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BIOCHEMICAL ESTIMATION OF WILD *GANODERMA LUCIDUM* COLLECTED FROM DIFFERENT AGRO-CLIMATIC ZONES OF HARYANA

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ABSTRACT

Ganoderma lucidum, commonly known as Rieshi or Lingzhi is a white-rot fungus of division basidiomycetes. *G. lucidum* is an important species from economical and medicinal point of view. Wild *G. lucidum* samples collected from four agro-climatic zones of Haryana state were oven-dried and analyzed for proximate, mineral and anti-nutrient compositions. The samples contained moisture content in the range of 75-80%, ash content (4-10%), crude fiber (20-38%), crude fat (3-5%), crude protein (18-22%) and carbohydrates (28-54%). Total soluble sugars were comparable within the range of 10.5-11.5%. No available calcium was found to be present whereas total iron and total zinc were present in the range of 3-6 ppm and 0.9-2 ppm respectively. Anti-nutrients, phytic acid and oxalic acid were found on the lower side in the range 1.7-3% and 0.5-0.9% respectively. The presence of these essential nutrients revealed the potential of *G. lucidum* to be used as a good diet supplement.

KEYWORDS: **Medicinal mushroom, *Ganoderma lucidum*, Neutraceutical, Bio-active components, basidiomycetes**

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INTRODUCTION

Mushrooms are higher fungi belonging to division Ascomycota and Basidiomycota. There are more than 38,000 varieties of mushrooms out of which about 2,000 varieties are fit for human consumption and about 200 varieties have therapeutic (medicinal) properties[1]. Out of the numerous species of mushrooms, *Ganoderma lucidum* is an important species, both from economical and medicinal point of view[2,3]. Commonly known as Lingzhi (mushroom of herb and immortality) in Korea and China, *G. lucidum* is found growing on dead or dying trees as a parasite[2,4]. *G. lucidum* is a reddish-brown coloured white-rot fungus of division basidiomycetes[5]. Light, temperature and humidity are mainly responsible for the variation of shape of fruiting bodies of *G.lucidum*[6]. From ancient times, mushrooms have been used by mankind. Earlier the mushrooms were consumed to combat the nutritional deficiency due to food shortage. Now-a-days, the usage of mushrooms shows an altered trend and lot of research work is being carried out on the chemical composition of mushrooms[1]. *G. lucidum* finds an important place due to its medicinal properties as it contains more than 400 bio-active elements. It is a non-edible medicinal mushroom, but its consumption as health enhancer is miraculous. It has been nominated as fungus of the year (2013) by the Journal "Mycology"[2,3]. *G. lucidum* has been used as drug for years in Asian countries[6,7]. India has a long recorded history of occurrence and usage of mushroom varieties rich in nutritional and medicinal values[4]. *G. lucidum* has been reported to possess anti-tumor, anti-oxidant, cholesterol lowering, detoxification, blood pressure lowering, anti-microbial, immune-modulating, blood glucose lowering and anti-allergic effects[2,8,9,10,11,12].

Most mushrooms, including *G. lucidum* are mainly composed of water, carbohydrate, crude fat, crude fibre and crude protein. Triterpenoids, steroids, glycoproteins, phenols and polysaccharides constitute the bioactive components of *G.lucidum*[4,9,10,12,13]. These components are helpful in maintaining good health and meeting nutritional requirements[7]. The biochemical composition and the pharmacological action of *G. lucidum* may vary depending on the origin, strain and cultivating conditions[2,14]. *G. lucidum* are grown and used as medicine and feed supplement in the form of tablets, powder and capsules[4,7]. India has variable ecological conditions which favour high biodiversity. *G. lucidum* from Haryana state are still unexplored and their nutritional and health benefits are not studied. Also the variation in occurrence according to the agro-climatic zones is not known. Some of them may be found to own high nutritional value and bioactive compounds with pharmaceutical applications. The objective of this study was collection, identification and biochemical analysis of wild *G. lucidum* collected from four different agro-climatic zones of Haryana state and explores their medicinal importance.

MATERIALS AND METHODS

Chemicals

All the chemicals used were of analytical grade. The chemicals were purchased from Hi-media and Qualigens fine chemicals- Fisher Scientific.

Collection of wild Ganoderma lucidum

The wild, matured reddish-brown fruiting bodies of *G. lucidum* were collected in wet rainy months found growing on tree trunks in the year 2012 and 2013. The collection was done from four agro-climatic zones of Haryana state. The north-eastern zone comprises of Panchkula, Yamunanagar, Ambala, Karnal and Kurukshetra districts. The south-western zone comprises of Sirsa, Hisar, Fatehabad and Bhiwani districts. The central zone comprises of Sonepat, Jhajjar, Rohtak, Panipat and Jind districts. Faridabad, Gurgaon and Rewari districts fall under south-eastern zones. 3-4 samples from each place were collected, making a total of 64 samples. Four wild *G. lucidum* samples collected from each agro-climatic zone of Haryana state are shown in Figure 1.

Sample preparation for analysis

Wild fruiting bodies thus collected were allowed to dry in hot air oven (40°C) and finally crushed to powdered form for further biochemical analysis.

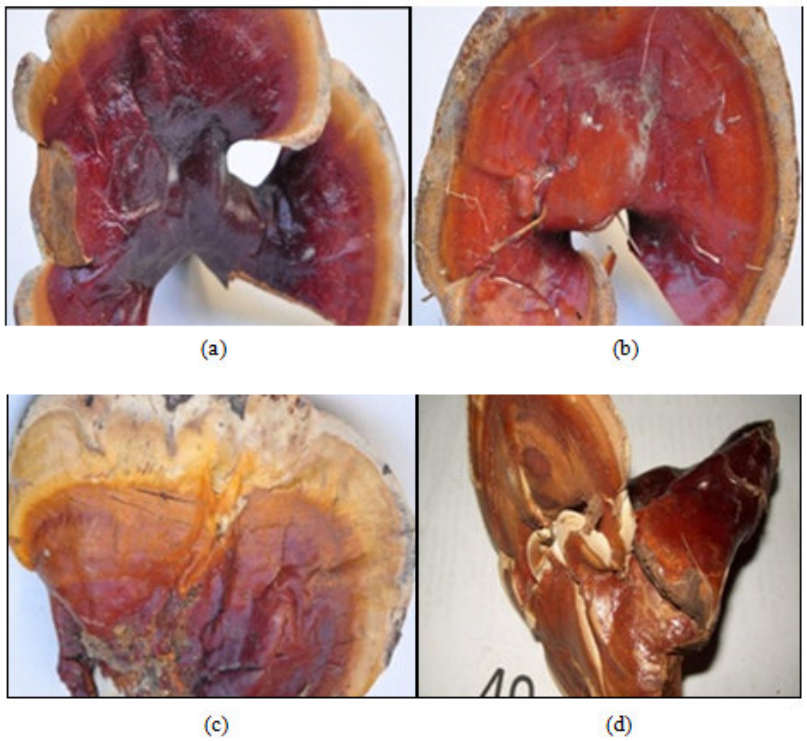

Figure 1

Wild Ganoderma lucidum samples collected from four agro-climatic zones of Haryana state; (a) North-east, (b) South-west, (c) Central zone and (d) South-east zones

Proximate composition

All experimentations were performed in duplicates for all the 64 samples collected.

Moisture, ash content and crude fiber

Moisture content, ash content and crude fiber was estimated by employing the standard method of analysis (AOAC, 1995)[15].

Crude fat

Crude fat was estimated using Soxhlet method (AOAC, 1995) [15]. Petroleum ether of boiling range 40-60°C was used as a solvent.

Crude protein

Crude protein was determined by Microkjeldahl method (AOAC, 1995) [15].

Total carbohydrates

Total carbohydrates were determined by using the following formula:

Total carbohydrate = 100 – (crude protein + crude fat + crude fiber + ash)

Available carbohydrates

Total soluble sugars

Total soluble sugars were estimated by the method of Yemm and Willis, (1954)[16].

Reducing sugars

Reducing sugars were estimated by Somogyi's modified method (Somogyi, 1945) [17].

Non-reducing sugars

Non-reducing sugars were determined by using the following formula:

Non-reducing sugars= (Total soluble sugars – reducing sugars)

Total minerals

Calcium, iron and zinc

Total calcium, iron and zinc in acid digested (HNO_3 : $HClO_4$ :: 5:1, v/v) samples were determined by the atomic absorption spectrophotometer following the method of Lindsey and Norwell (1969) [18].

Anti-nutrients

Phytic acid

Phytic acid was determined by the method of Haug and Lantzsch (1983)[19].

Oxalic acid

Oxalic acid was analyzed by the method of NIN (1980) [20].

Data analysis

Mean and standard deviation were calculated using Microsoft Office Excel to determine the representative values of each parameter for respective zones. One way ANOVA was performed using online calculation on www.easycalculation.com to find the variance between and within the samples in the zones. Graphs were constructed using MS Excel.

RESULTS

Collected samples were identified at Integrated Mushroom Project, HAIC, Murthal. The agro-climatic zones of Haryana state used for the sample collection were as per Haryana Government to improve horticultural products, 2002. The samples were properly packed, labelled and stored at room temperature. Different parameters were estimated to determine the proximate, mineral and anti-nutrient compositions. The biochemical parameters which were estimated include moisture content, ash content, crude fiber, crude fat, crude protein, total carbohydrates, available carbohydrates (total soluble sugars, reducing sugars, non-reducing sugars), minerals (Fe, Zn, Ca) and anti-nutrients phytic acid and oxalic acid. So, total 14 parameters were estimated for 64 wild *G. lucidum* samples.

Table 1

Proximate, mineral and anti-nutrient compositions of Ganoderma lucidum collected from the agro-climatic zones of Haryana state

ZONES: COMPONENTS	North-east	South-west	Central	South-east
MOISTURE (%)	78.73 ± 0.42	75.35 ± 0.48	79.93 ± 0.23	74.69 ± 0.62
ASH CONTENT (%)	7.82 ± 0.22	6.89 ± 0.18	9.70 ± 0.13	3.66 ± 0.15
CRUDE FIBER (%)	33.98 ± 0.69	29.42 ± 0.42	37.60 ± 0.36	19.67 ± 0.32
CRUDE FAT (%)	3.37 ± 0.15	3.89 ± 0.13	2.78 ± 0.11	4.80 ± 0.13
CRUDE PROTEIN (%)	21.14 ± 0.13	20.17 ± 0.12	21.97 ± 0.27	17.92 ± 0.28
TOTAL CARBOHYDRATES (%)	33.69 ± 0.82	39.64 ± 0.21	27.97 ± 0.54	53.95 ± 0.13
TOTAL SOLUBLE SUGARS (%)	11.12 ± 0.21	11.14 ± 0.25	11.36 ± 0.24	11.12 ± 0.21
REDUCING SUGARS (%)	2.36 ± 0.02	2.11 ± 0.06	2.49 ± 0.04	2.36 ± 0.02
NON-REDUCING (%)	8.76 ± 0.22	9.03 ± 0.27	8.88 ± 0.23	8.76 ± 0.22
TOTAL IRON (ppm)	4.84 ±0.11	3.64 ± 0.15	5.92 ± 0.11	2.80 ± 0.20
TOTAL ZINC (ppm)	1.23 ± 0.10	1.00 ± 0.06	1.50 ± 0.19	0.86 ± 0.04
TOTAL CALCIUM (ppm)	NP	NP	NP	NP
PHYTIC ACID (%)	2.42 ± 0.18	2.45 ± 0.12	2.78 ± 0.11	1.83 ± 0.11
OXALIC ACID (%)	0.64 ± 0.02	0.66 ± 0.04	0.78 ± 0.05	0.58 ± 0.04

**Data shown is mean composition ± standard deviation, expressed as g/100g sample dry weight of G. lucidum (%) and parts per million (ppm) from respective zones of Haryana state; NP= Not Present.*

The values represented in Table 1 are the average value ± standard deviation of the estimated parameters of the respective agro-climatic zones of Haryana state, namely north-east, south-west, central and south-east zones. The results of proximate and anti-nutrient compositions have been expressed as g/100g sample dry weight of *G. lucidum* and the results of total minerals content have been expressed as parts per million (ppm).

Figure 2
Proximate composition of Ganoderma lucidum collected from the agro-climatic zones of Haryana state

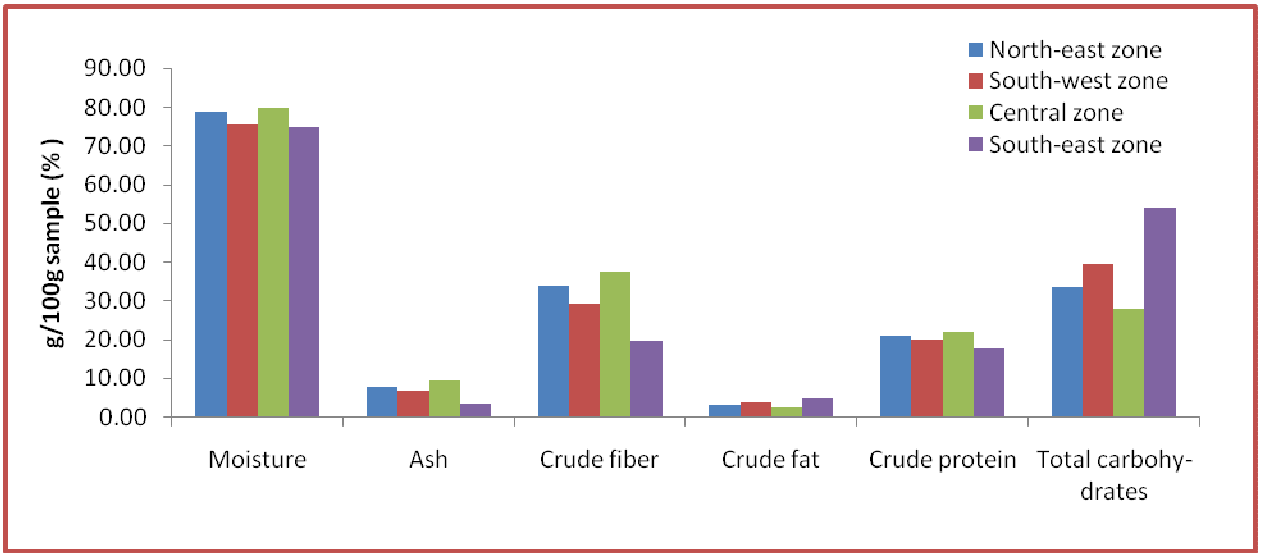

Figure 3
Sugar Content (%), Total minerals (ppm) and Anti-nutrient (%) composition of Ganoderma lucidum collected from the agro-climatic zones of Haryana state

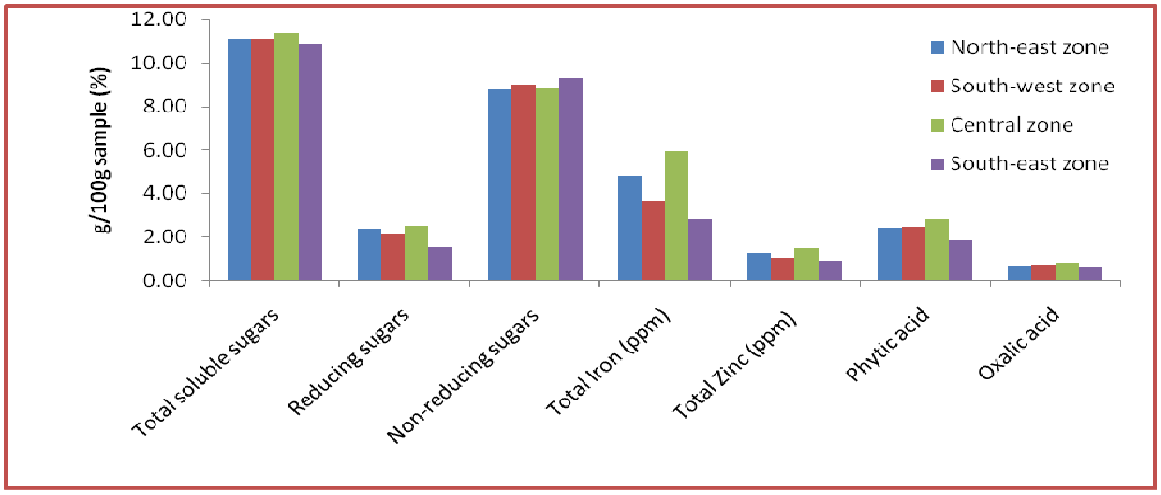

Figure 2 and 3 show the graphical comparison of the compositional analysis performed on the *G. lucidum* samples. Comparison between the zones has been shown, the *G. lucidum* samples from the central zone were found to be comparatively more potent to be used as health supplement, followed by the samples from the north- east zone of Haryana state. Moisture content ranged from 75-80%. The samples from four different zones showed significant differences in their biochemical composition, ash content in the range of 4-10%, crude fiber (20-38%), crude fat (3-5%), crude protein (18-22%) and carbohydrates (28-54%). Total soluble sugars were comparable within the range of 10.5-11.5%. No available calcium was found to be present whereas total iron and total zinc were present in the range of 3-6 ppm and 0.9-2 ppm respectively. Anti-nutrients, phytic acid and oxalic acid were found on the lower side in the range 1.7-3% and 0.5-0.9% respectively. Central zone of Haryana state was found to have maximum amount of ash content, crude fiber, crude protein, carbohydrates and

Fe and Zn contents. The presence of anti-nutrients was found to be negligible. Mineral content was also low in the regional *G. lucidum* samples. One way ANOVA was performed to analyze if there were some differences within the samples of a zone, leading to overall differences. It was found that no significant variance existed within the samples from a zone, (MS_{within} = 0.01-0.2).

DISCUSSION

Wild *G. lucidum* samples from four agro-climatic zones of Haryana state and were analyzed for proximate, mineral and anti-nutrient compositions which showed significant differences in the biochemical composition. These bio-molecules play an important role in the maintenance and proper functioning of vital organs of human body. The moisture content ranged from 74-80 %, maximum average value being 79.93% ± 0.23 of the samples from the central zone. The value was lower than that of some edible mushrooms reported in earlier studies[20]. The difference in the moisture content between the zones might be due to environmental factors, i.e. temperature and relative humidity during growth of mushrooms[21]. The ash content was observed maximum in central zone of Haryana state, 9.70 % ± 0.13 from the range 3-10%. The estimated values are consistent with earlier studies on *G.lucidum*[7,9,12]. These results were also in agreement with studied on other mushrooms like *Agaricus bisporus* (7.01%)[22]; *Calocybe indica* (7.43%), *Pleurotus sajor-caju* (6.32%), *Flammulina velutipes*(7.40%)[23]. The crude fiber showed maximum value 37.60 % ± 0.36 in samples from Central zone. Similar values have been reported by various workers with *G.lucidum*[7,9,12] and on other mushrooms also[22,23]. The results of crude fat (minimum 2.78 % ± 0.11) and crude protein (maximum 21.97% ± 0.27) estimated were consistent with earlier studies[7,9,12,21,22]. Mushrooms are known for their low fat contents[24]. The carbohydrate content was found to be in the range 27-54%, maximum being 53.95 % ± 0.13 which was lower than that reported in an earlier study[7] but the amount was higher than that reported in an another study[11] on *G. lucidum*. The same range was observed in the case of other mushrooms such as *Agaricus bisporus,* the most popular edible mushroom, *Calocybe indica*[22] and *Lentinus edodes*[23]. The maximum values of total soluble, reducing and non-reducing sugars were 11.36%, 2.49% and 8.88% respectively in the samples of central zone. However, non-significant difference was observed in sugar contents of all the four zones.

Very low parts per million (ppm) concentrations of minerals (Fe and Zn) were present in all the collected *G. lucidum* samples of Haryana state. Calcium was found to be absent. This observation is in contradiction with earlier reported studies by various workers on *G. lucidum*[7,9]. Non-availability might be due to phytates[25] and oxalates[26] which form complex with calcium and reduces the bioavailability. Low availability of Zinc might also be due to anti-nutrients present in mushroom samples[27]. High amount of phytic acid and oxalic acid in dietary items may cause health problems if utilized unprocessed[8]. Phytic acid chelates mineral elements like Calcium, Iron, Zinc and Magnesium, thus reducing the availability[28]. The anti-nutrients, phytic acid and oxalic acid were found to be on a lower side in the range 1.7-3% and 0.5-0.9% respectively, in all the wild *G. lucidum* collected, maximum being 2.78 % ± 0.11 and 0.78 % ± 0.05 respectively in the central zone. Similar levels of phytic acid and oxalic acid (2.43%±0.09, 0.57%±0.06 respectively) have been reported[7]. The amounts of ash content, crude fat, crude protein, carbohydrate content, phytic acid and oxalic acid from the *G. lucidum* collected from Haryana state were consistent with the work reported for the biochemical analysis of plants and herbs[29]. *G. lucidum* shows variations in biochemical composition. The variability may be due to differences in the origin, strain and cultivation conditions[2,14]. The observation of the present study supports the comparable performance of *G. lucidum* to plants and herbs to be utilized as a source of feed supplement. The use of *Moringa oleifera* leaves and *G. lucidum* has been successfully studied and reported in improvement of health of poultry due to the presence of these essential nutrients[7,9,29].

Wild samples of *G. lucidum* from Central zone of Haryana state were found to contain a maximum average amount of ash content, crude fiber, crude protein, carbohydrates, Iron, Zinc and minimum amount of crude fat, phytic acid and oxalic acid. So, the *G. lucidum* samples from Central zone showed the maximum potential to be used as a health food, followed by the samples of North-east zone, then samples from the South-west zone of Haryana state. The samples of *G. lucidum* from the South-east zone of Haryana state were found to show the minimum presence of above mentioned bio-molecules, as clear from Table 1 and graphical comparison given in Figure 2 and Figure 3. The future prospective of the study is to utilize the potential of *G. lucidum* of Haryana state as a feed supplement at a large scale for health improvement purposes.

CONCLUSION

This is the first comprehensive study for the analysis of biochemical composition of *Ganoderma lucidum* from different agro-climatic zones of Haryana state. The wild *G. lucidum* samples collected from Haryana state contained appreciable amounts of carbohydrate, protein, fiber, which are nutritional requirements to improve health and growth performance. The samples from the central zone contained the maximum values of carbohydrates, protein and fiber and lowest crude fat content, followed by the north-eastern zone. The anti-nutrients and total minerals present in the wild *G. lucidum* were found to be low. The present study reveals the potential of *G. lucidum* of Haryana state to be used as good diet supplement which is of the prime concern in the growing area of neutraceutical and pharmaceutical industries.

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