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Phytochemical Screening and Antimicrobial Activity of Kenyan Mushroom *Ganoderma lucidum*

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Authors' contributions

This work was carried out in collaboration among all authors. Author ES designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DLB, SO, NPK and LS managed the analyses of the study. Authors ES and DLB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To screen the Kenyan *Ganoderma lucidum* for phytochemicals and to investigate it's potential in the management of some disease-causing microbes.
Study Design: Hexane, ethyl acetate and methanol extraction; phytochemical study and anti-microbial activities were analysed on Kenyan *G. lucidum* mushroom.
Place and Duration of Study: Masinde Muliro University Science laboratories from January 2019 and March 2019.
Methodology: Sequential extraction of dried samples was done using hexane, ethyl acetate and methanol. The crude extracts were assayed against *Escherichia coli, Klebsiella pneumoniae, Methicillin-Resistant Staphylococcus aureus (MRSA), Pseudomonas aeroginosa, and Creptococcus neoformans* using agar well diffusion method. Ampicillin and nystatin were respectively used as controls for bacteria and fungi. Zones of inhibition measured signified the antimicrobial activity.

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Results: The following group of compounds were present in the mushroom; terpenoids, carbohydrates, phenolic compounds, glycosides and polyoses. Hexane and methanol extracts showed significant activity at (P= 0.022) against MRSA while ethyl acetate extract was active against *Streptococcus pyogenes* (P=0.05).
Conclusion: Kenyan *Ganoderma lucidum* contains terpenoids, carbohydrates, phenolic compounds, glycosides, flavonoids and phytosterols. Crude extracts (hexane, ethyl acetate and methanol) were active against *MRSA* and *S*treptococcus *pyogenes.*

Keywords: Ganoderma lucidum; phytochemical analysis; antimicrobial.

ABBREVIATIONS

C. neoformans : *Creptococcus neoformans*
DMSO : *Dimethylsulphoxide*
E. coli : *Escherichia coli*
EtOAc : *Ethyl acetate*
MeOH : *Methanol*
MHA : *Muller Hinton Agar*
MRSA : *Methicillin Resistant Staphylococcus aureus*
PDA : *Potato Dextrose Agar*

1. INTRODUCTION

Ganoderma lucidum is a worldwide medicinal mushroom that is either cultivated or grows naturally on the bark of trees. Kenyan *Ganoderma lucidum* mushroom is conk-like or kidney-like in shape. It is wood textured with 5 – 20cm in diameter and has a shiny surface which is black brown. In nature, it grows on dead or dying trees (Fig. 1). The Chinese *G. lucidum* is also known as "Lingzhi" is red-vanished, kidney-shaped mushroom [3]. *Lingzhi* is soft, cork-like and flat and the pores on the underside may be white or brown [3]. The two mushrooms are different in morphology and appearance since they grow in different ecological zones.

Phytochemicals from *Ganoderma lucidum* mushroom species all over the world have been known to have medicinal properties and contain physiological active constituents [1-2]. For instance, *G. lucidum* is known to contain more than 100 types of polysaccharides showing strong immunomodulating activities [1-4]. The most vital phytochemicals have been triter-penoids with anti-inflammatory, antitumorigenic, and hypolipidemic activities [4-7]. Other compounds reported from *Ganoderma lucidum* include the isolation of various Ganoderic acids [8-9,10], esters [10], alcohols [11] and lactones [12]. So far, there are little reports on either chemical constituents or biological activity of Kenyan *Ganoderma lucidum,* and therefore this research work sought to investigate the chemical and antimicrobial activity of this species.

Fig. 1. *Ganoderma lucidum* from Kakamega, Kenya

2. MATERIALS AND METHODS

2.1 Collection and Identification

Ganoderma lucidum was cultivated and obtained from a mushroom farm in Kakamega County. The county lies at an altitude of 1500-1600m above sea level and is 358 km west of Nairobi, Kenya. The mushroom species were identified by a herbarium staff at the East African Herbarium National Museums of Kenya in Nairobi where a voucher specimen is deposited and identification number EAHNMK 261 assigned to the species.

2.2 Extraction of *Ganoderma lucidum*

2.2.1 The mushroom sample

The collected fresh mushroom sample was cleaned, air dried for 7 days, ground into a fine powder using an electric grinder and stored under dry conditions ready for analysis.

2.2.2 Solvent extraction and analysis

A quantity of 1 kg of the powdered mushroom was soaked in 3L of hexane at room temperature and pressure for 48 hours. The mixture was then filtered and the obtained filtrate concentrated under reduced pressure using a rotary evaporator, resulting in 6.20 g of a dark gummy

semi-solid, a 0.5% yield from the dry weight of the mushroom. The dried mushroom material was soaked in ethyl acetate and then filtered. The filtrate was concentrated in a rotor evaporator under similar conditions as hexane extract to obtain a semi-solid light brown ethyl acetate extract (4.11 g). The same mushroom that was soaked in ethyl acetate was dried and soaked in methanol. Filtration was then concentrated using a rotor evaporator to obtain a dark brown methanol extract (10.03 g). Hexane, ethyl acetate and methanol extracts were investigated for phytochemicals and antimicrobial activities.

2.3 Phytochemical Screening

Standard procedures were used as described by [13-17]. These tests were based on visual observation of colour or precipitate formation after the addition of specific reagents. Crude extracts of hexane, ethyl acetate and methanol were analyzed for the presence of active phytochemicals.

2.3.1 Test for terpenoids

Salkowski test: About 0.5 g of hexane extract was placed in a clean test tube. A few drops of concentrated sulphuric (VI) acid were added to the extract in the test tube and shaken. On standing, the lower layer turned yellow at first then read later to indicate the presence of triterpenoids. The procedure was repeated for ethyl acetate and methanol extracts. Terpenoids were present in hexane, ethyl acetate and methanol extracts.

2.3.2 Test for saponins

A small amount of each extract was shaken with little quantity of water, and then if the foam was produced and persisted for 10 minutes, it confirmed the presence of saponins. All the three extracts did not produce the foam. This showed that the extracts did not have saponins.

2.3.3 Test for carbohydrates

Fehling's test: About 0.5 g of hexane extract was placed in a test tube and Fehling`s A and B solution added to it and heated. This test gave an orange-red precipitate in hexane, ethyl acetate and methanol extracts indicating the presence of reducing sugars.

2.3.4 Test for glycosides

Keller – Killiani test: About 10 ml of glacial acetic acid, 2.0ml ferrous chloride and 0.5 ml of concentrated sulphuric (VI) acid were to the extract. Acetic acid layer showed a blue colour which indicated the presence of glycosides.

Ammonia test: About 5 drops of 1% v/v NH_3 solution was added to about 0.5 g of hexane extract in a test tube and observations made. The procedure was repeated for 0.5 g of ethyl acetate and 0.5 g of methanol extracts. A yellow colouration was observed in hexane, ethyl acetate and methanol extracts.

2.3.5 Test for flavonoids

Ferric chloride test: About 100 g dry mushroom of *G*. *lucidum* was extracted in ethanol and then filtered. This was also general phytochemical test since it did not involve the other three extracts. The blackfish green colour was observed and this indicated that flavonoids were present in the mushroom.

Lead acetate test: Ethanol extract was prepared by soaking about 20 g of the dry mushroom in about 50 ml of ethanol. The reaction of the alcoholic solution of the mushroom extract (ethanol extract) with 10% lead acetate in a test tube gave a white precipitate. This indicated the presence of flavonoids.

2.3.6 Test for alkaloids

Mayer`s test: About 1.0 ml of Mayer`s reagent (potassium mercuric iodide) was added to 1ml of each crude mushroom extract. Formation of white precipitate was observed for hexane, ethyl acetate and methanol extracts. This showed the presence of alkaloids in the extracts.

2.3.7 Test for tannins

0.5 g of powdered mushroom material was boiled in 20 mL of distilled water in a test tube and then filtered by gravity. Then 0.1% $FeCl_3$ was added to the filtered samples and observed. Brown colouration indicated the presence of tannins in hexane, ethyl acetate and hexane extracts.

2.4 Bioactivity Tests of Crude Extracts from *Ganoderma lucidum*

2.4.1 Culture of test micro-organisms

The selected test microorganisms (*Escherichia coli, Klebsiella pneumonia, Streptococcus pyogenes* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) were resuscitated by culturing them on Mueller Hinton Agar (MHA) and incubated at 37°C for 18 hours.

The microorganisms were later transferred to Mueller Hinton Broth and incubated at 37°C for 6 hours for a uniform and young culture for the antibacterial tests. The *Cryptococcus neoformans* was also cultured in Potato Dextrose Broth and incubated at 28°C for 5 days ready for the bioassay test.

2.4.2 Antibacterial tests

Mueller Hinton agar was prepared according to the commercial instructions given. Mueller Hinton Agar media was then sterilized by autoclaving at a temperature of 121°C and pressure 15psi for 15 minutes. Using a 6 mm diameter coke borer, wells were then made through the agar in the dishes (well diffusion method) [18]. The concentrations of 100 mg/ml in Dimethylsulphoxide (DMSO) of crude extracts (hexane, ethyl acetate and methanol) and control (Ampicillin) were prepared. Only the 100 microlitre aliquot was dispensed into each well using micropipettes and left at 4°C for 4 hours for the sample to completely diffuse into the media. The samples were run in replicates of three. The test microorganisms (*Escherichia coli, Klebsiella pneumonia, Streptococcus pyogenes and Methicillin-Resistant Staphylococcus aureus* (*MRSA)* were then uniformly spread on each plate and incubated at 37°C for 24 hours. Zones of bacterial growth inhibition were observed and the diameter of each was measured in millimetres to assess the antimicrobial activity. The values obtained were used to determine the antibacterial activity by comparing the test and standard microorganisms.

2.4.3 Antifungal tests

Potato Dextrose Agar was prepared based on the commercial procedures described. Nystatin was used as the control for the fungi. Concentrations of 100 mg for both crude extracts and 10 mg for the control (Nystatin) were prepared by dissolving their masses in one millilitre of dimethylsulphoxide (DMSO). The samples were done in replicates of three. The fungus, *Cryptococcus neoformans*, was introduced into the Petri dishes by streaking with a sterilized wire loop. It was allowed to stay untouched for about four hours to diffuse in the media at 4°C. All the inoculated Petri dishes were then incubated at 28°C. They were allowed to stay there for about 24 hours before observations were made.

3. RESULTS

3.1 Phytochemical Results

The phytochemical results were as shown in Table 1.

3.2 Bioactivities of Crude Extracts against the Selected Pathogenic Microorganisms

All crude extracts and controls were subjected to selected micro-organisms. Commercial antimicrobials Ampicillin and Nystatin acted as positive controls for bacteria and fungi respectively. Results are shown in the Table.

4. DISCUSSION

4.1 Discussion for Phytochemical Results

From Table 1 above, terpenoids, saponins, carbohydrates, glycosides and flavonoids were not present in all the three extracts. Phenolic compounds were present in ethyl acetate and methanol extracts but absent in hexane extract. Carbohydrates in form of polysaccharides have also been isolated from other

Table 1. Phytochemical screening of Kenyan *Ganoderma lucidum*

No.	Secondary metabolites	Hexane extract	Ethyl acetate extract	Methanol extract
2	Terpenoids	+	+	+
3.	Saponins	-	-	-
4.	Alkaloids	-	-	-
5.	Carbohydrates	+	+	+
6.	Phenolic compounds (tannins)	-	+	+
7.	Glycosides	+	+	+
8.	Flavonoids	+	+	+

Key: Positive (+), Negative (-)

Table 2. Antimicrobial activities of crude extracts against selected pathogenic microorganisms

Micro-organism	Amp/Nys	Hexane extract	Ethyl acetate extract	Methanol extract	*P* –value
E.coli	NI	NI	NI	NI	
P. aeruginosa	NI	NI	NI	NI	
K. pneumoniae	NI	NI	NI	NI	
MRSA	31±0.33	13.3±0.33	10.3±0.33	10.3±0.33	*P*= 0.022
S. Pyogenes	40.3±0.33	10.3±0.33	11.7±0.33	NI	*P* = 0.05
C. neoformans	30.7±0.33	NI	NI	NI	*P* = 0.05

Key: Amp: Ampicillin, Nyst: Nystatin, MeOH: (Methanol), EtOAc: (Ethylacetate); NI: No inhibition

Fig. 2. Bioactivity of extracts for *against MRSA (*Kirby-Bauer disc sensitivity method*)*

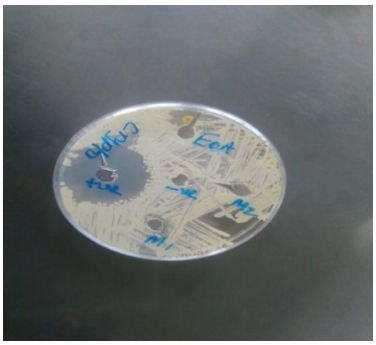

Fig. 3. Bioactivity of extracts against Streptococcus pyogenes

Ganoderma lucidum species. Polysaccharides have been known to have strong immunomodulating activities anti-inflammatory, antitumorigenic and hypolipidemic activity. Therefore, the Kenyan *Ganoderma lucidum* species could be a natural source of important phytochemicals for the management of inflammation and tumour [3]. *Ganoderma lucidum* is known to produce triterpenoids and steroids related compounds [8,9,10]. Total flavonoid and phenolic compounds with antioxidant activity were previously reported from *Ganoderma lucidum* extracts [20]. This, therefore, indicates the importance of *Ganoderma lucidum* as an antioxidant mushroom.

4.2 Discussion for Bioassays

The results in Table 2 showed significant activity against *MRSA* (*Methicillin Resistance Staphylococcus aureus*, (p=0.022), *Streptococcus pyogenes(p=0.05) and Creptococcus neoformans* (p = 0.05). None of the extracts inhibited *P. aeruginosa, E. coli* and *K. Pneumoniae.* This implies that the concentrations of bioactive compounds in the crude extracts might have been low, absent or antagonistic to each other to cause bioactivity. This outcome compares favourably with the previous findings [19].

5. CONCLUSION

The solvent crude extracts from the mushroom possessed the following phytochemicals; terpenoids, carbohydrates, phenolic compounds, glycosides and flavonoids. The crude extracts from the mushrooms showed significant activities against the tested pathogenic micro-organisms; *MRSA* and *Streptococcus pyogenes*.

6. RECOMMENDATIONS

Since phytochemicals were observed in the mushroom, isolation of the constituent compounds to be investigated and their efficacy against microbes determined. Other concentrations of crude extracts should be applied and the use of other antibiotics and antifungal as controls to be considered.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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