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Nutritional and Biochemical Pattern of *Ganoderma lucidum* Powder Ethanol Extract

T. Pushpa*[1], G. Senthilkumar[2] and V. Ampikapathi[3]

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A B S T R A C T

Ganoderma lucidum is one of the most important medicinal mushrooms utilized as food, feed and as medicine since time immemorial. In the current scenario, this mushroom is utilized as an antioxidant, anti-inflammatory, anticancer and antimicrobial agents. In this study *Ganoderma lucidum* Powder is used to study proximate analysis, secondary metabolite analysis by preliminary chemical analysis, quantitative analysis method, FT-IR and GC-MS methods. The results of present study revealed that powder extract contains carbohydrates, crude fat, proteins, minerals and secondary metabolites. FT-IR result showed different alkene and multiple chromophores. GC-MS chromatogram of the ethanol extract showed 15 peaks indicating the presence of fifteen compounds with the retention time range between 10.035 and 40.105. The nutritional values of the mushroom species studied here could potentially be used in well-balanced diets and as sources of bioactive compounds. Further studied are needed to confirm bioactivity of phytochemicals like steroids, flavonoids, alkaloids and phenolic compounds present in the extracts of *Ganoderma lucidum*.

***Key words*:** *Ganoderma lucidum*, Proximate analysis, Secondary metabolites, FT-IR and GC-MS

Ganoderma lucidum is one of the basidiospore bearing polypore fungi predominantly grows in tropical regions. It is a double walled fungus with efficient antimicrobial properties. This fungus belongs to the family Ganodermataceae [1]. Now a days, people suffer from varieties of metabolic disorders as well as microbial infections. Imbalance of body's metabolites leads to reduced immune activities. Incidence of cancer, metabolic disorders like diabetes mellitus, fungal infections, MDR bacterial infections are increased day by day [2-3]. To overcome these problems peoples using drug from the nature, in this line *Ganoderma lucidum* is selected and studied for its chemical potentials. This fungus is considered as a medicinal fungus and supported with few scientific studies. This study concentrates on the chemical and nutritional potential of *Ganoderma lucidum* powder with reference to primary metabolites, secondary metabolites along with proximate analysis like moisture, ash, carbohydrates proteins and lipids.

MATERIALS AND METHODS

Collection of Ganoderma lucidum

* **T. Pushpa**
✉ ammupushpa4390@gmail.com

[1-2] Department of Microbiology, AVVM Sri Pushpam College (Affiliated to Bharathidasan University, Thiruchirappalli), Poondi, Thanjavur - 613 503, Tamil Nadu, India

[3] P.G. Department of Botany, AVVM Sri Pushpam College (Affiliated to Bharathidasan University, Thiruchirappalli), Poondi, Thanjavur - 613 503, Tamil Nadu, India

Ganoderma lucidum was collected as wild from the paddy fields of Thiruvarur (District), Tamil Nadu and identified and authenticated in the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. The selected strains were multiplied on potato dextrose agar (PDA) petri plates and slant culture was also maintained for further analysis.

Preparation for mushroom ethanol extract

Ten grams mushroom powder was mixed with 50 ml of ethanol in a beaker and it was placed on a shaker for 24 hours. The aqueous solutions were filtered through Whatman (No.1) filter paper and then it was placed on the rotary evaporator vacuum, for 15 minutes at 37 °C. Then the residue was dissolved with 10 ml of dimethyl sulfoxide and stored at 40 °C for further analysis [4].

Proximate analysis of ganoderma powder

Moisture content, lipid content, Protein, Ash Content, total Carbohydrate were assessed using standard methods [5]. A NOVA 400 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) with an air/acetylene flame and respective hollow-cathode lamps was used for absorbance measurements. Wavelengths, slits and lamp current used for the determination of six elements were 213.9 nm, 0.5 nm, 4.0 mA (zinc); 422.7 nm, 1.2 nm, 4.0 mA (calcium); 324.8 nm, 1.2 nm, 3.0 mA (copper); 589.0 nm, 0.8 nm, 3.0 mA (sodium); 248.3 m, 0.2 nm, 6.0 mA (iron) and 766.5 nm, 0.8 nm, 4.0 mA (potassium), respectively. The results for mineral contents were expressed as mg/100 g DW [6].

Qualitative secondary metabolite analysis

Preliminary phytochemical like tannins, saponins, flavonoids, terpenoids, steroids, triterpenoids, alkaloids, anthraquinones, polyphenols cardiac glycosides coumarins were analyzed by using standard procedure Lala [7], Sofowara [8] and Harborne [9-10].

FT - IR spectroscopic analysis

FTIR analysis was performed using Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm^{-1} and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation. The peak values of the FT-IR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

GC-MS analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0.32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 270 °C; ion-source temperature 200 °C. The oven temperature was programmed from 40 °C (isothermal for 2 min), with an increase of 8 °C/min, to 150 °C, then 8 °C/min to 250 °C, ending with a 20 min isothermal at 280 °C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 [11]

Statistical analysis

Statistical analyses were conducted using GraphPad Prism (San Diego, CA, USA) version 5.02 for Windows. All the determinations were carried out in triplicate and data were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

The powder of *Ganoderma lucidum* is subjected for proximate analysis (Table 1). The results revealed that moisture content of powder was found to be 7.2%. This indicated the powder is fully dried. Fresh fruiting body contains more than 90% moisture content. Juice can be obtained as like vegetables and fruits. The protein, carbohydrate and fat contents of the whole plant were 16.3±0.16 mg/g, 28.6±0.12mg/g and 1.40±0.13 mg/g, respectively, which is in line with the report given by Borchers *et al*. [12]. Fernandes *et al*. [13] reported that fat content of this medicinal mushroom could be responsible for anti-inflammatory action. *G. lucidum* contained 3.10±2.76 mg/g of total ash 3.10±2.76 mg/g, which indirectly indicated the availability of high content of minerals [14]. Results of this study also reported the low level of ash value and it reveals low impurities and also indicated the availability of minerals like copper, manganese, magnesium, calcium, Mineral analysis revealed the presence of minerals like potassium (4.03±0.35mg/g), followed by calcium (30.5±0.32mg/g), magnesium (5.40±0.23mg/g) and iron (3.00±0.21mg/g), sodium, manganese, copper, phosphorus like minerals also detected in *Ganoderma lucidum* powder (Table 2). The high sodium potassium ratio indicated in this powder and considered this as a good nutrient for hypertention [15-16]. Highest quantity of Calcium in this powder could be used to maintain proper nerve transmission, muscle contraction, glandular secretion as well as mediating vascular contraction and vasodilation [17]. Many people in India showing Iron deficiency related problem due to chronic bleeding, infections, inadequate intake of bioavailable iron, deficiencies of folic acid, vitamin A or vitamin B_{12}, pregnancy, increased requirements throughout growing periods and menstrual losses in women during reproductive age. This could be compensated by taking this fruiting body as a food or nutraceutical or medicine. Copper and zinc are the essential trace elements that are needed only in minute amounts by the human body for important biochemical functions. The levels of copper and zinc are closely interrelated and its imbalanced availability leads to the risk of cardiovascular system disorders. Powder of *G. lucidum* showed recommended zinc/copper ratio, which reduce zinc and copper imbalance in human tissues [18-19].

Table 1 Proximate contents of *Ganoderma lucidum*

Proximate content	Quantity (mg/g)
Ash	3.10±2.76
Carbohydrate	28.6±0.12
Crude fiber	21.1±10.0
Crude Fat	1.40±0.13
Crude Protein	16.3±0.16
Moisture content of powder	07.2%

Table 2 Mineral analysis of *Ganoderma lucidum*

Minerals content	Quantity (mg/g)
Calcium	30.5±0.32
Magnesium	5.40±0.23
Iron	3.00±0.21
Zinc	1.40±0.57
Potassium	4.03±0.35
Phosphorus	2.36±0.36
Copper	2.05±0.23
Manganese	0.79±0.23
Sodium	2.28±0.02
Sulphur	1.19±0.00

Table 3 Qualitative analysis of bioactive compounds of *Ganoderma lucidum*

Bioactive compounds	Powder content
Alkaloids	+
Coumarins	+
Flavonoids	+
Glycoside	-
Phenols	+
Phlobatannins	-
Quinones	-
Saponin	-
Steroids	+
Tannin	+
Terpenoids	+

Secondary metabolite analysis revealed that the extracts of this mushroom fungus showed the presence of alkaloids, flavonoids, phenols, steroids and terpenoids in both the extracts (Table 3). Alkaloids, coumarins, flavonoids, phenols steroids, tannins and terpenoids were found in the extracts of *Ganoderma lucidum*. Quantification of secondary metabolites revealed the presence of higher quantities of alkaloids (5.70±0.09mg/g), flavonoids (2.59±0.08mg/g), phenols (1.41±0.09mg/g), steroids (8.02±0.28mg/g) and terpenoids (2.29±0.02mg/g) (Table 4).

Table 4 Quantitative analysis of bioactive compounds of *Ganoderma lucidum*

Bioactive compounds	Quantity (mg/g)
Alkaloids	5.70±0.09
Flavonoids	2.59±0.08
Phenols	1.41±0.09
Steroids	8.02±0.28
Terpenoids	2.29±0.02
Bioactive compounds	Quantity (mg/g)

Table 5 FT – IR analysis of phytocompounds of *Ganoderma lucidum*

Group frequency cm^{-1} of the sample	Functional group assignment
3327.40	Amines, Imines (=N–H); one bands
2940.92	Hydrocarbon chromophore, C–H Stretching, alkane
2831.62	Aldehydes, C–H Stretching vibration
1658.04	C–C Multiple bond stretching, Alkene, disubstituted, gem
1447.44	Hydrocarbon chromophore, C–H Bending, Alkane, CH_2 and CH_3
1111.98	Sulfur compounds, C=S Stretching vibrations
1019.24	Halogen compounds, C–X Stretching vibrations C–F
638.81	Halogen compounds, C–X Stretching vibrations C–CI

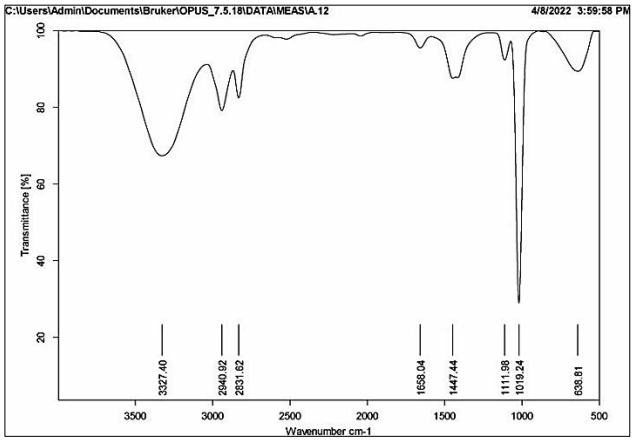

Fig 1 FT-IR pattern of *Ganoderma lucidum* powder

FT-IR analysis indicated the availability of amines, aldehyde, alkenes, Sulphur compounds, double bond and triple bond stretching, CH_3, CH_3 groups which also indicated the availability of terpenoids and steroids. The various functional groups observed in the different extracts probably indicate the presence of carbohydrates, carotenoid, glycogen, amino acids, amides, starch, calotropin, calotropogenin, phosphates, lipids, glycogen and cellulose. Spectral differences are the objective reflection of componential differences. By using FT-IR spectrum, we can confirm the functional constituent's presence in the given parts and extract, identify the medicinal materials from the adulterate and even evaluate the qualities of medicinal materials.

Table 6 GC – MS pattern of *Ganoderma lucidum*

Retention time	Name of the compounds	Molecular name	Molecular weight
10.035	benzoic acid, 2,5-Bis(Trimethylsiloxy)-, Trimethylsilyl Ester $$ Benzoic Acid, 2,5-Bis[(Trimethylsilyl)OXY]	C16H30O4Si3	370
13.970	Cyclohexasiloxane, dodecamethyl-	C12H36O6Si6	444
17.590	1,3-Diphenyl-1-((Trimethylsilyl)OXY)-1(Z)-Heptene	C22H30OSi	338
26.120	Dibenz[a,h]anthracene, 5,12-diphenyl-	C34H22	430
33.110	1,3-Dioxepane, 5,6-Bis[(3,4-Dimethoxyphenyl)Methyl]-2,2-Dimethyl-, (5R-Trans)- $$ (8R,8'R)-9,9'-Epoxy-3,4,3',4'-Tet	C25H34O6	430
33.465	Phenol, 5-methoxy-2,3,4-trimethyl-	C10H14O2	166
34.965	1,2-Benzenedicarboxylic Acid $$ 1,2-Benzenedicarboxylic Acid, Bis(2-Ethylhexyl) Ester $$ 1, 2-Benzenedica	C24H38O4	390
37.590	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C24H38O4	390
38.400	Methyl octyl phthalate	C17H24O4	292
39.525	4-Bromobutyric acid, 3-methylbutyl ester	C9H17BrO2	236
39.740	N-[1-(4-Fluorophenyl)-6,6-Dimethyl-2,4-Dioxo-3-(Trifluoromethyl)-2,3,4,5,6,7-Hexahydro-1H-Indol-3-YL]-2-Thi	C22H18F4N2O3S	466
39.795	C D Homo-Isoconessimine	C23H38N2	342
39.835	1,2-Cyclohexanedicarboxylic acid, di(3-fluorophenyl) ester	C20H18F2O4	360
39.915	1,3,5-Benzetriol, 3TMS derivative	C15H30O3Si3	342
40.105	9-Bromononanoic acid	C9H17BrO2	236

GC-MS chromatogram of the ethanol extract showed 15 peaks indicating the presence of fifteen compounds with the retention time range between 10.035 and 40.105 (Table 6). The active principles in the extract were confirmed based on retention time (RT), molecular formula, molecular weight (MW) and structures. The phytochemical compounds with their retention time (RT), molecular formula, molecular weight (MW) and structures are presented in (Table 6). The first compound identified with less retention time (10.035minb) was benzoic acid. The present GC-MS analysis result showed various long chain and short chain fatty acids, which shows some beneficial effect on cancer, autoimmune and inflammatory diseases, besides its ability to facilitate wound healing and may improve the immune response associated to a more successful elimination of pathogens such as bacteria and fungi, by interfering in many components of immune system such as macrophages, lymphocytes and neutrophils [20]. It also showed the presence of phenols, aldehydes and terpenoids, which shows Antioxidant, antiscorbutic, anti-inflammatory, antinociceptive, anti- mutagenic, wound healing property [21-22]. The bioactive compound oxirane observed at retention time 9.669, 9.783 and 9.841 exhibited bactericidal fungicidal and sporicidal activities. Chemical analysis using GC-MS revealed the availability of phenols, benzoates, phthalates, fatty acids,

Cyclohexasiloxane, 1,3-Dioxepane and anthracene like chemicals which showed various known biological effect. Cyclohexasiloxane is in general used in the skin glowing ointments or facial creams.

Ganoderma lucidum contains carbohydrates like glucon, which exhibits antimicrobial activity. Various anti-tumour polysaccharides from medicinal mushrooms are being re-evaluated in terms of their clinical efficacy. Such compounds would be expected to function by mobilizing the body humoral immunity to ward off viral, bacterial, fungal and protozoal infections resistant to current antibiotics. Polysaccharide Krestin has been shown to induce potent antimicrobial activity against Escherichia coli, Listeria monocytogenes and Candida [23-24]. Brizuela *et al*. [25] succeeded in the isolation and identification of Pleuromutilin, a diterpene that is especially useful for the treatment of mycoplasma infections in animals and served for the development of the first commercial antibiotic of basidiomycete origin. With the development of new fermentation and purification technologies, basidiomycetes are again receiving attention as potential sources of new classes of antibiotics [26-28]. The antimicrobial activity of aqueous and ethanol extracts from *Ganoderma lucidum* against nine UTI MDR bacterial strains, all the extracts provide positive assays against gram positive and gram-negative UTI isolates. Gram-positive bacteria were more sensitive than gram-negative bacteria to fungal extracts. This study also incurred insights from the reports of Lakshmi Priya and Srinivasan [29], Kumiko *et al*. [30], Chelladurai and Uma [31]. They also stated antimicrobial potentials of mushroom fungi against different microbial species.

Components of Mushrooms stimulates the immune system which may result in various therapeutic effects. Mushrooms have anticancer, liver protective, analgesic, sedative, anti-radiation and anti-ulcer properties. It also used by the peoples with diabetic, hypertension, obesity as it is a low-calorie, high protein diet with almost no sugars and starch. This also used as an antioxidant agent [32-33]. Components of *G. lucidum* prevents tissue thereby, it reduces inflammation via preventing the formation of inflammatory mediators. Elsayed *et al.* [34] reported that mushroom compounds showed anti-inflammatory activity by reducing histamine and serotonin production. They also stated that extracts also block cyclooxygenase enzyme there by prevents inflammation. Flavonoids, tannins and phenolic compounds are responsible anti-inflammatory, antibacterial, anti-viral activity. Secondary metabolites could be responsible for all biological activities, which is also supported by many scientists from all over the world. Glycosides or steroids [35-36], monoterpenoids [37], sesquiterpenes [38], diterpenes [39], terpenoids [40], flavonoids [41], glycosides [36], steroids [41], cyclohexyl ethanoids [40], Anthocyanins [42] could be responsible for anti-inflammatory activity. Antimicrobial activity of mushroom fungi could be due to the presence of high molecular weight and low molecular weight compounds [43-45]. Phenolic acids and related compounds such as phydroxybenzoic and cinnamic acids [46]. Rahman *et al*. [47] identified in *Ganoderma lucidum* also revealed activity against different fungi species. *Ganoderma* is an antifungal protein isolated from *Ganoderma lucidum* with activity against phytopathogenic fungi.

CONCLUSION

Ganoderma lucidum is very capable medicinal and nutraceutical source as a result of the abundance of pharmacological active compounds obtained from fruit bodies, mycelium and spores those are potentially health promoting agents. Due to its abundance of several bioactive compounds that have nutritional and medicinal effects and are present in all parts of the fungus. The results of present study revealed that powder extract contains carbohydrates, crude fat, proteins, minerals and secondary metabolites. FT-IR result showed different alkene and multiple chromophores. GC-MS chromatogram of the ethanol extract showed 15 peaks indicating the presence of fifteen compounds with the retention time range between 10.035 and 40.105. The nutritional values of the mushroom species studied here could potentially be used in well-balanced diets and as sources of bioactive compounds. Further studied are needed to confirm bioactivity of phytochemicals like steroids, flavonoids, alkaloids and phenolic compounds present in the extracts of *G. lucidum*.

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