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RESEARCH ARTICLE

In-vitro Evaluation of Antimicrobial Activity of *Ganoderma lucidum*

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Abstract

Ganoderma sp. provides bioactive compounds that claim to posses antibacterial activity. The aim of this research is to know antimicrobial activity of various extract of *Ganoderma lucidum*. Fruiting bodies of *Ganoderma lucidum*. were extracted by maceration method using 50 % aceton, 50 % ethanol, methanol and boiling water, The antimicrobial activity of various solvent extracts (50µg/ml) of *Ganoderma lucidum* was tested against six species of bacteria: *Escherichia coli* (MTCC-443), *Staphylococcus aureus* (MTCC-187), *Bacillus subilis* (MTCC-1789) *Salmonella typhi* (MTCC-531) and *Pseudomonas aeruginosa* (MTCC-779), *Vibrio cholera* (MTCC 1068). Acetone extract exhibited maximum antibacterial activity (31.60±0.10), The acetone extract (1000mg/ml) possess strong antifungal activity against 5 fungal strains which were tested by using poisoned food technique The strains used for antifungal activity consisted of *Aspergillus niger* FCN#34, *Curvularia lunata* FCN #12, *Fusarium oxysporum* FCN #80 ,*Alternaria alternata* FCN #15 *Drashelaria* sp. FCN #31.

INTRODUCTION

Ganoderma lucidum, a mushroom, is one of the most famous traditional Chinese medicinal herb bio-products of Mushroom have multi beneficial effects for human welfare. Medicinal mushrooms are widely used as traditional medicinal ingredients for the treatment of various diseases and related health problems. Most of the medicinal extracts from mushroom are different forms of polysaccharides which strengthens the immune system with little or no side effect. Medicinal mushroom research has focused on discovery of compounds that can modulate positively or negatively the biological response of immune cells. One interesting aspect of its performance is antimicrobial effect due to the extracts derived from this mushroom which contain bacteriolytic enzyme, lysozyme and acid protease (Klaus & Miomir, 2007).

The activities of G. lucidum are mainly due to polysaccharides and/or triterpenoids of the fungus. Some of the triterpenoids showed antioxidant, anticancer and antimicrobial properties (Lin *et al*, 2003, Prasad *et al*, 2008,Quereshi *et al* 2010) found that extracts of *Ganoderma lucidum* from the exposed dead trunk and roots of Mangifera indica had antibacterial activity. *Ganoderma* species like any other fungi grow wild on living or dead or dying wood log of hardwood and sometimes on dead roots. Typically found at the base of living hardwoods or occasionally on the stumps or roots of a wide range of deciduous hosts (Chang and Mshigeni, 2001). Mushroom fruit-bodies are complex structures, both morphologically and more physiologically with undoubted variations in chemical composition from batch to batch. In this research we used *Ganoderma lucidum* that grow on dead wood of Samanea saman (Jacq.) Merr. (Fabaceae) generally called as a rain tree. Rain tree is a folk remedy for cold, diarrhea, headache, intestinal ailment and stomache. The aim of this research is to know antibacterial activity of various extract of *Ganoderma lucidum* against test pathogen. Fruiting bodies of *Ganoderma* were extracted by micro Kjeldahl apparatus using 50 % acetone, 50 % ethanol, methanol and boiling water, respectively.Therefore, G. lucidum products with different triterpenes and polysaccharides or combinations of these two groups are most likely

to result in different pharmacological activities (Leung *et al*, 2002). A new class of compounds with nutritional and medicinal features extractable from either the mycelium or the fruiting bodies of mushrooms have been referred to as "mushroom nutraceuticals". G. lucidum is rich in mushroom nutraceutical components with potential therapeutic values (Chang & Buswell, 1996).
The aim of present work was to carry out in-vitro experiments to screen antimicrobial potential of different extracts of *Ganoderma lucidum*.

Material and methods:

Collection of Sample

The wood decant fungal species of *Ganoderma lucidum*. was collected from TFRI Campus, Jabalpur from the exposed dead wood or trunk of Mangifera indica. The freshculture of G. lucidum was obtained by tissue culture technique (Oei, 2005).

Extraction of bioactive compounds from fruiting bodies of G. lucidum:

In the present study, the mushroom material was grounded to a fine powder with the help of pestle and mortar. Ten gram of mushroom powder was subjected to Soxhlet extraction using micro Kjeldahl apparatus (ASGI, India) for 10 hours using 100 ml each of the following solvents viz., ethyl alcohol, methanol,acetone and distilled water. The extracts were recovered by filtration and kept at 35^0C for further analysis (Dulger & Gonuz, 2004). All the solvent extracted fractions were subjected to in vacuo desiccation at 40ºC in a rotary vacuum evaporator
(Buchi R - 300 Rotavapor, Buchi Co. Germany) to remove any traces of solvents and to obtain residues. The test residues were prepared as stocks using distilled water (40µg/ml) and were tested for their antimicrobial activity.

Microorganismstested-

In vitro antimicrobial susceptibility test was performed using a set of microbes such as Gram negative, Gram positive bacteria and filamentous fungi which included both human clinical pathogen and laboratory control strains. The panel consisted of- Bacillus subtilis, (MTCC 1789) *Escherichia coli* , (MTCC 443) Pseudomonas aeruginosa, (MTCC 779) Salamonella typhi , (MTCC 531) *Staphylococcus aureus* , (MTCC 187) *Vibrio cholera* , (MTCC 1068) Antibacterial activity was measured in terms of Inhibition Zone size (in mm) obtained after the incubation at 37ºC±2ºC for 24 hours. The strains used for antifungal activity consisted of *Aspergillus niger* FCN#34, *Curvularia lunata* FCN #12, *Fusarium oxysporum* FCN #80 ,*Alternaria alternata* FCN #15 *Drashelaria* sp. FCN #31. Antifungal activity was measured in poison food technique obtained after the incubation at 28ºC±2ºC for 7 days. The percentage of mycelial inhibition was calculated by following formula:

$$\% \text{ inhibition} = \frac{dc-dt}{dt} \times 100$$

dc = average diameter of fungal colony in control sets.
dt = average diameter of fungal colony in treated sets.

Antibacterial Assay:

The antibacterial assay was performed by Disc diffusion method using paper disc as reservoir. 10 ml Nutrent Agar media was inoculated with 100 µl of inoculums test bacteria and poured in to Petridis. Each 0.5 mg extract was diluted with 1 ml dimethylsulfooxide (DMSO). Ten microlitre extract was put in paper disc (every disc contained 500 mg the extract). The Petri dishes were incubated 24 hours at 37ºC, for each bacterial test. The results were obtained by measuring the diameter of zone of inhibition.
Minimum inhibitory concentration (MIC) is defined as the lowest concentration which results in maintenance or reduction of inoculums viability over a period of 24 hours. DMSO as a negative and Gentamycin as positive control were prepared in the mictrotiter well plates with Nutrent Broth as a diluents. The plates were incubated at 37˚C. The least concentration of extract or standard drug (Gentamycin) showing no visible growth after 24 hours was taken as MIC.

Antifungal activity:

The method of was adopted to evaluate the effect of *Ganoderma* extract on the growth of fungus. 20 ml of sterilized and cooled (40^0C) growth media (PDA) with desired concentration of antibiotic (Gupta and Banerjee, 1970) were poured into pre-sterilized petriplate. Requisite amount of different concentrations of extracts were added into the plates. The assay plates rotated clockwise and anticlockwise to ensure an even distribution of the extract into the

medium. In control plates the medium was subjected with respective solvents. After the solidification of agar medium, a disc (5 mm diameter) of test fungal strain from 7 days old culture was placed aseptically in the centre of each plate. The assay plates were incubated at 28±2^{0}C for 7 days. The experiment was run in triplicate.

Results

Result of the antimicrobial activity of different extracts of concentration (50 µg/ml) of G. lucidum was determined by disc diffusion and Poison food technique method as shown in the Table1.and Table 2
G. lucidum was found as the most effective and strong antibacterial when tested against a panel of human pathogenic and foods contaminating bacteria by using filter paper disc diffusion the antibacterial activity of methanolic acetone, acetone & Distilled water extract was found to be more sound. Inhibitory effect of these extract against Bacteria was also remarkable.

It is apparent from the table that acetone extract of the strain possessed strong antibacterial activity which was most inhibitory against E.coli (23.06±0.11). the acetone extract was equally inhibitory against *Bacillus subilis* (21.00±0.00) and *Salmonella typhi* greatly reduce in case of Pseudomonas aeruginsa (10.20 ± 0.05). Vibro cholera (17.60±0.05) and *Staphylococcus aureus* (18.00±0.11) at the same concentration methanolic extract of G. lucidum was equally inhibitory to all the bacterial strain and exhibited and antibacterial effect maximum inhibition halos were observed against E.coli (20.00 ± 0.23) and equally Staphylocous aureus (18.00 ±0.20) Bacillus subillis (18.00± 0.21). The water and ethanolic extract were found to be not much effective against all the strains.

Result of the antifungal activity of different concentration G. lucidum suppress the growth of pathogenic fungi differently. The acetone extract possess strong antifungal activity against 5 fungal strains which were tested by using poisoned food technique. More over, the antifungal activities of almost all the extracts were found to be concentration dependent. Water extract completely failed to inhibit 100% growth of any of the fungi even it was very high concentration (1000mg/ml) which has been tested.

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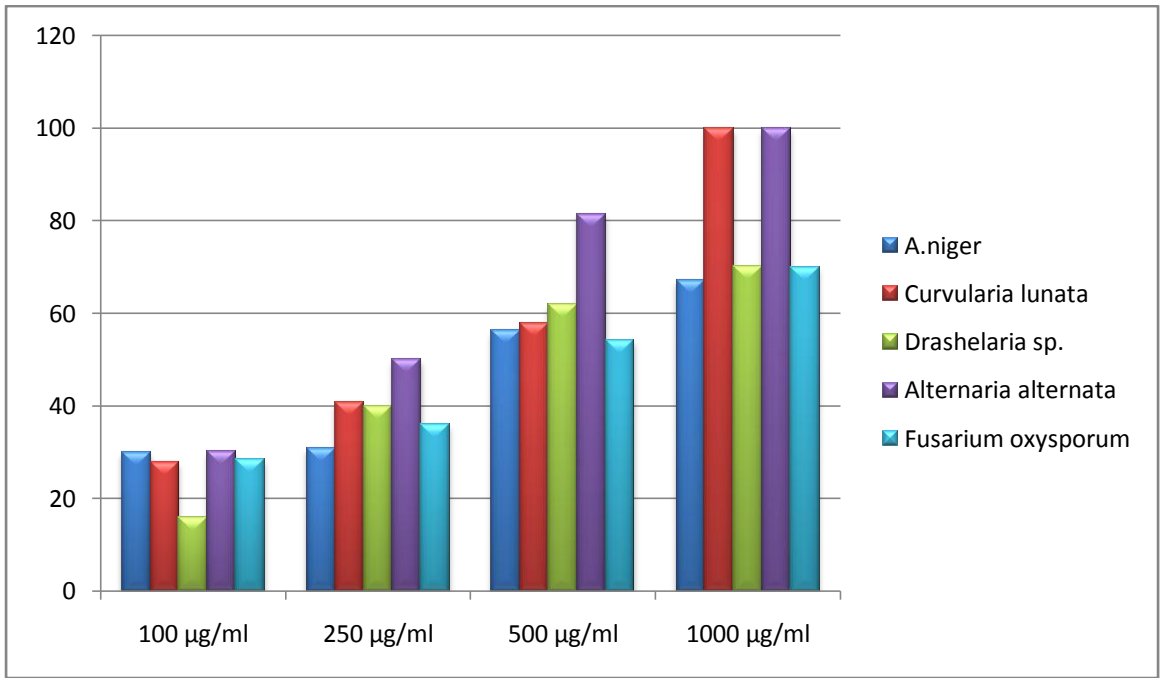
Table no.1 Evaluation of antibacterial activity of different extracts of G.lucidum by filter paper disc diffusion method

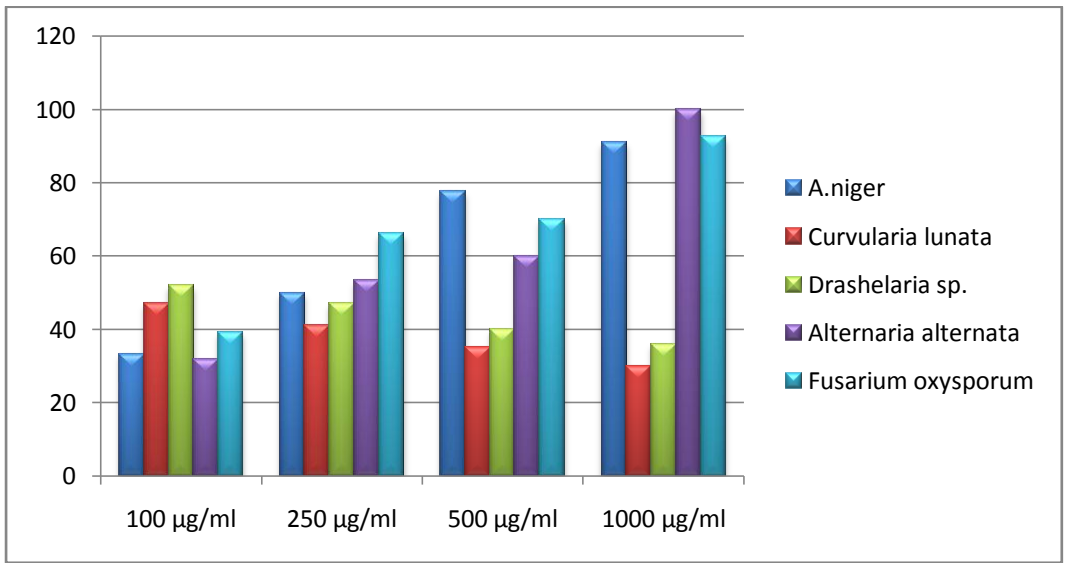
Test organism	Zone of inhabitation (mm)				
	Methanol (50 µg/ml)	Ethanol (50 µg/ml)	Acetone (50 µg/ml)	Distilled water (50 µg/ml)	Gentamycin sulphate (50 µg/ml)
Staphylococcus aureus (MTCC187)	18.00 ± 0.20	9.00 ± 0.15	18.00 ± 0.11	8.00 ± 0.04	31.00 ± 0.03
Bacillus subilis (MTCC1789)	18.00 ± 0.21	10.60 ± 0.37	21.00 ± 0.00	8.00 ± 0.33	30.00 ± 0.04
Pseudomonos aeruginosa (MTCC779)	10.00 ± 0.36	11.60 ± 0.15	10.20 ± 0.05	6.20 ± 0.32	32.00 ± 0.02
Vibrio cholera (MTCC1068)	15.80 ± 0.22	12.60 ± 0.20	17.60 ± 0.05	9.60 ± 0.25	29.80 ± 0.10
Salmonella typhi (MTCC531)	17.00 ± 0.12	10.00 ± 0.01	20.60 ± 0.19	7.00 ± 0.06	34.00 ± 0.01
E- coli (MTCC443)	20.00 ± 0.23	10.66 ± 0.30	23.60 ± 0.11	7.30 ± 0.20	32.30 ± 0.01

Graph no.2 Evalution of antifungal activity of different extract of *Ganoderma lucidum* by poison food method

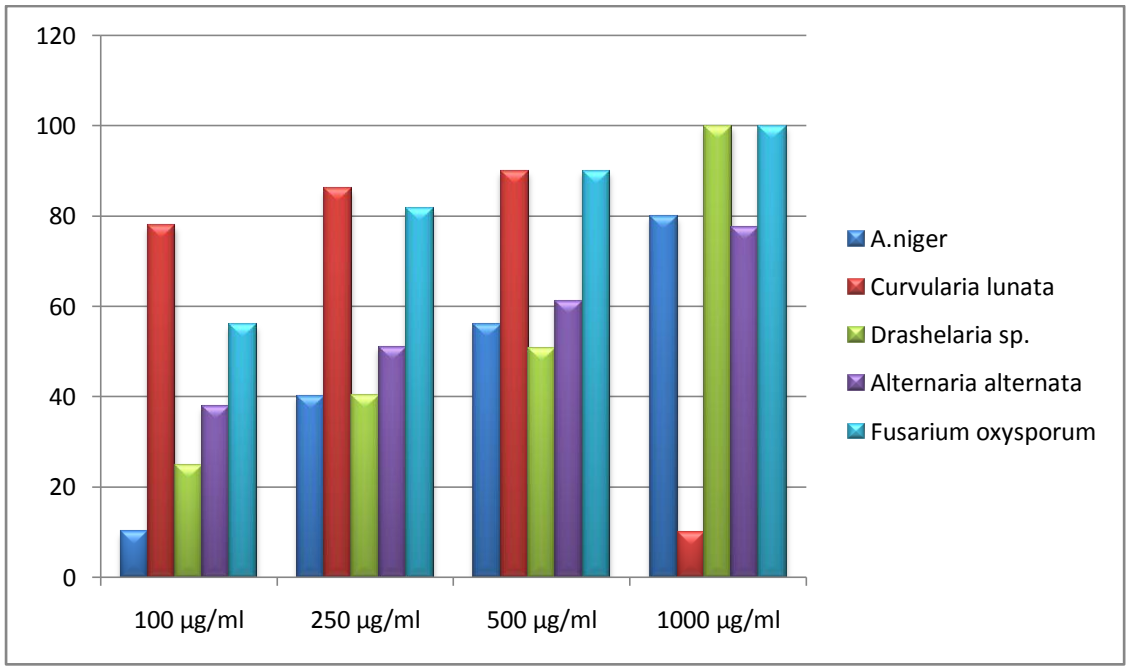
Graph no .2 (1) Evalution of antifungal activity of Methanolic extract of *Ganoderma lucidum* by food poison method



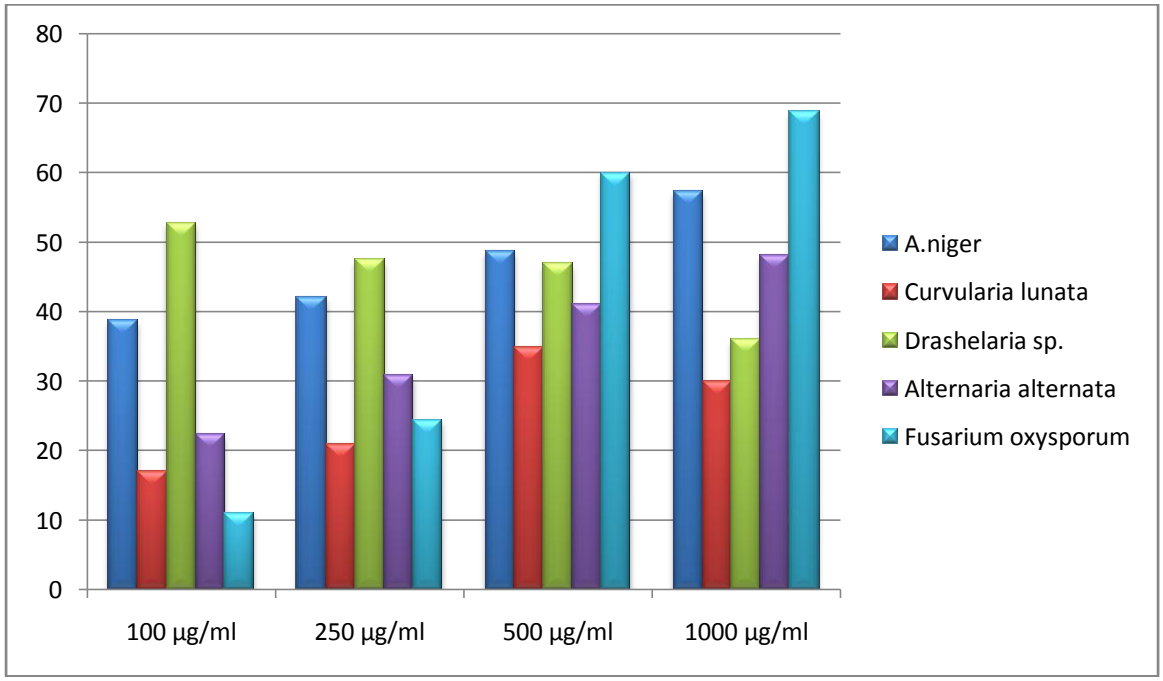
Graph no .2 (2) Evalution of antifungal activity of Ethanolic extract of *Ganoderma lucidum* by food posion method



Graph no .2 (3) Evalution of antifungal activity of Acetone extract of *Ganoderma lucidum* by food posion method



Graph no .2 (4) Evalution of antifungal activity of Water extract of *Ganoderma lucidum* by food poison method



Discussion

Chu *et al*., 2005 reported antifungal peptide designated pleurostrin which exhibited antifungal activity against F. oxysporum. *Alternaria alternata* and *Drashelaria* sp. A. variety of antifungal proteins and peptides have been isolated and purified from fruiting body of *Ganoderma* mushrooms.

Many antimicrobial compounds such as terpenes, lectins, polysaccharides etc. act on the bacterial cytoplasmic membrane (Lin & Chou, 1984; Yang *et al*, 2002). Various extracts of G. lucidum have been found to be equally effective when compared with gentamycin sulphate. (Dulger & Gonuz 2004) reported the antimicrobial properties of 4 different extracts of macrofungus (Cantharellus cibarius) against 50 important human pathogens. He observed good antimicrobial activity with ethanol and acetone extracts against most of the pathogens. Cowan (1999) reported that the most active components are generally water insoluble, hence it is expected that low polarity organic solvents would yield more active extracts. In the present study the aqueous extract exhibited least antibacterial activity than the organic extracts. According to Gao *et al* 2003, *Ganoderma lucidum* and other *Ganoderma* species more often in combination with chemotherapeutic agents have been used to treat various bacterial diseases. Its polysaccharide components were found to be the bioactive principle which play an important role in antibacterial activity. Smania *et al* (2007) observed maximum antibacterial activity of methyl australate, a derivative from G. lucidum against E.coli and P.aeruginosa followed by S. aureus. While least zone
of inhibition was recorded for Bacillus species.(Klaus & Miomir 2007) have studied the influence of various extracts isolated from G. lucidum on E. coli, Bacillus species, S. aureus and Salmonella species. The aqueous fruiting body extract showed maximum zone of inhibition against Bacillus species while least zone of inhibition was reported for E. coli and Salmonella species. (Yoon *et al* 1994) investigated the bioactivity of aqueous extracts from the fruiting body of G.
lucidum and found that the extracts also exhibited inhibitory activity towards Bacillus species. Extracts from G. applanatum (Smania *et al* 1999) and G. pfeifferi (Mothana *et al* 2000) have been shown to possess significant antibacterial activity against E. coli. (Sheena *et al* 2003) reported that methanol extract of G.lucidum showed remarkable antibacterial activity against E. coli, Salmonella species and B. subtilis. Keypour *et al* (2008) investigated the antibacterial activity of a chloroform extract of G. lucidum from Iran. The results of disc diffusion tests showed that
the acetone extract had growth inhibitory effects on B. subtilis and S. aureus.

Conclusion

The results from the present study supported the usage *Ganoderma* lucidum fruiting body as an ideal bio-pharmaceutics and suggested that the acetone and methanolic extract possessed strong antimicrobial activity. All the extracts in this study exhibited strong antifungal activity. This might be due to presence of rich phytochemical constituents such as phenols, flavonoids and ascorbic acid. This study is strongly suggestive that *Ganoderma* lucidum can be used as antimicrobial agent in the development of new drug for the different bacterial and fungal pathogenesis in humans

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