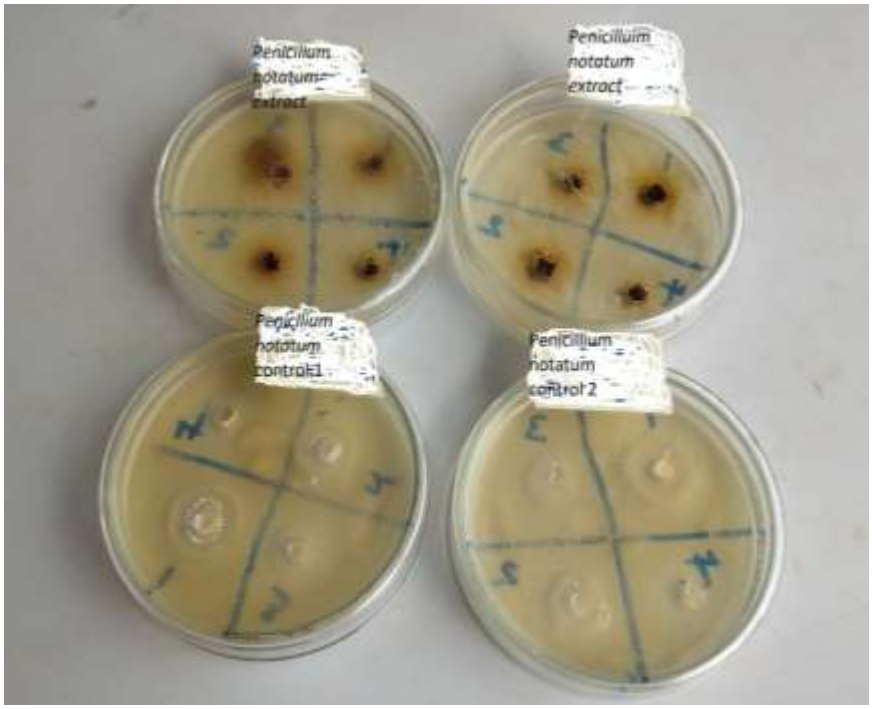

Fig 3: *Penicillium notatum* extract and control

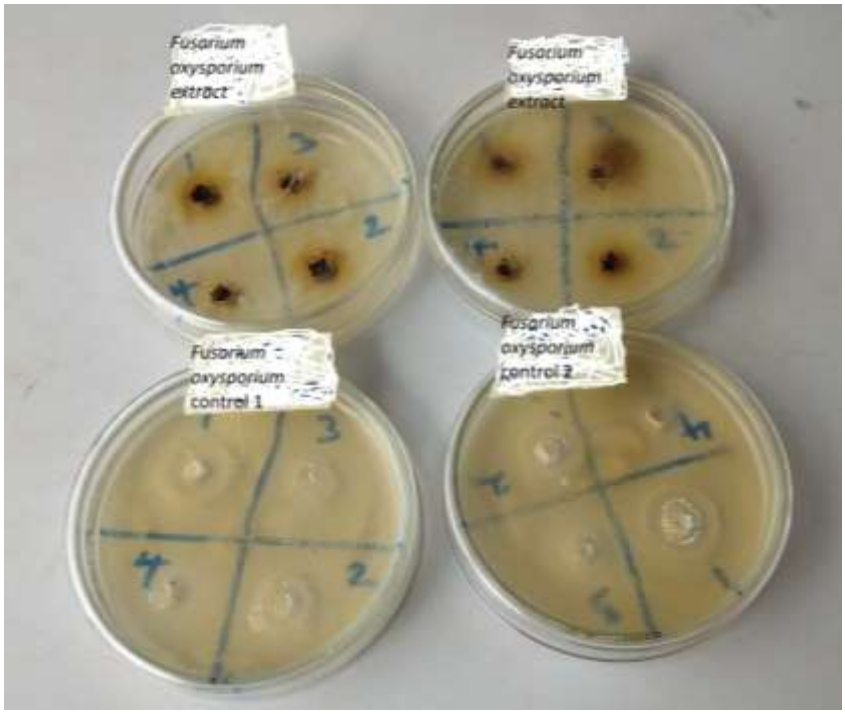

Fig 4: *Fusarium oxysporium* extract and control

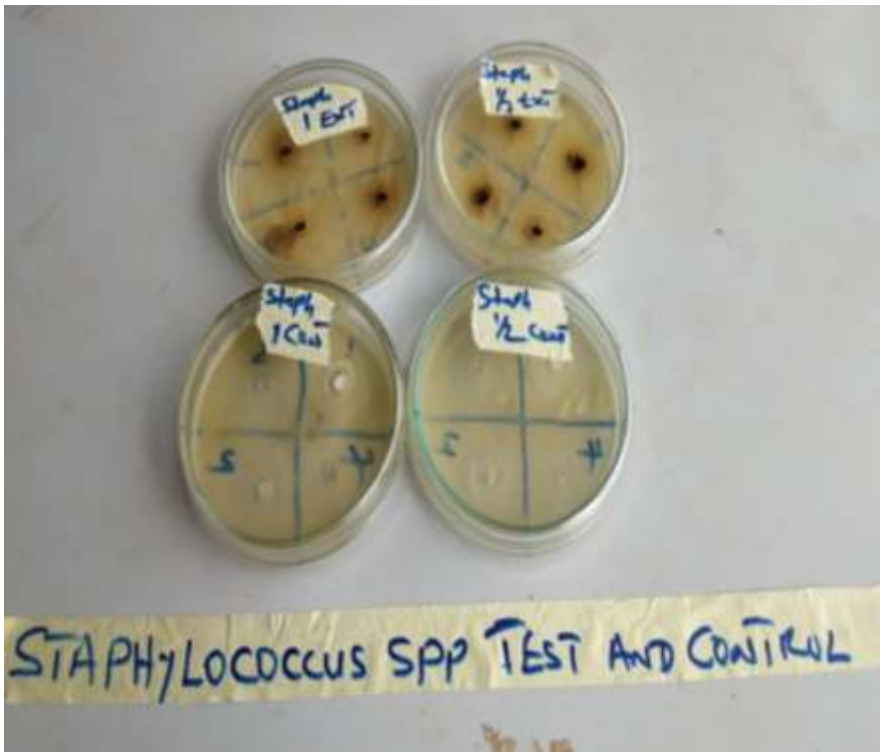

Fig 5: *Staphylococcus aureus* extract and control

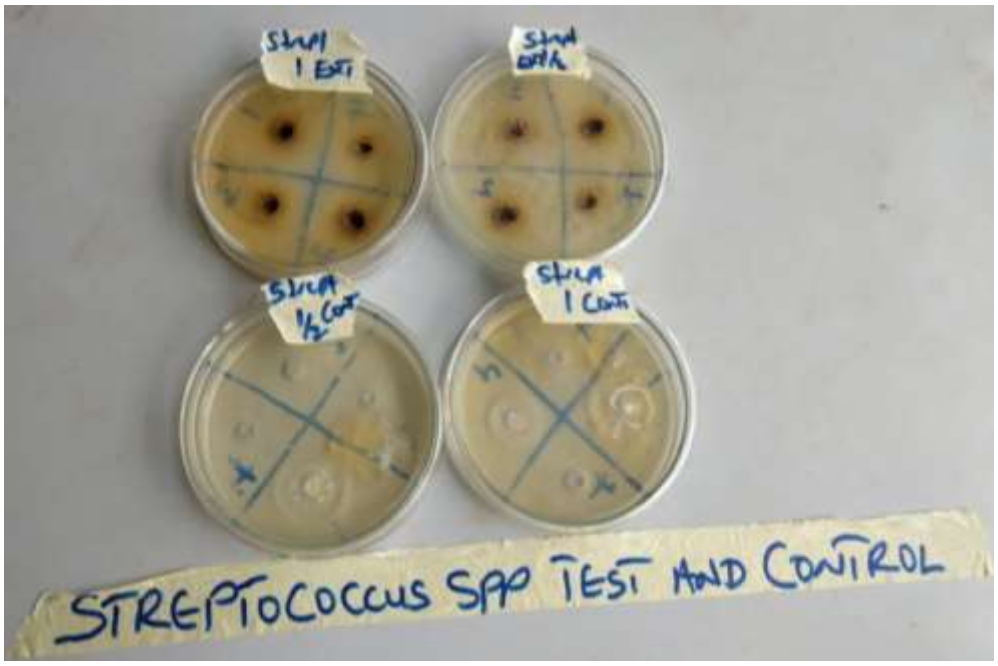

Fig 6: *Streptococcus pneumoneae* extract and control

Fig 7: *Pseudomonas aeruginosa* extract and control

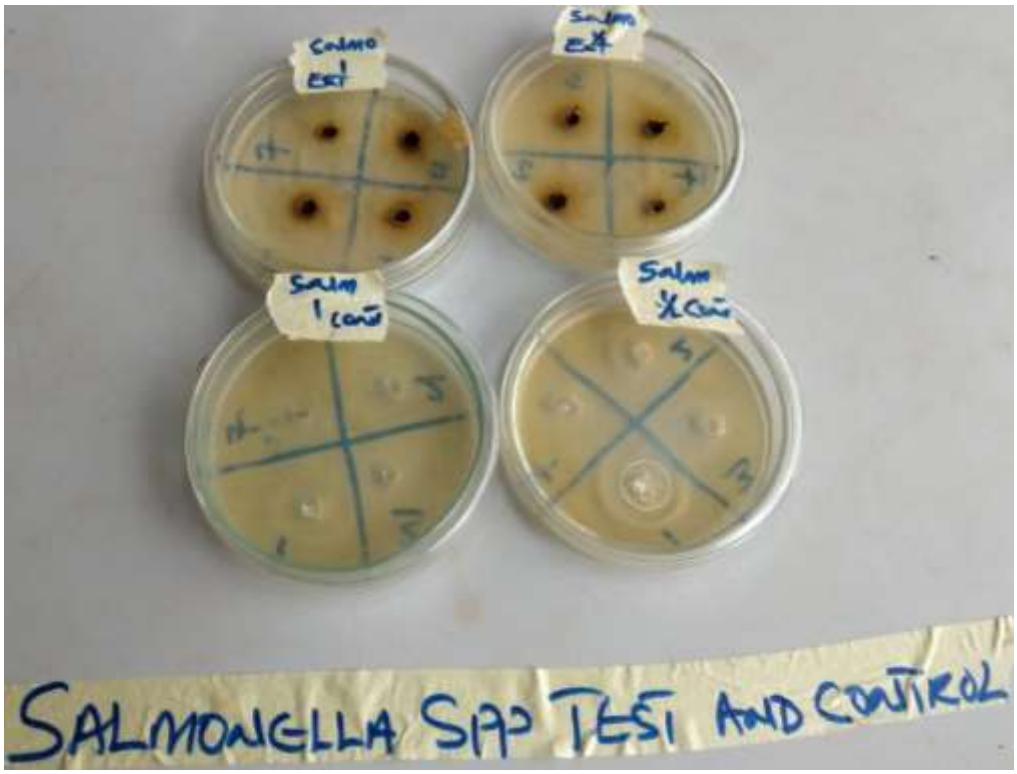

Fig 8: *Salmonella typhi* extract and control

Fig 9: A = newly emerged *G. lucidum* from the substrate, B = older dried harvested *G. lucidum*

There was increase in the minimum inhibition concentration (MIC) value of the extract on *Staphylococcus aureus* and *Streptococcus pneumonia* when compared with that of the control drug used in this study. This could be attributed to high resistance of these organisms to conventional antibiotic because of frequent abuse. Odo *et al.* (2020) had observed *Staphylococcus aureus* as one of the frequently encountered resistant bacteria pathogens. *Streptococcus pneumonia* that causes respiratory system infections has also been found to be resistant to many antibacterial agents due to frequent abuse of antibiotic (Cheesbrough, 1985). The MICs of *G. Lucidum* extract and that of the conventional drug that were statistically similar for *Pseudomonas aeruginosa* and *Staphylococcus aureus* showed that *G. lucidum* extract could serve as an alternative source for production of noble antibacterial agents against both Gram negative and Gram-positive pathogens of plants and animals. Odo *et al.* (2020) had also observed a high growth inhibition on *P. aeruginosa* and *Staphylococcus aureus* using methanol extract of *G. latifolia* plant leaves.

Therefore, for safety, availability of raw materials, and economic convenience, the use of methanol extract of *G. lucidum* for the treatment of plant and animal diseases caused by *Pseudomonas* like bacterial spot of tomatoes would be highly encouraged. The MIC of the control drug that was lower in all the pathogens when compared with that of the extract except on *P. aeruginosa,* showed the efficacy of the control drug over the tested bacterial pathogens. Agboke *et al.* (2005) also observed that increased MIC is always associated with decreased efficacy of antimicrobial agent. In this case, the lesser efficacy of the extract as seen from the higher MIC when compared with that of the control drugs may have resulted from its crude nature. However, the extract although crude, was able to inhibit both fungal and bacterial pathogens tested. This showed that *G. lucidum* extract can be classified as a broad-spectrum agent and could be recommended as a noble source of a raw material for production of effective antifungal and antibacterial agents for treatment of both plant and animal diseases.

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