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Physicochemical and Total Phenolic Content of Fruiting Body Powder of *Ganoderma Lucidium* from Daxen Agritech , Himachal Pradesh

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ABSTRACT

The present study aims to investigate the physicochemical analysis of *Ganoderma lucidium* fruiting body powders were from Daxen Agritech India Pvt .Ltd, Himachal Pradesh. *Ganoderma lucidium* has been used for hundreds of years as a health promotion and treatment strategy. A number of bioactive compounds have been characterized from *Ganoderma lucidium* which are found to be responsible for their pharmacological potential. So a preliminary screening was done to reveal the bioactive constituents of *Ganoderma lucidium*. The phytochemical constituents extract was evaluated by using solvent like methanol and aqueous extract. The Phytochemical screening reveals that the Methanol extracts were a rich source of phytoconstituent containing alkaloids, carbohydrates, glycosides, Flavanoids, Saponins, phenol, Triterpenoids and steroids. Whereas, water extract contain Saponins, tannins and phenol. Among the two solvents used for extraction, Methanol extract showed more number of Phytoconstituents followed by water extract. Methanol extract have higher solubility for more active phytochemical constituents in *Ganoderma lucidium*. Analysis for proximate constituent showed moisture contents , crude fibre crude fat, crude protein ,carbohydrates, nitrogen. The methanolic extract of *Ganoderma lucidium* showed highest amount of phenolic content. The presence of these essential physicochemical constituent implies that it can be used for its medicinal values in health care systems.

Keywords: *Ganoderma lucidium*, physicochemical, bioactive compounds, Methanol, phenolic, proximate analysis.

I. INTRODUCTION

The herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants as a primary health care modality (Kamaraj *et al.*, 2012). Phytochemicals are abundant, locally renewable, user-friendly and environmental safe, and attracts low capital (Anjorin *et al*.,2009). Mushroom contains a vast amount of compounds among which are polysaccharides and triterpenes. Polysaccharides are regarded for their anti tumour and immunostimulating activities and are majorly composed of glucans (Kao *et al*., 2013). Terpenes or terpenoids are amongst the compounds responsible for the medicinal, culinary and fragment uses of aromatic and medicinal plants (Dorman *et al*., 2000).An extensive review by described the isolation, characterization and biological activity of terpenoids found in fungi and plants.

Ganoderma lucidium in Chinese term called as Lingzhi , The name represents a combination of spiritual potency and essence of immortality, and is regarded as the "herb of spiritual potency,'' symbolizing success, well-being ,divine power, and longevity. Lingzhi has been recognized as a medicinal mushroom for over 2000 years, and its powerful effects have been documented **in** ancient scripts (Wasser 2005). The fungus is cosmopolitan with interwoven hypha that forms a lamellaless mycelium employed in the treatment of various medical condition ranging from cancer, tumour,

wound healing, hypotension, microbial infections, to inflammatory condition (Min *et al*.,2000) and(Zhou *et al*.,2002), (Odey *et al* .,2012).Numerous investigations have proved that medicinal plants as well as microorganisms contain diverse classes of compounds such as tannins, alkaloids, flavanoids, steroids, terpenoids, phenols etc(Chitemerere and Mukanganyama,2011). The study aims to qualitatively determine the phytochemical components , total phenolic content and proximate analysis of *Ganoderma lucidium* fruiting body found in Daxen Agrotech India Pvt Ltd , Himachal Pradesh.

II. MATERIALS AND METHODS

Plant Materials

The fruiting body powder of *Ganoderma lucidium* was collected from Daxen Agrotech India Pvt Ltd, Himachal Pradesh. Fresh fruiting bodies of *Ganoderma lucidium* were kept for further analysis.

Extraction of bioactive compounds of *Ganoderma lucidium*

Aqueous extraction

The aqueous extract of fruiting body of *Ganoderma* were prepared by transfer of one gram(1g) of the fruiting bodies powder to 50 ml capacity of sterile wide-mouthed screw-capped bottles.10 ml of sterile de-ionized distilled water was added to the powdered samples which were allowed to soak for 24 hours at room temperature, after heating the extracts for 2 hour at 100°C.The mixtures were then centrifuged at 2000 rpm for 10 minutes at 4°C.The supernatants were filtered through a sterile funnel containing sterile Whatman filter paper (No.1) and then filter sterilized using 5ml sterile syringe with 0.2 membrane filter.

Solvent extraction

Air dried powder of *Ganoderma lucidium* fruiting body powder was extracted by using soxhlet apparatus .10 g of fruiting powder was taken in a paper cone and placed in soxhlet apparatus.100 ml of solvent (methanol and water) was taken in the round bottom flask attached to this setup . Solvents get vapourized and rises up to the condenses back into the liquid and falls into the sample in the cone and extract certain compounds falls into the round bottom flask. Methanol extract of *Ganoderma lucidium* fruiting body powder was golden brown colour and aqueous extract was dark brown colour.

Phytochemical screening

Qualitative physicochemical screening were carried out using the methods of Savithramma *et al*., 2011, Onyeike and Osuji., 2003 and others. The extracts were then subjected to qualitative chemical tests for various phytoconstituents like alkaloids, flavanoids, carbohydrates, reducing sugars, tannins and phenolic compounds, cardiac glycosides, terpenoids, anthraquinones, saponins, volatile oils and steroids.

The test were based on the visual observation of colour change or precipitate formation after the addition of specific reagent.

Detection of Alkaloids
Mayer's test

To a few ml of extract, one or two drops of Mayer' s reagent were added by the side of the test tube. A white creamy precipitate indicated the test as positive.

Preparation of Mayer's Reagent

Mercuric chloride (1.358g) was dissolved in 60 ml of water and KI (5g) was dissolved in 10 ml of water. The solutions were mixed and made up to 100 ml with water.

Wagner's test

To a few ml of extract ,few drops of wagner's reagent were added by the side of the test tube. A reddish brown precipitate confirmed the test as positive.

Preparation of Wagner's Reagent

Iodine (1.27 g) and KI (2 g) were dissolved in 5ml of water and made up to 100 ml with distilled water.

Detection of Carbohydrates
Mohlisch's Test

To 2 ml of extract , two drops of alcoholic solution of α -naphthol was added, the mixture was shaken

well and 1 ml of conc.H2SO4 was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates

b)Fehling's Test

I ml of extract was boiled on water bath. To this, 1 ml of Fehling solutions A and B were added. A red precipitate indicated the presence of sugar. Fehling's solution A: CuSO4 (34.66g) was dissolved in distilled water and made up to 500 ml using distilled water. Fehling's solution B: Potassium sodium tartarate (173g) and NaOH (50g) was dissolved in water and made up to 500 ml.

c) Benedict's Test

To 0.5 ml of extract, 1 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 mins. A characteristic coloured precipitate indicated the presence of sugar.

Benedict's Reagent

Sodium citrate (173g) and Na2CO3 (100g) were dissolved in 800 ml of distilled water and boiled to make it clear. CuSO4 (17.3g) dissolved in 100 ml distilled water was added to it.

d) Barfoed's Test

To 1 ml of extract, 1 ml of Barfoed's Reagent was added and heated on a boiling water bath for 2 min. Red precipitate indicated the presence of sugar.

Barfoed's Reagent

Copper acetate (30.5g) was dissolved in 1.8 ml of glacial acetic acid.

e) Test for **Glycosides**
Legal's Test

To the extract, few drops of 10% NaOH were added to make it alkaline. Then freshly prepared sodium nitroprusside was added to the solution. Presence of blue colouration indicated the presence of glycosides in the extract.

f) Detection of protein
Millon's Test

To 2 ml of extract, few drops of Millon's reagent were added. A white precipitate indicated the presence of protein.

g) Detection of Aminoacid
Ninhydrin Test

To the extract add 0.25% Ninhydrin reagent. Boiled for few minutes . formation of blue colour indicates the presence of Aminoacid.

h) Detection of Flavanoids

To 5 ml of water was dissolved in 2 g of the extract in a test tube. Few drops of sodium hydroxide solution were added to the resulting solution. A yellow colour indicated the presence of flavanoids..

i)Detection of Phytosterols
Libermann-Buchards test

The extract was mixed with 2 ml of acetic anhydride. To this1 or 2 drops of concentrated sulphuric acid was added slowly along the sided of the test tube. An array of colour change shows the presence of phytosterols.

j) Detection of Saponins

The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. 2cm layer of foam indicates the presence of saponins.

k) Detection of Tannins

Ferric chloride Test

About 0.5 mg of dried powdered samples was boiled in to 20 ml of water in the test tubes then filtered. A few drops of 0.1 % ferric chloride was added and observed for brownish green or blue black colouration.

l) Detection of Phenolic compound

The extract was diluted to 5 ml with distilled water. To this a few drops of neutral 5% ferric chloride solution was added. A dark green color indicates the presence of phenolic compounds.

m) Detection of Triterpenoids and Steroids

Libermann - Burchard's Test

Extract was treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added from the sides of the test tube, showed a brown ring at the junction of two layers and the upper layer turning green showed the presence of Steroids and formation of deep red colour indicated the presence of triterpenoids.

Salkowski's test

Extract was treated with few drops of conc. Sulphuric acid, shaken well and allowed to stand for some time, red colour at the lower layer indicated the presence of Steroids and formation of yellow coloured lower layer indicated the presence of Triterpenoids.

n)Detection of Anthraquinones

To 5g of the extract 5 ml of benzene was added and shaken well properly until it dissolved. 5 ml of 10% of ammonia solution was then added to the filtrate .Pink, red, or violet coloration in the ammoniacal (lower) phase indicates the presence of free hydroxyl anthraquinones

Determination of total phenolic compound

(Almey *et al .,2011)* 1 ml of extracts (125-1000µg/ml) of methanol and water was mixed thoroughly with 5 ml of Folin- Ciocaltea reagent. After 5 min 4 ml of 7.5% sodium carbonate (Na_2CO_3) was added, allowed to react for 1 hr at room temperature. The absorbance was measured at 765 nm against a blank having all the reagents excluding the sample using spectrophotometer. Samples were measured in triplicates. This procedure was repeated 5 times for each extract. The total phenols were quantified by the standard curve obtained. Standard curve of gallic acid solution (10, 20,40,60,80 & 100 µg/ml) was prepared using the similar procedure from which the regression formula was derived. Total phenol values were expressed as mg of gallic acid equivalents (GAE)/g of dry extract. The total content of phenolic compounds in plant extract and in different fractionates in Gallic acid equivalents (GAE)was calculated by the following formula

$$C=(c*v)/m$$

Where, C=total content of phenolic compound mg/g extract in GAE,C=the concentration of gallic acid established from the calibration curve mg/ml, V=the volume of extract ml, m=the weight of pure Ganoderma fruiting body methanolic extract gm.

The total phenolic contents of the test fractions were calculated using the standard curve of Gallic acid (y=0.005*-0.191,R2=0.814). Then the absorbance at 765nm was determined. These data were used to estimate the phenolic contents using a standard curve obtained from various concentration of Gallic acid. Total phenol content was expressed as mg of Gallic acid equivalent.

Proximate analysis

(Ogbe *et al.,* 2012*)*Analysis for proximate contents of the dried powder of *Ganoderma lucidium* was done by methods described by American Organisation for Analytical chemistry – AOAC. The sample was weighed (0.1g) and was analysed for moisture contents, carbohydrates, crude fiber, Crude protein, Total ash, Crude fats (lipids).Nitrogen was analyzed by Kjeildal method as described by American Organisation for Analytical Chemisty.

III. RESULTS

The phytochemical analysis of *Ganoderma lucidium* in the methanolic extract reveals the presence of alkaloids, carbohydrates, glycosides, flavanoids, saponins, phenol, triterpenoids and steroids. Protein, amino acids, Phytosterols, tannins and anthraquinones were not detected in extract.). The total phenol content of methanol extract and water extract of Ganoderma lucidium with total phenol content of162.4mg/g and 34.6mg/g. Methanol extract had the highest amount among the samples in this study. In proximate analysis the chemical compositionaremoisture(9.7%), crudefat(2.2%), reducingsugar(1.7%), crudefibre(29.9%), carbohydrate(32%), nitrogen(21.4%), polysaccharides (25%), crude protein (13.2%) and crude ash (3%).

Table 1. Phytochemical constituents of *Ganoderma lucidium* from Methanol and Water

Phytochemical	Tests	Observations from solvent	
		Methanol	Water
Alkaloids	Mayer's	+	-
	Wagner's	+	-
Carbohydrates	Molisch's	+	-
	Fehling's	-	-
	Benedict's	-	-
	Barfoed's	-	-
Glycosides	Legal's	+	+
Protein	Millon's	+	-
Amino acid	Ninhydrin	-	-
Flavanoids	Sodium hydroxide	+	-
Phytosterols	Libermann-Burchard's	-	-
Saponins	Foam	+	+
Tannins	Ferric chloride	-	+
Phenol	Ferric chloride	+	+
Triterpenoids	Libermann-Burchard's	+	-
steroids	Salkowski	+	-
Anthraquinones	Ammonia	-	-

+ present
- Absent

Table 2. Total phenolic content of *Ganoderma lucidium*

Concentration (µg/ml)	Absorbance Gallic acid	Best fit Equation	R^2 value	Absorbance of Ganoderma lucidium methanol extract	Absorbance of Ganoderma lucidium Water extract
250	1.124	Y=0.005x-0.191	0.814	162.4	34.6
200	0.694			101	31.2
150	0.468			90.2	29
100	0.315			88.2	21.4
50	0.254			80.8	19.7

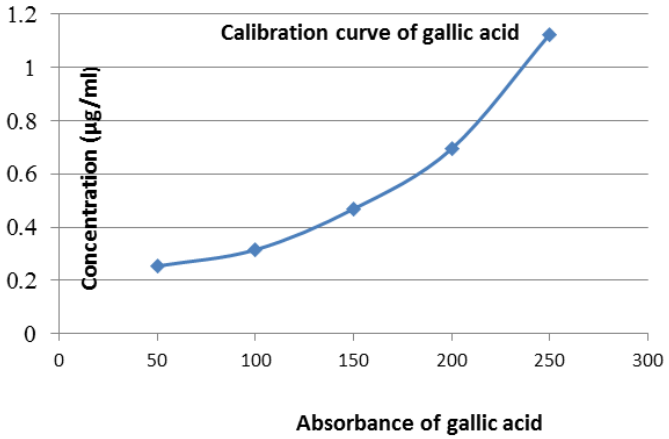

Figure 1

Table 2. Proximate Analysis

Parameter	Contents (%)
Moisture	9.7
Crude fat	2.2
Reducing sugar	1.7
Crude fibre	29.9
Carbohydrates	32
Nitrogen	21.4
Polysaccharide	25
Crude Protein	13.2
Crude ash	3

IV. Result and Discussion

The physicochemical analysis of *Ganoderma lucidium* disclosed the presence of major Phytoconstituents viz., alkaloids, carbohydrates, glycosides, flavanoids, saponins, phenols, triterpenoids and steroids. Among the two solvents used for extraction methanol extract showed more number Phytoconstituents followed by aqueous extract. This is in agreement with the findings of Jonathan and Fasidi. 2003 that bioactive secondary metabolites of mushrooms extracted may be different depending on the extractive solvent. Different solvents have been reported to have the capacity to extract different phyto constituents depending on their solubility or polarity in the solvent.

The presence of alkaloids in the *Ganoderma lucidium* powder explains its antibacterial activity, since this physicochemical is reported to have anti-bacterial activity (Idowu *et al*., 2003). The phenol, Flavanoids, found in this study are known to be source of plant based antioxidants which can protect the nerves, heart, liver and other organs and tissues.this antioxidant property may be responsible for reduction of hepatic damage (Lakshmi *et al*., 2006).Carbohydrates are known to inhibit colonization of pathogenic microbial flora in the intestines, hence, the elimination of these pathogens from the gut system accompanied by improve immunity (Gun *et al*.., 2003).

The presence of tannins in methanol extract fractions which can complex with the metal ions and macromolecules such as proteins and carbohydrates (Dei *et a*l., 2007) obtained in the powdered sample can be utilized in weight reduction management. Saponins, as secondary metabolites can be found as hydrophilic can be found as hydrophilic glycoside moiety combined with a lipophyllic triterpenes derivative to form a therapeutically cardio-active agent in form of steroid-saponins and triterpenoids saponins.(Dei *et al*., 2007). Saponins are also reported to have anti-inflammatory, expectorant and immune stimulating effects (Ray Sahelian, 2012).Triterpenoids are the bitter tasting physicochemical which gives the extract its bitter taste, the triterpenoids are said to form complexes with steroids(sterols) to provide the said anti-inflammatory effects of this wild mushroom an equally, its anti-bacterial property. Steroids was also observed in this study , this can explain the claims of analgesic effects of the mushroom. This findings agrees with that of Ko *et al.*, 2008.who reported that steroids found from *Ganoderma lucidium* includes 0.3-0.4% which has anti-inflammatory activity. These steroids are also precursors of Ganoderic acid and protease inhibitors.

Methanol extract obtained in this study might have higher solubility for constituents. Water was observed in this study to be poor solvent compared to other solvents used. This is in line with research findings that some phytochemicals are more soluble in alcohol than water. This also confirmed the suggestion of Fujita *et al*., 2005 who suggested that methanol was better than water as extracting solvent. The Methanol extract were found to extract the maximum active components being solvents that have low polarity in accordance with previously reported literature(Shamaki *et al.*, 2012).

Analysis for proximate chemical composition in percentages, from 0.1g of the crude powder of the

mushroom was observed. The high fiber, low moisture , total ash contents implies is and high absorption rate to provide energy requirements for cellular and gastrointestinal functions. Agree with Carbohydrates and low levels of fat. Their presence in finding agrees with that of Ogbe *et al*.,(2008) which suggest the high nutritional values of this mushroom. Wasser, (2005) reported that more than 100 polysaccharides are found in *Ganoderma lucidium* and these polysaccharides are considered to contribute to the bioactivity of the mushroom. Protein suppress the allergic responses (Muller *et al*., 2000, Muller *et al*.,2006).

Low fat content , as reported in other plants (Ogbe *et al*., 2012) shows the health benefits of this mushroom and stressing its nutritional value, it is reported that extract from this mushroom has cholesterol lowering properties in Hamsters and Mini pigs(Berger et al., 2004).The Nitrogenous components provide essential requirement for nucleosides formation in the body, these amino acids are important components of DNA and RNA that are useful in cellular function and cell differentiation ,thus, its mitogenic capacity (Wasser,2005).

V. Conclusion

In the present study of *Ganoderma lucidium* fruiting body powder suggests that methanol extracts contain rich phytochemical constituents such as alkaloids, carbohydrates, glycosides, flavanoids, saponins, phenols, triterpenoids and steroids and also extract of *Ganoderma lucidium* harvested from Daexan , Himachal Pradesh demonstrate appreciable quantities of carbohydrates ,Crude protein, Crude fats (Lipids),and essential mineral element required by the body for normal function of organs and tissues. The present findings will be extended for purification, characterization and wound healing activity; it can be used as therapeutic agent for bacterial disease in future.

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