

Original article

In vitro* antioxidant activity of different extract of *Ganoderma lucidum

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Abstract

Free radicals are implicated for many diseases including diabetes mellitus, arthritis, cancer, ageing etc. In the treatment of these diseases, antioxidant therapy has gained a great importance. Different extract (Hot water, Hydro alcoholic, Chloroform and Petroleum Ether) of *Ganoderma lucidum* was studied for its in vitro antioxidant activity using different models viz. DPPH radical scavenging, ABTS radical scavenging, FRAP assay and Superoxide Radical Scavenging Assay. The results were analyzed statistically by the regression method. Its antioxidant activity was estimated by IC50 value. The results showed that the Hot water and Hydro alcoholic extracts of *Ganoderma lucidum* showed potent antioxidant activity than the Chloroform and Petroleum Ether extracts. The antioxidant potency of different extracts has been expressed as in order of RGHW>RGHA>RGCH>RGPEt In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. The antioxidant property may be related to the polyphenols and flavonoids present in the extract. These results clearly indicate that *Ganoderma lucidum* is effective against free radical mediated disease.

Key words DPPH, FRAP, *Ganoderma lucidum*, Superoxide, Flavanoids.

Introduction

Free radicals [reactive oxygen species (ROS)] are an entire class of highly reactive molecules derived from the metabolism of oxygen. Moreover, these radicals can cause extensive damage to cells and tissues, during infections and various degenerative disorders, such as cardiovascular disease, aging, and neurodegenerative diseases like Alzheimer's disease, mutations and cancer.^{2,5,9} Although many anti-oxidant defence systems consisting of enzymatic (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and nonenzymatic (ascorbic acid, glutathione and α -tocopherol) compounds can maintain the balance between free radical generation and protection from damage by these radicals but these anti-oxidant systems do not provide complete protection from against ROS under conditions of severe oxidative stress.⁶ In Asia growing as a parasite or saprotroph on a wide variety of trees. In nature, Lingzhi grows at the base and stumps of deciduous trees, especially maple. Today, Lingzhi is effectively cultivated both indoors under sterile conditions and outdoors on either logs or woodchip beds.

Over 300 reports have been published concerning the chemical constituents of *G. lucidum* and related species. The fruiting body, mycelia and spores of *G. lucidum* contain approximately 400 different bioactive compounds, which mainly include triterpenoids (Ganoderic acid, Ganosporeric acid A.) [8], polysaccharides (β -D-glucans, [9] Heteropolysaccharides and Glycoprotein, water-soluble β -D-glucans), nucleotides (Adenosine and 5-deoxy-5-Methylsulfynyladenosine), proteins/peptides (Lingzhi-8 (LZ-8)). *G. lucidum* also contains sterols, amino acids, soluble proteins, oleic acid, cyclo-octasulfur, an ergosterol peroxide (5,8-epidioxy-ergosta-6,22E-dien-3-ol) and the cerebroside (4E',8E)-N-D-2'-hydroxystearoyl-1-O- β -D-glucopyranosyl-9-methyl-4-8-sphingadienine and (4E,8E)-N-D-2'-hydroxypamitoyl-1-O- β -D-glucopyranosyl-9-methyl-4-8-sphingadienine. [10,11,12] Lingzhi may possess anti-tumor, immunomodulatory and immunotherapeutic activities, supported by studies on polysaccharides, terpenes and other bioactive compounds isolated from fruiting bodies and mycelia of this fungus. It has also been found to inhibit platelet aggregation, and to lower blood pressure (via inhibition of angiotensin-converting enzyme, cholesterol, and blood sugar [13,15]).

Material & Methods

Preparation of plant extracts:

Samples of *Ganoderma lucidum* (GL) were collected from the district. Almora, Dehradun, UK & identified by Department of Botany, FRI, Dehradun. The freshly cut GL were dried in the drying room with active ventilation at ambient temperature. 500g of powdered of *Ganoderma lucidum*. Has been taken. Fractionation has been done according to increase polarity as Petroleum ether, Chloroform, Hydro alcoholic

and Hot water. The obtained extract was filtered and evaporated by using vacuum evaporator under 40C to give different %yield [JRPET: 13.92gm, JRCH: 19.68gm, JRHA: 42.6gm, JRHW: 163.32gm. of dried crude extract.

Determination of antioxidant activity of different extracts of the *ganoderma lucidum* in vitro:

DPPH radical scavenging assay:

To the ethanolic solution of DPPH (100 μ M) an equal volume of test drug was added, dissolved in distilled water at various concentrations (10 μ g to 50 μ g/ml). After incubation for 20 minutes at room temperature absorbance was recorded at 517nm. Blank was carried out in the same manner without the drug. For rate kinetic study absorbance was taken immediate after addition of DPPH up to 500 seconds. The experiment was conducted in triplicate.

$$\text{DPPH Scavenged (\%)} = \frac{A_{\text{CONTROL}} - A_{\text{TEST}}}{A_{\text{CONTROL}}} \times 100$$

Where A_{control} is the absorbance of the control reaction and a_{test} is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the *Ganoderma lucidum* extracts were expressed comparing with standard.¹

ABTS radical cation decolonization assay:

To the reaction mixture containing 0.3 ml of ABTS radical, test compound was added at various concentrations from 10 μ g to 500 μ g/ml in suitable solvent in a final volume of 2.5 ml make up with phosphate buffer, pH 7.4 (20mM). Blank was carried out without drug. Absorbance was recorded at 734nm immediate after the addition of ABTS radical. The experiment was repeated thrice in triplicate.

Ferric reducing antioxidant potential (FRAP) assay:

Various concentrations of the extracts in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml) and incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

Where A_{Test} is absorbance of test solution; A_{Blank} is absorbance of blank. The antioxidant activity of the *Ganoderma lucidum* extracts were compared with the standard.

Superoxide radical scavenging assay:

To the reaction mixture containing 0.5 ml of various concentrations of drug (10µg to 500µg

/ml), 0.2 ml of NBT and 1ml of alkaline DMSO were added. Blank was carried without drug, only

$$\% \text{ increase in Reducing Power} = \frac{A_{\text{TEST}}}{A_{\text{BLANK}}} \times 100$$

DMSO in was added. Absorbance was recorded at 560 nm. The experiment was repeated thrice in triplicate.

Results and discussion

Several concentrations ranging from 10–600 µg/ml of all four extract of *Ganoderma lucidum* were tested for their antioxidant activity in different in vitro models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the models. The percentage scavenging and IC50 values were calculated for all models given in table. 1.

Table: 1 Comparison of IC₅₀ values of different extracts of *Ganoderma lucidum*

SNo.	Model	IC ₅₀ value of Hot Water extract (µg/ml)	IC ₅₀ value of Hydro alcoholic extract (µg/ml)	IC ₅₀ value of Chloroform extract (µg/ml)	IC ₅₀ value of Peroleum Ether extract (µg/ml)
1	DPPH Radical Scavenging Assay	182.6	238.6	274.3	347.4
2	ABTS Radical Cation decolonization Assay	148.8	224.6	288.4	327.2
3	Ferric Reducing Antioxidant Potential Assay	98.8	204.3	288.6	336.3
4	Superoxide Radical Scavenging Assay	201.8	254.4	301.6	499.3

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing¹⁵.

The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm¹⁰ (Fig. 1).

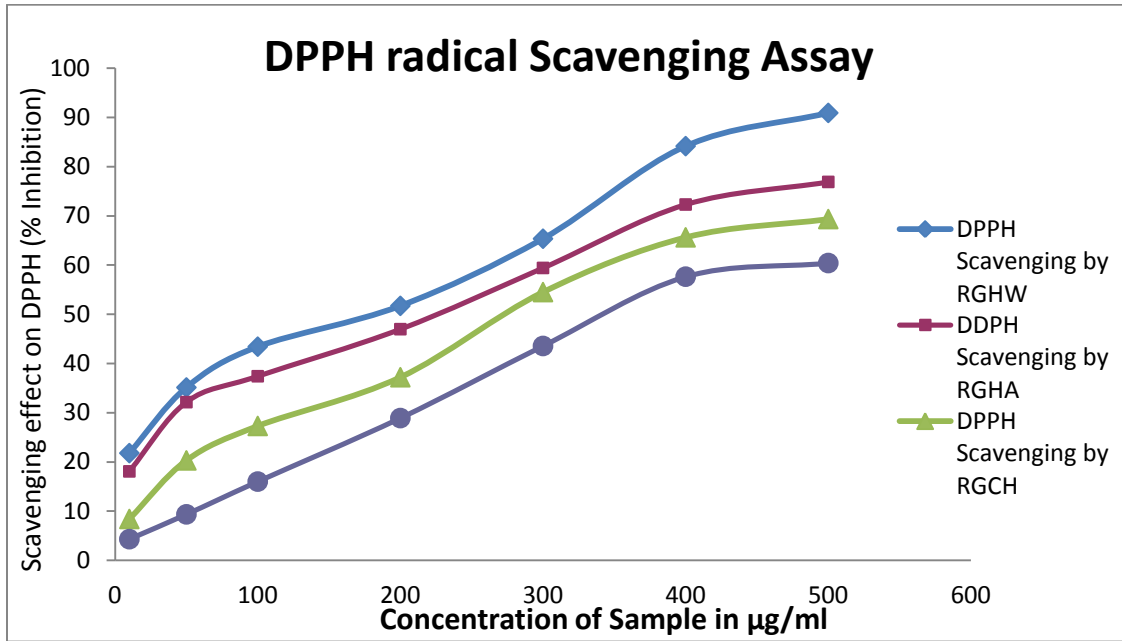


Figure 1: Dose response curve of DPPH radical scavenging assay of various extracts isolated from *Ganoderma lucidum*.

The antioxidant activity of the extract by this assay implies that action may be by either inhibiting or scavenging the ABTS radicals since

both inhibition and scavenging properties of antioxidants towards this radical have been reported in earlier studies⁸ (Fig. 2).

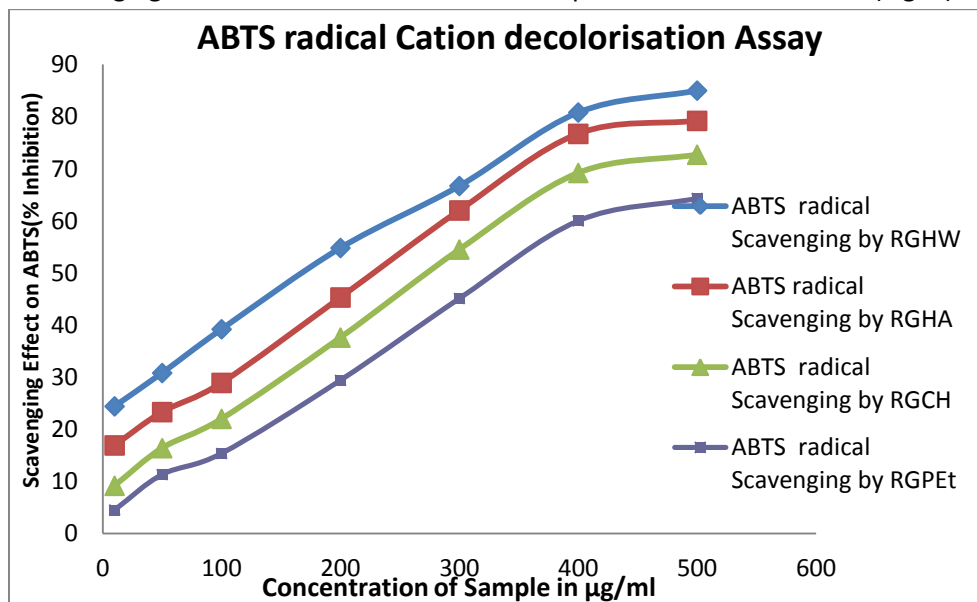


Figure 2: Dose Response Curves of ABTS cation scavenging activity of various extract fractions isolated from *Ganoderma lucidum*.

The potency of ferric ion reducing antioxidant potency has been observed. The result indicated free radical scavenging activity of the

extracts in dose dependent manners, which are shown in (Fig. 3).¹²

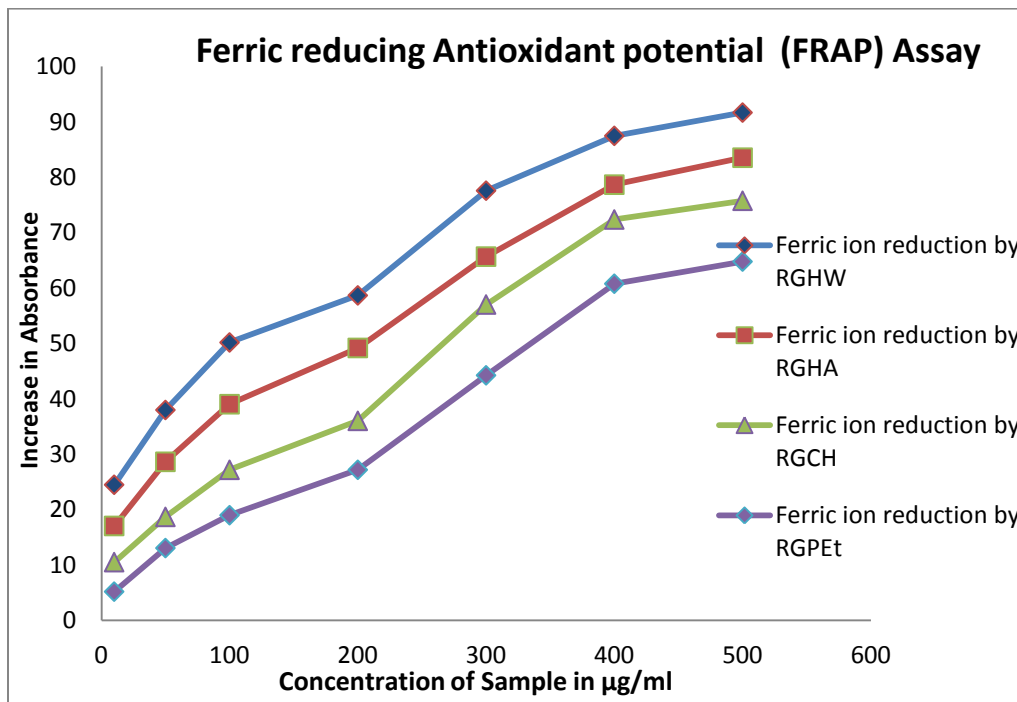


Figure 3: Dose Response Curves of reducing power of various extract fractions isolated from *Ganoderma lucidum*.

Superoxide can cause oxidation or reduction of solutes depending on their reduction potential. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes, which catalyse

the breakdown of superoxide radical¹³. In our study, alkaline DMSO used for superoxide generation indicates that *J.regia* is a potent superoxide scavenger (Fig. 4).

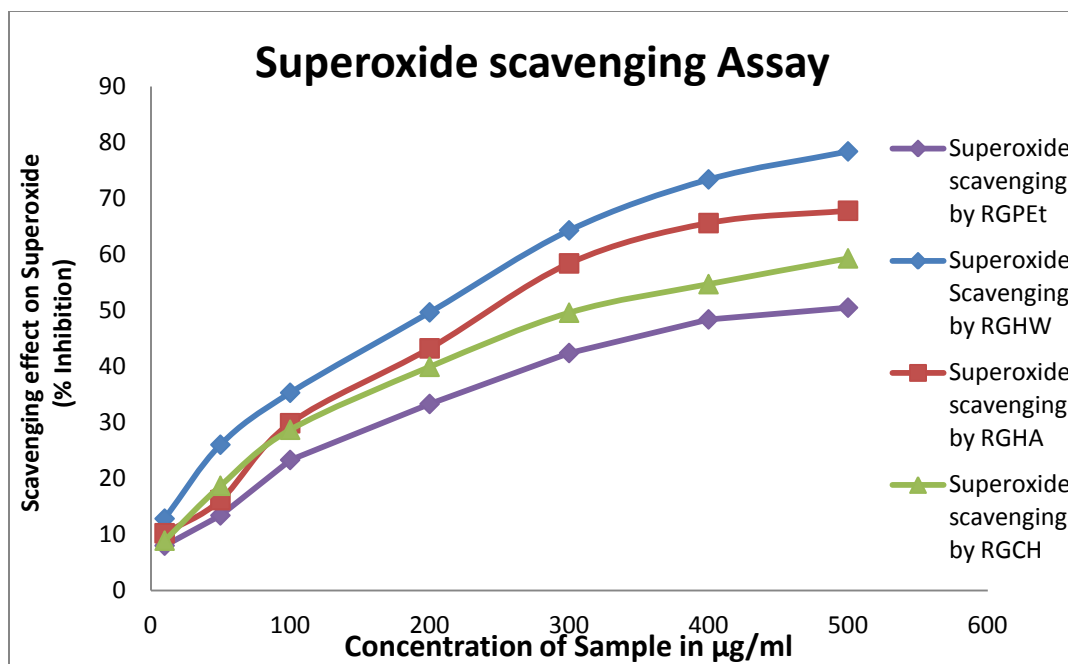


Figure 4: Dose Response Curve of Superoxide Radical Scavenging Assay of various extracts isolated from *Ganoderma lucidum*.

Conclusion

The results of the present study show that the extract of *Ganoderma lucidum* exhibits the greatest antioxidant activity through the scavenging of free radicals which participate in various pathophysiology of diseases including ageing. This observation also shows presence of a great amount of polyphenols.

Acknowledgement

The authors sincerely thank CMJ University, Meghalaya, Aasam and Dev Bhoomi Institute of Pharmacy and Research for providing the necessary facilities to carry out this research work.

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