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Studies on Analysis of few secondary metabolites and antimicrobial activity of *Ganoderma lucidum*

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ABSTRACT

Objective: To evaluate the phytochemical studies and antimicrobial activity study the secondary metabolites of *Ganoderma lucidum* using HPTLC analysis and also assess the antimicrobial activity of selected mushroom against the selected pathogens of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumoniae*. **Methods:** *Ganoderma lucidum* was collected in our College Campus during rainy season. Phytochemical analysis of flavonoids and phenolic compounds were analysed by HPTLC method. Further the powder of *Ganoderma lucidum* was used by antimicrobial activity was evaluated against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumoniae.* Acetone, ethanol and methanol used as extractive solvents. **Results** Six kinds of flavonoids and four kinds of phenolic compounds were reported in *Ganoderma lucidum*. Maximum antibacterial activity of crude extracts of methanol extracts *Ganoderma lucidum* (24 ± 0.666mm) shows high level of antibacterial activity against *Klebsiella pneumoniae.* . Acetone and ethanol extracts shows good antibacterial activity (17±0.666mm, (20±0.577) respectively, against *Pseudomonas aeruginosa, Escherichia coli*. Gram negative bacteria such as *Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae* were most susceptible than the gram positive bacteria (*Staphylococcus aureus*). The zones of inhibition in both the extracts are less than the methanolic extracts against the pathogens. **Conclusion** *Ganoderma lucidum* is now mostly used in neutraceuticals products throughout the world. Further investigation is also required to go through the detailed analysis of bioactive compounds in mushroom.

KEYWORDS*:* HPTLC- High Performance Thin layer Chromatography, *Ganoderma lucidum, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae.*

1. INTRODUCTION

Mushroom belongs to a group of plants known as fungus, which are separated from other plants and animals. Mushrooms can be defined as macrofungus with a distinctive fruiting body which can either epigeous or hypogeous. Many of the currently available antifungal drugs have undesirable side effects and lead to the rapid development of drug resistance causing profound effect on human health. Traditional medicine has a huge treasury of herbs and remedies that can be tapped as a source for obtaining antifungal agents from the plant, animal and fungal kingdom after scientific research. Mushrooms need to produce antibacterial and antifungal compounds to survive in their natural environment. Antimicrobial compounds isolated from many mushroom species proved to be a benefit for humans. Like plants, mushrooms accumulate a variety of secondary metabolites including phenolic compounds, polypeptides, terpenes and steroids [1]. Mushrooms have been reported as therapeutic foods that are useful in preventing diseases such as hypertension, hypercholesterolemia and cancer due to their chemical composition [2].

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Ganoderma lucidum is a Basidiomycetes fungus belonging to those family polyporaceae. *Ganoderma lucidum* as a medicinal mushroom for its antimicrobial, antiviral properties. [3] *Ganoderma lucidum* contain a variety of chemical substances, terpenoids and more than 100 types of polysaccharides. The major compounds such as ganoderic acid, triterpenes and polysaccharides are having more potential therapeutic values [4].

Ganoderma lucidum has been reported to contain many immuno regulating compounds and called as longevity mushroom in Korea. Low molecular weight fraction of *Ganoderma lucidum* showed significant anti HIV activity without affecting host T-cells [5]. *Ganoderma lucidum* used as neutraceutical, a new class of compounds with potential therapeutic values which are extracted from mycelium or fruiting bodies of

mushrooms [6]. Recently glycoprotein (containing 82.8% carbohydrate and 17.2% protein) obtained from the mycelium culture broth of *Ganoderma lucidum* exhibited an increase in the antitumor activity capacity of mice. The extracts from different sources have different functions. It has been widely reported that the antitumor and anticancer Effects of the polysaccharides are based on the enhancement of the host's immune system rather than direct cytocidal effects.[7]

2. MATERIALS AND METHODS

2.1 Collection of Mushroom

The selected mushroom of *Ganoderma lucidum* was collected from the fence region of animal house of our College during the rainy season. It was collected from the living tree of the exposed root region of *Albizia lebbeck* L.Wild. (Mimosaceae) by hand picking.

2.2 Identification of Mushroom

The collected mushrooms were identified by the help of morphological characters of the colour, size, shape, shining appearance, texture, hardness, margin, concentric rings, striations and pores in the ventral side of the basidiocarp. The mushrooms were identified by the artificial key to differentiate the species by [8, 9, 10, 11]

2.3 Preparation of Mushroom Powder

The fresh fruit bodies of *Ganoderma lucidum* was manually cleaned with a small painting brush to remove all extraneous particles and sliced into smaller pieces by the help of knife. Later it was further dried at 45-60^{0}C for 48 hrs in hot air oven. Then the dried mushroom was ground into powder. The mushroom powders were carefully stored in polythene bag and then it was analysed for phytochemical analysis and antibiotic assay.

2.4 Phytochemical studies

Phytochemical screenings for major constituents were under taken using standard qualitative methods. [12]

2.5 Analysis of Phenolic and Flavonoid Content

The mushroom powder was subjected for the analysis of flavonoid and phenolic content by HPTLC techniques.

2.6 Preparation of mushroom extracts

One gm of mushroom powder was mixed with 10 ml of acetone, ethanol, methanol separately in separate beaker and it was shaked for 24 hrs [13]. The extract was filtered through what man No.1 filter paper and then it was placed in the rotatory evaporator vaccum for 15 minutes at 37^{0}C. Then the residue was dissolved with 10 ml of dimethyl sulfoxide and stored at 40^{0}C for further analysis.

2.7 Selection of microbes

The human pathogens of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumoniae* were obtained from the Department of Microbiology, ANJA College, Sivakasi. The above selected organisms were characterized by the following staining, motility and biochemical tests.

2.8 Assessment of antibacterial activity (well diffusion method)

Antibacterial activity was evaluated by well diffusion method by [14] using the solvents of acetone, ethanol and methanol. The four wells were prepared in the medium using sterile cork borer (5mm) carefully without damaging the agar. The four wells were loaded with control using respective solvent, standard antibiotic, 100µl and 150µl of solvent extract respectively. Likewise separate plates were prepared with separate wells for the solvents of acetone, methanol and ethanol for selected microorganism. The diameter of the zone of inhibition was measured in mm using scale. After the 24 hours of incubation the antibiotic assay was assessed separately for acetone, methanol and ethanol extracts of selected mushroom for separate microbes.

2.9 Statistical analysis

All the results of zone of inhibition against the selected microorganisms were statistically analysed with F test using one way ANOVA.

3. RESULTS AND DISCUSSION

The preliminary phytochemical tests result indicates the presence of phenolic compounds, flavonoids, coumarins, tannin, sugar and alkaloids (Table-1). It is positively correlated with the findings of [13]. A wide range of potentially beneficial phenolic compounds could be natural substrates for oxidative enzymes such as peroxidases or polyphenol oxidases which are present in high levels in mushrooms [15]. *Ganoderma lucidum* having different triterpenes and polysaccharides. The *Ganoderma lucidum* have many biologically active components like triterpenes, polysaccharides, ganoderic acids. In addition to the biological activities its shows antimicrobial, antiviral, antioxidant, antitumour, immunomodulatory and anticancer properties [16]. The *Fomes fomentarius* shows secondary metabolites such as phenols, flavonoids and tannins etc. [17] Very few bioactive proteins such as lectin and a ribonuclease have been isolated from *Ganoderma lucidum* [18]. *Ganoderma lucidum* products with different triterpenes and polysaccharides are most likely to result in different pharmacological activities.

Table1: Kinds of secondary metabolites in the selected mushroom (Qualitative methods)

Secondary metabolites	*Ganoderma lucidum*
Phenolic compounds	+
Flavonoids	+
Coumarins	-
Tannin	+
Sugar	+
Alkaloids	+

Key words: **+** indicates presence; **-** indicates absence

The methanolic extracts of mushroom powder samples were subjected to analyse the secondary metabolites of flavonoids and phe-

nolic substances by using HPTLC methods. The result of flavonoid contents were given in the Table-2. As per the results, *Ganoderma lucidum* having 6 kinds of flavonoids with peak area of 1130.1, 707.9, 1234.5, 352.5, 2971.3, and 10096.6 (Fig-1) Among the 6 types, the 6 th flavonoids become high (10096.6) peak area and the 4th flavonoids become less (352.5) peak area .In this mushroom unknown, compounds are not reported.

Table 2: Peak area of flavonoid content in *Ganoderma lucidum*

Track	Peak	Height	Area	Assigned substance
Ganoderma lucidum	1	271.7	1130.1	Flavonoid 1
Ganoderma lucidum	2	31.2	707.9	Flavonoid 2
Ganoderma lucidum	3	30.9	1234.5	Flavonoid 3
Ganoderma lucidum	4	15.1	352.5	Flavonoid 4
Ganoderma lucidum	5	57.2	2971.3	Flavonoid 5
Ganoderma lucidum	6	217.0	10096.6	Flavonoid 6

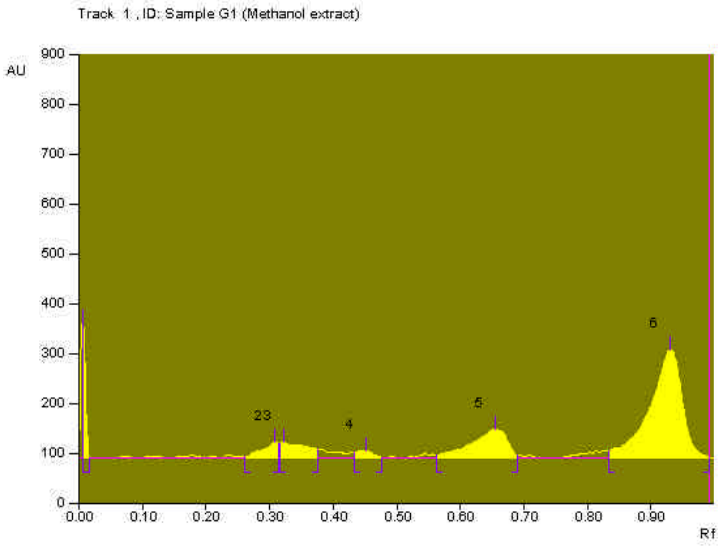

Figure 1: Densitogram display of methanolic extracts of *Ganoderma lucidum* for detection of flavonoid compounds.

As per the HPTLC result, *Ganoderma lucidum* reported (Table-3) the presence of four kinds of phenolic compounds ie 1 to 4 with the peak area of 791.3, 178.8, 1899.2, and 8255.6. (Fig.2)Highest peak area of 8255.6 found in 4th phenolic compound. However, it also reported the unknown compounds with peak area of 650.9, 937.2 and 13942.5. The unknown compounds with highest peak area of 13942.5 which is more than the peak area 4th kinds of phenolic compounds.

Table 3: Peak area of phenolic content in *Ganoderma lucidum*

Selected mushroom	Peak	Height	Area	Assigned substance
Ganoderma lucidum	1	38.2	791.3	Phenolic 1
Ganoderma lucidum	2	11.3	178.8	Phenolic 2
Ganoderma lucidum	3	650.9	650.9	Unknown
Ganoderma lucidum	4	1899.6	1899.2	Phenolic 3
Ganoderma lucidum	5	8255.6	8255.6	Phenolic 4
Ganoderma lucidum	6	937.2	937.2	Unknown
Ganoderma lucidum	7	13942.5	13942.5	Unknown

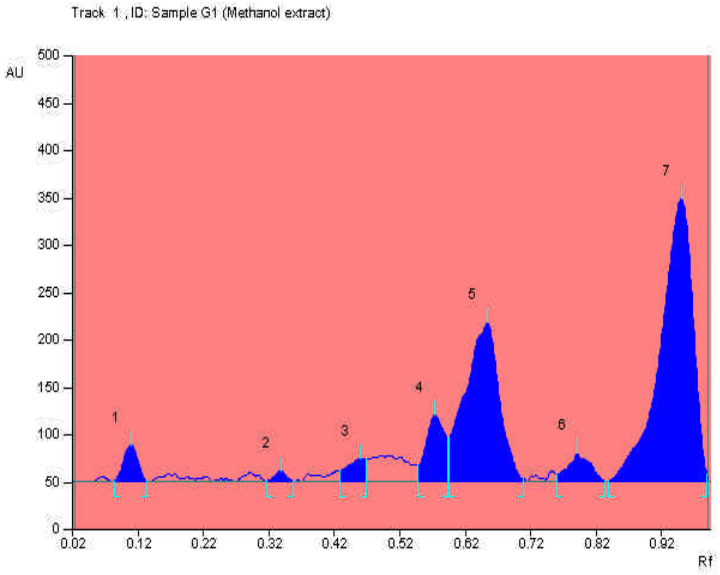

Figure.2 Densitogram display of methanolic extracts of *Ganoderma lucidum* for detection of phenolic compounds.

It is positively correlated with the findings of [13] Majority of the fungi produce similar secondary metabolites. *Ganoderma lucidum* contain polysaccharide which can be useful as natural health promotor against bacteria, parasites and viruses. The mushroom revealed the presence of saponins, alkaloids flavonoids and tannin [19, 20].

The mushroom powders are used for the assessment of antibacterial activity by using the solvents of acetone, ethanol and methanol. The similar type of various solvents used by various mushroom powders. [21, 22] Acetone, ethanol, methanol by [23]. Ethyl acetate, ethanol, acetone and volatile oil extracts are also analysed by [24]. Methanolic and alcoholic extracts are worked by and [25].

Antimicrobial activity was analysed by well method and agar disc diffusion method using Muller hinton agar medium [26]. The results of the zone of inhibition in Acetone, ethanol and methanol extracts of *Ganoderma lucidum* is given in the (Table-4 and Fig.3, Fig.4,Fig.5) In well method, the methanol extracts of *Ganoderma lucidum* have high level of antimicrobial activity (24 ± 0.666mm) at 150 µl concentration against *Klebsiella pneumoniae*. It is positively correlated by the findings of [27] the moderate activity was displayed by acetone and ethanol extracts (17±0.666mm, (20±0.577) at 150 µl concentration respectively, against *Pseudomonas aeruginosa, Escherichia coli*. The zones of inhibitions were also moderately differ from each other when compare with control and standard antibiotics. As per the comparative results, (Fig.6) methanolic extract of *Ganoderma lucidum* showed remarkable antibacterial activity against *Escherichia coli*. This similar work was supported by [22] because it may contain the solubility of the secondary metabolites of alkaloid, flavonoids, phenol, etc.

Table 4: Comparision of zone of inhibition against the slected microbes in the various solvent extracts of *Ganoderma lucidum*

Pathogens used	*Ganoderma lucidum* (Zone of inhibition in mm)					
	Acetone		Ethanol		Methanol	
	100µl	150 µl	100µl	150 µl	100µl	150 µl
Escherichia coli	***13mm±0.577***	***15mm±0.881***	***9mm± 0.577***	***20mm±0.577***	***13mm±0.666***	***22mm±0.881***
Pseudomonas aeruginosa	7mm±0.333	17mm±0.666	7mm±0.333	15mm±0.577	7mm±0.333	18mm±0.577
Staphylococus aureus	8mm±0.333	16mm±0.333	6mm±0.577	14mm±0.333	10mm±0.577	16mm±0.881
Klebsiella pneumoniae	9mm±0.333	15mm±0.881	8mm±0.333	17mm±0.577	14mm±0.333	24mm±0.666

± *indicates the standard error, N=5.*

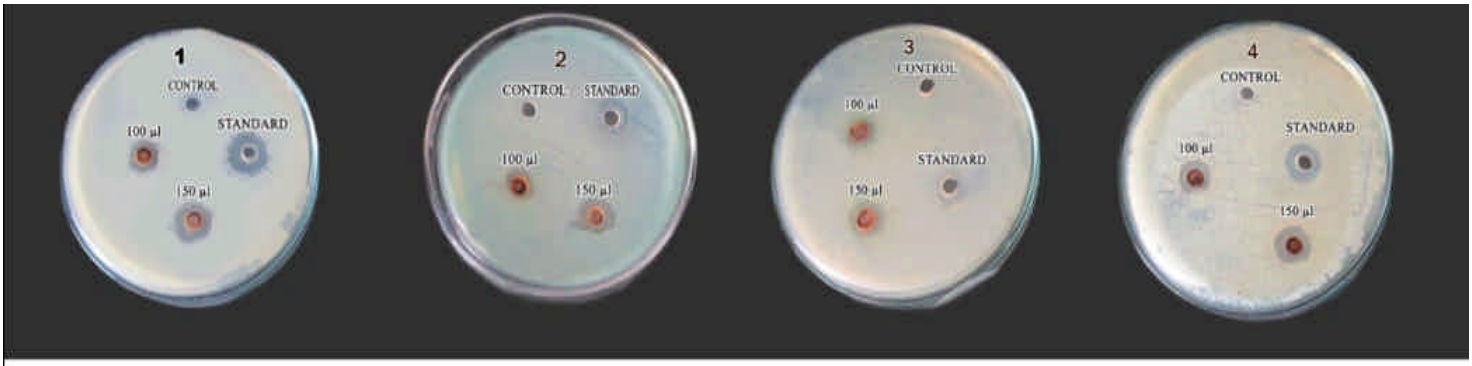

Figure 3 : ANTIBIOTIC ASSAY - ZONE OF INHIBITION IN ACETONE EXTRACTS OF *GANODERMA LUCIDUM* AGAINST SELECTED PATHOGENS

1. *ESCHERICHIA COLI,2.PSEUDOMONAS AERUGINOSA 3.STAPHYLOCOCCUS AUREUS 4.KLEBSIELLA PNEUMONIAE.*

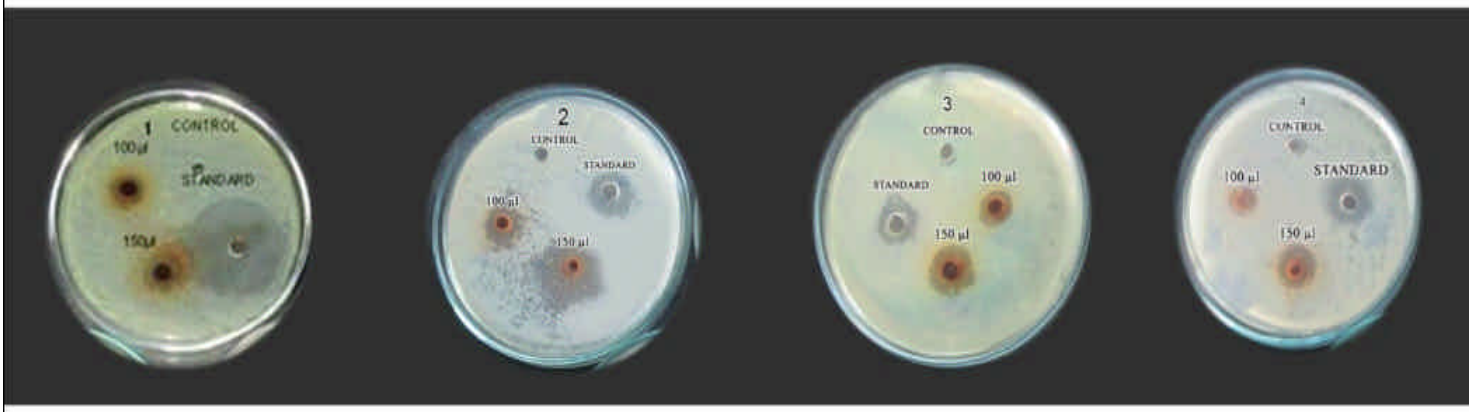

Figure 4 : ANTIBIOTIC ASSAY - ZONE OF INHIBITION IN ETHANOL EXTRACTS OF *GANODERMA LUCIDUM* AGAINST SELECTED PATHOGENS

1. *ESCHERICHIA COLI,2.PSEUDOMONAS AERUGINOSA 3.STAPHYLOCOCCUS AUREUS 4.KLEBSIELLA PNEUMONIAE.*

Figure 5 : ANTIBIOTIC ASSAY - ZONE OF INHIBITION IN METHANOL EXTRACTS OF *GANODERMA LUCIDUM* AGAINST SELECTED PATHOGENS

1. *ESCHERICHIA COLI,2.PSEUDOMONAS AERUGINOSA 3.STAPHYLOCOCCUS AUREUS 4.KLEBSIELLA PNEUMONIAE.*

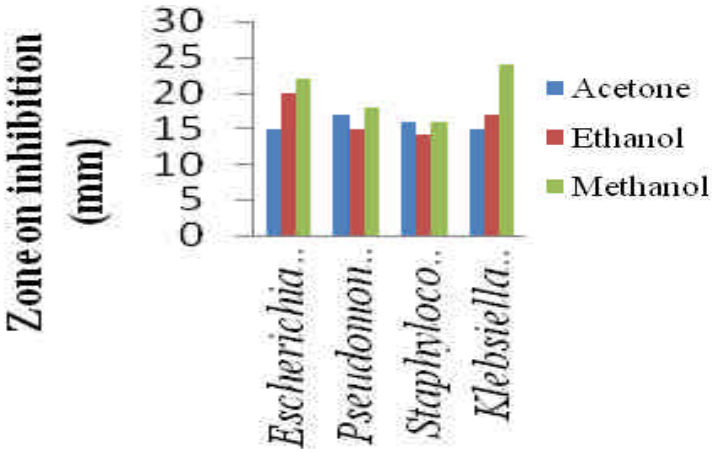

Figure 6: Antibiotic assay – Zone of inhibition against selected microbes in the selected solvent extracts of *Ganoderma lucidum* at 150µl concentration.

As per the statistical analysis the zone of inhibition in the solvent extracts of methanol shows significant mean values against the *Ganoderma lucidum* and selected pathogens found to have an appreciable effect of antibiotic activity.

4. 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 etc., One species possess a high variety of bioactive compounds and therefore of pharmacological effects. The best example is *Ganoderma lucidum*, which not only contains > 120 different triterpenes but also poly saccharides, proteins and other bioactive compounds. The spectrum of detected the pharmacological activities of mushrooms is very broad. Dependent on increasing knowledge about chemistry, biotechnology and molecular biology of mushrooms as well as an improvement of screening methods, a rapid increase in the application of mushrooms for medicinal purpose can be expected. In the opinion of chang, mycelial products are "wave of the future" because they ensure standardized quality and year around production. Therefore the *Ganoderma lucidum* is now mostly used in neutraceuticals products throughout the world. Further investigation is also required to go through the detailed analysis of bioactive compounds in mushroom. Each and every mushroom are having specific characters of distribution toxicity and phyto chemistry which may leads to the way for the production of new kinds of drugs.

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