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Determination of total phenolic content and antioxidant activity of *Ganoderma lucidum* collected from Dang district of Gujarat, India

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ABSTRACT

The main aim of this study is to determine the total phenolic content (TPC) and the antioxidant activity of the four extracts (methanol, ethyl acetate, chloroform, methanol: ethyl acetate) of *G. lucidum*. Results clearly showed that the methanolic extract had the highest TPC followed by Methanol: ethyl acetate, chloroform and ethyl acetate extract. The antioxidant activity of the extracts was determined with the help of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay, 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical scavenging activity assay and Ferric Reducing Antioxidant Power (FRAP) assay. For the DPPH assay, the lowest IC_{50} was shown by the methanol extract, thus having the highest antioxidant activity and for the ABTS assay, again the lowest IC_{50} was shown by the methanol extract. For the FRAP assay, the reducing ability of the ethyl acetate extract was found to be the highest. A positive correlation between the total phenolic content and the three antioxidant assays was observed for each extract. This *in vitro* study suggested that *G. lucidum* could be used as a potent natural antioxidant.

KEYWORDS

Ganoderma lucidum;
Antioxidant activity;
TPC;
DPPH assay;
ABTS assay;
FRAP assay;
Correlation.

INTRODUCTION

Oxygen being one of the most prevalent elements in the earth's crust is required by all living organisms (except anaerobic bacteria) for energy production. This energy is produced in the body by the oxidation of food materials[1]. This continuous process of oxidation leads to the production of free radicals which along with the free radicals produced from external sources (pollution, cigarette smoke, radiation, medication) create a condition known as oxidative stress which causes damage to relevant molecules like DNA, proteins, carbohydrates and lipids in the body[2]. This process plays a major part in the development of chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases. Even though almost all organisms possess antioxidants and several enzyme systems like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase to protect them from oxidative damage, these systems are inadequate to prevent the damage entirely. Hence antioxidant supplements or foods containing high concentrations of antioxidants are needed which may help scavenge free radicals and reduce oxidative damage. Also, antioxidants can be obtained from external sources like food

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and supplements. However, certain synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been found to cause negative health effects. Hence, there has been a tendency to substitute them with much safer naturally occurring antioxidants[3].

G. lucidum has been used for hundreds of years as a health promotion and treatment strategy. A number of bioactive compounds have been revealed from *G. lucidum* which are found to be responsible for the pharmacological potential of the mushroom. Thus the antioxidant activity of *G. lucidum* was described in this study.

Various constituents of *G. lucidum* especially polysaccharides and triterpenoids have shown *in vitro* antioxidant activities. In a study done by Heleno et al., (2012)[4], the phenolic extract of the fruiting body of *G. lucidum* gave high DPPH scavenging activity, reducing power, β-carotene bleaching inhibition and lipid peroxidation inhibition through TBARS formation inhibition.

In one study, the crude exopolysaccharide of the fruiting bodies of *G. lucidum* showed high antioxidant activity which indicated the beneficial effects of mushroom polysaccharides as antioxidants[5].

When different extracts of *G. lucidum* were tested for antioxidant activity by the FRAP (Ferric reducing ability of plasma) assay, the reducing ability of dichloromethane extract was observed the highest, followed by aqueous, methanol, ethyl acetate and hexane extracts[3].

In the present study, the total phenolic content and the antioxidant activities of the methanol, ethyl acetate, chloroform and methanol: ethyl acetate extracts of *G. lucidum* were studied with the help of Folin-Ciocalteu method and DPPH, ABTS and FRAP assays respectively.

EXPERIMENTAL

Preparation of extracts

The fruiting bodies of *Ganoderma lucidum* were obtained from Waghai village (20.7667°N, 73.4833°E), district Dang. The extraction method of Kamra and Bhatt, 2012 with certain modifications was used. The dried fruiting bodies were ground to a fine powder using a domestic blender. For preparing the extracts, methanol, ethyl acetate, chloroform, methanol: ethyl acetate (50:50, v/v) were used as solvents to obtain the pharmacologically active compounds from the mushroom. For every 1 gram (g) of powder, 50 milliliter (ml) of solvent was used and was subjected to extraction using a reflux apparatus. After the completion of extraction, the supernatant was filtered through Whatman #1 filter paper. All solvent extracted fractions were evaporated to dryness to obtain residues. The residues were reconstituted using their respective solvents (except for the chloroform residue, which was reconstituted in methanol) to obtain stock solutions having concentration of 10 mg/ml. The extracts were stored at 4ºC in air tight containers. The antioxidant activities of the different extracts were carried out by preparing working solutions of different concentrations (125µg/ml, 250µg/ml, 500µg/ml, and 1000µg/ml) from the stock solution.

Determination of total phenolic content (TPC)

Folin-Ciocalteu phenol reagent consists of a mixture of the hetero-poly phosphomolybdic and phosphotungstic acids in which the molybdenum and tungsten are in the 6^+ state. On reduction with certain reducing agents, molybdenum blue and tungsten blue are formed, in which the mean oxidation state of the metals is between 5 and 6. Total phenols were determined according to the colorimetric reaction with the Folin-Ciocalteu reagent by the modified method of Singleton & Rossi, 1965[6].

1 ml of the extracts (125-1000 µg/ml of methanol, ethyl acetate, chloroform and methanol: ethyl acetate extracts) was mixed thoroughly with 5 ml of Folin-Ciocalteu reagent. After 5 minutes, 4 ml of 7.5% sodium carbonate (Na_2CO_3) was added and allowed to react for 1 hr at room temperature. The absorbance was measured at 765 nm against a blank having all the reagents excluding the sample using spectrophotometer. Samples were measured in triplicates. This procedure was repeated 5 times for each extract. The total phenols were quantified by the standard curve obtained. Standard curve of gallic acid solution (10, 20, 40, 60, 80 and 100 µg/ml) was prepared using the similar procedure from which the regression formula was derived.

Total phenol values were expressed as mg of gallic acid equivalents (GAE)/g of dry extract.

Determination of antioxidant capacity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity assay

DPPH is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole which gives it its deep violet colour. When a solution of DPPH is mixed with an antioxidant that can donate a hydrogen atom, then this gives rise to the reduced form of DPPH (non-radical form). This is characterized by the loss of the violet colour and the formation of a pale yellow colour. The degree of decolourization is measured by the decrease in the absorbance and thus indicates the scavenging potential of the antioxidant compound. The assay was carried out according to the modified method of Blois (1958)[7].

2 ml of 0.1mM solution of DPPH in methanol was mixed with 1 ml of the extracts at different concentrations (125-1000 µg/ml of methanol, ethyl acetate, chloroform and methanol: ethyl acetate extracts). The mixture was then incubated at room temperature for 30 min in the dark. The control was prepared by mixing 2 ml of DPPH solution with the respective solvent. The absorbance was measured against a blank at 517 nm using spectrophotometer. Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity. The antioxidant activity of the extract was expressed as IC_{50}, which was defined as the concentration (µg/ml) of extract that inhibits the formation of DPPH radicals by 50%. IC_{50} values were obtained from linear regression analysis. Ascorbic acid was used as the standard. Samples were prepared and measured in triplicates. The percentage of scavenging activity of each extract on DPPH radical was calculated as % inhibition of DPPH (I%) using the following equation:

$$I\% = [(A_o - A_s)/A_o] \times 100$$

where A_o is the absorption of control and A_s is the absorption of the tested extract solution.

ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)] free radical scavenging activity assay

The method is based on the generation of a blue/green $ABTS^{\bullet+}$ chromophore via the reaction of ABTS and potassium persulfate. The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 734 nm. The assay was carried out using the modified method of Re *et al.*, (1999)[8].

The $ABTS^{\bullet+}$ stock solution was prepared by reacting ABTS aqueous solution (7 mM) with 2.45 mM aqueous solution of potassium persulfate in equal quantities; the mixture was allowed to stand in the dark at room temperature for 12-16 hrs before use. The working solution of $ABTS^{\bullet+}$ was obtained by diluting the stock solution in methanol to give an absorbance of 0.70 ± 0.02 at 734 nm. Then, 2.7 ml of $ABTS^{\bullet+}$ solution was mixed with 0.3 ml of the extracts at different concentrations (125-1000 µg/ml of methanol, ethyl acetate, chloroform and methanol: ethyl acetate extracts). The mixture was then incubated at room temperature for exactly 7 min in the dark. The control was prepared by mixing 2.7 ml of $ABTS^{\bullet+}$ solution with the appropriate solvent. The absorbance was measured against a blank at 734 nm using spectrophotometer. The antioxidant activity of the extract was expressed as IC_{50}, which was defined as the concentration (µg/ml) of extract that inhibits the formation of ABTS radicals by 50%. IC_{50} values were obtained from linear regression analysis. BHT was used as the standard. Samples were prepared and measured in triplicates. The percentage of scavenging activity of each extract on $ABTS^{\bullet+}$ was calculated as % inhibition (I%) using the following equation:

$$I\% = [(A_o - A_s)/A_o] \times 100$$

where A_o is the absorption of control and A_s is the absorption of the tested extract solution.

Determination of ferric reducing antioxidant power (FRAP)

This method is based on the ability of the sample to reduce Fe^{3+} to Fe^{2+} ions. At low pH, in the presence of TPTZ, ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous (Fe^{2+}-TPTZ) form with the formation of an intense blue colour having an absorption maximum at 593 nm. The method described by Benzie and strain (1996)[9] was followed.

2.9ml of the FRAP reagent was mixed with 0.1 ml

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of the extracts at different concentrations (125-1000 µg/ml of methanol, ethyl acetate, chloroform and methanol: ethyl acetate extracts). The mixture was then incubated at 37ºC for 30 min in the dark. The absorbance was measured at 593 nm against a blank having all the reagents excluding the sample using spectrophotometer. Increased absorbance of the reaction mixture indicates an increase of reduction capability. Samples were measured in triplicates. This procedure was repeated 5 times for each extract. Ascorbic acid was used as the standard. Standard curve of ascorbic acid solution (10, 20, 30, 40, 50 µg/ml) was prepared using the similar procedure from which the regression formula was derived. Results were expressed in µg of ascorbic acid equivalents (AAE)/ml of extract.

Statistical analysis

All determinations of total phenols and antioxidant capacity by DPPH, ABTS, FRAP assay were conducted in triplicates. Experimental data were expressed as mean ± SD of three replicates. Analysis of variance and significant difference among means were tested by one way ANOVA. Statistical signiûcance was accepted at a level of 5%. $P < 0.05$ was regarded as signiûcant and $p < 0.01$ was very signiûcant. Correlations between the TPC and antioxidant assays were found out with the help of Microsoft Excel 2007 (Roselle, IL, USA).

RESULTS AND DISCUSSION

Determination of total phenolic content

TABLE 1 shows the mean total phenolic content (TPC) of the extracts measured using the GAE equation of $y= 0.012x + 0.039$ ($R^2 = 0.996$). Results clearly show that the methanolic extract has the highest TPC (83.67484± 28.17 mg GAE/g dry extract) followed by Methanol: ethyl acetate (73.9096± 6.96 mg GAE/g dry extract), chloroform (41.81646± 1.96 mg GAE/g dry extract) and ethyl acetate extract (33.833± 7.66 mg GAE/g dry extract).

ANOVA analysis showed significant differences between TPCs of the methanol and ethyl acetate extract ($p <0.01$), methanol and chloroform extract ($p <0.01$), ethyl acetate and chloroform extract ($p <0.05$), ethyl acetate and methanol: ethyl acetate extract ($p <0.01$) and chloroform and methanol: ethyl acetate extract ($p <0.01$). The results suggest that the TPC varied significantly from one extract to another.

TABLE 1 : Mean total phenolic content of the extracts

Solvent	Total phenol (mg/g dry extract)
Methanol	83.67484
Ethyl acetate	33.833
Chloroform	41.81646
Methanol:ethyl acetate	73.9096

Phenolic plant compounds fall into several categories; simple phenolics, phenolic acids (derivatives of cinnamic and benzoic acids), coumarins, flavonoids, stilbenes, tannins, lignans and lignins[1]. Phenolics with only few hydroxyl groups are found to be soluble in ether, chloroform, ethyl acetate, methanol, and ethanol[10].

The results suggested that extraction by methanol gave the highest phenolic content as compared to the other solvents thus proving methanol to be a suitable solvent for the extraction of phenolic compounds. The reason could be that methanol has the ability to inhibit the reaction of polyphenol oxidase that causes the oxidation of phenolics and also evaporates with ease as compared to other solvents[11]. Therefore, the results showed that different extracting solvents influenced different yields of TPC.

In a study done by Li *et al.*, 2012[12], the total phenolic content in the ethanol-soluble acidic components (ESAC) from the fruiting bodies of *G. atrum* was found to be 75.80±5.67 mg/g ESAC, which could have been related to the antioxidant activity of the mushroom.

DPPH free radical scavenging activity assay

TABLE 2 shows the % inhibition of DPPH radicals by the four extracts. Results clearly show that among the four extracts, methanol extract has the highest % scavenging activity for all the concentrations (125, 250, 500, 1000 µg/ml) followed by methanol: ethyl acetate, chloroform and ethyl acetate extracts. The IC_{50} values were obtained from linear regression analysis. The lower the IC_{50}, the higher the antioxidant power. The lowest IC_{50} was shown by the methanol extract (142.84±71.84 µg/ml) thus having the highest antioxidant activity. The remaining extracts had higher IC_{50} values as compared to methanol. Their IC_{50} values in the increasing order are: Methanol: ethyl acetate (163.14±3.45 µg/ml), chloroform (197.02±0.96 µg/ml)

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TABLE 2 : % inhibition of DPPH (I%) by the extracts

Concentration (µg/ ml)	% inhibition of DPPH (I%)			
	Methanol	Ethyl acetate	Chloroform	Methanol:ethyl acetate
125	48.64±4.18%	22.46±0.84%	33.52±0.04%	44.52±0.68%
250	66.971±6.97%	48.21±0.85%	55.5±3.54%	60.00±1.41%
500	86.435±4.56%	68.75±0.52%	80.28±1.77%	83.14±1.55%
1000	91.69±2.25%	86.68±0.95%	89.73±0.42%	90.43±1.57%

and ethyl acetate (285.81±7.45 µg/ml). Ascorbic acid was used as reference as a radical scavenger. The IC_{50} of ascorbic acid was found to be 139.76±0.16 µg/ml which was the lowest amongst all the extracts. Significant difference ($p<0.01$) between the IC_{50} values of the ethyl acetate and chloroform extract, ethyl acetate and methanol: ethyl acetate, chloroform and methanol: ethyl acetate extract was observed.

The results showed that the methanol extract had the highest % scavenging activity at all the concentrations (125, 250, 500, 1000 µg/ml) followed by methanol: ethyl acetate, chloroform and ethyl acetate extracts. The lowest IC_{50} was observed in the methanol extract (142.84±71.84 µg/ml) thus having the highest antioxidant activity.

When these results were compared to the results obtained by other reports, it was found that the above obtained results gave a better scavenging activity when compared to other reports. In one study done by Mau *et al.*, in 2005[13], the methanolic extract from *G. tsugae* showed DPPH• scavenging activity of 88.4% at 5 mg/ml which is lower than the results obtained from methanolic extract of *G.lucidum;* which showed DPPH• scavenging activity of 91.69% at 1 mg/ml. In another study done by Mau *et al.,* in 2005, the DPPH• scavenging effect of fruiting body methanol extract of *Ganoderma lucidum* was about 45% at a concentration of 500 µg/ml. whereas the results obtained in the present study was much higher for the 500 µg/ml fruiting body methanol extract and was found to be 86.435% thus proving *G.lucidum* to be a potent antioxidant.

Also, in a study done by Chen *et al.*, in 2008[14], the purified polysaccharides from the dried fruiting bodies of *G. atrum* scavenged 76.9% of the DPPH free radicals at a dose of 1 mg/ml. The scavenging activity of methanolic extracts from *G. tsugae* exhibited 42 and 75% radical scavenging activity at a concentration of 200 and 500 ppm, respectively[15]. In a study done by Heleno *et al.*, (2012)[4], the phenolic extract of the fruiting body of *G. lucidum* gave high DPPH• scavenging activity (EC_{50} 0.14 mg/ml).

Methanol thus proved to be a better solvent probably because it could dissolve phenols, flavonoids, triterpenoids and glycosides. This shows that the extracting solvents are likely to influence measurement of antioxidant activities of the extracts.

ABTS free radical scavenging activity assay

TABLE 3 shows the % inhibition of ABTS radicals by the four extracts. Results clearly show that among the four extracts, methanol extract has the highest % scavenging activity for all the concentrations (125, 250, 500, 1000 µg/ml) followed by methanol: ethyl acetate, chloroform and ethyl acetate extracts. The IC_{50} values were obtained from linear regression analysis. The lower the IC_{50}, the higher the antioxidant power. The lowest IC_{50} was shown by the methanol extract (223.666±1.87 µg/ml) thus having the highest antioxidant activity. The remaining extracts had higher IC_{50} values as compared to methanol. Their IC_{50} values in the increasing order are: Methanol: ethyl acetate (225.78±9.19 µg/ml), chloroform (234.32±12.58 µg/ml) and ethyl acetate

TABLE 3 : % inhibition of ABTS (I%) by the extracts

Concentration (µg/ ml)	% inhibition of ABTS			
	Methanol	Ethyl acetate	Chloroform	Methanol:ethyl acetate
125	34.44±1.19%	30.34±0.03%	33.9±2.83%	34.25±0.35%
250	48.75±0.21%	44.56±4.3%	47.73±0.62%	48.06±0.06%
500	76.32±4.7%	70.00±1.41%	71.27±1.03%	73.94±2.74%
1000	96.72±0.38%	92.00±2.83%	94.04±2.88%	96.30±2.62%

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(260.58±16.54µg/ml). The IC_{50} for standard (BHT) was found to be 124.26±0.37 µg/ml. No significant differences in ABTS•+ scavenging potential could be determined among the IC_{50} values of the extracts.

The ABTS method is based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm. The ABTS radical cation is generated by the oxidation of $ABTS^{\bullet+}$ with potassium persulfate, its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 734 nm. The absorbance was always found to decrease with the increase in the concentrations of the extract.

All the tested extracts of *G. lucidum* exhibited effectual radical cation scavenging activity. The results showed that the methanol extract had the highest % scavenging activity at all the concentrations (125, 250, 500, 1000 µg/ml) followed by methanol: ethyl acetate, chloroform and ethyl acetate extracts. The lowest IC_{50} was observed in the methanol extract (223.666±1.87 µg/ml) thus having the highest antioxidant activity.

Methanol thus proved to be a better solvent probably because it could dissolve phenols, flavonoids, triterpenoids and glycosides. This shows that the extracting solvents are likely to influence measurement of antioxidant activities of the extracts.

In one study, the crude exopolysaccharide extract of the fruiting bodies of *G. lucidum* showed high antioxidant activity which indicated the beneficial effects of mushroom polysaccharides as antioxidants. The ABTS scavenging activity of the crude exopolysaccharide was found to be (63.75 ± 2.47 %)[5].

Determination of ferric reducing antioxidant power (FRAP)

TABLE 4 shows the reducing ability of the four extracts expressed in µg of ascorbic acid equivalents (AAE)/ml of extract. All the extracts of *G.lucidum* at different concentrations exhibited strong antioxidant activity. All values increased with the increasing concentrations (125, 250, 500, 1000 µg/ml) of every extract. The reducing ability of the ethyl acetate extract was found to be the highest (121.075, 354.375, 645.3, 1120.25 µg AAE/ml of extract) among all the extracts followed by Methanol: ethyl acetate (42.275, 115.45, 216.15, 605.15 µg AAE/ml of extract), methanol (31.75, 110.45, 291.875, 497.8095 µg AAE/ml of extract) and chloroform extract (31.325, 76.5, 220.25, 431.008 µg AAE/ml of extract). Thus the phytoconstituents present in *G. lucidum* act as strong reducing agents and hence strong antioxidants. Significant difference was observed in all the concentrations between the methanol and ethyl acetate extract ($p<0.03$ and $p<0.05$). Also significant difference was observed in all the concentrations between the ethyl acetate and chloroform extracts ($p<0.01$ and $p<0.03$) and in all the concentrations between the ethyl acetate and methanol: ethyl acetate extracts ($p<0.05$).

TABLE 4 : Ferric reducing antioxidant power of the four extracts

Concentration (µg/ ml)	FRAP (µg AAE/ml of extract)			
	Methanol	Ethyl acetate	Chloroform	Methanol: ethyl acetate
125	31.75±16.41	121.075±77.26	31.325±12.29	42.275±50.68
250	110.45±7.03	354.375±187.67	76.5±9.62	115.45±95.05
500	291.875±49.56	645.3±321.83	220.25±26.14	216.15±235.39
1000	497.8095±57.73	1120.25±529.14	431.008±68.74	605.15±118.82

In the FRAP method, the presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the absorbance at 593 nm. FRAP measures the overall antioxidant activity or reducing potential, as an indication of host's total capacity to withstand free radical stress[16].

All the extracts of *G.lucidum* at different concentrations exhibited strong antioxidant activity. All values increased with the increasing concentrations (125, 250, 500, 1000 µg/ml) of every extract. The reducing ability of the ethyl acetate extract was found to be the highest among all the extracts followed by Methanol: ethyl acetate, methanol and chloroform extract. Thus the phytoconstituents present in *G. lucidum* act as strong reducing agents and hence strong antioxidants.

In one study, when different extracts of *G. lucidum*

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were tested for antioxidant activity by the FRAP (Ferric reducing ability of plasma) assay, the reducing ability of dichloromethane extract was observed the highest, followed by aqueous, methanol, ethyl acetate and hexane extracts[3].

In a study done by Li *et al*., 2012[12] the FRAP value of the ethanol-soluble acidic components (ESAC) from the fruiting bodies of *G. atrum* was found to be 0.031±0.0021 mmol/g which proved the reducing power of the mushroom.

Correlation between total phenolic content and antioxidant activity

The total phenolic content of each extract was correlated with the antioxidant activity. The correlations are represented in TABLE 5. The total phenolic content of each extract was correlated with the antioxidant activity. Correlations between the TPC vs DPPH (r^2= 0.838, 0.91, 0.765, 0.826), TPC vs ABTS (r^2 = 0.978, 0.970, 0.929, 0.936), TPC vs FRAP (r^2 = 0.993, 0.997, 0.986, 0.978) was found. The correlation of TPC vs FRAP was found to be the strongest.

The phenolic derivatives compounds are the vital antioxidants which exhibit scavenging efûciency on the free radicals; reactive oxygen species which are numerous and widely distributed in the plant kingdom. Recent studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants in vitro than vitamins E or C, and thus might contribute significantly to the protective effects *in vivo*. The antioxidative effect of phenolics is due to radical scavenging activity, reducing activity, and an indirect effect arising from chelation of prooxidant metal ions[17]. Hence Correlations between the TPC and antioxidant assays were conducted. The correlation of TPC vs FRAP was found to be the strongest.

Results revealed that there is a strong and significant positive correlation between TPC and all the antioxidant assays for every extract. This showed that as the concentration of phenols increased in the extracts, the antioxidant activity also increased, thus suggesting that the phenols could be the major antioxidants present in *G lucidum* that are responsible for its high *in vitro* antioxidant activity.

In the study done by Li *et al*., in 2012[12], the phenolic content of the ethanol-soluble acidic components (ESAC) from the fruiting bodies of *G. atrum* was found to be correlated to the antioxidant activity of ESAC.

Many more studies have been done with other plants where the total phenolic content has been correlated with the antioxidant activity of the compound.

In a study done by Rawat *et al*., in 2011[18], the total phenolic content of *Myrica esculenta* was found to be positively correlated with its antioxidant activity.

In another study done on *Ranunculus marginatus* by Kaya *et al.*, in 2010[19], proved a positive correlation between the total phenolic content of the extracts and their antioxidant activity.

Also the study conducted by Sreeramulu and Raghunath in 2010[20] on certain roots, tubers and vegetables showed that the antioxidant activity of these plants was correlated with their total phenolic content, thus proving that the phenols could be responsible for the antioxidant activities of these plants.

TABLE 5 : Correlation between total phenolic content and antioxidant activities

Correlation	r^2 values for different extracts			
	Methanol	Ethyl acetate	Chloroform	Methanol: ethyl acetate
TPC vs DPPH	0.838	0.914	0.765	0.826
TPC vs ABTS	0.978	0.970	0.929	0.936
TPC vs FRAP	0.993	0.997	0.986	0.978

CONCLUSION

A major concern these days are free radicals which are produced in the body by the process of oxidation by normal cellular mechanisms or derived from external sources. Many diseases such as cancer, cardiovascular diseases, neurodegenerative diseases, rheumatoid arthritis, atherosclerosis, hypertension and AIDS are believed to be related to production of excessive amounts of ROS in the body. Free radicals have also been known to cause oxidative rancidity in foods rich in lipids. Antioxidants are molecules which can protect the body from free radicals so that the vital molecules

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(DNA, lipids and proteins) are not damaged. In the last few years the focus has been on the use of natural and safer antioxidants because the synthetic antioxidants have been found to be unsafe. Thus the antioxidant activity of *G. lucidum* was described in this study.

Phenolic compounds are important secondary metabolites widely found in plants which have been found to possess strong antioxidant activity. Thus an attempt was made to study the relationship between the total phenolic content and antioxidant activity of the mushroom.

From this study it could be concluded that the methanol extract was found to have the highest yield and was also found to extract the maximum phytoconstituents being a polar solvent. This proved methanol to be an appropriate solvent for extraction. The extracts of *G. lucidum* showed strong antioxidant activity thus proving it to be a potent antioxidant. A positive correlation between the antioxidant activity and phenolic content could be the reason for it's high antioxidant activity. However, further studies are needed for the isolation and identification of individual phenolic compounds and also in vivo studies are needed for the better understanding of their mechanism of action to scavenge the free radicals. Further studies could also be done to isolate and purify the extracts to determine the components responsible for the activity of these extracts. This study suggests that *G. lucidum* could play a vital role in reducing the oxidative stress and could also help to prevent certain neurodegenerative diseases.

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