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Evaluation of polyphenolic contents and antioxidant activity of wildly collected *Ganoderma lucidum* from central Himalayan hills of India

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ABSTRACT

Ganoderma lucidum, a unique woody and spongy mushroom, belonging to family Ganodermataceae is widely distributed throughout the world. It is a highly medicinal mushroom which is being consumed for its pharmaceutical value rather than as food. To evaluate the polyphenolic contents (flavanoids) of both methanolic and aqueous extracts, and evaluate its anti- oxidant activity against ABTS [2, 2' –azinobis (3-ethylenebenzothiazoline-6-sulphonate)] and DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals. Phenolic contents were determined by using Ci0calteu method and aluminium clod method respectively using quercetin catechol as a standard. Extract containing predominant amount of phenolic was evaluated for its anti- oxidant activity against ABTS and DPPH free radicals. And its activity was compared with quercetin and ascorbic acid. In this study, G. lucidum's methanloic extract was found to contain 9.245 ± 0.184 mg Catec hol Eq/gm sample dry weight of total phenolics and 2.140 ± 0.132 mg Quercetin Eq/gm sample dry weight of flavanoids which was quite higher than its aqueous extract. It was also found to possess anti- oxidant activity with IC_{50} value of 1.162 ± 0.016 mg/ml against DPPH and 0.555 ± 0.009 mg/ml against ABTS free radicals. These findings demonstrate that G. lucidum has a potent anti- oxidant activity which can be used in formulation of many anti- oxidant products. It can be use as a source of phenolics, and can be regarded as a potent herb for various ailments caused by oxidant species.

Key words: *Ganoderma lucidum*, phenolics, flavanoids, DPPH, ABTS

INTRODUCTION

Ganoderma lucidum (W.Curt.:Fr.) P.Karst (Lingzhi or Reishi, Ganodermatacea, higher Bsidomycetes) is a high value medicinal mushroom, which is well known for nutraceutical and pharamaceutical properties promoting human health. Accordingly, it is widely used for nutritional supplement and broad spectrum high medicinal value functional food and source of more than 400 physiologically beneficial bioactive molecules [1]. Some important applications of such nutaraceuticals include anti-allergic-activities [2], antitussive properties, bronchitis-preventive effect including regeneration of bronchial epithelium, anti-inflammatory, antibacterial, antitumor activities [3], enhancing myocardial metabolism, and lowering blood pressure [4]. The presence of various polyphenolic contents and untapped bioactive components, such as beta- and hetero-beta-glucans, ling zhi-8 protein, and ganoderic acids (triterpenes) have also been observed in *G.lucidum* [5].

The major aspect of *G. lucidum* products is to inhibit reactive oxygen species (ROS). The scavenging of free radicals is the prime factor for controlling degenerative or pathological process of various serious ailments in the human body, such as aging [6], cancer [7], Alzheimer's disease [8], heart diseases [9], neurodegenerative disorders, atherosclerosis, cataracts, and inflammation [10].

The present study was considered to evaluate the antioxidant behaviour of *G. lucidum* grown under stressed cold and humid climate conditions of the Central Himalayan Hills (2000 m MSL) of Indian subcontinent. Two types of in-vitro assays, DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS [2, 2' –azinobis (3-ethylenebenzothiazoline-6-

sulphonate)] were performed. Total phenolic contents (TPC), total flavonoid contents (TFC) were determined, to evaluate their effects on free-radical scavenging capabilities of *G. lucidum* using different extracts.

MATERIALS AND METHODS

Collection and Authentication

Wild samples of *Ganoderma lucidum* were collected from the local regions of Pithoragarh, Uttarakhand, India. It was authenticated by the Mycology department, DIBER, field station, Pithoragarh. Samples were then air dried, lyophilized and crushed to powdered form.

Twenty grams of each of the samples was extracted with 200 ml of methanol and water, separately, at room temperature, with agitation for 24 – 30 hr. Methanolic extracts were filtered off using Whatman no. 1 filter paper, and concentrated under reduced pressure up to dryness below 35°C. Water extracts were freeze-dried.

Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2' –azinobis-3 ethylenebenzothiazoline - 6 - sulphonate ammonium salt (ABTS), catechin, quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA); Folin-Ciocalteu phenol reagent, potassium ferricyanid, ascorbic acid, vannilin, and sodium carbonate were purchased from Merck Chemical Supplies (Darmstadt, Germany). All the other chemicals used, including the solvents, were of analytical grade.

Determination of Total Phenolic contents

Total phenolic contents in the extracts were determined by the modified Folin Ciocalteu Method [11]. 1.0 mg/ml for each sample of both water and methanolic extract. was mixed with 5 ml Folin Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4ml (75 gm/l) of sodium carbonate. The tubes were vortexed for 30 seconds and allowed to stand for 30 minutes, at room temperature, for color development. Absorbance was then measured at 765 nm using the Double beam PC based Labomed UV-Vis spectrophotometer. Total phenolic contents were expressed as milligram Catechol Equivalent/g (mgCE/gm) of sample dry weight using the following equation based on the calibration curve: $Y= 0.015x + 0.013$ $R^2= 0.966$; where, x was the absorbance and y was the mgCE/gm of sample dry weight.

Determination of Total Flavonoid contents

Flavonoids were estimated using Aluminium chloride colorimetric method [12]. 1.0 mg/ml of sample extract of both water and methanolic extract was mixed with 0.1 ml 10% aluminium chloride, 0.1 ml of 1.0 M potassium acetate and 2.8 ml of distilled water. The reaction mixture was vortexed for 30 seconds ,allowed to remain at room temperature for 30 minutes, and then the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared using quercetin as standard. Flavonoid contents expressed as mg Quercetin equivalent/gm (mgQE/gm) of sample dry weight using the following equation based on the calibration curve, $Y= 0.014x + 0.029$ $R^2= 0.0992$; where, x was the absorbance and y was the mgQE/gm of sample dry weight).

DPPH Radical-Scavenging Activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activities of the extracts [13]. Both methanolic and water extracts were having their own pale brown colorations therefore absorbance was corrected accordingly in the experiment. A different concentration of each extract was added, at an equal volume, to methanolic solution of DPPH (0.1 Mm). After 30 minute, at room temperature, the absorbance was recorded at 517nm. The experiment was repeated for three times. Accorbic acid and sample, which is required to scavenge 50% of DPPH free radicals. Percentage inhibition was calculated as,

$$\% \text{ scavenged DPPH radical} = [(Abs_{control} - Abs_{sample}] / Abs_{control}] \times 100$$

Where, Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract/standard.

ABTS Radical-Scavenging Activity

Free radical scavenging capacity of *G.lucidum* was also assessed with the help of 2,2'-azinobis [3-ethylbenzthiazoline] -6-sulfonate (ABTS) assay [14]. 7.0 mM ABTS solution in methanol and 2.45 Mm potassium persulfate solution in water were prepared as separate stock solution in equal quantities, and allowing them to react for 12 hrs, at room temperature, in the dark. The solution was then diluted by mixing 1.0 ml of ABTS solution and appropriate volume of methanol, to obtain the absorbance 0.702± 0.001 units, at 734nm. Mushroom extracts (0.1

ml) were allowed to react with 1.0 ml of the ABTS scavenging capacity for the extracts was compared with ascorbic acid and quercetin.

% scavenged ABTS radical =$[(Abs_{control} – Abs_{sample})]/ (Abs_{control})] \times 100$

Where, Abs control I the absorbance of ABTS radical + methanol; Abs sample is the absorbance of ABTS radical + sample extract/standard.

RESULTS

Total Phenolic contents

The total phenolic content in methanolic extracts of *G.lucidum* was found to be 9.2 mg Catechol Equivalent/gm dry weight of *G.lucidum* (Table 1).

Total Flavonoid contents

The total flavonoid contents in methanolic extracts of wild *G.lucidum* was found to be 2.14 mg Quercetin Equivalent/gm dry weight *G. lucidum* (Table 1).

Table 1: Total phenolics and flavonoids in *G. lucidum*

Extracts	Total Phenolics (mgCatechol Eq/gm)	Total Flavonoids (mgQuercetin Eq/gm)
Methanol	9.245 ± 0.184	2.140 ± 0.132
Aqueous	8.442 ± 0.104	1.841 ± 0.084

DPPH Radical Scavenging Activity

In DPPH assay, IC_{50} value for methanolic extract of wild *G.lucidum* was found to be 1.162 mg/ml (Table 2). The graph of *G. lucidum* extract was also compared to the standard graph of Quercetin and Ascorbic acid given in Figure 1b and 1c.

Figure 1 : In vitro DPPH radical scavenging activity of a) *G. lucidum* extract b) Quercetin and c) Ascorbic acid

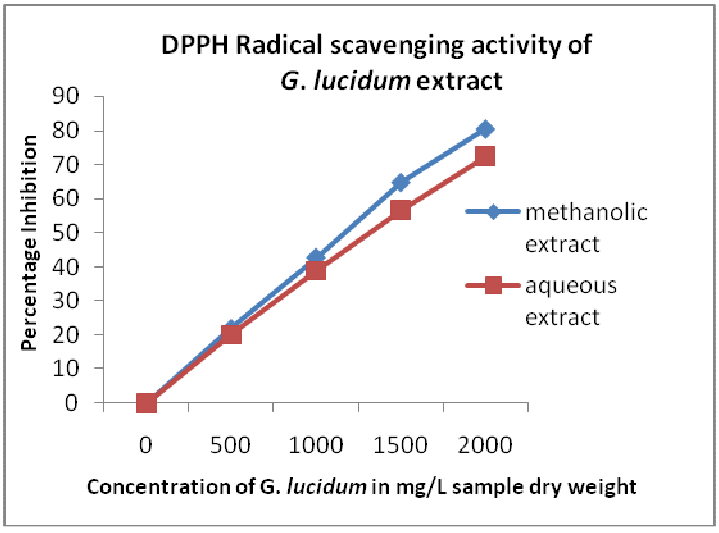

Figure 1a. In vitro free radical scavenging pattern of *G. lucidum* extracts with DPPH.

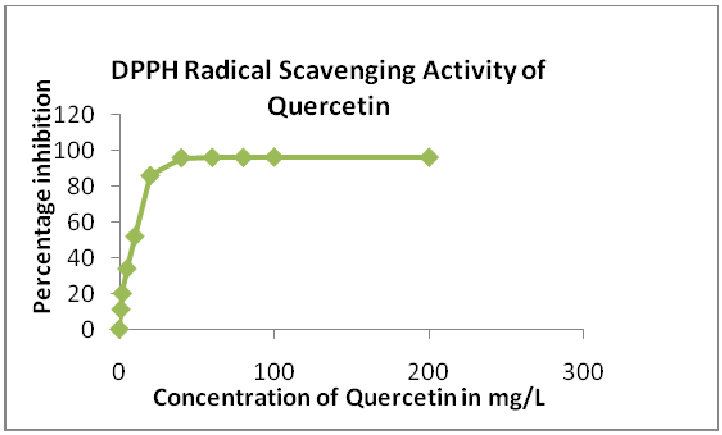

Figure 1b. In vitro free radical scavenging pattern of Quercetin with DPPH.

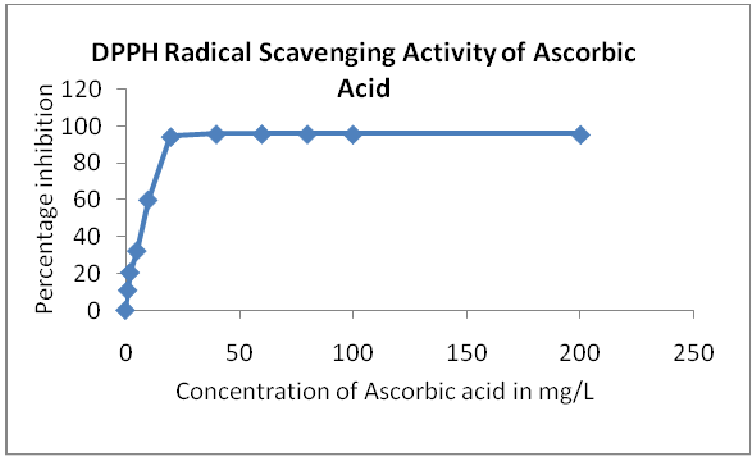

Figure 1c. In vitro free radical scavenging pattern of Ascorbic acid with DPPH

ABTS Radical Scavenging Activity

IC_{50} values for methanolic extract of wild *G. lucidum* was 0.555 mg/ml, for ABTS assay (Table2). The graph of *G. lucidum* extract was also compared to the standard graph of Quercetin and Ascorbic acid given in Figure 2b and 2c.. All the findings were statistically significant.

Figure 2 : In vitro ABTS radical scavenging activity of a) *G. lucidum* extract, b) Quercetin and c) Ascorbic acid

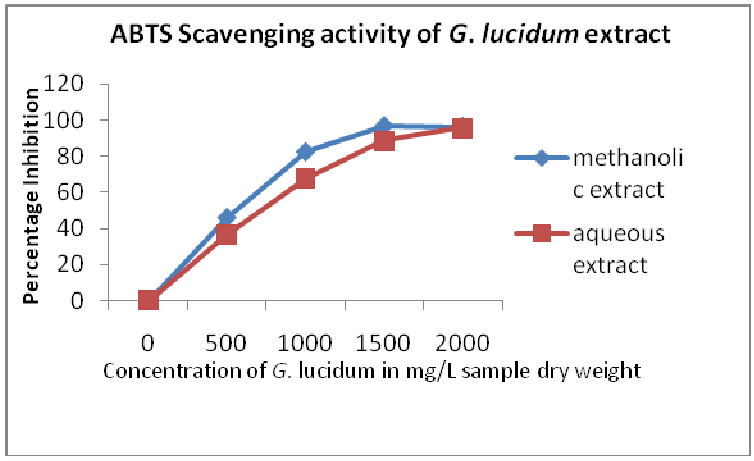

Figure 2a. In vitro free radical scavenging pattern of *G. lucidum* extracts with ABTS.

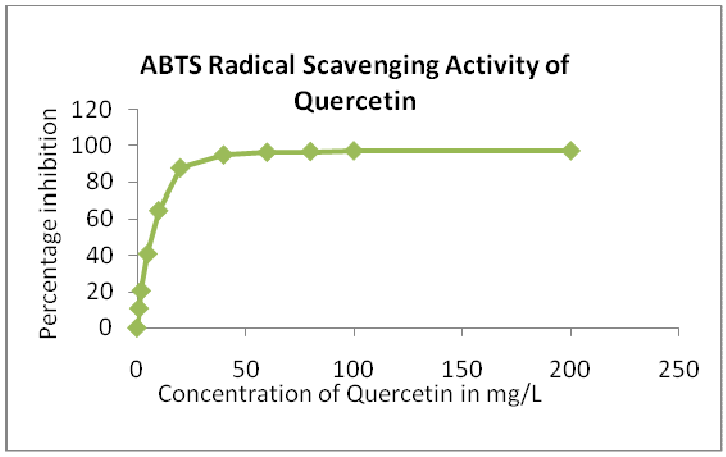

Figure 2b. In vitro free radical scavenging pattern of Quercetin with ABTS.

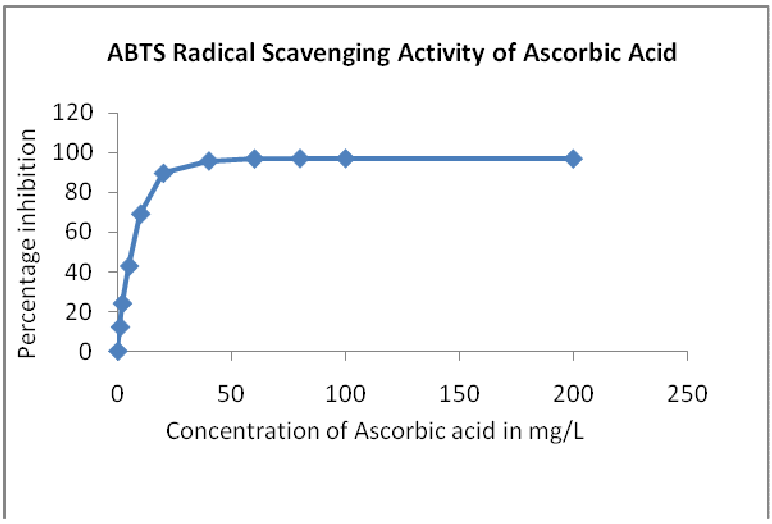

Figure 2c. In vitro free radical scavenging pattern of Ascorbic acid with ABTS.

Table 2 : IC_{50} values of *G. lucidum* (GL) and standard anti- antioxidants in against in vitro DPPH and ABTS radical systems.

Sample	DPPH radical system	ABTS radical system
GL methanolic extract	1.162 ± 0.016 mg/ml	0.555 ± 0.009 mg/ml
GL aqueous extract	1.309 ± 0.012 mg/ml	0.716 ± 0.006 mg/ml
Quercetin	9.479 ± 0.246 µg/ml	6.943158± 0.135 µg/ml
Ascorbic acid	8.210 ± 0.328 µg/ml	6.385 ± 0.253 µg/ml

IC_{50} is the minimum concentration of the extract that inhibits the absorbance of free radicals up to 50% from their initial absorbance. Data are expressed as means ±SD (N=3). IC_{50} values were calculated from linear regression analysis.

DISCUSSION

Phenolic compounds form a major class of phytochemicals, which are responsible for inhibiting the oxidative damage caused by free radicals generated inside of our body [15]. The concentration of phenolic compounds was higher in the methanolic extracts, when compared with the aqueous extracts. The total phenolic content in methanolic extracts of *G.lucidum* clearly demonstrates that it can be considered as a better source of polyphenols.

Flavonoids are well-known dietary biochemical agents, which show pH dependent antioxidant behaviour in human body. These molecules are also effective for cardiovascular system and work as cardio protective agents [16]. The concentration of phenolic compounds was higher in the methanolic extracts, when compared with the aqueous extracts. The total flavonoid contents in methanolic extracts of wild *G.lucidum* implies that extracts with the higher concentration of total phenols is also having more concentrations of flavonoids.

DPPH radical scavenging activity of the extract was found to be increased in dose dependent manner (Figure 1a). The scavenging effect increased with the concentration of extract up to 2.0 mg/ml dry weight of sample. At and above this concentration, more than 80% scavenging of free radicals was observed, and further no sufficient increase

in radical scavenging was observed. Therefore, at this concentration level, *G. lucidum* extract is supposed to show appropriate beneficiary effects to the human health.

The DPPH scavenging activity of the extract is dependent on various biochemicals besides the polyphenolic contents [17]. Radical scavenging involves exchange of a hydrogen atom or electron [18]. There might be some other chemical moieties other than the phenolics, which play important role in DPPH radical scavenging [19]. Experimental results are often influenced by Radical systems, and therefore it is worthwhile to analyze the extracts for free radical scavenging activity against ABTS free radical. Methanol and aqueous extracts of *G. lucidum* were evaluated comparatively for their scavenging capacities. The extracts showed significant ABTS scavenging capacity in dose dependent manner (Figure 2a). Furthermore, extracts exhibited the scavenging potential more than 75% above the concentration 2.0 mg/ml, which is comparable to standard antioxidants like ascorbic acid and quercetin (Figure 2) at this concentration. IC_{50} values for methanolic extract of wild *G. lucidum* was 0.555 mg/ml, for ABTS assay (Table 2).

CONCLUSION

The study of anti- oxidant activity of *G. lucidum*, collected from stressed environmental conditions of Central Himalayan Hills revealed that the samples have a high potential of free radical scavenging and high phenolic constituents. Good extractability of bioactive antioxidants i.e., flavonoids and phenolic contents in the methanol was also established in this study. Methanolic extract has been found to possess higher free radical scavenging activity as compare to the free radical scavenging activity shown by the water extract of *G. lucidum* in both ABTS [2, 2' – azinobis (3-ethylenebenzothiazoline-6-sulphonate)] and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical environment.

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REFERENCES

[1] S.P. Wasser, A.L. Weis, *Int J Med Mushrooms*, **1999**,1, 31-62.
[2] T. Mizuno, 4th International Symposium on *Ganoderma lucidum*, Seoul, Korea, **1992**, 21- 31.
[3] J.T. Xie, C.Z. Wang, S. Wicks, J.J. Yin, J. Kong, J. Li, Y.C. Li, C.S. Yuan, Exp Oncol. **2006**; 28:25–29.
[4] S.T. Chang, Mushroom Research and Development- Equality and Mutual benefit, In: DJ Royes, editors. *Mushroom Bilology and Mushroom Products*, Pennsylvania, **1996**, 1-10.
[5] U. Lindequit, Structure and biological activity of triterpenes, polysaccharides and other constituents of *Ganoderma lucidum*. In: BK Kim, IH Kim and YS Kim, editors. *Recent advances in Ganoderma lucidum research*, Pharmaceutical society,Seoul, Korea, **1995**, 61-92.
[6] J. Burns, P.T. Gardner, D. Matthews, G.G. Duthie, M.E. Lean, A. Crozier, *J Agric Food Chem*, **2001**, 49, 5797-08.
[7] Y. Cai, Q. Luo, M. Sun, H. Corke, *Life Sci*, **2004**, 74, 2157-84.
[8] M.A. Smith, G. Perry, P.L. Richey, L.M. Sayre, V.E. Anderson, M.F. Beal, *Nature*, **1996**, 382, 120-21.
[9] A. Luximon-Ramma, T. Bahorun, A.M. Soobrattee, O.I. Aruoma, *J Agric Food Chem*, **2002**, 50, 5042–47.
[10] O.I. Aruoma, *J Am Oil Chem Soc*., **1998,** 75, 199-12.
[11] K. Wolfe, X. Wu, R.H. Liu, *J Agric Food Chem*., **2003**, 51, 609–14.
[12] C. Chang, M. Yang, H. Wen, *J Food Drug Analaysis*, **2002**, 10, 178-182.
[13] T. Hatano, R. Edamatsu, A. Mori, Y. Fujita, T. Yashida, T. Okuda, *Chem Pharm Bull*, **1989**, 37, 2016-2021.
[14] R. Re, N. Pellegrini, A. Proteggente, M. Yang, *Free Radic Bio Med.*, **1999**, 26, 1231-37.
[15] L.R. Ferguson, *Mutat Res.*, **2001**, 475, 89– 11.
[16] S.M. Nabavi, M.A. Ebrahimzadeh, S.F. Nabavi, A. Hamidinia, A.R. Bekhradnia, *Pharmacologyonline*, **2008**, 2, 560-567.
[17] G.A. Agbor, J.E. Oben, J.Y. Ngogang, C. Xinxing, J.A. Vinson, *J Agric Food Chem*., **2005**, 53, 6819–24.
[18] P. Maisuthisakul, M. Suttajit, R. Pongsawatmanit, *Food Chem*., **2007**, 100, 1409–18.
[19] F.O. Jimoh, A.A. Adedapo, A.A. Aliero, A.J. Afolayan, *Pharm Biol*., **2008**, 46, 333–40.