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In Vitro antioxidant activity of methanolic extract of *Ganoderma lucidum* (Curt.) P. Karst

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Abstract
In this study antioxidant activity and bioactive compounds of methanolic extract of *Ganoderma lucidum* (Curt.) P. Karst. were analyzed. Significant antioxidant activity on inhibition of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was observed when compared to standard antioxidant like L-ascorbic acid. IC50 value of the extract was 2.25 mg/mL. Total phenolics, flavones and ascorbic acid levels were estimated following standard techniques. The results showed that the methanolic extract of *G. lucidum* possessed remarkable amount of antioxidant compounds and also good free radical scavenging effects against different free radicals. The study also revealed that *Ganoderma lucidum* compounds can be used as better antioxidant supplement of nutrients.

Keywords: Antioxidant, DPPH, *Ganoderma lucidum*, phenols, flavanoids, ascorbic acid

1. Introduction
Various physiological processes in living organisms usually produce oxygen-centered free radicals and other reactive oxygen species as byproducts. Oxidative damage caused by free radicals may be related to aging and different diseases, such as atherosclerosis, cancer and rheumatoid arthritis. Antioxidants protect cellular components from oxidative damage, which is likely to decrease risk of mutations and carcinogenesis and also protect immune cells, allowing them to maintain immune surveillance and response. Various components of *G. lucidum*, in particular polysaccharides and triterpenoids, show antioxidant activity *in vitro* [1-7]. Antioxidants from Lingzhi were found to be absorbed quickly after ingestion, resulting in an increase in the plasma total antioxidant activity of human subjects [8].
Armassa *et al* examined the antioxidative activity, and cytotoxic effect in breast cancer cell line of medicinal mushrooms extracts; *Lentinus polychrous* and *Ganoderma lucidum*[9]. They found that the extract from edible *L. polychrous* mushroom exhibited similar antioxidative activity and the total phenolic compounds to the *G. lucidum* extracts. Moreover, the extract from *G. lucidum* caused a 50% decrease in breast cancer cell viability. Agarwal *et al* studied *in vitro* antioxidant activity of various extracts (Hot water, Hydro alcoholic, Chloroform and Petroleum Ether) of *Ganoderma lucidum.* They found that the Hot water and Hydro alcoholic extracts of *Ganoderma lucidum* showed potent antioxidant activity than the Chloroform and Petroleum Ether extracts [10].

2. Material and methodology
2.1. Standards and Reagents
DPPH (2, 2-diphenyl-1-picrylhydrazyl), L-ascorbic acid, tannic acid, (+)catechin, and folin ciocalteu reagent were obtained from Sigma (St. Louis, MO, USA). All other chemicals and solvents were of analytical grade.

2.2. Sample collection and extract preparation
Fruiting bodies of *Ganoderma lucidum* were collected from various places of district Bilaspur H.P., India and identified at Department of Biosciences, H.P.U., Shimla (Figure 1). Fresh fruiting bodies of *Ganoderma lucidum* were sun-dried, cut into small pieces. A coarse powder was obtained using a mill. The methanolic extract of the sample was prepared as described by Pal *et al* with little modification [11]. For extraction, 50gm sample was extracted by stirring at 100 rpm with 100 ml methanol at 30° C for 24 hours and filtered through Whatman filter paper no.1. The residue was then extracted with two additional 100 ml portion of methanol, in a similar manner. The combined methanolic extract was evaporated by rotary evaporator at 40°C to dryness, re-dissolved in methanol to concentration of 20 mg/ml and stored in dark at 4°C for further use.

Fig 1: *G. lucidum* in its natural habitat.

2.3. Scavenging Ability on 2, 2-Diphenyl-1-picrylhydrazyl Radicals
The hydrogen atoms or electron donation ability of the extracts was measured from the bleaching of the purple coloured DPPH methanolic solution with little modification. 3 mL of various concentrations of the extracts in methanol was added to 1 mL of methanolic DPPH (final concentration of DPPH was 200 μM). The mixture was shaken vigorously

and allowed to stand for 30 min at room temperature and absorbance of the resulting solution was measured at 517 nm using spectrophotometer (Merck Spectroquant Pharo 100) [12]. Inhibition of the DPPH free radicals in (%) was calculated as

$$\text{Inhibition}(\%) = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

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

2.4. Determination of Antioxidant Components

2.4.1. Total Phenolic Contents of the Extract Was Determined following Makkar *et al* [13]

The reaction mixture was prepared by mixing 0.1 mL of the extract and 0.9 mL of double distilled water. 2.5 mL of sodium carbonate solution (20%) was added to it, followed by 0.5 mL of FCR (1 N). After 40 minutes at room temperature, absorbance was read at 725 nm. Tannic acid (0.5 mg/mL) was used to prepare standard curve. The results were expressed as mg of TAEs per g of the extracts.

2.4.2. The Amounts of Total Flavonoids (TFC) Were Determined Colorimetrically

A suitable aliquot 250 μL taken for estimation [14] was mixed with 1 mL of water in a test tube. At the start, 75 μL of 5% aqueous NaNO2 was added to *Ganoderma lucidum* in its natural habitat. the test tube; then after 5 min 150 μL of 10% $AlCl_3$ and after 6 min 500 μL of 1.0 M NaOH were added sequentially. Finally, 275 μL distilled water was added. The reaction mixture was mixed thoroughly. The absorbance was noted at 510 nm using a spectrophotometer. TFC, calculated using a standard calibration curve, were reported as (+) catechin equivalents (mg CE/g of the extract).

2.4.3. β-Carotene and Lycopene Were Determined according to the Method of Nagata and Yamashita[15]

The dried extract (100 mg) was vigorously shaken with 10 mL of acetonehexane mixture (4 : 6) for 1 minute and filtered through Whatman number 1 filter paper.The absorbance of the filtrate was measured at 453, 505, and 663 nm. The contents of β-carotene and lycopene were calculated according to the following:

$$\text{Lycopene}\left(\frac{\text{mg}}{100\text{mL}}\right) = -0.0458A_{663} + 0.372A_{505} + 0.0806A_{453}$$

$$\beta - \text{carotene}\left(\frac{\text{mg}}{100\text{mL}}\right) = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$$

2.4.4. The Ascorbic Acid Content Was Determined from Dried Methanolic Extract

A 100 mg of the extract was mixed with 1% metaphosphoric acid (10 mL) and incubated at room temperature for 45 min and filtered. 1 mL of filtrate was mixed with 9 mL of 2,6-dichloroindophenol and absorbance was recorded at 515 nm in 30 min against a blank. The ascorbic acid content was calculated using calibration curve of L-ascorbic acid. The results were expressed in terms of mg of ascorbic acid per g of extract.

2.5. Statistical Analysis

All the analyses were performed in triplicates and results were reported as means ± standard deviation (SD).

3. Results and Discussion

3.1. The mushroom extract showed positive antioxidant activity by fading the violet colour of DPPH solution to yellow and pale violet. The scavenging activities of radical were in direct proportion with the concentrations of the extract. As the concentration of extract was increased, the scavenging activity towards DPPH radicals was also increased. The results showing in Figure 2 clearly indicates the increase in % inhibition of DPPH free radical on increasing concentration.

IC50 value of methanolic extract of *Ganoderma lucidum* was 2.25 mg/mL compared with L-ascorbic acid IC50 = 0.063 mg/mL. The results of DPPH scavenging effect of methanolic extracts were higher than what was reported by Puttaraju *et al* [16].

3.2. Bioactive Components

The present studies also concentrate on five different bioactive components like phenols, flavonoids, ascorbic acid, β-carotene, and lycopene (Table 1). Phenolic compounds were found to be a major class of phytochemicals, which are responsible for inhibiting the oxidative damage caused by free radicals generated inside our body [17]. Total phenolic content in the methanolic extract was found to be 15.25 mg tannic acid equivalent per g of the extract. The total phenolic content in methanolic extracts of *Ganoderma lucidum* clearly exhibit that it can be considered as a better source of polyphenols. There are several reports which emphasized that the phenolic compounds acts as scavengers of free radicals and these compounds seem to be associated with antioxidant activity [18-22].

Flavonoids are also very important dietary biochemical agents, which are very effective for cardiovascular system and work as cardioprotective agents[23]. The total flavonoid contents in methanolic extracts of *Ganoderma lucidum* were found to be 1.253 mg catechin equivalent per g of the extract. Ascorbic acid was found in small amounts (1.05 mg/100 g). β-carotene and lycopene were found in very low amounts. Several references are available on insignificant quantities of ascorbic acid, lycopene, and β-carotene in methanolic extracts of fruiting bodies as naturally occurring antioxidant components [24, 25].

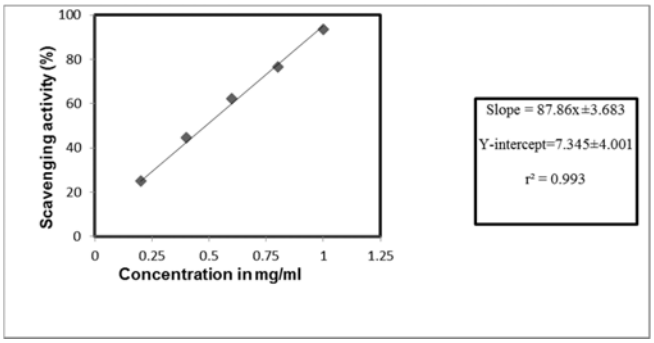

Fig 2: Scavenging activity of methanolic extract of *Ganoderma lucidum* using DPPH test.

Table 1: Bioactive components present in methanolic extract of *Ganoderma lucidum*

Bioactive components of *Ganoderma lucidum*	Quantity in mg/gm
Total Phenols	15.25±0.0243
Total Flavonoids	1.253±0.0212
Lycopene	0.325±0.004
β Carotene	0.025±0.033
Ascorbic acid	1.05±0.005

4. Conclusions

The results of the present study revealed that the methanolic extract of *Ganoderma lucidum* exhibited the significant antioxidant activity through the scavenging of free radicals which participate in various pathophysiology of diseases including ageing. Methanolic extract of this mushroom showed appreciable amount of phenols, flavones and ascorbic acid with potent free radical scavenging activity. Though *Ganoderma lucidum* is not edible, but its broad medicinal properties make this mushroom unique. The rich antioxidant contents make this mushroom ideal nutritional supplement with good medicinal properties.

5. Acknowledgments

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6. Conflict of interest

The authors declare that there is no conflict of interest.

7. References

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