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Biochemical esimation of wildly collected *Ganoderma lucidudm* from Central Himalayan Hills of India

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ABSTRACT

This study was undertaken to reveal the amount of various bio-molecules present in Ganoderma lucidum by utilising various standard chemical methods. These bio-molecules are very important for vital organs of human body for their proper functioning. All together ten biochemical parameters were estimated which include total carbohydrates, simple carbohydrates, polysaccharides, reducing and non- reducing sugars, proteins, ether extract, fibres, organic matter and ash content. Wild collected sample of G. lucidum from Central Himalayan Hills was found to contain a perceptible amount of bioactive molecules predominantly polysaccharides (29.25 ± 1.38%), protein (20.61 ± 1.04%) and fibres (34.46 ± 1.78%) which are responsible for its number of therapeutic activities.

Key words *Ganoderma lucidum*, bioactive molecules, carbohydrates, polysaccharides, fibres, nutraceutical.

INTRODUCTION

Ganoderma lucidum (W.Curst.:Fr.) P.Karst. (Higher Basidiomycetes) is a very high valuable medicinal mushroom, which is well recognized as a “miracle herb” due to its effectiveness in treating broad range of diseases and disorders. The fruiting body of *G. lucidum* is popularly known as “Reishi” in Japan and “Lingzhi” in China. Being as an oldest medicinal mushroom, it is used for more than 2,000 years in Traditional Chinese Medicine (TCM) [1]. Recent studies on this mushroom have demonstrated many interesting biological activities, including antitumour [2], hypotensive [3], cytoxicity [4], anticomplementary [5], antimicrobial [6], hepatoprotective [7], hypolipidemic [3], anti-diabetic [8] and anti-inflammatory effects [9].

G. lucidum as a high value medicinal mushroom contains a wide variety of biochemical substances, including more than 119 different types of triterpenes and several types of polysaccharides. A new class of compounds with nutritional and medicinal features extractable from either the mycelium or the fruiting bodies of mushrooms have been referred as “mushroom nutraceuticals’’. *G. lucidum* is rich in mushroom nutraceutical components with potential therapeutic values [10]. The active ingredients of *G. lucidum* includes high molecular weight polysaccharides, triterpenoids, oraganic germanium, adenosine, ganoderic essence, amino acids, vitamins, minerals, proteins and fibers, beta-glycan, hetroglcan, proteoglycan, nucleotides [11].

Pharmacological activities of *G. lucidum* have been attributed mainly due to two distinct classes of compounds viz. polysaccharides and triterpenes. The former class is having potent bioactivity. Many types of polyglycans have been detected in *G. lucidum*-neutral polysaccharides (beta-1-3, beta-1-6 homo-D-glycan) acidic glycan and polyglycan [12], arabinoxyloglucan, a highly branched heteroglucan a peptidoglycan [13]. But most important bioactive polysaccharids from *G. lucidum* beta-1-3, beta-1-6 D glucan beta-1-3-D-glucopyronan with 1-15 units of beta-1-6 monoglucosyl side chains [14]. It has been shown that high molecular weight beta-1-3, beta-6-D-glucans exhibit

antitumor activity. Molecular weight, solubility and antitumor activity seem to be positively correlated among themselves. Antitumor activity is also correlated with frequency of polysaccharide branching which varies with different stages of mycelial development [15]. Different extraction and purification procedures yield a variety of bioactive glycans. More than 80 triterpenoids and over 50 types of polysaccharides have been isolated from *G. lucidum* and a related species *G. tuage* [16]. The aim of this study is to determine the biochemical parameters of wildly collected sample of *G. lucidum* from the Central Himalayan region, and expose its significance.

MATERIALS AND METHODS

1. Collection and Authentication

Wild samples of *G. lucidum* were collected from various parts of the humid and dense forest areas of Central Himalayan Hills region located at an altitude of 2000 m MSL (Mean Sea Level). Collected samples were authenticated by the Mycology Department of Defence Institute of Bio-Energy Research, Pithoragarh. Samples were allowed to dry at room temperature (30-35°C) and also dried under lyophilisation and finally crushed to powdered form of samples. Powdered samples were then examined for biochemical composition.

2. Determination of Carbohydrates

Total carbohydrate content in *G. lucidum* was determined by Anthrone method [17]. 100 mg of the powdered sample was hydrolysed in 5.0 ml of 2.5 N HCl by keeping it in a boiling water bath for three hours. It was then neutralised with sodium carbonate until the effervescence was ceased. Volume was then finally made to 100 ml and centrifuged. 0.5 and 1.0 ml aliquots were taken from the supernatant for analysis. Meanwhile standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. Volume was made up to 1 ml including the samples. Then 4.0 ml of ice cooled anthrone reagent was added and heated for eight minutes in a boiling water bath. It was then cooled rapidly and finally the green to dark green colour was read at 630 nm. Standard graph was plotted by putting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph the amount of carbohydrate present in the sample tube was calculated.

2.1. Determination of Polysaccharides

Polysaccharide content in *G. lucidum* samples was measured by extracting all simple sugars with the help of hot 80% ethyl alcohol [17]. The extraction of simple sugars was carried out repeatedly until there was no coloration in elute by anthrone reagent. Now residue was hydrolysed with 2.5 N HCl to convert all polysaccharides into glucose. This glucose was treated with hot concentrated acid and anthrone reagent. The observed green coloration was read spectrophotometerically like total carbohydrate estimation.

2.2. Determination of Simple Carbohydrates

Simple Carbohydrates was calculated by subtracting the amount of polysaccharides from the total amount of carbohydrates present in the sample.

2.3. Determination of Reducing Sugars

Estimation of reducing sugars was carried out using Dinitrosalicylic acid method [18]. 100 mg of the sample was weighed and the sugars were extracted with hot 80% ethanol twice (5.0 ml each time). Supernatant collected was then evaporated on a boiling water bath at 80°C and then 10 ml water was added to dissolve the sugars. 0.5 to 3.0 ml of the extract was pipette out in test tubes and the volume was made to 3.0 ml with water in all the tubes, 3.0 ml of DNS reagent was added, heated the contents of the tubes till they were warm, 1.0 ml of 40% Rochelle salt solution was added. When the contents of the tubes were still warm, 1.0 ml of 40% Rochelle salt solution was added, mixtures were cooled and finally the intensity of dark red colour at 510 nm was read. And also a series of standards was run using glucose (0-500 µg) and finally plotted a graph. The amount of reducing sugars present in the sample was calculated by using the standard graph using D-glucose as a standard.

2.4. Determination of Non- reducing sugars

Non-reducing sugar content in *G. lucidum* was calculated by simply subtracting the amount of reducing sugars from the total amount of the simple sugars present in the sample taken.

3. Determination of crude proteins

Dried sample (2.0 g) was taken in 500 ml Micro-Kjeldahl's flask in which 10 g of digestion mixture (9.5 g of Potassium sulphate and 0.5 g of Copper sulphate) and 20 ml of concentrated Sulphuric acid were added. Small pieces of calcium carbonate were introduced into the flask to check the bumping. The flask was then kept on the electric heater till the colour of the digested material became light bluish-green. The flask was cooled overnight and the material was diluted with distilled water to make the aliquot of 250 ml in a volumetric flask. The aliquot (25 ml) was taken in 300 ml Micro-Kjeldahl's flask. To this 80 ml of saturated Sodium hydroxide solution was added and

kept on the electric heater with distillation unit connected through a trap to the condenser. The lower end of the condenser was dipped in a solution of 25 ml of 2% Boric acid containing Toshiro's indicator (Methyl red - 80 mg, Methylene blue - 20 mg and Methanol - 100 ml) in a 250 ml beaker. Distillation was carried out for 30 minutes during which all the ammonia released was trapped in the Boric acid solution [19]. Finally percentage of crude protein was obtained by multiplying the total nitrogen by 4.38. % of Crude Proteins = % of Nitrogen× 4.38 (For mushrooms)

4. Determination of Ether-Extract

Ether-extracts of Ganoderma samples were quantified using Soxhlet's apparatus [19]. Oven dried sample (5 g) was taken in a thimble of Whatman filter paper No. 1 and placed in the extractor. The extractor was connected with weighed oil flask below and the condenser above. Petroleum ether of boiling point 60 to 80°C was poured into the extraction tube with 60 ml. more than required for permitting siphon to the oil flask placed on the heater. Cold water was passed through the condenser during the extraction process. Extraction was carried out for 6 hours till the liquid was as clear as clean water. The flask was then disconnected and dried in a hot air oven at 100 ± 5°C for 4 to 6 hours till the ether was completely evaporated. It was cooled in a desiccator and weighed to a constant weight. The difference in the weight of oil flask after and before extraction denoted the ether extract of the sample.

5. Detremination of Crude fibres

Total amount of fibre was calculated by using the reported method [20].The sample after ether extraction was transferred from the thimble to a 500 ml round bottom reflex condenser flask. 200 ml of 1.25% Sulphuric acid solution was poured into the flask and was heated in a heating mantle. Cold water was allowed to flow through the condenser. After boiling for 30 minutes, the content of the flask was filtered through the muslin cloth. The residue on the cloth was washed with distilled water to remove the acid. The residue was transferred to the same flask and 200 ml of 1.25% Sodium hydroxide solution was poured into the flask. The content was boiled for 30 minutes and filtered through the same cloth. It was washed with distilled water to remove the alkali. The residue was transferred to a crucible and kept in a hot-air oven at 100 ± 5°C for drying. The crucible was cooled in a desiccator and weighed to a constant weight. The content was put in a muffle furnace at 600°C (ash was free of black particles) for 30 minutes, cooled in a desiccator and weighed. The loss in weight during ashing was the percentage of the crude fibre.

6. Ash value

It was calculated by using a standard method [21]. Dried sample (5.0 g) was taken in a weighed crucible and heated in a muffle furnace at 600 ± 15°C till the content was free of black particles. The crucible was cooled in a desiccator and weighed to a constant weight. The ash content was calculated by weighing the weight of crucible at different stage.

7. Determination of Organic matter

Organic matter was calculated by total sum of crude protein, ether extract, crude fibre and nitrogen free extract present in the sample [19].

8. Statistical Analysis

Results are expressed as mean ± Standard deviation (SD) of triplicate experiments. The data were subjected to one-way analysis of variance (ANOVA), and significant differences between means were determined statistically using Student's 't' test with the Open Stat software. $P < 0.05$ was assumed as the level of significance for experimental results.

RESULTS AND DISCUSSION

1. Carbohydrates

Carbohydrates are the energy providing nutrient substrates for life. *G. lucidum* was found to contain high amount of carbohydrates (40.14 ± 2.15%). More than 100 types of polysaccharides are known, which are present in the fruiting body, spores and mycelial submerged liquid culture of *G. lucidum.* They comprise one of the major sources of *G. lucidum*'s pharmacologically active compounds [22]. Most have a molecular weight ranging from 4×10^5 to 1×10^6 in the primary structure. In this study the percentage of polysaccharides was found 29.25%, apart from the favourable amount of reducing (1.19 ± 0.14%) and non- reducing sugars (10.31 ± 0.75%).

2. Proteins

In this study, *G. lucidum* was found to contain a high amount of proteins (20.61 ± 1.04%), the second next abundant bio-molecule after carbohydrates. Proteins are the basic nutrients for body building and body maintenance [23]. *G. lucidum* contains various bioactive protein molecules like LZ-8 protein molecule, which is very similar to variable region of the immunoglobulin heavy chain in its sequence and in its predicted secondary structure [22].

3. Ether extracts

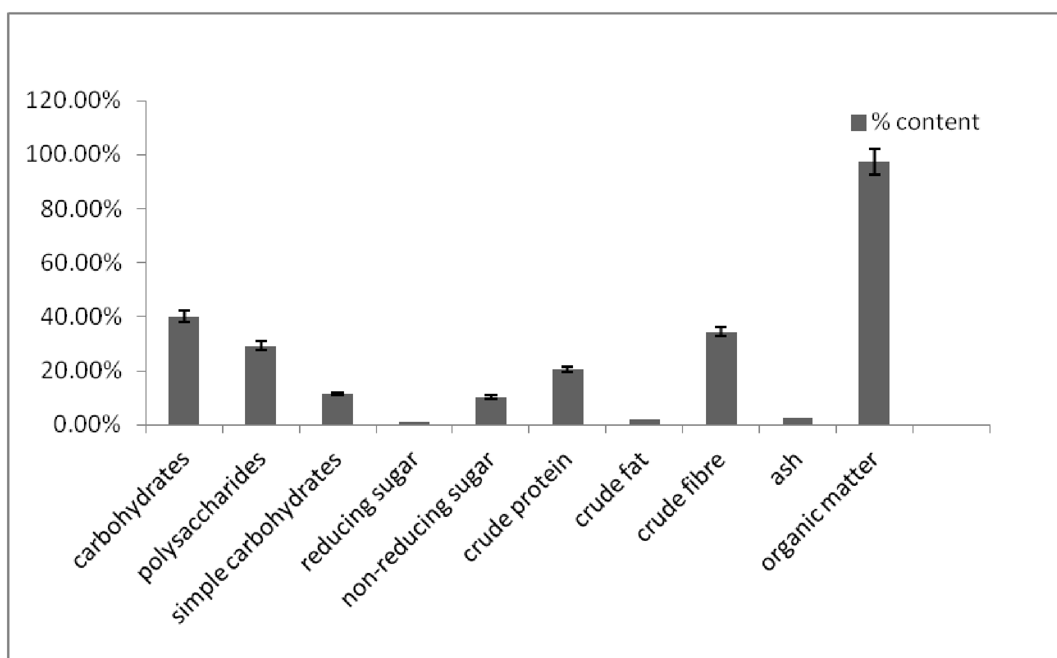
Ether extracts are composed of various non polar bio-molecules like fats and other lipid molecules. *G. lucidum* was also found to contain favourable amount of ether extract (2.08 ± 0.36%). Unsaturated fats are divided into monounsaturated fats and polyunsaturated fats, and both types are thought to have beneficial effects on cholesterol levels. Monounsaturated fats help lower LDL (bad) cholesterol while also boosting HDL (good) cholesterol. Omega-3 fatty acid is an "essential fatty acid," which cannot be manufactured by our bodies, so eating these foods is the only way to get them. Omega-3 fatty acids are thought to lower blood pressure, combat LDL (bad) cholesterol, fight inflammation and protect the brain and nervous system [22].

Table 1 Biochemical contents in g/100g sample dry weight of *Ganoderma lucidum*.

S. No	Biochemical	% Composition
1	Total Carbohydrates	40.14 ± 2.15%
2	Total Polysaccharides	29.25 ± 1.38%
3	Simple Carbohydrates	11.50 ± 0.86%
4	Reducing sugars	1.19 ± 0.14%
5	Non- reducing sugars	10.31 ± 0.75%
6	Crude proteins	20.61 ± 1.04%
7	Ether extract	2.08 ± 0.36%
8	Crude fibre	34.46 ± 1.78%
9	Total Ash	2.54 ± 0.42%
10	Organic matter	97.29 (avg.)

Data are expressed as means ± SD (N=3).

Fig. 1 Biochemical contents in 100 g dried sample of *G. Lucidum*



4. Fibres

The fibre content in *G. lucidum* was found to be very high (34.46 ± 1.78%). Fibre is a class of materials that are continuous filaments or are in discrete elongated pieces, similar to lengths of thread. A fibre is an elongated tapering thick-walled plant cell that imparts elasticity, flexibility, and tensile strength. They are very important in the biology of both plants and animals, for holding tissues together [24]. Fibres are very important for digestive tract as they increase the surface area of food and make the digestion process easier.

5. Ash value

When either organic compounds are decomposed or released at high temperature (600 ± 15°C), the remaining residue is the ash. This residue consists of oxides and salts containing anions such as phosphates, chlorides, sulfates, and other halides and cations such as sodium, potassium, calcium, magnesium, iron, and manganese [25]. Therefore Ash represents the mineral content of any drug sample. The ash content in *G. lucidum* samples was 2.54±0.42%, which indicates the significant amount of minerals in these samples [26].

6. Organic Matter
Organic matter contains all the carbon containing compounds i.e., carbohydrates, proteins, fibres and fat. So, its higher value indicates the presence of higher amount of all the carbon containing compounds. *G. lucidum* was found to contain high amount of organic matter (97.29g/100g dried material), indicating the presence of carbohydrates, proteins, fibres and fat.

CONCLUSION

Biochemical study of *G. lucidum* revealed the importance of this miracle herb in the growing realm of nutraceutical and pharmaceutical industries. The perceptive amounts of bio-molecules present in *G. lucidum* are found to be very useful in living bodies. As, its pharmacological activity is mainly attributed to the presence of polysaccharides, and in this study it was found that the amount of polysaccharides present was 29.25 ± 1.38% and therefore it is worthwhile to conclude that *G. lucidum* can be a very useful herb in treating various ailments. Its protein content (20.61 ± 1.04%) and fibrous content (34.46 ± 1.78%) makes this herb so valuable and can be regarded as a health food. Its high amount of carbohydrate content (40.14 ± 2.15%) indicates that it can also be used as a source of energy, as carbohydrates are the storage form of energy. Its ash value indicates the presence of significant amount of minerals in this herb.

In view of the above, it is worthwhile to say that *G. lucidum* is a very potent high valued medicinal mushroom, which can be taken as a daily health supplement in the form of drinks and capsules. There is a lot of scope for the future research on the various aspects of its artificial cultivation, biochemistry, pharmacology and pharmacognosy. Biometabolites like polysaccharides, fibres, phenolic compounds and alike materials of *G. lucidum* will be the key future driving force in the realm of green pharmacology and pharmacognosy.

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