



Collection, Identification, Phytochemical analysis and Phytotoxicity test of Wood inhabiting Fungi *Ganoderma lucidum* (Curt.Fr.)P.Karst.

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ABSTRACT

Plan: In this study 10 strains of mushroom *Ganoderma lucidum* were collected from various places and decided to screen their phytotoxicity activity and phytochemical constitution.

Preface: The popular mushroom *Ganoderma lucidum* (Reishi) was a bracket fungus and has been widely used for the promotion of health and longevity in Asian countries. The dried powder of *G.lucidum* was popular as a cancer chemotherapy agent in ancient China. *G.lucidum* clearly demonstrates anticancer activity in experiments with cancer cells and has possible therapeutic potential as a dietary supplement or alternative therapy for breast and prostate cancer. And also the fruiting bodies of *Ganoderma lucidum* (Polyporaceae) are a well known Chinese crude drug identified as a phytotoxic agent. However, since *G.lucidum* was available from different sources, it is advisable to test its biological activity.

Methodology: *G.lucidum* strains collected was isolated and identified with potato dextrose agar medium. Then the secondary metabolites were qualitatively studied through phytochemical analysis.

Outcome: Significant root length inhibition was observed at 100ppm and 200ppm. Similarly seed germination was also significantly inhibited at the concentration 100ppm and 200ppm extracts. The mean data of root length inhibition by ethanol extract in 100ppm was 0.393 and 0.208 in 200ppm. Likewise the mean data of seed germination inhibition by ethanol extract in 100ppm was 15 and 0.208 in 200ppm. Overall results supported that *Ganoderma lucidum* clearly supported the wider medicinal uses and established its anticancer activity.

Key words: Bracket fungus, *Ganoderma lucidum*, Phytochemicals, Radish seed, Seed Germination, Phytotoxicity.

1. INTRODUCTION

Medicinal mushrooms are mushroom used in the practice of medicine. Many species of mushrooms have been used in folk medicine for thousands of years.¹ Medicinal mushrooms are now the subject of study for many ethno botanists and medical researchers. It is estimated that there are approximately 1.5 million species of mushrooms in the world of which 70,000 species are described. About 10,000 of the known species belongs to the macro fungi of which about 5000 species are edible and over 1,800 species are considered to have medicinal properties².



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G.lucidum has been used for promotion of vitality, longevity, prevention and treatment of various human diseases in China and other Asian countries.³ It was used for the treatment of asthma, diabetes, altitude sickness, cardiovascular disease, AIDS and Cancer.^{4, 5} *G.lucidum* appears to be very safe since oral administration of the extracts does not display and toxicity^{6,7} and its merits have been investigated as a potential prophylactic agent for human health.⁸ *G.lucidum* and related species have the longest historical medicinal usage dating back at least four thousand year. In Japan it was called “keishi or mushroom of immortality”. Traditionally it has been used widely in the treatment of hepatopathy. The ability of some mushrooms to inhibit tumor growth and enhance some aspects of the immune system has been a subject of research for approximately 50 years.⁹

Lingzhi was the name of *G.lucidum* and its close relative *Ganoderma tsugae*. *G.lucidum* enjoys special veneration in Asia, where it has been used as a medicinal mushroom in traditional Chinese medicine for more than 4000 years, making it one of the oldest mushrooms known to have been used in medicine.¹⁰

The word lingzhi, in Chinese, means “herb of spiritual potency” and has also been described as “mushroom of immortality”. Because of its presumed health benefits and apparent absence of side-effects, it has attained a reputation in the East as the ultimate herbal substance.¹¹

The mechanisms by which *G.lucidum* may affect cancer are unknown and they may target different stages of cancer development: through inhibition of angiogenesis (formation of new, tumor-induced blood vessels, created to supply nutrients to the tumor) mediated by cytokines, cytotoxicity, inhibiting migration of the cancer cells and metastasis, and inducing apoptosis of tumor cells.¹²

2. MATERIALS AND METHODS

2.1. Collection

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

2.2. Isolation of *G. lucidum*¹³

Tissue pieces of *Ganoderma* fructification were surface sterilized with tap water and 0.1% mercuric chloride solution, then rinsed for 2min in distilled water. Fruiting bodies of *G.lucidum* isolates were inoculated onto potato dextrose agar (PDA) medium. After inoculation the fungal cultures were purified using pure culture technique and stock culture was maintained in PDA slants for further studies.

2.3. Identification

For confirmation of identification, taxonomic keys and descriptions were consulted¹⁴. Descriptions of basidiomycetes were made according to their macro, micro and cultural features by using standard manuals such as Manual of soil fungi¹⁵, Dematiaceous Hyphomycetes¹⁶. Colony color, morphology, hyphal structure, spore size and spore bearing structures were identified and compared.

2.4. Microscopical observation

Morphological observations mainly followed by different methods.¹⁷ Lacto phenol cotton blue staining was used as the mounting medium. Microscopic characters were observed using a light microscope. For microscopic observation dermic elements were carefully examined and measured in thin sections perpendicular to the pileus surface.

2.5. Grinding

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.¹⁸

2.6. Extract preparation

Various extracts of the experimental samples were prepared according to the methodology of Indian pharmacopoeia.¹⁹ 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.

Qualitative screening of phytochemicals from mushroom extract^{20, 21}

S.No	Phytochemicals	Chemicals added
1.	Tannins	2ml ethanol extract+3ml D.H ₂ O+ 2drops 5% FeCl ₃
2.	Alkaloids	200mg mushroom powder+10ml methanol+1% HCl
3.	Saponins	5ml extract+ 5ml D.H ₂ O
4.	Cardiac glycosides	2ml filtrate+1ml glacial acetic acid+ FeCl ₃ +Con.H ₂ SO ₄
5.	Terpenoids	2ml filtrate+2ml acetic anhydride+ Con.H ₂ SO ₄

The extracts were subjected to various tests to screen phytochemicals tannins, alkaloids, saponins, cardiac glycosides and terpenoids.

2.7. Radish seed phytotoxicity assay

To evaluate phytotoxic properties of extract of *Ganoderma lucidum*, radish seed phytotoxicity assay were done for this purpose.²²

2.8. For root length determination

Whatman No: 1 filter paper kept on petridish and 5ml extracts (100ppm and 200ppm) were added separately. Filter paper was dried at room temperature for reducing extra solvent. 5ml DDW was added and then 20 radish seeds were placed on petri dishes followed by tightly sealed and incubated at 23±2°C. Root length was measured after 1, 3 and 5 days of interval. Only DDW containing petridish was used as control. Each assay was carried out in three times.

2.9. For seed germination determination

This part of the determination was similar to that of earlier determination. Here also two concentration that (100ppm and 200ppm) were used. Germinated seeds were counted after every day up to 5 days. Each experiment was carried out in three times.

3. RESULTS AND DISCUSSION

Table: 1 collection of *Ganoderma lucidum* from various places.

S.No	Location	Substrate	Strain no
1	Paeraiyur	<i>Bambusa vulgaris</i>	VAMNG-I
2	Mulaiyur	<i>Bambusa vulgaris</i>	VAMNG-II
3	Kudikadu	Red Soil	VAMNG-III
4	Cholapuram	<i>Morinda oleifera</i>	VAMNG-IV
5	Aadudhurai	<i>Cocos nucifera</i>	VAMNG-V
6	Pandhanallur	<i>Syzygium cumini</i>	VAMNG-VI
7	Sirkali	<i>Musa paradisiacal</i>	VAMNG-VII
8	Ammachatthiram	Barks	VAMNG-VIII
9	Valangaiman	Barks	VAMNG-IX
10	Sundhara Perumal Kovil	Alluvial Soil	VAMNG-X

3.1. Microscopical structure of spores and hyphae of *G.lucidum*

Basidiomata annual, stipate brilliantly laccate, mycelia mat white to light cream scattered, more or less extensive, much branched thin walled hyphae often described as “witches broom”. Aerial mycelium and the lateral branches may become very short. Thick walled hyaline, branched aseptate 1.5-3.0mm, Ellipsoid to avoid, $8.5-12.5 \times 5.5-7.5\mu\text{m}$. In these findings the microscopical structure of mycelium was aerial and has thick walled hyaline.

3.2. Morphological identification

The structure of the pileal crust and cortex are usual characters in the taxonomy of Ganodermataceae. The former character occurs mainly in *Ganoderma* and *Amauroderma*. Fruitbodies of *Ganoderma* mostly have hymenoderm, and anamixoderm.²³ For species identification, however, hyphal characters are often useful.²⁴ The naturally produced basidiocarps of *G.lucidum* shows various morphological characteristics; sessile, stipitate, imbricate, non- imbricate.

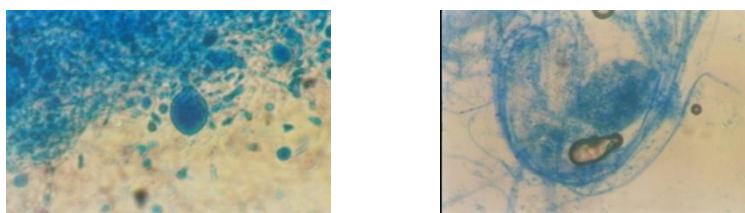


Fig: 1.Spores and hyphae Of *G.lucidum*



Fig. 2. Fruiting body of *G.lucidum*



Fig. 3. Pure culture of *G.lucidum*

G.lucidum was a basidiomycete's mushroom with pores underneath the cap. The basidiocarp was dark red to brown with a lacquered appearance but often cream or yellowish towards the margin, corky or woody and circular to semicircular to fan shaped. The stalk was dark brown to black, hard and eccentric, but sometimes the stalk was absent. The hymenophores compose of tiny circular pores, white and smooth surface. The spore was ovoid with double layer.

3.3. Cultural identification

Growth rate moderate to rapid 1.5-3.5/week, covering petridish in 12-15 days Advancing zone white hyaline even appressed. Mycelial mat white to light cream, scatered, more or less extensive. Hyphae in the advancing zone hyaline, thin walled. Texture of mycelia mat appressed farinaceous felty.

Table: 2 Phyto chemical analyses on *G.lucidum* extract

S.No	Phytochemicals	Observation	Inference
1.	Tannins	Blue black precipitate	(+)
2.	Alkaloids	Brownish red precipitate	(+)
3.	Saponins	Froth formation	(-)
4.	Cardiac glycosides	Green blue precipitate	(+)
5.	Terpenoids	Blue green ring	(+)

(+) Indicates presence of phytochemicals (-) Indicates absence of phytochemicals

Preliminary phytochemical screening of *G.lucidum* unprocessed powder, reveals the presence of phytochemicals which are absent in the fractionated extracts, this was a possible explanation to metabolites formation in the body when extract of this mushroom was ingested and formed metabolites may be responsible for the bioactive properties of this mushroom.²⁶ In these study alkaloids, cardiac glycosides, tannins, terpenoids shows positive results and saponin shows negative results. The presence of alkaloids in the mushroom powder explains anti-bacterial activity; since this phytochemical was reported to have anti-bacterial activity. The presence of tannins which can complex with the metal ions and macromolecules such as proteins and carbohydrates obtained in the powdered sample can be utilized in weight reduction management.²⁷ Mushroom extracts of *G.lucidum* were prepared and phytochemical analysis under taken using various methods to identify the constituents of the mushroom²⁸.

Triterpenoids are the bitter tasting phytochemical which gives the extract its bitter taste, the terpenoids are said to form complexes with steroids to provide the said anti-inflammatory effects of this wild mushroom and equally, its anti-bacterial activity. Presence of these phytochemical elements indicates that Indian medicinal mushrooms like *G.lucidum* are potential sources of anti-tumor activity. *G.lucidum* possess profound antioxidant and anti tumor activity. In these study alkaloids, cardiac glycosides, tannins, terpenoids shows positive results and saponin shows negative results.

3.4. Radish seed Phytotoxicity assay & Seed germination

Table 1, 2 and Fig 1, 2 shows the inhibitory effect of *G.lucidum* against radish seeds. Statistical analysis proved that root length was significantly inhibited by the extracts at both the concentrations 100ppm and 200ppm.

In another cases, seed germinations were also significantly inhibited by the both concentrations 100ppm and 200ppm. These results were analyzed compared with control. These observations may be accomplished due to the presence of active biological compounds.

Table: 3. Analysis of mean data of root length inhibition by ethanol extract of *Ganoderma lucidum*

Variables & concentration	root length(mm)	Days	Day 1	Day 3	Day 5
Control	14.315	Control	8.56	13.63	20.75
100ppm	0.393	100ppm	0.25	0.37	0.59
200ppm	0.208	200ppm	0.08	0.21	0.33

Table: 4 Analysis of mean data of Seed Germination, inhibition by ethanol extract of *G. lucidum*

Variables & Concentration	%Seed Germination	Days	Control	100ppm	200ppm
Control	85	Day 1	7	0	0
100ppm	15	Day 2	9	0	0
200ppm	0.208	Day 3	12	1	0
		Day 4	14	2	1
		Day 5	17	3	2

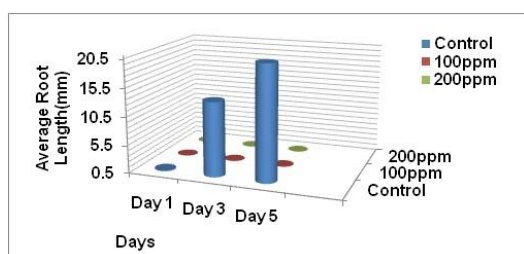


Fig.3

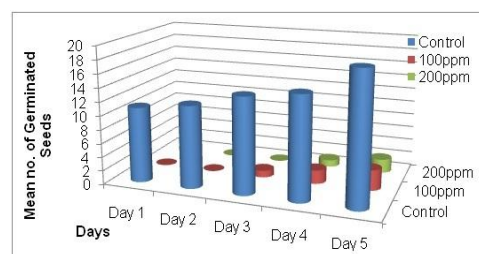


Fig.4

Fig: 3. Histogram shows regular root length inhibition by the ethanol Extract at two different concentrations (100ppm and 200ppm) of *G. lucidum*. Data compared with control.

Fig: 4. Histogram shows phytotoxicity assay on radish seed germination percentage at two different concentrations (100ppm and 200ppm) of *G. lucidum*. Data compared with control.

4. CONCLUSION

Since 1970s numerous mushroom fungi have been increasingly used as a source of medicinal compounds and therapeutic adjuvants or health food supplements. It contains phytochemical compounds and also the phytotoxicity assay may be accomplished due to the presence of active biological compounds. In drug discovery, the major secondary metabolites are of potential medicinal interest.

Drug discovery is the key attempt of our age to overcome many life-threatening diseases like cancer. Plant-based compounds have been playing an important role in the development of several clinically useful anticancer agents i.e., including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan and etoposide derived from epipodophyllotoxin.²⁹

Different studies have shown many secondary metabolites as a source of bioactive compounds with allelo chemical potential have great chemical diversity and are involved in many metabolic and ecological processes.³⁰

In drug discovery, the major secondary metabolites (terpenoids, phenolics and alkaloids) are of potential medicinal interest. The mentioned structure diversity is reflected in a variety of biological activities as, for instance, inhibitors of enzymes and antitumor, immunosuppressive and anti parasitic agents.³¹

These findings may be attributed to the nature of biological active compounds and their strong solubility with appropriate solvent. The clinical study indicated that *G.lucidum* was well tolerated and could improve the immunity. However, sometimes it is often better to use alcohols or hydro alcoholic solutions after partial lipid removal³². Many researchers have already been used ethanol as a solvent for evaluating cytotoxicity, phytotoxicity, antibacterial, antitumor activity in several plant species.^{33, 34} However, intensive and extensive investigations are needed to establish their therapeutic potential. Further study is required for isolating specific compound.

Ganoderma lucidum is the valuable medicinal mushroom for its multipurpose uses. The present study has been undertaken to identify this plant as a source of phytotoxic agent for giving a basic platform for anti cancer studies. Specifically ethanol extract of *G.lucidum* has been studied using different bioassay for this purpose³⁵.

The experimental findings reveal that *G.lucidum* occurring in South India has potential effect for anticancer activity. At present we are collaborating with another laboratory to study the potential of *Ganoderma lucidum* extract in complimentary cancer therapy through a clinical study of cell lines and Wister albino rats analysis.

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