

Research Article

CHARACTERIZATION OF *GANODERMA LUCIDUM*: PHYTOCHEMICAL AND PROTEOMIC APPROACH**Rakhee¹, Niroj Kumar Sethy¹, Anuja Bhardwaj¹, Vijay Kumar Singh¹, Raj Kishore Sharma², Renu Deswal³, Kalpana Bhargava¹, Kshipra Misra^{1*}**¹Department of Biochemical Sciences, Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, Delhi 110054, India²Department of Chemistry, University of Delhi, Delhi 110007, India³Molecular Plant Physiology and Proteomics Laboratory, Department of Botany, University of Delhi 110007, India

Abstract: The present study provides an extensive qualitative phytochemical analysis, proximate composition and proteome study on Indian isolate of medicinal mushroom *Ganoderma lucidum* (*G. lucidum*). Regardless of reported health benefits and economic importance, there is relatively little information available about its qualitative phytochemical analysis, proximate composition and proteome study especially of Indian isolates of *G. lucidum*. Therefore, a thorough qualitative phytochemical analysis was carried on powdered whole body of *G. lucidum* fruiting body (GLF_{wb}), *G. lucidum* mycelium (GLM_{wb}) and their aqueous extracts (GLF_{Aq} and GLM_{Aq}), respectively. These were prepared by accelerated solvent extraction technique (ASE) which showed the presence of several bioactive components such as alkaloids, amino acids, proteins, carbohydrates, flavonoids, phenols, gums, mucilages and saponins. We estimated chemical composition of the medicinal mushroom GLF_{wb} and GLM_{wb} in terms of its carbohydrate (35.23±0.05%, 70.51±0.24%) content, crude fiber (33.53±0.00%, 5.37±0.02%), fat (2.29±0.003%, 2.62±0.24%), moisture (3.38±0.01%, 7.43±0.01%), protein (23.12±0.001%, 11.88±0.01) and total ash (2.45±0.001%, 7.43±0.01%). Besides, protein identification of both crude GLF_{wb}, GLM_{wb} and their aqueous extracts GLF_{Aq} and GLM_{Aq} by SDS-PAGE followed by MALDI-TOF/TOF analysis revealed the presence of various types of highly abundant proteins such as DNA replication licensing factor Mcm7 [*Monascus purpureus*], adenylate cyclase [*Coccidioides immitis* str. *Silveira*], ATP synthase f chain, mitochondrial precursor [*Coccidioides immitis* C735 delta SOWgp], malate dehydrogenase [*Ajellomyces capsulatus* G186AR], AFR634Wp [*Ashbya gossypii* ATCC 10895], myosin [*Ajellomyces capsulatus* G186AR], kinesin family protein [*Ajellomyces dermatitidis* SLH14081], UDP-N-acetylglucosamine pyrophosphorylase [*Scheffersomyces stipitis* CBS 6054], ZYRO0D01518p [*Zygosaccharomyces rouxii*], hsk1-interacting molecule 1 [*Cryptococcus neoformans* var. *neoformans*], DNA repair protein Rad35 [*Cryptococcus gattii* WM276] and others.

Keywords: *Ganoderma lucidum*; phytochemical analysis; proximate composition; proteome analysis.

Note: Coloured Figures and Supplementary Information available on Journal Website in "Archives" Section

Introduction

G. lucidum (Ling Zhi, Reishi mushroom) is a mushroom very commonly used as a traditional

Chinese medicine (TCM) and has been economically significant species mostly in the far east Asian countries such as China, Japan and Korea for over 4000 years (Sanodiya *et al.*, 2009). The genus *Ganoderma* is related to polypore macrofungi and mostly found in tropical and subtropical areas. It is saprobic fungus grows on living or deadwood as well as occasionally on soils ascending from the buried roots during high humidity monsoon season.

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It is a well-known basidiomycete and has been referred to as the "Mushroom of Immortality and God of herbs" (Al-Obaidi *et al.*, 2016; Haq *et al.*, 2014). Of late, mushrooms have created lot of interest mainly in protein rich functional food. On account of their known medicinal properties they are reported to be used as prophylactic agents in various health related problems such as antitumor, immuno-modulatory, cardiovascular, respiratory, anti-hepatotoxic, central nervous system related diseases, coronary heart diseases, arteriosclerosis, hepatitis, arthritis, nephritis, bronchitis, asthma, hypertension, cancer, gastric ulcer, antitumor activity, cardiovascular effects, liver related problems and treatment of HIV infections etc. (Al-Obaidi, 2016; Bishop *et al.*, 2015; Cheng & Sliva, 2015; Cilerdzic *et al.*, 2014; Kao *et al.*, 2016; Mahajna *et al.*, 2009; Wang & Ng, 2006; Xu *et al.*, 2016; Gogoi & Sarma, 2012; Jat *et al.*, 2015; Joseph, 2009; Thakur *et al.*, 2007).

This mushroom contains several distinctive secondary metabolites such as fatty acids, flavonoids, nucleobases, polyphenols, polysaccharides, steroids, and vitamins etc. (Batra *et al.*, 2013; Joseph *et al.*, 2011; Khan *et al.*, 2015; Kirar *et al.*, 2015; Lakshmi *et al.*, 2003; Pillai *et al.*, 2008; Rathor *et al.*, 2014). Though there are numerous studies available in the literature confirming the application of *G. lucidum* as pharmacologically active food however, a little information has been reported till date about a systematic qualitative and quantitative fingerprinting specially for Indian cultivated variety.

Therefore, in our study, we carried out a comprehensive qualitative phytochemical and proximate analysis along with SDS-PAGE followed by MALDI-TOF/TOF of *G. lucidum*. The proteomic one dimensional approach (1-DGE) combined with mass spectrometry (MS) has been used for identifying soluble proteins isolated through accelerated solvent extraction technique (Yinet *et al.*, 2012). These proteins may be further studied for their bio-efficacy evaluation and other medicinal applications.

Materials and methods

Herbal material collection

The similar quality of *G. lucidum* was used in this study as was used by others in previous studies (Bhardwaj *et al.*, 2016). The material was procured

from M/s Aryan Mushroom (Delhi, India). *G. lucidum* was crushed into fine powder using grinder and stored in air tight polythene bag at room temperature until required for use.

Extract preparation

Accelerated Solvent Extraction system (ASE 350) equipped with a solvent controller unit from Dionex Corporation (Sunnyvale, CA, USA) was used in aqueous extracts preparation of fruiting body and mycelium at room temperature in triplicate manner. The aqueous extracts were lyophilized in lyophilizer (Allied frost FD-5, Delhi, India) and lyophilized extract was stored at 4°C until use.

Chemical composition analysis

All the four samples (GLF_{Wb}, GLM_{Wb}, GLF_{Aq} and GLM_{Aq}) were investigated thrice for qualitative photochemical analysis. The analysis was carried out for various compounds using standard methods such as: alkaloids (Mayer's test), carbohydrates (Molish's test, Fehling's test and Benedict's test), saponins (Foam test), proteins, amino acids (ninhydrin test), phenolic compounds and flavonoids (lead acetate test) (Khandelwal, 2008; Raaman, 2006; Shah *et al.*, 2014).

Proximate analysis

Proximate analysis as a quantitative evaluation of phyto-constituents of the dried powder of both GLF_{Wb} and GLM_{Wb} was carried out using standard procedures as described by Raghuramulu *et al.* (2003). The samples were analyzed for proximate parameters such as carbohydrates, crude fiber, crude protein, fat moisture contents and total ash. The moisture content was obtained by drying the samples in a halogen moisture analyzer (Wensar HMB100, New Delhi, India) at 100°C until circulation was accomplished; ash, from burn up residue obtained at 550°C after 8 h by using muffle furnace (Metrex, New Delhi, India); crude protein by the Kjeldahl method (kes-125/kelvac/distyl-em/kel freeze, Chennai, India) (Hsu *et al.*, 2002) and soxhlet extraction method with petroleum ether was used for the gravimetric determination of fat content (Sanmee *et al.*, 2003). The total carbohydrate was calculated as: 100% - [%moisture+%ash+%crude protein+%fat+%fibre] (Güner *et al.*, 1998; Mattila *et al.*, 2002).

Extraction of proteins

Protein extraction was carried out for whole body GLF_{wb} and GLM_{wb} powder as well as lyophilized powder of *G. lucidum* aqueous extract (GLF_{Aq} and GLM_{Aq}). All four samples were ground thoroughly to a fine powder in liquid nitrogen by ceramic mortar and pestle. Protein was extracted using tris-glycerol assay and protein quantification by Bradford assay. Both samples were ground with extraction buffer (tris-pH 7.4, 100mM + glycerol 30%) in ratio of sample and buffer 1:10 followed by centrifugation for 20minutes at 12,000 rpm and finally discarded the residue/debris with the collection of the supernatant. Protein pellets were prepared using Pro-Q method. In this method, methanol was added to 150 μ l supernatant of all the samples, further $CHCl_3$ and Milli-Q were sequentially added to this and vortexed after each step. Protein discs were formed after centrifugation at 12,000rpm for 5minutes at 4 $^{\circ}$ C and were washed by methanol with centrifugation at same rpm for 5minutes. After that these pellets were dried on ice and used for protein separation (Jiang *et al.*, 2015).

Protein separation by one-dimensional gel electrophoresis (1-DGE)

One-dimensional well established SDS-PAGE gel electrophoresis technique (Weber & Osborn, 1969) was applied on 15% polyacrylamide gel for the separation of proteins. Bromophenol blue was added to the sample in a ratio 1:3 and then boiled for 5 minutes at 95 $^{\circ}$ C prior to gel electrophoresis. 30 μ l of both the samples were loaded then gel was stained with Coomassie brilliant blue R-250 and the separated proteins were visualized. Prestained SDS-PAGE standards of lower molecular weight range (14-97 kDa) were used as molecular marker.

In-gel trypsin digestion

For protein digestion, Coomassie brilliant blue R-250 stained 1-D gels were washed for 20min with Milli-Q and differential bands were manually cut out and subjected to in-gel digestion. Briefly, the whole-body *G. lucidum* fruiting body gel was cut into five sections, zone first (GLF_{W-1}), zone second (GLF_{W-2}), zone third (GLF_{W-3}), zone fourth (GLF_{W-4}) and zone fifth (GLF_{W-5}) and similarly its aqueous extract (GLF_{Aq}) gel was cut into five sections, zone first (GLF_{A-1}), zone second (GLF_{A-2}), zone third (GLF_{A-3}), zone fourth (GLF_{A-4}) and zone fifth (GLF_{A-5}) (Figure 1).

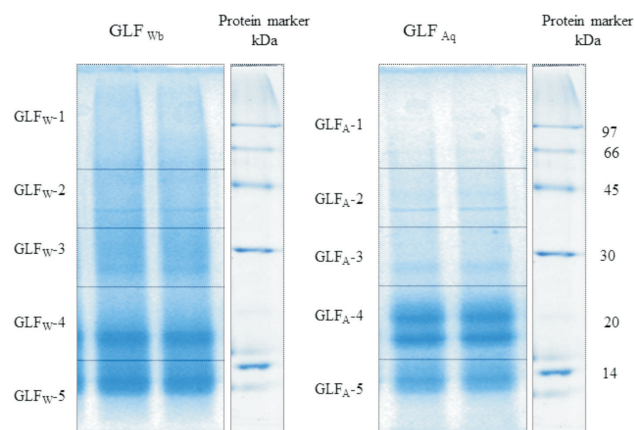


Figure 1: SDS-PAGE band profile of proteins (MW ranging from 14kDa- 97kDa) in both GLF_{wb} and GLF_{Aq} . The grids indicate how the gel bands (GLF_{W-1-5} , GLF_{A-1-5}) were cut for mass spectrometry. The right side of the figure indicates the molecular weight of the markers (kDa).

The whole body *G. lucidum mycelium* gel was cut into five sections, zone first (GLM_{W-1}), zone second (GLM_{W-2}), zone third (GLM_{W-3}), zone fourth (GLM_{W-4}) and zone fifth (GLM_{W-5}) and similarly its aqueous extract (GLM_{Aq}) gel was cut into five sections, zone first (GLM_{A-1}), zone second (GLM_{A-2}), zone third (GLM_{A-3}), zone fourth (GLM_{A-4}) and zone fifth (GLM_{A-5}) (Figure 2) and each sectioned gel fragment was washed thrice with Milli-Q followed by destaining with reagent 30mM potassium ferricyanide and acetonitrile (1:1). The destained gel pieces were washed thrice with Milli-Q and then with a mixture of Milli-Q/ACN/10mM NH_4HCO_3 (1:0.5:0.5) followed by washing with

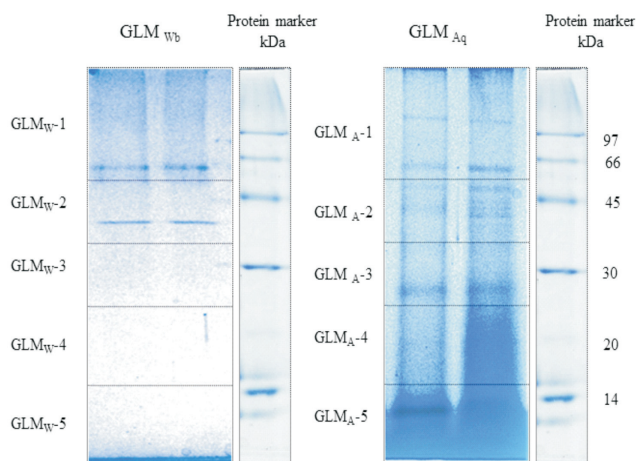


Figure 2: SDS-PAGE band profile of proteins (MW ranging from 14kDa- 97kDa) in both GLM_{wb} and GLM_{Aq} . The grids indicate how the gel bands (GLM_{W-1-5} , GLM_{A-1-5}) were cut for mass spectrometry. The right side of the figure indicates the molecular weight of the markers (kDa).

ACN. The washed gel pieces were dried using Speed-Vac concentrator (SAVANT SC250EXP, Delhi, India). In-gel tryptic digestion was performed overnight with incubation at 37°C. The supernatant was collected and washed by 50% ACN followed by reaction termination by acidification with 0.1% TFA solution (Ahmad *et al.*, 2013). The microcentrifuge tubes were sonicated, dried over Speed-Vac and supernatants were collected for MALDI-TOF/TOF analysis.

Mass spectrometry

MALDI-TOF/TOF was accomplished using ABSciex 5800 TOF/TOF system (ABSciex, USA). The tryptic digested peptides of each zone were mixed with an acidic solid matrix of α -cyano-4-hydroxy cinnamic acid (CHCA) of 10mg/ml concentration for peptide mass fingerprinting. This matrix was prepared in 70% acetonitrile (ACN) and 0.01% trifluoroacetic acid (TFA). 0.5 μ L of each digested protein and prepared matrix were mixed together and manually spotted onto a plate (384 opti-TOF 123mm x 81mm stainless steel (Applied Biosystem, ABSciex, USA) and subsequently dried at ambient temperature. The peptide mass spectra were recorded in positive ion reflectron mode using the above remarked mass spectrometer fortified with a 384-sample scout source and after each pulsed extraction the ion acceleration voltage was 29,000 V. The MS and MS/MS data were recorded repeatedly on the MALDI-TOF/TOF system per peptide mass fingerprinting (PMF) spectrum with the use of three most abundant peptide signals. In the Post Processing section, monoisotopic peak list was generated and True peptide mass list was created using the smoothing function by Protein pilot software version 3.2 (ABSciex). Database search with the peptide masses was completed against Global proteome server (GPS) explorer workstation installed with the MASCOT search engine (Matrix science) to search the database of the National Center for Biotechnology Information (NCBI) with following search parameter: Mass Tolerance: ± 250 ppm species, *Homo sapiens*; maximum number of missed cleavages was set to 1 for all the samples.

Statistical analysis

All the mean values and their standard deviations were calculated using Excel (Microsoft® Excel® 2016 MSO (16.07426.1015) 32-bit.

Results

All the studies including qualitative and proximate proteomics study were performed on the whole-body *G. lucidum* fruiting body, *G. lucidum* mycelium and their lyophilized aqueous extract obtained as per the method detailed in materials and methods.

Chemical composition analysis

Results of phytochemical analysis as presented in Table 1 clearly demonstrated the presence of alkaloids, amino acids, carbohydrates, flavonoids, phenolic compounds, proteins and saponins in all the four samples GLF_{Wb}, GLF_{Aq}, GLM_{Wb} and GLM_{Aq}. Interestingly, phytosteroids could be identified only in GLF_{Wb} and GLM_{Wb} but were absent in GLF_{Aq} and GLM_{Aq} sample as confirmed by Liebermann-Burchard's test.

Proximate analysis

The proximate chemical composition of GLF_{Wb} was found to be 2.29 \pm 0.003% fat, 23.12 \pm 0.001% protein, 35.23 \pm 0.0% carbohydrate, 33.53 \pm 0.0% crude fibre, 3.38 \pm 0.01% moisture and 2.45 \pm 0.01% ash. On the other hand, GLM_{Wb} contained 2.62 \pm 0.24% fat, 11.88 \pm 0.01% protein, 70.51 \pm 0.24% carbohydrate, 5.37 \pm 0.02% crude fiber, 7.43 \pm 0.01% moisture and 2.48 \pm 0.01% ash. The results are shown in Table 2.

Identification of proteins using MALDI-TOF-TOF

The protein profiling of all the four samples was analyzed using 1D SDS-PAGE as depicted in Figure 1. The characterization of protein zones by MALDI-TOF/TOF indicated the presence of several proteins with above 40 mascot score in all the samples, GLF_{Wb}, GLM_{Wb}, GLF_{Aq} and GLM_{Aq} (Supplementary information). The comparative protein analysis showed eleven proteins in GLF_{Wb}, thirty-five proteins in GLF_{Aq}, twenty-six proteins in GLM_{Wb} and twenty-six proteins in GLM_{Aq}.

Comparison of all the four samples (GLF_{Wb}, GLM_{Wb}, GLF_{Aq} and GLM_{Aq})

The results from supplementary information were compiled in the form of Venn diagram (Figure 3) which was drawn as per the method described elsewhere (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) to compare all the four samples with each other in terms of their identified proteins. Venn diagram clearly depicted the presence of three common proteins namely short-chain

Table 1
Phytochemical composition of all the four samples (GLF_{Wb}, GLF_{Aq}, GLM_{Wb} and GLM_{Aq})

S.No. Phytochemical tests	Observation			
	GLF _{Wb}	GLF _{Aq}	GLM _{Wb}	GLM _{Aq}
1. Alkaloids				
a. Mayer's test	+ve	+ve	+ve	+ve
2. Carbohydrates				
a. Molish's test	+ve	+ve	+ve	+ve
b. Fehling's test	+ve	+ve	+ve	+ve
c. Benedict's test	+ve	+ve	+ve	+ve
3. Saponins				
a. Foam test	+ve	+ve	+ve	+ve
4. Proteins and Amino acids				
a. Million's test	-ve	-ve	-ve	-ve
b. Biuret test	-ve	-ve	-ve	-ve
c. Ninhydrin test	+ve	+ve	+ve	+ve
5. Phytosteroids				
a. Liebermann-Burchard's test	+ve	-ve	+ve	-ve
6. Phenolic compounds and flavonoids				
a. Ferric chloride test	-ve	-ve	-ve	-ve
b. Lead acetate test	+ve	+ve	+ve	+ve
c. Magnesium and Hydrochloric acid reduction test.	-ve	-ve	-ve	-ve

+ve Present -ve Absent

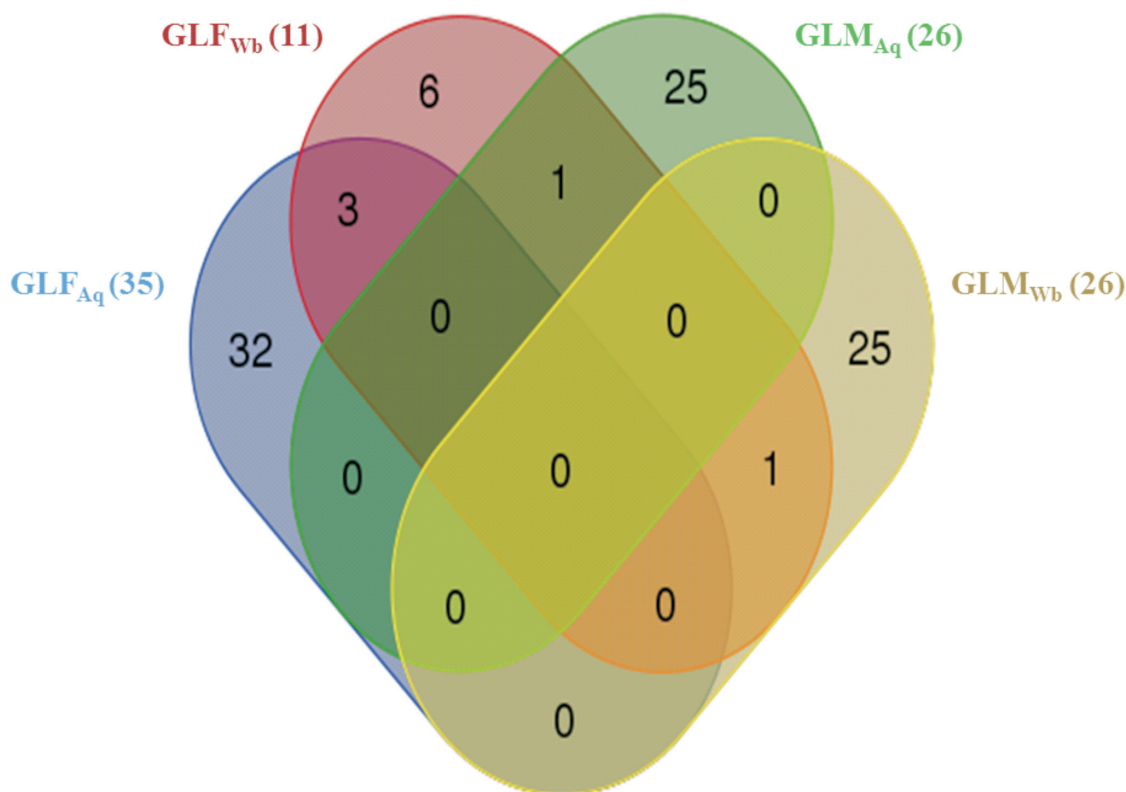


Figure 3: Venn diagram demonstrated the distribution of proteins among all the four samples (GLF_{Wb}, GLM_{Wb}, GLF_{Aq} and GLM_{Aq})

Table 2
Chemical composition of GLF_{wb} and GLM_{wb}

Parameters	Contents percent (%)±SD	
	GLF _{wb}	GLM _{wb}
Moisture	3.38±0.01	7.43±0.01
Total ash	2.45±0.001	2.48±0.01
Crude protein	23.12±0.001	11.88±0.01
Fat	2.29±0.003	2.62±0.24
Crude fiber	33.53±0.00	5.37±0.02
Carbohydrate	35.23±0.05	70.51±0.24

dehydrogenase, dynactin and nuclear distribution protein nudF 2 in both the crude samples GLF_{wb} and GLM_{wb}. However, presence of one common protein namely myosin was found in between GLF_{wb} and GLF_{Aq}. Another protein namely kinesin was present in between GLF_{Aq} and GLM_{Aq}.

Discussion

The medicinal and pharmacological activities of *G. lucidum* depend on the abiotic factors like altitude, temperature, humidity as well as abiotic factors like substrate which may in turn account for the presence of differential levels of primary and secondary metabolites (Morath *et al.*, 2012). Therefore, for the first-time, we ventured to evaluate a detailed comparative characterization of Indian variety of *G. lucidum* in terms of proximate composition analysis, phytochemical and proteome analysis between four selected samples GLF_{wb}, GLM_{wb}, GLF_{Aq} and GLM_{Aq}.

There are different classes of phytochemicals found in all the samples with characteristic biological activities such as anti-inflammatory, anti-microbial activities, anti-fungal and anti-cancer and presence of amino acids in the samples is furthermore reported to have the property of quenching the deleterious radicals (Yue *et al.*, 2008; Hong *et al.*, 2004; Eo *et al.*, 2000). However, the presence of phytosteroids in aqueous extracts (GLF_{Aq} and GLM_{Aq}) of the samples showed their effectiveness towards decreasing the cholesterol levels and the potential to restrain lung, stomach, ovarian and breast cancers. The presence of these phytochemicals indicated the medicinal importance of *G. lucidum*, which is very much in alignment with the earlier reported literature (Ren *et al.*, 2016).

The results of proximate analysis of GLF_{wb} and GLM_{wb} clearly revealed the presence of high protein

(21.12±0.001%, 11.88±0.01%), carbohydrate (35.23±0.05%, 70.51±0.24%) and low fat content (2.29±0.03%, 2.62±0.24%). The less moisture content (3.38±0.01%, 7.43±0.01%) suggested the high shelf life of the sample as also previously confirmed by Azanha and Faria (Azanha and Faria, 2005). Ash content (2.45±0.001%, 2.48±0.001%) is mostly studied in terms of mineral content of the original food. Presence of 33.53±0.00%, 5.37±0.02% crude fibre in GLF_{wb} and GLM_{wb} sample will improve the digestibility resulting in promotion of health benefits such as reduction of blood cholesterol etc (Goyal *et al.*, 2016).

All dried mushrooms are good source of quality proteins which could be used as supplementary diet for health purposes (Naknaen *et al.*, 2015). In this study, the proteins were first determined in phytochemical (qualitative) and proximate (quantitative) analyses which ascertained the enrichment of the samples with considerable amount of proteins. These observations encouraged us to carry out the proteome analysis for the identification of different kind of proteins present in all the four samples GLF_{wb}, GLM_{wb}, GLF_{Aq} and GLM_{Aq}.

Proteomic analysis using 1-DGE along with MALDI-TOF/TOF has been found as a powerful tool for the protein identification in fungi. One-dimensional gel electrophoresis method, an important and advantageous technique has been used to separate numerous proteins of molecular weight ranging from 14-97 kDa (Supplementary information) which are already found in different kinds of conventional medicinal products (Panda & Swain, 2011; Petrovska *et al.*, 2004). The protein profiles of GLF_{wb}, GLM_{wb}, GLF_{Aq} and GLM_{Aq} explored the similarities and differences in the four samples studied. Figure 1 and 2 showed more number of protein bands in GLF_{Aq} and GLM_{Aq} as compared to GLF_{wb} and GLM_{wb}. This may be attributed to the sample preparation protocol. It has been reported that use of ASE method for sample preparation provides higher yield and concentrates the constituents (Richter *et al.*, 1996). Higher number of proteins in GLF_{Aq} and GLM_{Aq} also suggests that aqueous *G. lucidum* (mycelium and fruiting body) is a rich source of edible proteins. Very limited studies are available for protein isolation, identification and quantification from *G. lucidum*. These proteins may further be evaluated and quantified using 2-DE coupled with MALDI-TOF/TOF analysis.

Proteome analysis of the selected samples of *Ganoderma lucidum* identified several proteins. The high abundant proteins in all the samples were segregated according to their biological functions (Supplementary information) and are discussed below in detail.

DNA replication licensing factor MCM7 is a protein that has been found in GF_W-2 encoded by the MCM7 gene in humans and is used in highly conserved mini-chromosome maintenance hence, significant for the initiation of eukaryotic genome replication. It may be involved in the formation of replication forks and in the recruitment of other DNA replication linked proteins (Sun *et al.*, 2016).

Myosin covers a family of ATP-dependent motor proteins present in GF_A-5 that are best known for their use in muscle contraction and their involvement in a wide range of other mobility processes mostly based on actin as stated by Pollard and Korn, 1973.

Malate dehydrogenase is a protein found in GF_W-2 which catalyses the oxidation of malate to oxaloacetate using the reduction of NAD⁺ to NADH hence may be involved in human metabolic process such as citric acid cycle, gluconeogenesis etc. (Iñiguez *et al.*, 2016).

Kinesins are microtubule (MT) filamentous protein identified in GM_W-1 powered by the hydrolysis of adenosine triphosphate (ATP) and the active movement of kinesins helps in numerous cellular functions for example mitosis, meiosis, transport of cellular cargo and in axonal transport etc. (Brendza *et al.*, 2000).

DNA repair protein Rad50 present in GM_A-2 is involved in DNA double-strand break repair. The Gene knockout studies of the mouse homolog of Rad50 propose that it is important for cell growth and viability.

UDP-N-acetylglucosamine pyrophosphorylase is an enzyme identified in GF_A-1 which catalyzes the chemical reaction and aminosugars metabolism (Mio *et al.*, 1998).

Hsk1-interacting molecule 1 protein present in GM_A-2 is known to participate in binding catalytic activity as established by Johnston *et al.*, 2012.

Conclusion

The phytochemical analysis suggested that all the four samples (GLF_{Wb}, GLM_{Wb}, GLF_{Aq} and GLM_{Aq})

are rich in flavonoids, phenolic compounds, steroids, saponins, alkaloids and low in fat content. Proximate analysis of GLF_{Wb} and GLM_{Wb} also highlighted the importance of *G. lucidum* as an edible nutritionally rich mushroom. 1-D SDS PAGE gel electrophoresis along with MALDI TOF/TOF of GLF_{Wb}, GLM_{Wb}, GLF_{Aq} and GLM_{Aq} confirmed the presence of several biologically active functional proteins. These proteins may further be evaluated for their characteristic biological efficacies.

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Abbreviations

G. lucidum, *Ganoderma lucidum*; GF, *G. lucidum*; GLF, *G. lucidum* fruiting body; GLM, *G. lucidum* mycelium; GLF_{Wb}, whole body of GLF; GLF_{Aq}, aqueous extract of GLM; HA, High altitude; ASE, Accelerated Solvent Extraction; rpm, revolution per minute; 1-DGE, one-dimensional gel electrophoresis; GLF_W-1, zone first of GLF_{Wb}; GLF_W-2, zone second of GLF_{Wb}; GLF_W-3, zone third of GLF_{Wb}; GLF_W-4, zone fourth of GLF_{Wb}; GLF_W-5, zone fifth of GLF_{Wb}; GLF_A-1, zone first of GLF_{Aq}; GLF_A-2, zone second of GLF_{Aq}; GLF_A-3, zone third of GLF_{Aq}; GLF_A-4, zone fourth of GLF_{Aq} and GLF_A-5, zone fifth of GLF_{Aq}; GLM_W-1, zone first of GLM_{Wb}; GLM_W-2, zone second of GLM_{Wb}; GLM_A-1, zone first of GLM_{Aq}; GLM_A-2, zone second of GLM_{Aq}; GLM_A-3, zone third of GLM_{Aq}; GLM_A-4, zone fourth of GLM_{Aq}; GPS, Global proteome server; ACN, acetonitrile; NH₄HCO₃, ammonium bicarbonate; CHCl₃, chloroform; CHCA, *o*-cyano-4-hydroxy cinnamic acid; NCBI, National Center for Biotechnology Information; PMF, peptide mass fingerprinting; TFA, trifluoro acetic acid; TCM, traditional Chinese medicine.

Conflict of interest

The authors declare that there is no conflict of interest.

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