Original Research Article

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

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ABSTRACT

Aim: To screen the Kenyan *Ganoderma lucidum* for bioactive phytochemicals and to investigate its potential in management of some disease causing microbes.

Study design: Hexane,ethylacetate 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, ethylacetate and methanol. The crude extracts were assayed against *Escherichia coli, Klebsiella pneumoniae, Methicillin Resistant Staphylococcus aureus (MRSA), Pseudomonas aeroginosa, and Creptococcus neoformans* using disc diffusion method. Ampicillin and nystatin were respectively used as controls for bacteria and fungi. Zones of inhibition measured signified the antimicrobial activity. The following groups of compounds were present in the mushroom; steroids, triterpenoids, carbohydrates, phenolic compounds, glycosides, flavonoids, phytosterols and polyoses.

Results: The following group of compounds were present in the mushroom; steroids,

triterpenoids, carbohydrates, phenolic compounds, glycosides, phytosterols and polyoses. Hexane and methanol extracts showed significant activity at (P= 0.022) against MRSA while ethylacetate extract was active against Streptococcus pyogenes (P=0.05). Bacteria strains *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and the fungi, *Cryptococcus neoformans* were insensitive to all the extracts.

Conclusion: Kenyan *Ganoderma lucidum* contains steroids, triterpenoids, carbohydrates, phenolic compounds, glycosides, flavonoids and phytosterols. Crude extracts (hexane, ethylacetate and methanol) were active against some pathogenic microbes *MRSA*, *S. pyogenes* and *C. neoformans*.

Keywords: *Ganoderma lucidum,* phytochemical analysis, antimicrobial

1.INTRODUCTION

Ganoderma lucidum is not only tropical equatorial but also worldwide medicinal mushroom that is either cultivated or grows naturally on the bark of trees. Polysaccharides, peptidoglycans and triterpenoids are the three major physiologically active constituents of *Ganoderma lucidum* [1]; [2]. It is known to contain more than 100 types of polysaccharides showing strong immunomodulating activities [3]. Terpenes have also been found to have anti-inflammatory, antitumorigenic, and hypolipidemic activities [4]. Among the reported chemical analyses of Chinese *Ganoderma lucidum* include the isolation of various Ganoderic acids [5]; [6]; [7], esters [7], alcohols [8] and lactones [9]. So far, there are no reports on either chemical constituents or biological activity of Kenyan *Ganoderma lucidum* and this made the researcher to carry out chemical and antimicrobial analysis on the Kenyan *Ganoderma* species.

2. MATERIAL 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 was identified by a herbarium staff at the East African Herbarium National Museums of Kenya in Nairobi where a voucher specimen was deposited and identification number EAHNMK 261 assigned to the *Ganoderma* species.

2.2 Extraction of *Ganoderma lucidum*

2.2.1 Preliminary Extraction

The collected mushroom was cleaned, air dried for 7 days, ground into fine powder using an electric grinder and stored under suitable 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 distilled 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 6.20g of a dark gummy semi-solid, a 0.5% yield from the dry weight of the mushroom. The resulting residue was taken through sequential extraction with ethylacetate and lastly with methanol following the same procedure as with hexane. The resulting crude extracts were weighed and recorded. Ethyl acetate weighed 4.11g while methanol was 10.03g

2.3 Phytochemical screening

Standard procedures were as described by [10]; [11]; [12], [13]; [14]. These tests were based on visual observation of colour or precipitate formation after addition of specific reagents. Crude extracts of hexane, ethylacetate and methanol were analyzed for the presence of useful phytochemicals.

Salkowski test: About 0.5g of hexane crude extract of Kenyan *Ganoderma lucidum* 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, if the lower layer turned red, it indicated the presence of steroids. If the lower layer turned golden yellow, it indicated the presence of triterperoids. The procedure was repeated for ethylacetate and methanol extracts.

Fehlings test: About 0.5g of hexane extract was placed in a test tube and Fehling`s A and B solution added to it and heated. If it gave an orange - red precipitate, reducing sugar would be present.

Anthraquinone test: About 0.5g of dry powdered mushroom material was extracted with either ammonia or caustic soda by filtration. If its filtrate or aqueous layer showed pink colour, it indicated the presence of glycosides.

Keller – Killiani test: This is for cardiac glycosides. The dry mushroom material was extracted with chloroform. Then glacial acetic acid, 2.0ml ferrous chloride and 0.5 ml of concentrated sulphuric (VI) acid were added. If acetic acid layer showed blue colour, it indicated the presence of glycosides.

Ammonia test: A few drops of 1% NH_3 solution was added to an extract to be tested in a test tube. A yellow colouration was observed if flavonoid compounds were present.

Ferric chloride test: If alcoholic solution of the mushroom extract (Ethanol extract) reacted with freshly prepared $FeCl_3$. If it gave black fish green colour, it showed that flavonoids were present.

Lead acetate test: The reaction of alcoholic solution of the mushroom extract (ethanol extract) with 10% lead acetate in a test tube. If it gave white precipitate, this indicated the presence of flavonoids.

**Mayer`s test:**1.0 ml of Mayer`s reagent (potassium mercuric iodide) was added to 1ml of the crude mushroom extract. Formation of white precipitate was a positive indicator for the presence of alkaloids.

Tannins test; 0.5g 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 for brownish green or a blue – black colouration. This colour change showed the presence of tannins.

2.4 Bioactivity tests of Crude extracts from *Ganoderma lucidum*

3.4.1 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.

3.4.2 Antibacterial tests

19 g of Mueller Hinton Agar (MHA) media was dissolved in 500ml of distilled water in a 500ml conical flask and heated to boiling to dissolve the media completely. Mueller Hinton Agar media was then sterilized by autoclaving at a temperature of 121°C and pressure 15psi for 15 minutes. The media was then left to cool to 45 °C and dispensed into sterile plates of 22 mm diameter of 20mL per plate and the agar allowed to solidify. Using a 6 mm diameter coke borer, wells were then made through the agar in the dishes (well diffusion method) [15]. Concentrations of 100 mg/ml for crude, isolated compounds of hexane, ethyl acetate and methanol solvents and 10 mg/ml for control (Ampicillin) were dissolved in dimethylsulphoxide (DMSO). The reconstituted fractions were serially diluted in tenfold ratios. 100µl from each 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 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. Each plate had a similar concentration but with different bacteria streaked on each. Zones of bacterial growth inhibition were observed and diameter of each was measured in millimeters to assess the antimicrobial activity. The values obtained were used to determine the antibacterial activity by comparing the test and standard microorganisms.

3.4.3 Antifungal tests

9.8 g of Potato Dextrose (PDA) agar media was dissolved in 200ml distilled water and placed in a 500ml conical flask. The mixture was heated to boiling to dissolve the media completely. The PDA agar media was sterilized by autoclaving at 121°C at 15psi for 15 minutes. Sterilized media was left to cool to 45°C and dispensed into petri dishes. The media was then left petri dishes so as to solidify on the petri dishes. Nystatin was used as control for the fungi. Concentrations of 100

µg/ml for both crude extracts, isolated compounds and 10mg/µl for the control (Nystatin) were prepared by dissolved in one milliliter of dimethylsulphoxide (DMSO). The prepared concentrations (100mg/ml) of crude extracts (hexane, ethyl acetate and methanol), isolated compounds and control (Ampicillin) were varied by tenfold serial dilutions for each sample using micropipettes. The fungus (*Cryptococcus neoformans*) was introduced into the petri dishes by streaking with a sterilized wire loop. They were allowed to stay untouched for about four hours to diffuse in the media. All the inoculated petri dishes were then taken into the incubator at 28°C. They were allowed to stay there for about 24 hours before observations were made.

3. RESULTS AND DISCUSSION

3.1 Phytochemical results

Crude extracts weighed as follows; hexane 6.18 g, ethyl acetate 4.11g while methanol was 10.03g. Crude extracts from *Ganoderm alucidum* were used in phytochemical screening and antimicrobial activities. The phytochemical screening performed on the mushroom *Ganoderma lucidum* crude extracts was based on visual observation of coloured complex formation according to [10];[11]; [12]; [13]; [14].

Table 1: Phytochemical screening of Kenyan *Ganoderm alucidum*

No.	Secondary metabolites	Test Name	Hexane	Ethyl acetate	Methanol
1.	Steroids	Salkowski test	+	+	+
2.	Triterpenoids	Salkowski test	+	+	+
3.	Saponins	Foam test	-	-	-
4.	Alkaloids	Ferric chloride test	-	-	-
		Mayer`s test	-	-	-
		Hager`s test	-	-	-
5.	Carbohydrates	Fehlings test	+	+	+
6.	Phenolic compounds (tannins)	Braemer`s test	-	+	+
7.	Glycosides	Keller – Killiani test	+	+	+
		Anthraquinone test (Caustic soda)	+	+	+
		Anthraquinone test (ammonia)	+	+	+
8.	Flavonoids	Ferric chloride test	+	+	+
.		Lead acetate test	+	+	+
9.	Phytosterols	Salkowski test	+	+	+
10.	Polyoses	Molisch test	-	+	+

Key: Positive result is represented as (+), negative result is represented as (-)

From the table above, steroids, triterpenoids, saponins, carbohydrates, glycosides, flavonoids and phytosterols were present in all the three extracts. Phenolic compounds and polyoses were present in ethylcetate and methanol extracts but absent in hexane extract. Carbohydrates in form of polysaccharides have also been isolated from other *Ganoderma*

lucidum species. Polysaccharides have been known to have strong immunomodulating activities [3]. Kenyan *Ganoderma lucidum* gave positive test for carbohydrates. If tested for immunomodulating activity, it could be a source of medicine. Triterpenoids have also been confirmed in other *Ganoderma lucidum* species to be useful anti-inflammatory, antitumorigenic and hypolipidemic activity. Kenyan *Ganoderma lucidum* has again tested positive for triterpenoids. This again shows that triterpenoids from this mushroom can be used as a good source of medicine.

Table 2: Phytochemical screening of Indian *Ganoderma lucidum* from Waghai village

No.	Phytochemical	Test	EtOAc	MeOH	MeOH:EtOAC
1.	Steroids	Salkowski`s test	+	+	+
2.	Triterpenoids	Salkowski`s test	+	+	+
3.	Saponins	Foam test	-	-	-
4.	Alkaloids	Mayer`stest	-	-	-
		Wagners test	-	-	-
		Dragendorf test	-	-	-
5.	Carbohydrates	Molisch test	+	+	+
		Fehlings test	+	-	-
		Benedict test	-	-	-
6.	Phenolic cpds/ tannins	Ferric chloride test	+	+	+
		Lead acetate test	+	-	+
7.	Glycosides	Keller-Killiani test	+	+	+
8.	Flavonoids	Alkaline reagent test	-	-	-
9.	Phytosterols	Liebmann-Burchards reagent	-	-	-

Key: Positive result is represented as (+), negative result is represented as (-)

From the table above, it shows that Indian *Ganoderma lucidum* has similar group of phytochemicals as those found in the Kenyan species; steroids, triterpenoids, carbohydrates, phenolic compounds or tannins and glycosides [16]. The only difference is that flavonoids and phytosterols are present in the Kenyan species while the same compounds are absent in the Indian species. Unlike the Kenyan species, the Indian variety lack flavonoids and phytosterols [16]. *Ganoderma lucidum* from foot hills of Marudhamalai, India have been known to contain triterpenoids, steroids, flavonoids and alkaloids. The mushroom lacks saponins, glycosides and carbohydrates [17]. Phytochemicals from *Ganoderma* lucidum mushrooms all over the world have been known to be medicinal. The most vital phytochemicals have been triterpenoids and polysaccharides. Hexane extracts from Russian *Ganoderma lucidum* contains terpenoids only while ethylacetate extracts from the same mushroom contains flavonoids, terpenoids and phenols [18].

3.2 Bioactivities of crude extracts and controls against antimicrobial tests

All crude extracts and controls were subjected to various micro-organisms. Commercial antimicrobials ampicillin and nystatin acted as controls for bacteria and fungi respectively. Results are shown inthe table below.

Table 3: Antimicrobial statistical data for crude extracts, isolated compounds and controls

Micro-organism	Amp/Nys	Hexane extract	EtOAc extract	MeOH extract	*P* -value
E.coli	**0**	**0**	**0**	**0**	
P. aeruginosa	**0**	**0**	**0**	**0**	
K. pneumoniae	**0**	**0**	**0**	**0**	
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**	**0**	***P* = 0.05**
C. neoformans	**30.7±0.33**	**0**	**0**	**0**	***P* = 0.05**

Key: Amp(Ampicillin), Nyst(Nystatin), MeOH (Methanol), EtOAc (Ethylacetate*)*

The results above showed that all extracts showed inhibition against *MRSA* (*Methicillin Resistance Staphylococcus aureus*). *Streptococcus pyogenes* was inhibited by all the tests applied except methanol extract. None of the extracts inhibited *P. aeruginosa, E. coli* and *K. pneumoniae.* Increasing the concentrations of crude extracts and compounds isolated could cause high inhibition against test organisms. Screening results are shown in table 2. The results in the same table showed that hexane and methanol extracts exhibited significant inhibitions against MRSA (*p*=0.022) and ethyl acetate against *Streptococcus pyogenes* (p=0.05). *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* did not respond to any of the extracts including the controls used. *E. coli*, *P. aeruginosa, Klebsiella pneumonia* did not respond to all the crude extracts (hexane, ethylacetate and methanol) as shown in table 2. Non responsive effectsof micro-organisms against crude extracts (hexane, ethyl acetate and methanol), and controls does not mean that they cannot respond totally to the tests applied. Synergy could have been a factor that might have led to the negative effect. Concentrations of bioactive compounds in the crude extracts and controls might have been low. Another factor is that there might be complete absence of compounds that could cause inactivity.

Ampicillin, an antibacterial control acted against Methicillin Resistant *Staphylococcus aureus* (*MRSA*) (p = 0.022) and *Streptococcus pyogenes* (p = 0.05) while nystatin, an antifungal control acted against *Creptococcus neoformans* (p = 0.05). Ethylacetate extract from Indian *Ganoderma lucidum* acted against *MRSA* at 25 mm while methanol extract from the same Indian mushroom was against *E.coli* and *P. aeruginosa* at 1.25mg/ml and 6.25mm respectively [16]; [17].

Table 4: Antimicrobial statistical data for crude extracts of Indian *Ganoderma lucidum*

Minimum Inhibitory Concentration (mg/ml)			
Organism	**Ethyl acetate**	**Methanol**	**MeOH:EtOAc**
Staphylococcus aureus	2.5	ND	2.5

Escherichia coli	ND	1.25	ND
Pseudomonas aeruginosa	ND	0.625	ND

4. CONCLUSION

Phytochemical analysis of crude extracts (hexane, ethylacetate and methanol) showed that steroids, triterpenoids, carbohydrates, phenolic compounds, glycosides, flavonoids and phytosterols were present in Kenyan *Ganoderma lucidum*. Saponins and alkaloids were not identified in this extracts. Crude extracts (hexane, ethylacetate and methanol) were active against some antimicrobials *MRSA*, *S. pyogenes* and *C. neoformans*. However, *E. coli*, *P. aeruginosa, Klebsiella pneumoniae* did not respond to all the crude extracts under investigation.

5. RECOMMENDATIONS

Bioactive responsible compounds should be isolated from the extracts of *Ganoderma lucidum*. The mushroom could produce compounds that could have medicinal value. Antimicrobial and bioactivities should also be done to the isolated compounds.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

C. *neoformans – Creptococcus neoformans*

DMSO - Dimethylsulphoxide

E. coli – Escherichia coli

EtOAc- Ethyl acetate

G. lucidum – Ganoderma lucidum

K. pneumonia – Klebsiella pneumonia

MeOH - Methanol

MHA – Muller Hinton Agar

MRSA – Methicillin Resisistant *Staphylococcus aureus*

P. aeruginosa – Pseudomonas aeruginosa

PDA – Potato Dextrose Agar

S. pyogenes – Streptococcus pyogenes