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ANTIOXIDANT ACTIVITY, ANTIBACTERIAL ACTIVITY AND TOTAL PHENOL AND FLAVONOID ANALYSIS OF *GANODERMA LUCIDUM*

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ABSTRACT

Ganodermalucidum fungus is broadly used as a nutritional medicine due to several medicinal properties like energy enhancing, antioxidant, antimicrobial, and immunostimulatory and anticancer. Phytochemical of methanolic extract were studied. Total phenolic and flavonoids of methanol extract were observed using Gallic acid and quercetin standard curve. Total phenols content (154.16±4.21 mg GAE/g dry extract) and total flavonoids (29.09 ± 0.05 mg QE/g) were recorded. The antioxidant activity of methanol extract was observed using(DPPH) 1, 1-diphenyl-2-picrylhydrazylfree radical scavenging activity assay, (ABTS) 2,2.-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) free radical scavenging activity assay as well as (FRAP) Ferric reducing antioxidant power assay. In DPPH assay, $IC_{50}$of methanol extract (143.23±68.79µg/ml), for ABTS assay, again lowest IC_{50}value (212.542±1.14 µg/ml) were recorded therefore having highest antioxidant activity and the FRAP assay, reducing ability of methanol extract 485.5186±46.53 µg AAE/ml was found. The antimicrobial activity of *G.lucidum* methanolic extract were tested against Gram positive bacteria *Bacillus cereus* (MTCC-430) and *Staphylococcus aureus* (MTCC-96) and Gram negative bacteria *Escherichia coli*(MTCC-1687), *Pseudomonas aeruginosa* (MTCC-2453), *Klebsiella pneumonia* (MTCC-3384).The highest zone of inhibition activity was investigated against *Staphylococcus aureus*(14.0 mm, zone of inhibition diameter).This study also discovered that *G.lucidum* compounds could be use for better antioxidant nutrient supplement.

INTRODUCTION

Ganodermalucidum has high medicinal properties; it has good pharmaceuticals and nutraceutical properties, which are helping human health. *Ganoderma* is usually used for dietary food supplement, high medicinal and it has more than 400valuable bioactive molecules **(S.P.** Wasser, A.L. Weis 1999). Several significant applications of nutaraceuticals such as anti-inflammatory, anti-allergic-activities, antitumor activities, antitussive properties, bronchitis-preventive effect including regeneration of bronchial epithelium, enhancing myocardial metabolism, lowering blood pressure and antibacterial (T. Mizuno, 1992; S.T. Chang, 1996; J.T. Xie et al, 2006). In *Ganodermalucidum* various kind of polyphenolic contents and available bioactive compounds like ganoderic acids,

ling zhi-8 protein and beta- and hetero-beta-glucans have been detected too (U. Lindequit, 1995).*Ganodermalucidum*has been investigated various pharmacological properties such as anti-viral, immuno-modulating, anti-atherosclerotic, anti-diabetic analgesic, chemopreventive, radio-protective, sleep-promoting, hypo-lipidemic, anti-fibrotic, hepato-protective, antioxidative and radical-scavenging, hypoglycaemic, antiulcer and anti-aging properties(Zhou, S.H et al.,2002; Smith, J. et al.,2002;Yuen JW et al.,2005; Vickers, A.,2000)

*G. lucidum*contains bioactive molecules such as phenols, steroids Terpenoids, nucleotides, glycoprotein's, steroids and Polysaccharides these are extracted from mycelium, fruit body and spores. The most significant bioactive compounds of *G. lucidum* is flavonoids and phenolic compounds (Galor et al. 2011; Yuen and Gohel 2005). In additional, *G. Lucidum* showed great antioxidant activity due to crude exo polysaccharide of fruiting bodies which showed the valuable effects of polysaccharides as antioxidants (S.Mahendran et al., 2012).While diverse extracts such as aqueous, methanol, ethyl acetate, hexane and dichloromethane of *G. Lucidum* were confirmed for antioxidant activity by the DPPH and FRAP (Ferric reducing ability of plasma) assay (A.Kamra et al., 2012). In current years, various potential sources of natural antibiotics have been using for numerous infectious infections, frequently viral, fungal and bacterial. In the searches of new medicinal plants for new anti-microbial agents are more serious in the countries such India where infectious diseases origin from bacteria are not only wild but bacterial agents are emerging an growing resistance against antibiotics **(Kamaraj *et al.*, 2012).** In this study, phytochemical analysis of methanolic extract and total phenolic and flavonoid content analysis of *G.lucidum.* The antimicrobial activity against different pathogenic microorganism of methanolic extract of *G.lucidum* by using well diffusion method was estimated. The antioxidant activity was determined by 1, 1-diphenyl-2-picrylhydrazyl(DPPH) free radical scavenging activity assay, Ferric Reducing Antioxidant Power (FRAP) assay and 2,2.-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical scavenging activity assay.

MATERIALS AND METHODOLOGY

Sample Collection : *Ganodermalucidum* fruiting bodies were collected from different places of district Bilaspur H.P., INDIA.

Extract Preparation: *Ganodermalucidum* was sun-dried and crushed to powder form. The methanolic extract of the sample was prepared **(Pal et al., 2010).**For extraction, 20gm sample and 100 ml methanol was stirring at 100 rpm with at 30^{o} C for 24 hours and filtered by Whatman filter paper no.1. The methanolic extract was evaporated by rotary evaporator at 40^{o}C, residues redissolved in methanol (20 mg/ml).

Phytochemical Screening of Extract: Primary phytochemical screening tests can be done for founding chemical composition of *G.lucidum* methanolic extract. The extract was tested for qualitative analysis of various phytochemicals includes Alkaloids, Phenolic compounds, Flavonoids,, Saponins, Carbohydrates, Reducing sugars, Steroids, Cardiac glycosides, Terpenoids, Anthraquinones and Tannins. These all tests were performed by previously described methods **(**Raaman, 2006; De, S et al., 2010; Shamaki et al., 2012). Analysis of Total Phenolic Content: Total Phenolics content was reported by the Folin-Ciocalteu method (Makkar et al., 1993) with certain change. 200 µl of extract and 1 ml Follin-Ciocalteu reagent were mixed and after 3 min 1 ml of 7.5% of sodium carbonate solution was added. The mixture was mixed well and kept to stand for 2 h in dark condition. The absorbance was observed in triplicate using UV-VIS Spectrophotometer at 515nm. A blank was prepared and observed. A gallic acid standard calibration curve was obtained from several concentrations of gallic acid. The results were expressed as GAE (gallic acid equivalents/g) of the extract.

Analysis of Total Flavonoid content: The Total Flavonoids content was reported by UV-VIS spectrophotometrically **(Quettier-Deleu et al., 2000)**. 4 ml distilled water was added to 1ml of extract then 1 ml of 5%sodium nitrate was added. After 5 min. 1 ml of 10% aluminium chloride was added. Allowed to settle for 5 min then 2 ml of NAOH was added. Tests were performed in Triplicates. The absorbance was observed at 510 nm and the absorbance of blank was recorded similarly. Total flavonoids content was examined by quercetin standard curve (10-180 mg/ml). The absorbance were used and expressed as milligrams of quercetin equivalents (QE/g of extract).

DPPH Radical-Scavenging Activity: Free radical-scavenging activities of *G.lucidum* was determined using 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) (T. Hatano et al., 1989). Methanolic extract was having pale brown color. The extract was added with diverse concentration an equal volume to DPPH (0.1 mM). After 30 minutes absorbance was observed at 517nm. The experiments were done in triplicates. Sample and Ascorbic acid which is necessary to scavenge 50% free radicals of DPPH. Percentage of scavenging was calculated by following equation

% scavenged DPPH radical= [(Abs control – Abs sample] / Abs control] × 100

Where, Abs control = absorbance of DPPH radical + methanol, Abs sample = absorbance of DPPH radical +extract.

ABTS Radical-Scavenging Activity: Free radical scavenging capability of *G.lucidum*was calculated by 2,2'-azinobis [3-ethylbenzthiazoline] -6-sulfonate (ABTS) assay **(R. Re et al., 1999)**. 2.45 mM potassium persulfate solution in water and 7.0 mM ABTS solution in methanol were prepared in equal quantities. In dark condition at room temperature for 12 hours kept them to react. After then mixture was diluted by 1.0 ml of ABTS solution with proper volume of methanol to get the absorbance 0.702± 0.001 units at 734nm. 0.1mL of extract was permitted to react 1.0 ml of ABTS scavenging capacity for the extract was compared with ascorbic acid and quercetin.

% scavenged ABTS radical =[(Abscontrol – Abssample)]/ (Abscontrol)] × 100

Where, Abs control = absorbance of ABTS radical + methanol, Abs sample = absorbance of ABTS radical +extract.

FRAP Ferric Reducing Ions Activity: This method is used for determined capability of the sample to reduce Fe3+ to Fe2+ ions. This method was described by Benzie and strain (1996)**.** At low pH, presence of TPTZ, ferric-tripyridyltriazine (Fe3+-TPTZ) compound is reduced ferrous (Fe2+-TPTZ) form with an intense blue colour and having absorption at 593 nm.2.9ml of FRAP reagent was mixed 0.1 mlof extract at diverse concentrations (125-1000 µg/ml). Then mixture was incubated at 37ºC for 30 min in dark condition. The absorbance were recorded at 593 nm. Samples were performed in triplicates. As standard ascorbic acid was used. Results were calculated in µg of ascorbic acid equivalents (AAE)/ml of extract.

Antimicrobial assay: The Gram positive species *Bacillus cereus* (MTCC-430) and *Staphylococcus aureus* (MTCC-96)and Gram negative species *Escherichia coli* (MTCC-1687),*Pseudomonas aeruginosa* (MTCC-2453), *Klebsiella pneumonia*(MTCC-3384) were obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh. Antimicrobial activities of the *G.lucidum* extract was reported using agar well diffusion method. Nutrient agar was poured into Petri plates and 100µl of bacterial suspension spread over the media. 8mm diameter wells were punched in the agar using gel puncture. 30µl of extract was introduced into wells directly. Thepetri plates were incubated at 37ºC for 24 hours. After incubation, inhibition zones was formed on the medium zone of inhibition calculated in mm. Streptomycin was used as standard antibacterial agent respectively. The diameter of the zone of inhibition were recorded in millilitres (mm). Distilled water used as negative control. All tests were accomplished in triplicate.

RESULT AND DISCUSSION

Phytochemical Screening: The phytochemical analysis results were showed in Table 1.Phenols, Terpenoids, Carbohydrates, glycosides and flavonoids were present in extract. Hence, Alkaloid, Saponins and Protein were not present methanol extract. Carbohydrates have also been extracted in polysaccharides from *Ganodermalucidum*. Polysaccharides have shown strong immunomodulating activities, hypolipidemic activity, anti-inflammatory and antitumorigenic.

Table 1 Phytochemical Analysis of *Ganodermalucidum*

S.N.	SECONDARY METABOLITES	METHANOL EXTRACT
1	Alkaloids	-ve
2	Phenols	+ve
3	Flavonoids	+ve
4	Carbohydrates	+ve
5	Glycosides	+ve
6	Terpenoids	+ve
7	Saponins	-ve
8	Protein and Amino acid	-ve

Total Phenol Content: Table 2 showed the mean Total Phenolic Content (TPC) of the extract measured by GAE equation. Methanolic extract has showed highest Phenol content (154.16±4.21mg GAE/g dry extract) Phenolics with few hydroxyl groups are found to be soluble in methanol **(A.A.Almey et al., 2010)**. The results recommended that extraction using methanol as solvent gave the highest phenolic content thus showing methanol to be aappropriate solvent for extraction of phenolic compounds.

Total Flavonoid Content: The total flavonoid content was calculated in *G.lucidum*(29.09 ± 0.05 mg QE/g), it was higher than previously recorded value of 10.82 mg QE/mg **(Raseta et al., 2016)**. Results were showed in Table 2. According to studies, high flavonoids content offer shield against oxidative defences and oxidative stress like enzymes and vitamins **(Tripathy et al., 2014)**.

Table 2 Total Phenolic and Flavonoid Content of *G. lucidum* methanol extract

SOLVENT	TOTAL PHENOL (mgGAE/g dry extract)	TOTAL FLAVONOID (mgQE/g dry extract)
Methanol	154.16 ± 4.21	29.09±0.05

DPPH Radical Scavenging Activity: In DPPH assay, IC_{50} value of *G.lucidum* methanol extract was recorded (143.23±68.79 µg/ml).The IC_{50} value was got by linear regression analysis. Table 3 shows the % inhibition of DPPH radicals. Methanol extract has the highest% scavenging activity. The lower IC_{50} shows higher antioxidant power.

ABTS Free Radical Scavenging Activity: Table 3 showed % inhibition of ABTS radicals by extract. Methanol extract has shown highest %scavenging activity. Methanol extract (212.542±1.14µg/ml)shown lowest IC_{50}, hence having highest antioxidant activity. The IC_{50} value was determined from linear regression analysis. Thus methanol showed to be a well solvent due to it could dissolve flavonoids, phenols, triterpenoids and glycosides.

FRAP Ferric Reducing Antioxidant Power: Reducing ability of the methanol extracts shown Table 3. Reducing ability expressed in µg of ascorbic acid equivalents (AAE)/ml of extract. The reducing ability of the methanol (485.5186 µg AAE/ml)of extract recorded. Thus the phytochemical present in *G. Lucidum* shows strong antioxidant and strong reducing ability.

Table 3 DPPH, ABTS and FRAP analysis result of methanol extract of *G. lucidum*

	DPPH	ABTS	FRAP
IC50(µg/ml)	143.23± 68.79	212.542± 1.14	
% of inhibition	92.78±3. 32%	94.83±0.2 9%	

Ferric Reducing Ability (µg AAE/ml)			485.5186± 46.53

Antibacterial Activity : The antimicrobial activities of *Ganodermalucidum* against the Gram positive and negative organisms were showed in Tables 4.The methanol extractshowed great antimicrobial activity. The highest inhibition against *Staphylococcus aureus*(14.0 mm)was recordedin the methanolic extract.

Table 4. Antimicrobial Activity of *Ganodermalucidum*

SN.	Test Organisms	Zone of Inhibition (In mm)
1	*Bacillus cereus*	**13.0**
2	*Staphylococcus aureus*	**14.0**
3	*Escherichia coli*	**6.0**
4	*Pseudomonas aeruginosa*	**12.0**
5	*Klebsiellapneumonia*	**1.0**

CONCLUSION: In this study concluded that *Ganodermalucidum* has higher antioxidant activity. Methanol extract of *G.lucidum* shown positive results of different phytochemical analysis such as Phenols, Flavonoids, Carbohydrates and Glycosides. Total phenolic and Flavonoid were determined. Total phenol content of methanol extract154.16 ± 4.21 mgGAE/g dry extract and total flavonoids 29.09±0.05 mgQE/g dry extract were recorded. Which is having great antioxidant capacity Antioxidant capacity were determined using DPPH, ABTS and FRAP assays. In this study also performed antibacterial activity against gram positive and negative bacteria. Hereby methanol extract showed highest antibacterial activity against *Staphylococcus aureus (*14.0mm) while lowest inhibition showed against *Klebsiella pneumonia.*

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