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COMPARATIVE STUDY OF ANTIOXIDANT ACTIVITIES OF CULTIVATED AND WILD *GANODERMA LUCIDUM* (W. CURT. FR.) P. KARST APHYLLOPHOROMYCETIDEAE FROM NORTH WESTERN INDIAN HIMALAYAS

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ABSTRACT
Ganoderma lucidum is a high value medicinal mushroom. Many active substances with immuno-modulating effects have been isolated from *G. lucidum*. The fruiting bodies collected from tree trunk of *Dalbergia sissoo* was identified and isolated using 2.0% Malt-Extract Agar (MEA) medium and mushroom was cultivated over the substrate wheat straw. Fruiting bodies were analyzed for different phytochemicals and antioxidant activities. The findings indicated that cultivated *G. lucidum* contained higher phenolics (28.27 mg GAE/g dry wt.) in comparison to wild (24.21 mg GAE/g dry wt.) mushroom fruiting body. Results also showed higher radical scavenging activity on DPPH (10-81.21%) and ABTS (29.34-99.45%), FRAP (98.17-382.01), total antioxidant activity (167.24-375.74), reducing power (0.10-0.40) and metal chelating activity (39.37-75.0%) in cultivated isolate. All the activities were found to be increased with increase in concentration both in cultivated and wild *G. lucidum*. In general cultivated isolate had higher antioxidant activities in comparison to wild one.

INTRODUCTION

Ganoderma lucidum (W. Curt. Fr.) P. Karst. is one of the most popular mushrooms known in Oriental medicine (Wasser, 2005). It is well known for its use as a remedy for many types of diseases and has a number of pharmaceutical effects-immuno-modulating, antiinflammatory, anti-cancerous, hypo-lipidemic, antihypertensive, wound healing, anti-aging, hypo-glycemic and anti-ulcer (Chang and Buswell, 1999; Liu, 1999; Zhou and Gao, 2002). It is known to contain various compounds, which can be categorized as water soluble, organic soluble and volatile compounds. The major compounds are proteins, polysaccharides, amino acids and terpenoids, each having their own outstanding medicinal and health effects (Mattila *et al.*, 2002; Pochanavanich, 2002; Wang *et al.*, 2002). A number of products prepared from *G. lucidum* are sold throughout the world as dietary supplements. The estimated global turnover of *G. lucidum* products was approximately $150 million with 175,000 distributors in 2011 (www.google.com/ Organo gold review, 2012). The global production of this mushroom was about 4900–5000 tons in 2002, of which 3800 tons were produced in China. Many countries like Malaysia, China and the United States (Stamets, 2005) have developed technologies for the cultivation of this mushroom. In India, market for *G. lucidum* based nutraceuticals is growing very rapidly and has crossed Rs. 100 crores annually through imports from Malaysia and China (Rai, 2008). The mushroom has very large climatic diversity and occurs in natural habitat of temperate to tropical regions of the world. Uttarakhand, a hill state of India, is gifted with a rich medicinal mushroom flora that includes *Ganoderma*, *Cordyceps* and *Auricularia* (Singh *et al.*, 2007a). 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, cancer, Alzheimer's disease, heart diseases, neurodegenerative disorders, atherosclerosis, cataracts, and inflammation.

Antioxidant properties of several specialty and edible mushrooms have been studied earlier (Mau *et al.*, 2002; Singh *et al.*, 2007b; Babu and Rao, 2013; Mishra *et al.*, 2013). There has been no report on antioxidant activities of cultivated *Ganoderma lucidum* grown under North-Western Indian Himalayan conditions. The paper deals with comparison of antioxidant properties viz. total phenolics, total antioxidant activity, reducing power, scavenging effects on radicals, and metal chelating activity of cultivated and wild *G. lucidum* isolates.

MATERIALS AND METHODS

Sample preparation

The fruiting bodies of *Ganoderma lucidum* was collected from tree trunk of *Dalbergia sissoo,* VPKAS experimental farm,

Hawalbagh, Almora (1250 m MSL), Uttarakhand, India during rainy season, identified and isolated using 2.0% Malt-Extract Agar (MEA) medium. The mushroom was cultivated over the substrate wheat straw as per standard methodology of Mishra and Singh (2008). The mushrooms cultivated on wheat straw were harvested, washed thoroughly under running tap water to remove adhering wheat straw and cut into small pieces. Sliced mushrooms were subjected to dry. The dried mushrooms were stored in sealed plastic container in a cool place until further analysis of different phytochemicals and antioxidant activities.

Extraction of mushroom samples

Mushroom sample (1.5 g) was extracted by stirring with 20 mL methanol at 25C°, 150 rpm/min for 12 h and filtered through Whatman filter paper No. 1. The extraction was repeated again as described earlier. The extracts were mixed, filtrated and diluted upto final volume of 50 ml with methanol. The extract solution stored in amber bottles at 4C° served as the working solution (30 mg/mL) for determination of total phenolics and antioxidant activity.

Determination of total polyphenol

Total polyphenol (TPP) in the mushroom samples was estimated by a colorimetric assay, based on procedures described by Singleton and Rossi with some modifications (Singleton and Rossi, 1965). Gallic acid was used to calculate the standard curve (1-80 μg/mL). The results were mean values $\pm$ standard error and expressed as mg of gallic acid equivalent (GAE)/g dry weight of mushroom.

Determination of radical scavenging activity on DPPH

Radical scavenging activity (RSA) on DPPH (2, 2-diphenyl-1-picrylhydrazyl) was determined by measuring the decrease in absorbance of methanolic DPPH solution at 515 nm in the presence of the extract (Brand-Williams *et al.*, 1995) with some modifications. Radical scavenging activity was calculated as a percentage of DPPH discoloration by the equation:

$$\text{RSA on DPPH (\%)} = [(A_{control} - A_{sample})/ (A_{control})] \times 100$$

Where, A_{sample} is the absorbance of the solution recorded during addition of extract/reference at a particular level, and $A_{control}$ is the absorbance of the DPPH solution without addition of extract.

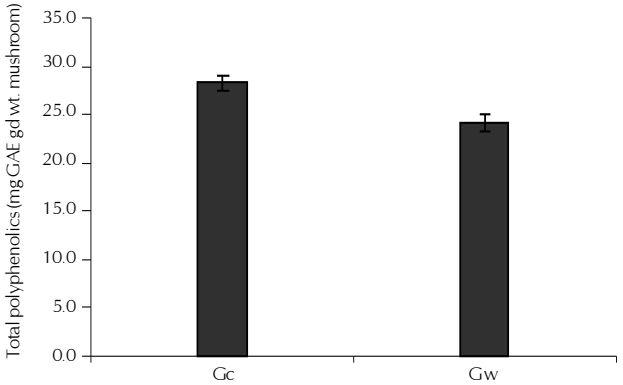

Figure 1: Total phenolics content (mg GAE/g dry weight mushroom) of cultivated and wild *G. lucidum*. All data are means of three (n = 3) independent measurements $\pm$ standard error

Determination of radical scavenging activity on ABTS

The radical scavenging activity (RSA) on ABTS [2,22 -azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] was determined by measuring the decrease in absorbance of methanolic ABTS solution at 745 nm in the presence of the extract (Arnao *et al.*, 2001). The percentage inhibition was calculated by the equation:

$$\text{RSA on ABTS (\%)} = [(A_{control} - A_{sample})/ (A_{control})] \times 100$$

Where, A_{sample} is the absorbance of the solution recorded during addition of extract/reference at a particular level and $A_{control}$ is the absorbance of the ABTS solution without addition of extract.

Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay was done according to the method of Benzie and Strain (1996) with some modifications. The FRAP value was determined by plotting a standard curve obtained by addition of ferrous sulfate heptahydrate (Merck, Darmstadt, Germany) to the FRAP reagent. The results were expressed in μM equivalent to $FeSO_4.7H_2O$ as FRAP value.

Determination of total antioxidant activity

The total antioxidant activity of mushroom extracts was estimated using the phosphomolybdenum method (Prieto *et al.*, 1999) based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of specific green phosphate /Mo (V) compounds. A standard curve of trolox (10-100ìM) was prepared and total antioxidant activity was expressed as ìM trolox equivalent.

Reducing power assay

The reducing power (RP) was determined according to the standard methodology (Huda-Faujan *et al.*, 2009). The absorbance was measured at 700 nm; higher absorbance indicates higher reducing power.

Determination of metal chelating activity

The chelating of ferrous ions by mushroom extract was determined by the modified method (Dinis *et al.*, 1994). The metal chelating activity (MCA) was calculated according to the following equation:

$$\text{Metal chelating activity (\%)} = [(A_{control} - A_{sample})/ (A_{control})] \times 100$$

Where, A_{sample} is the absorbance of the solution recorded during addition of extract at a particular level and $A_{control}$ is the absorbance of the $FeCl_2$ and ferrozine solution without addition of extract.

Statistical analysis

The statistical analyses were performed using the statistical package SPSS (Statistical Package for Social Science, SPSS Inc., Chicago, IL). Analyses of variance were performed by ANOVA and significance of each group was verified with one-way analysis of variance followed by Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Total polyphenols

Total phenolic contents for the analysed dried mushroom

samples evaluated by the Folin–Ciocalteu method are shown in Fig. 1. Results were expressed as mg GAE/g dry wt. mushroom sample. The amount of total phenolics in cultivated *G. lucidum* isolate was significantly higher (28.27 mg GAE/g dry wt.) than wild isolate (24.21 mg GAE/g dry wt.). Mau *et al.* (2005) also found the phenolic content in the range of 40.86-42.34 mg/g in case of *G. tsugae*. Total phenolic content in extracts of *Ganoderma lucidum* basidiocarps cultivated on wheat straw varied from 28.06 mg GAE/mg to52.15 mg GAE/mg (Cilerdzic *et al.*, 2014). The key role of polyphenols in neutralization of free radicals has been demonstrated in many previous studies. Although there are some data indicating that mushrooms represent a poor source of phenolic compounds (Yaltirak *et al.*, 2009), these compounds are important antioxidant agents in *Ganoderma* spp. (Cilerdzic *et al.*, 2013). The content of phenolic compounds could be used as an important indicator of antioxidant capacity. It has been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic compounds (Pan *et al.*, 2008).

Radical scavenging activity on DPPH and ABTS

Free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity (Pal *et al.*, 2013). The radical scavenging activity (RSA) of cultivated and wild *G. lucidum* isolate was tested against the DPPH and ABTS. RSA on DPPH and ABTS of cultivated *G. lucidum* isolate varied from 10.00-81.21% and 29.34-99.45%, respectively (Fig. 2a and b) and was found to be concentration dependent. RSA on DPPH and ABTS was higher in cultivated *G. lucidum* isolate (81.21 and 99.45, respectively at 25 mg/mL concentration); however, it was 60.00 and 80.53, respectively at 25 mg/mL concentration for wild *G. lucidum*. Scavenging effects of the acetonic, methanolic and aqueous extracts from the fruiting bodies of *P. salmoneostramineus* (Alam *et al.*, 2011a) and *P. citrinopileatus* (Alam *et al.*, 2011b) increased with increasing concentrations. Lo (2005) found that *A. bisporus, P. eryngii, P. ferulae* and *P. ostreatus* fruit bodies scavenged DPPH radicals by 46.6-68.4 per cent at 5.0 mg/mL. Mau *et al.* (2005) showed that the scavenging abilities on DPPH of hot water extracts from *G. tsugae* were in the range of 38.8-91.2%. With the presence of radical scavenging activity, consumption of *G. lucidum* products might be beneficial to protect human body against oxidative damage, which can be further developed into health related degenerative illnesses. ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain breaking antioxidants (Leong and Shui, 2002).

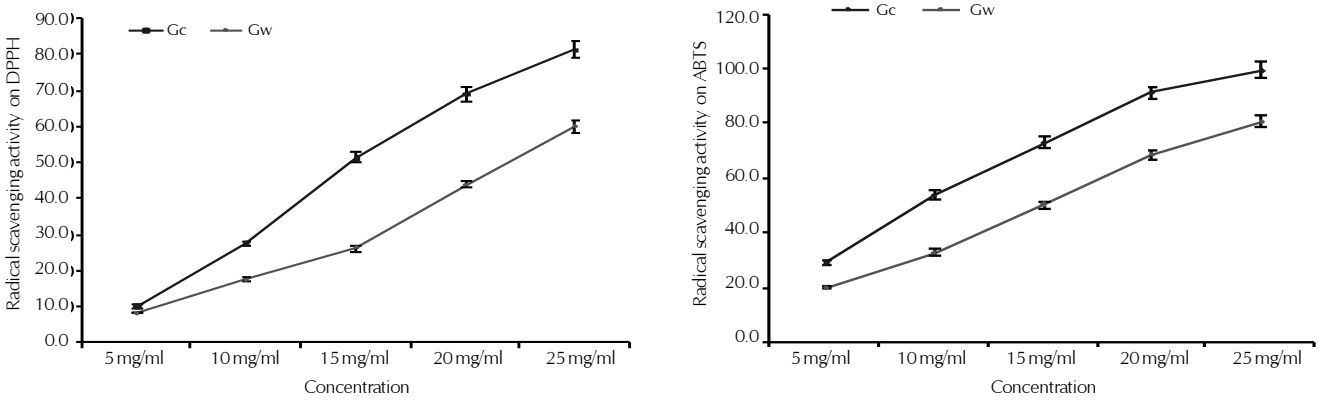

Figure 2a and b: Radical scavenging activity on DPPH and ABTS of cultivated and wild *G. lucidum*. All data are means of three (n = 3) independent measurements ± standard error

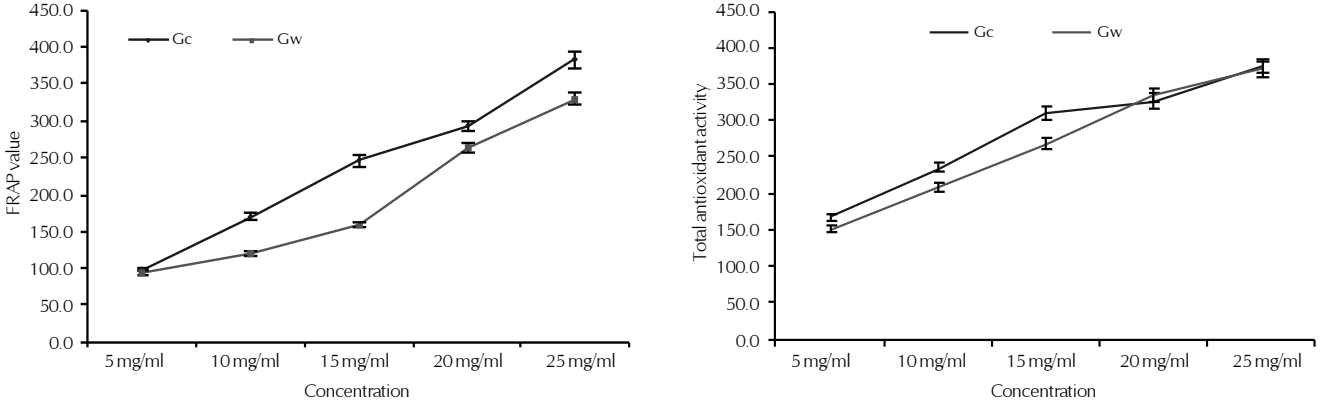

Figure 3: FRAP value (μM $FeSO_4$ equivalent) of cultivated and wild *G. lucidum*. All data are means of three (n = 3) independent measurements ± standard error

Figure 4: Total antioxidant activity of cultivated and wild *G. lucidum*. All data are means of three (n = 3) independent measurements ± standard error

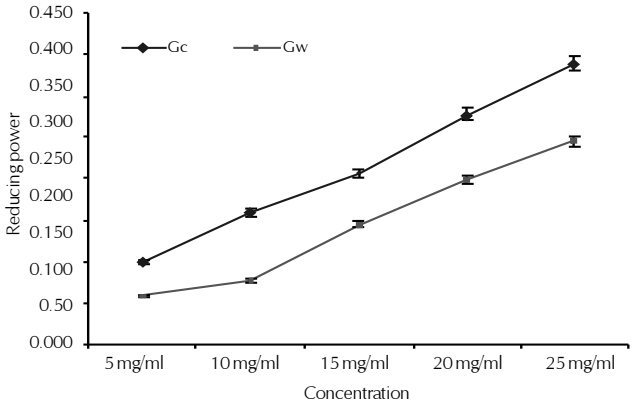

Figure 5: Reducing power of cultivated and wild *G. lucidum*. All data are means of three (n = 3) independent measurements ± standard error

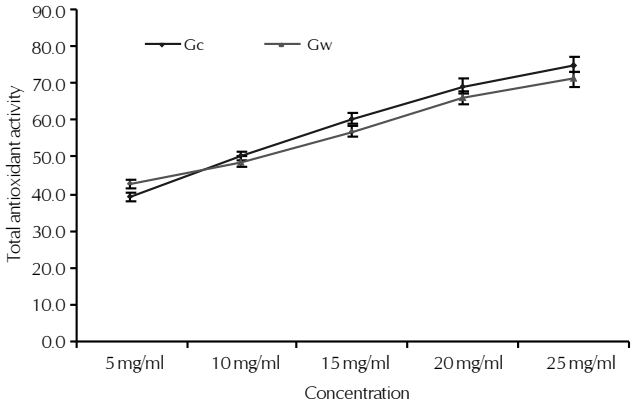

Figure 6: Metal chelating activity of cultivated and wild *G. lucidum*. All data are means of three (n = 3) independent measurements ± standard error

Ferric reducing antioxidant power (FRAP)

Both the samples showed increased FRAP with the increase in concentration (Fig. 3). At 25 mg/mL concentration, the ferric reducing power of cultivated *G. lucidum* was found to be higher (382.01 μM $FeSO_4$ equivalent). The results revealed that *G. lucidum* possessed hydrogen-donating capacity indicating the significant reducing power.

Total antioxidant activity

Total antioxidant activities of mushroom samples showed increased trend with increase in concentration (Fig. 4). Consistently for mushroom samples, cultivated *G. lucidum* showed higher antioxidant activity (375.74 μM trolox equivalent) at 25 mg/mL concentration, however, it was 371.32 μM trolox equivalent for wild *G. lucidum*. Lee *et al.* (2007) also reported increase in antioxidant activities of *Pleurotus citrinopileatus* with increased concentration.

Reducing power

Reducing power of *G. lucidum* was excellent and increased steadily with the increased concentration (Fig. 5). The reducing powers of cultivated *G. lucidum* were 0.40 at 25mg/mL concentration and it was significantly higher than wild isolate (0.25). Mau *et al.* (2005) reported higher reducing power from mature and baby Ling chih, mycelia and filtrate of *G. tsugae*. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity of the plants (Kumar *et al.*, 2013).

The high reducing power exhibited by the sample might be indicative of the hydrogen donating ability of the active species present in the extracts (Shimada *et al.,* 1992). Accordingly, both samples might contain higher amounts of reductone, which could react with free radicals to stabilize and block radical chain reactions.

Metal chelating activity

The metal chelating activity of mushroom samples on ferrous ions increased with increase in concentration (Fig. 6). The chelating activity cultivated *G. lucidum* (75.00% at 25 mg/mL concentration) was higher with wild *G. lucidum* (71.25%). The results are in accordance with the findings of Mau *et. al.* (2005) who reported that the hot water extracts from *G. tsugae* mature and baby Ling chih chelated 42.6 and 39.5% of ferrous ions at 20mg/ml, respectively. Chelating agent may serve as secondary anti-oxidants as they reduce redox potential and stabilize the oxidized forms of metal ions. Since ferrous ions are the most effective pro-oxidants in food system, the high ferrous ion chelating abilities of the dried mushroom samples would be beneficial. This study could provide valuable information to support *G. lucidum* as an excellent source of antioxidants in human diet.

The total phenolics and antioxidant activities of wild and cultivated form of *G. lucidum* were investigated in this study. Cultivated *G. lucidum* contained higher phenolics, higher radical scavenging activity on DPPH and ABTS, FRAP, total antioxidant activity, reducing power and metal chelating activity. High content of total phenols in cultivated *Ganoderma lucidum* might explain high antioxidant properties of cultivated *G. lucidum*. Therefore, cultivated *G. lucidum* could be a potential source of antioxidants and the consumption of mushroom might give certain level of health protection against oxidative damages.

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