



Studies on Antimicrobial Potential of Different Strains of *Ganoderma lucidum* (Curt.: Fr.) P. Karst.

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ABSTRACT

Bioproducts of mushrooms 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. The fruit bodies of *Ganoderma lucidum* were collected from various places in Tamil Nadu. Invitro antagonistic effect of the ethanol, di ethyl ether, chloroform, distilled water extracts of these macro fungi were tested against some disease causing micro organisms. Different extracts of these *Ganoderma lucidum* species exhibited various degree of inhibition against the test organisms. The widest inhibitory zone (18mm) were obtained with ethanol, Diethyl ether, chloroform extracts of VAMNG strain V against *E.coli*, *B.subtilis*, while the highest in vitro antifungal activity (16.8mm) was observed in di ethyl ether extract of VAMNG strain V against *A.niger*. The lowest zone of inhibition (5mm) was demonstrated with the aqueous extract of VAMNG-II strain against *B.subtilis* while the lowest invitro antifungal activity (3mm) was observed in aqueous extract of VAMNG-I against *F.oxysporum*. The implications of these findings were discussed.

Keywords: *Ganoderma lucidum*, extract preparation, antimicrobial activity, strain.

INTRODUCTION

Ganoderma species are regarded as higher fungi because the carpophores are visible enough to be seen with naked eyes. Although, the real organism comprises of intercellular microscopic bodies which could not be visualized with ordinary eyes.^{1,2} *G.lucidum*, a mushroom, is one of the most famous traditional Chinese medicinal herbs. 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.³ The mushroom attracts international attention as a valuable herb due to the wide variety of its biological activities, such as antitumor, immunomodulatory, cardiovascular, respiratory, antihepatotoxic and antinociceptive (active against pain) effect.^{4, 5} It's major compounds with significant pharmacological activities are ganoderic acid, triterpenes and polysaccharides. It is interesting that during the last three decades; more than 150 triterpenes^{6, 7} and more than 50 carcinostatic polysaccharides have been isolated and are known to be unique compounds in this mushroom. Therefore, *G. lucidum* products with different triterpenes and polysaccharides or combinations of these two groups are most likely to result in different pharmacological activities.⁸

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 values⁹.

Usually *Ganoderma lucidum* as immune booster especially when combined with other medicinal ingredients. Despite the important medicinal uses of

Ganoderma in Nigeria, information on the bacteria and fungi that they specifically controlled is scantily in Literatures. Therefore, the objective of this work is to shed light on specific pathogenic microorganisms which could be inhibited by this group of medically important mushrooms.

MATERIALS AND METHODS

Collection

Fruiting bodies of *G.lucidum* was collected from various places around Thanjavur, Thiruvarur and Nagappatinam districts. Then it was transported to laboratory using a clean poly ethylene bag.

Powderization

The sporocarps were cut in to small pieces, dried at 40° C for 48 hours and powdered. In each step, the plant material was dried to remove moisture and overcome the fungal contamination. The air-dried powder was stored in an air tight container for further use.¹⁰

Extract Preparation

Various extracts of the experimental samples were prepared according to the methodology of Indian pharmacopoeia.¹¹

The chemical nature and physical state of the mushroom powder make it difficult to dissolve in distilled water. So, the plant materials were soaked in distilled water for 24hrs. One gram of powder was dissolved separately in 10ml ethanol, diethyl ether, chloroform and distilled water in cleaned screw cap bottle for 24hrs. After 24hrs the dissolved extracts from the bottles were transferred to centrifugal tubes and centrifuged at 3000rpm for 10min. The centrifuged extracts (supernatant) were again



re-centrifuged and filtered with millipore filter. The filtered solvents with dissolved chemicals were concentrated and stored separately in refrigerated at 4°C.

Antimicrobial Activity

This research was aimed at screening anti-bacterial potency of the *Ganoderma* species used. The microorganisms were sub cultured into Petri dishes and incubated for 24 hrs at 37°C for bacteria and 72 hours at 28°C for fungi to obtain actively growing cultures at exponential phase. Determination for antibacterial activities of water, methanolic and ethanolic extracts were carried out using agar well diffusion methods.¹²

Test Organisms

The stored culture of bacteria *Escherichia coli*, *Bacillus subtilis* and pathogenic fungal strains *Aspergillus niger*, *Fusarium oxysporum* were used.

Antibacterial Studies

Bacterial Media (Muller Hinton Agar Media)

Thirty Six grams of Muller Hinton Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes. The solidified plates were bored with 6mm dia cork borer. The plates with wells were used for the antibacterial studies.

Antifungal studies

Fungal media (PDA)

Two Hundred gram of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm dia cork borer.

Well Diffusion Method

The prepared culture plates were inoculated with different bacteria and fungus by using plate method. Wells were made on the agar surface with 6mm cork borer. 20µl extracts from the strain VAMNG-I was poured into the well using sterile micro pipette. The plates were incubated at 37±2°C for 24 hours for bacterial activity and 48 hours for fungal activity. The plates were observed for the zone formation around the wells. Likewise all the strains were used to determine their antagonistic effect. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

RESULTS AND DISCUSSION

Antimicrobial Activity

Many antimicrobial compounds such as terpenes, lectins, polysaccharides etc. act on the bacterial cytoplasmic membrane.^{13, 14} Various extracts of *G. lucidum* have been found to be equally effective when compared with

gentamycin sulphate. Antimicrobial properties of 4 different extracts of macrofungus (*Cantharellus cibarius*) against 50 important human pathogens.¹⁵

The most active components are generally water insoluble, hence it is expected that low polarity organic solvents would yield more active extracts.¹⁶ In the present study the aqueous extract exhibited least antibacterial activity than the organic extracts.

For ethanolic extract, highest antibacterial activity (18mm) was demonstrated by the strain VAMNG-V against *E.coli*, *B.subtilis* while the highest antifungal activity was (16.8mm) observed in di ethyl ether extract of VAMNG-V against *A.niger*. The lowest antagonistic effect of the aqueous extract was observed with *Ganoderma lucidum* VAMNG-1 against *F.oxysporum*.

Generally water extract of all the studied strains demonstrated lower values of inhibitory zone compared with di ethyl ether, ethanol, and chloroform.

Strain VAMNG-I and VAMNG-II collected from *Bambusa vulgaris* shows lowest inhibitory effect against the bacterium *B.subtilis* and fungus *F.oxysporum*.

Among the 10 strains of *Ganoderma lucidum*, VAMNG-V from the substrate of *Cocos nucifera* has shown the most antagonistic effect. Thus average inhibition is 13.86. Maximum zone of inhibition was exhibited for *E.coli* while least zone of inhibition was recorded for *F.oxysporum*.

From this study, it was seen clearly that all the ten strains of *G. lucidum* used demonstrated high level of antimicrobial activities in different proportions. These results affirm the claims of traditional herbalists in the south western Nigeria that *Ganoderma* species could be used to treat some bacterial and fungal infections of man. *Ganoderma* species especially *G. lucidum* could be used as feed supplement to resist microbial infections and boost immune system in human beings.¹⁷

Table 1: Collection of *G. lucidum* from various places of Thanjavur, Thiruvarur and Nagappatinam districts.

Location	Substrate	Strain no
Paeraiyur	<i>Bambusa vulgaris</i>	VAMNG-I
Mulaiyur	<i>Bambusa vulgaris</i>	VAMNG-II
Kudikadu	Red Soil	VAMNG-III
Cholapuram	<i>Morinda oleifera</i>	VAMNG-IV
Adudhurai	<i>Cocos nucifera</i>	VAMNG-V
Pandhanallur	<i>Syzygium cumini</i>	VAMNG-VI
Sirkali	<i>Musa paradisiaca</i>	VAMNG-VII
Ammachatthiram	Barks	VAMNG-VIII
Valangaiman	Barks	VAMNG-IX
Sundhara Perumal kovil	Alluvial Soil	VAMNG-X

It was observed that *G. lucidum* extracts behaved differently in their antimicrobial effectiveness depending on the solvent used for extraction.



Ganoderma lucidum and other *Ganoderma* species more often in combination with chemotherapeutic agents have been used to treat various bacterial diseases.¹⁸ Polysaccharide components were found to be the bioactive principle which plays an important role in antibacterial activity. The bioactivity of aqueous extracts from the fruiting body of *Ganoderma lucidum* and found that the extracts also exhibited inhibitory activity towards *Bacillus* species.¹⁹

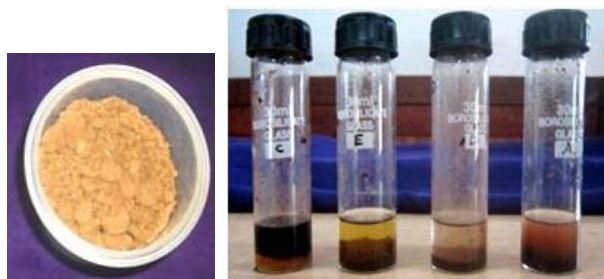


Figure 1: Powder & Extract of *G. lucidum*

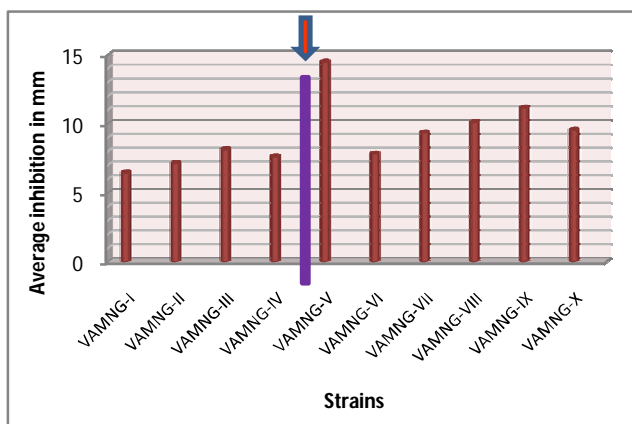


Figure 2: Flow chart of ten strains of *Ganoderma lucidum*

CONCLUSION

This piece of work demonstrates that mushrooms, similar to plants, have a great potential for the production of useful bioactive metabolites and that they are a prolific resource for drugs. The responsible bioactive compounds belong to several chemical groups very often they are polysaccharides or triterpenes. One species possess a high variety of bioactive compounds and therefore of pharmacological effects.

Resistance to antibiotics was emerging in a wide variety of organism and multiple drug resistant organisms pose a serious threat to the treatment of infectious diseases. Hence, mushroom derived antimicrobial substances have received considerable attention in recent years. It was apparent from the present study that mushroom extracts from *G. lucidum* could be employed to combat several diseases caused by pathogenic microorganisms.

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