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In vitro Antimicrobial and Antioxidant Properties of *Ganoderma lucidum E*xtracts Grown in Turkey

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Authors' contributions

This work was carried out in collaboration between all authors. Author GYC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DO and BA managed the analyses of the study and managed the literature searches. The mushroom sample was collected and identified by author HA. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To determine antimicrobial and antioxidative effects of *Ganoderma lucidum*.
Place and Duration of Study: Erciyes University, Faculty of Pharmacy, Pharmaceutical Biotechnology research laboratory,Kayseri, Turkey, between January to March, 2013.
Methodology: Antimicrobial inhibitory effects were carried out on the extracts using disc diffusion method. Antioxidant activities of the ethanolic and methanolic extracts from *G.lucidum* were evaluated by using 2,2-diphenyl-1-picrylhydrazyl [DPPH] radical scavenging, metal chelating, total flavonoid and total antioxidant activity assays. In addition, the amounts of phenolic compound, β-carotene and lycopene components in the extracts were determined.
Results: The antimicrobial effects of ethanolic and methanolic extracts of *G. lucidum* were tested against one species of Gram positive bacteria, two species of Gram-negative bacteria and two yeast. The highest inhibitory activity was determined against *Candida glabrata* RSKK 04019 [25±1 mm, inhibition zone diameter]. On the other hand, the lowest inhibitory activity was determined against *Candida albicans* ATCC 90028 and *Bacillus*

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subtilis RSKK 244 [10±1 and 10±0 mm, inhibition zone diameter]. DPPH radical scavenging effect was detected in the methanol extract [IC_{50} = 3.82±0.04 µg/mL] was higher than the ethanol extracts [IC_{50} = 7.03±0.07 µg/mL]. Compared to reference antioxidant, the methanol and ethanol extracts of *G.lucidum* provided a lower IC_{50} than butylated hydoxyanisole [BHA] [IC_{50} = 0.30±0.01 µg/mL]. Phenolic compounds were the major antioxidant component found in the mushroom extracts.
Conclusion: These results showed that *G. lucidum* may be used in pharmaceutical applications because of its effective antioxidant properties.

Keywords: Antimicrobial activity; Antioxidant capacity; G. lucidum.

1. INTRODUCTION

Ganoderma lucidum (Curtis) P. Karst. [common names: Reishei, Lingzhi] is a species of basidiomycetes that belongs to Ganodermataceae of polyporales [1]. *G. lucidum* is an edible mushroom that has been used for centuries in Traditional Chinese Medicine for its health promoting properties [2]. Its extracts are used world-wide as ingredients in health foods, herbal medicines and dietary supplements and have been used as anti-cancer and antioxidant agents and for prevention and treatment of various other diseases [3], such as hypertension, bronchitis, arthritis, neurasthenia, hepatopathy, chronic hepatitis, nephritis, gastric ulcer, tumorigenic diseases, hypercholesterolemia, immunological disorders, scleroderma, cardiovascular disease, AIDS and cancer [4]. Its antitumor and immune enhancing properties, along with no cytotoxicity, raise the possibility that it could be effective in preventing oxidative damage and resulting disease [5].

Oxidation processes are essential to many living organisms for the production of energy to fuel biological processes. The uncontrolled production of reactive oxygen species [ROS] and the unbalanced mechanism of antioxidant protection result in the onset of many diseases and accelerate ageing [6]. Oxidative damage plays a significantly pathological role in human diseases. Cancer, emphysema, cirrhosis, atherosclerosis, and arthritis have all been correlated with oxidative damage [7]. Oxygen-centred free radicals and other reactive oxygen species, that are continuously produced in vivo, result in cell death and tissue damage. The antioxidants in human diets are of great interest as possible protective agents to help human body reduce oxidative [8]. They act to prevent lipid oxidation in food and to decrease the adverse effects of reactive species [both ROS and reactive nitrogen species] on normal physiological functions in humans [9].

The medicinal value of this mushroom lies in some chemical substances that produce a definite physiological effect on human body. The most important of these bioactive constituents of mushroom is phenolic compounds and flavonoids [10]. Among these, flavonoids are the most ubiquitous group of secondary metabolites demonstrating a wide range of biochemical and pharmacological effects, including anti-inflammatory [11], antibacterial [12], antifungal [13], antioxidant [14] and anticarcinogenic [15]. Biological and pharmacological properties of *G. lucidum* are their anti-tumor activities, including the effects on cell cycle arrest, inhibition of cell proliferation and induction of apoptosis in human colon carcinoma and breast cancer cell lines. Several reports exist on the action of *G. lucidum* extract inhibited growth of several cancer cell lines including the human prostate and bladder cancer lines [3].

The potential medicinal value and wide acceptability of *G. lucidum* have attracted intense interest in the search for pharmacological compounds from these edible mushrooms. *G. lucidum* appears to be very safe because oral administration of the extract does not display any toxicity and merits investigation as a potential preventive agent in humans [5].

In this study, the antimicrobial activity of ethanolic and methanolic extracts of *G.lucidum* by using agar disc diffusion method against some pathogens was evaluated and the contents of scavenging effects on radicals, total antioxidants and chelating effects on ferrous ion were determined. Besides, this study is also designed to determine contents of phenolic compounds, total flavanoid, β-carotene and lycopene of ethanolic and methanolic extracts from *G. lucidum* .

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Anhydrous sodium carbonate, Folin–Ciocalteu phenol reagent, iron[II] sulfate heptahydrate [$FeSO_4 \cdot 7H_2O$], methanol, ethanol, acetone, glacial acetic acid and n-hexane were purchased from Merck [Darmstadt, Germany]. EDTA, 2,2-diphenyl- 1-picrylhydrazyl [DPPH], 3-[2-pyridyl]-5,6-bis[4-phenylsulfonic acid]-1,2,4-triazine [ferrozine], iron [II] chloride [$FeCl_2$], gallic acid, aluminium chloride hexahydrate, potassium acetate butylated, rutin, hydroxyanisole [BHA] and 2,6- di-tert-butyl-4-methylphenol [BHT] were purchased from Sigma-Aldrich GmbH [Steinheim, Germany]. All other chemicals were analytical grade and obtained from either Sigma or Merck.

2.2 Macrofungi Materials

G. lucidum A-3708 macrofungi material was collected in October 2011 in Koycegiz from Mugla in Turkey. The identification of macrofungi materials was confirmed by a taxonomist, Dr. Hakan Allı, in the Department of Biology, Mugla Sıtkı Koçman University, Mugla, Turkey.

2.2.1 Preparation of the extracts

Collected macrofungus material was dried in the shade and ground in a grinder with a 2-mm-diameter mesh. Approximately 25 g of dried and powdered macrofungus material was extracted with ethanol [96% extra pure] and methanol by using Soxhlet apparatus at 60ºC for 3 hours. The extract was filtered and concentrated under vacuum at 75ºC by using a rotary evaporator [Laborota 4000, Heidolph]. The extract was stored at 4ºC until used within a maximum period of 1 week.

2.2.2 Antimicrobial activity

2.2.2.1 Test pathogens

Test pathogens. Escherichia coli ATCC 25922, *Salmonella enteritidis* ATCC 13076, *Bacillus subtilis* RSKK 244, *Candida albicans* ATCC 90028 and *Candida glabrata* RSKK 04019 were used as test pathogens were obtained from Erciyes University, Faculty of Pharmacy, Pharmaceutical Biotechnology research laboratory culture collection. Nutrient Broth [NB] and Yeast Extract Peptone Dextrose [YEPD] Broth were used for culturing of test

microorganisms. All strains were stored at −20ºC in the appropriate medium containing 10% glycerol and regenerated twice before use in the manipulations.

2.2.2.1.1 Inhibitory effect by the disc diffusion method

The disc diffusion method was employed for the determination of the antimicrobial activity of the essential oil [16]. Bacterial cultures were grown at 37ºC for 24 h in Nutrient Broth. *C. glabrata* and *C. albicans* was cultured in YPD Broth. The culture suspensions were adjusted by comparing against 5.0 McFarland. One hundred microlitres of suspension of the test microorganisms were spread on solid media plates. Filter paper discs [6 mm in diameter] were impregnated with 20 µl of the extract which is dissolved 1:50 vol/vol in (50%) DMSO and placed on the inoculated plates, which were stored at 4ºC for 2 h and then incubated for 24 h. The diameters [mm] of the inhibition zones were measured after 24 hours of incubation. Antibiotic discs of erythromycin [15 µg/disc] and nystatin [30 µg/disc] were also used as positive controls.

2.2.3 Antioxidant activity

2.2.3.1 DPPH radical scavenging assay

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of a methanol solution of DPPH using the method of Blois [17]. The extract solutions (different concentrations between 0.25-1 mg/mL) were added to a 0.004% methanol solution of DPPH. The mixture was shaken vigorously and left to stand at room temperature for 30 minutes in the dark. Then the absorbance was measured at 517 nm against a blank with a spectrophotometer [model U-1800, Hitachi, Tokyo, Japan]. Inhibition of DPPH free radical as a percentage [I%] was calculated according to the formula:

$$I\% = [[A_{Control} - A_{Sample}] / A_{Control}] \times 100$$

where $A_{Control}$ is the absorbance of the blank and A_{Sample} is the absorbance of the test compound.

Tests were carried out in triplicate. BHA and BHT were used as positive controls.

2.2.3.2 Determination of bioactive component contents

Phenolic compounds of the extracts were analyzed using Folin–Ciocalteu reagent according to the method of Singleton and Rossi [18] using gallic acid as standard, with some modifications [19]. The extract solutions [0.1 mL] were mixed with 0.2mL of 50% Folin–Ciocalteu reagent. The mixture was allowed to react for 3 minutes, and 1mL of aqueous solution of 2% Na_2CO_3 was added. Then, the mixture was vortex-mixed vigorously. At the end of incubation for 45 minutes at room temperature, absorbance of each mixture was measured at 760 nm. The same procedure was also applied to the standard solutions of gallic acid. Phenolic compounds were expressed as mg of gallic acid equivalents/g of the extracts.

β-Carotene and lycopene were determined according to the method of Nagata and Yamashita [20]. The dried extract [100 mg] was vigorously shaken with 10 mL of acetone–hexane mixture [4:6 vol/vol] and filtered through disposable filters [pore size, 0.45 µm;

Millipore]. The absorbance of the filtrate was measured at 453, 505 and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations:

$$\begin{aligned}\text{Lycopene [mg=100 mL]} = &[-0{:}0458 \cdot A663] \\ &+[0{:}372 \cdot A505] \\ &-[0{:}0806 \cdot A453] \\ \text{β- Carotene [mg=100 mL]} = &[0{:}216 \cdot A663] \\ &-[0{:}304 \cdot A505] \\ &+[0{:}452 \cdot A453]\end{aligned}$$

where A453, A505 and A663 represent the absorbance at 453, 505 and 663 nm, respectivley. The results were expressed as mg of carotenoid/g of extract.

2.2.3.3 *Determination of total flavonoid content*

The total flavonoid content was determined according to the aluminium chloride colorimetric method [21]. Each mushroom extracts [2 mL, 0.3 mg/mL] in methanol were mixed with 0.1 mL of 10% aluminium chloride hexahydrate, 0.1 mL of 1 M potassium acetate and 2.8 mL of deionized water. After the 40 minutes incubation at the room temperature, the absorbance of the reaction mixture was determined spectrophotometrically at 415 nm. Rutin was chosen as a standard [the concentration range: 0.005 to 0.1 mg/mL] and the total flavonoid content was expressed as milligram RE per g of dry extracts. The absorbance at 415 nm = 14.171 c_{rutin} [mg/mL] + 0.0461, R^2 = 0.9991.

2.2.3.4 Metal chelating activity on ferrous ions [Fe^{2+}]

Metal chelating activity was determined according to the method of Decker and Welch [22] with some modifications [19]. In brief, 0.5mL of the mushroom extracts was mixed with 0.05mL of 2mM $FeCl_2$ and 0.1mL of 5mM ferrozine. The mixture was diluted to the desired total volume with the solvent. Then, the mixture was shaken vigorously and left standing at room temperature for 10 minutes. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm.

The percentage of inhibition of ferrozine– Fe^{2+} complex formation was calculated using the formula given below:

$$\text{Scavenging effect [\%]}=[[A_{Control} - A_{Sample}] / A_{Control}] \times 100$$

where $A_{Control}$ is the absorbance of the ferrozine–Fe^{2+} complex and A_{Sample} is the absorbance of the test compound.

EDTA was used for comparison

2.2.3.5 Total antioxidant activity-ferric thiocyanate method

The antioxidant activity of MEGL and EEGL was determined according to the ferric thiocyanate method in linoleic acid emulasion [23]. For stock solutions, 10 mg of MEGL and EEGL was dissolved in 10 mL deionized water. Then, the solution, which contains different concentration of stock MEGL and EEGL solution samples [250-1000 µg/mL] in 2.5 mL of potassium phosphate buffer [0.04 M, pH 7.0], was added to 2.5 mL of linoleic acid emulsion in potassium phosphate buffer [0.04 M, pH 7.0]. The mixed solution [5 mL] was incubated at

37ºC in glass flask. At regular intervals during incubation, 0.1 mL aliquot of the mixture was diluted with 3.7 mL of ethanol, followed by the addition of 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in 3.5% hydrochloric acid. The peroxide level was determined by reading the absorbance at 500 nm in a spectrophotometer [CHEBIOS s.r.l. UV-VIS Spectrophotometer]. During the linoleic acid oxidation, peroxides are formed, which oxidize Fe^{+2} to Fe^{+3}. The latter ions form a complex with thiocyanate and this complex has a maximum absorbance at 500 nm. These steps were repeated every 12 h until the control reached its maximum absorbance value. Therefore, high absorbance indicates high linoleic acid emulsion oxidation. Solutions without added samples were used as blanks. All data on total antioxidant activity are the average of duplicate experiments. The percentage inhibition of lipid peroxidation in linoleic acid emulsion was calculated by following equation:

$$\text{Inhibition of lipid peroxidation [\%]} = 100 - [[A_{Sample}/A_{Control}] \times 100$$

Where $A_{Control}$ is the absorbance of the control reaction and A_{Sample} is the absorbance in the presence of the sample of MEGL, EEGL or standard compounds.

2.2.4 Statistical analysis

All experiments were done in triplicate, and mean values are presented. The results were expressed as mean±SD values. Statistical analyses were performed using SPSS version 11.0 [SPSS, Chicago, IL, USA]. Pearson's correlation analysis was used to determine the statistical significance of differences between the values. The analysis was used for comparisons of phenolic compounds and the antioxidant activity of the extracts. The level of statistical significance was taken at $P< 0.05$

3. RESULTS AND DİSCUSSİON

3.1 Antimicrobial Activity

The antimicrobial effects of ethanolic and methanolic extracts of *G.lucidum* were tested against one species of Gram positive bacteria, two species of Gram-negative bacteria and two yeast. As summarized in Table 1, the inhibition zones of *G.lucidum* which were obtained against all test pathogens were in the range of 10–25 mm. The highest inhibitory activity was determined against *C.glabrata* RSKK 04019 [25±1 mm, inhibition zone diameter]. On the other hand, the lowest inhibitory activity was determined against *C.albicans* ATCC 90028 and *B. subtilis* RSKK 244 [10±1 and 10±0 mm, inhibition zone diameter]. Our results indicate that G.lucidum contains antimicrobial constituents inhibiting Gram-positive bacteria, Gram negative bacteria and yeast. Ofodile et al. [24] reported that the crude n-hexane:diethyl ether, chloroform:acetone and methanol extracts of four species of Ganoderma *(Ganoderma colossum (Fr.) C. F. Baker, G. resinaceum Boud., G. lucidum (*cf.) (Curtis) P. Karst. and *G. boninense (cf.) Pat.),* from Nigeria, were tested for antimicrobial activity. The three solvent extracts of all the species of Ganoderma were active against *Pseudomonas syringae* and *Bacillus subtilis,* whereas none of the extracts were active against *Cladosporium herbarum.* In addition, *Ganoderma* spp. had antifungal activity against the dermatophyte *Trichophyton mentagrophytes* and the yeast *C. albicans* [25]. Sridhar et al. [26] reported that the methanolic extract of *G.lucidum* showed the antifungal activity 30 mm inhibition zone was recorded from 200 mg of extract against *Mucor indicus* and 3mm by 50 mg of extract against *Aspergillus flavus*. Recently, more studies demonstrated that *G.*

lucidum and other *Ganoderma* species contained antimicrobial constituents that are able to inhibit gram-positive and/or gram-negative bacteria [27,28]. The aqueous extract from the carpophores of *G. lucidum* inhibited 15 types of gram-positive and gram-negative bacteria at different extent using the serial broth dilution method, with the most potent inhibition against *Micrococcus luteus* [minimum inhibitory concentration (MIC) 0.75 mg/mL)] [28]. Also, it was suggested by that Ganoderma species especially *G. lucidum* could be used as feed supplement to resist microbial infections and boost immune system in human beings [29]. Jonathan and Awotona reported that *in vitro* antagonistic effect of the ethanol, methanol and distilled water extracts of G*. lucidum, G. applanatum* and *G. australe* were tested against some disease causing microorganisms. Both crude and pure extracts of these *Ganoderma* species exhibited various degree of inhibition against the test organisms. The widest inhibitory zone (20.3mm) were obtained with the crude methanolic extract of *G. lucidum* against *Proteus mirabilis* while the highest in-vitro antifungal activity (24.3mm) was observed in the crude ethanolic extracts of *G. lucidum* against *Aspergillus niger*. The lowest zone of inhibition (2.3mm) was demonstrated with the aqueous extract of *G. australe* against *Escherichia coli* and 2.7mm with purified extract of *G. australe* against *Penicillum oxalium*. The minimum inhibitory concentration (MIC) for the ethanol extract ranged between 1.7 and 5.0mg/mL for bacteria and between 2.0 and 6.0mg/mL for fungi [30].

Table 1. Antimicrobial activity of G.lucidum extracts

Test pathogens	Inhibition zone		Diameter [mm]	
	Ethanol	Methanol	Erithromycin	Nystatin
B. subtilis RSKK 244	13±1	10±0	ND	20±0
E. coli ATCC 25922	16±0	NI	ND	9±0
S. enteritidis ATCC 13076	12±0	12±0	ND	9±0
C. glabrata RSKK 04019	NI	25± 1	33±0	ND
C. albicans ATCC 90028	10±1	NI	22±0	ND

NI: No inhibition; ND: Not determined

3.2 The Yield of the Extracts

The yield of *G. lucidum* two different extracts and the concentration of phenolic compounds are shown in Table 2, the yields obtained from various extracts of *G. lucidum* varied considerably. Differences in the yield of extracts might be attributed to the availability of different extractable components.

Table 2. The yields and the antioxidant compounds content of the mushrooms extracts[a]

Extracts	Yields (% w/w)	Phenolic compounds (mg of gallic acid equivalents/g extract)	Total flavanoid contents [mgRE/g]	β -Carotene content (mg/g)	Lycopene content (mg/g)
EEGL	11.7	49.52±0.68	10.66±0.69	0.425±0.004	0.075±0.003
MEGL	3.2	46.21±1.83	10.21±1.19	0.224±0.004	0.038±0.002

EEGL: Ethanol extract of G.lucidum, MEGL: Methanol extract of G. lucidum.

[a] Values represent averages ± standard deviations for triplicate experiments. Values in the same column with different superscript uppercase letters are significantly ($p < 0.05$) different.

3.3 Antioxidant Activity of the extracts

3.3.1 DPPH radical scavenging assay

Antioxidants react with DPPH, which is a stable free radical and convert it to 1,1-diphenyl-2-picryl hydrazine. The degree of discolouration indicates the radical-scavenging potential of the antioxidant [31]. This method is based on the reaction of DPPH that is characterized as a preformed stable free radical with a deep violet colour and any substance that can donate a hydrogen atom to DPPH reduces it to a stable diamagnetic molecule. The effects of phenolic compounds on DPPH scavenging are thought to be due to their hydrogen donating ability. It was reported that the decrease in the absorbance of the DPPH caused by phenolic compounds are due to scavenging of the radical by hydrogen donation, which is visualized as a discoloration from purple to yellow. The reduction of the DPPH was followed *via* the decrease in absorbance at 517 nm [32]. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. This test is a standard assay in antioxidant activity studies and offers a rapid technique for screening the RSA of specific compounds or extracts [33]. In this study, DPPH percentage scavenging activities of the mushroom extracts and the synthetic antioxidant were measured at different concentrations ranging between 0.25 and 1.0 mg/mL [Fig. 1]. The 50% inhibitory concentration [IC_{50}] values for DPPH scavenging activities of the mushroom extracts, BHA compared and shown in Table 3, as calculated from the percentage inhibition versus log concentration of the extract curves. The IC_{50}, meaning the concentration of antioxidant needed to decrease [by 50%] the initial substrate concentration, is a parameter widely used to measure the antiradical efficiency. The lower IC_{50} values show the higher antioxidant activity [19]. With regard to IC_{50} values of scavenging abilities on DPPH radicals, the effectiveness was in a descending order: $BHA_{methanol}$ >$BHA_{ethanol}$ > the methanol extract> the ethanol extract. DPPH radical scavenging effect was detected in the methanol extract [IC_{50} = 3.82±0.04 µg/mL] was higher than the ethanol extracts [IC_{50} = 7.03±0.07 µg/mL]. Compared to reference antioxidant, the methanol and ethanol extracts of *G.lucidum* provided a lower IC_{50} than BHA [IC_{50} = 0.30±0.01 µg/mL] .These results showed the methanol extract has more effective scavenging ability on DPPH radicals than the ethanol extracts. But, neither methanol extract nor ethanol extract has more activity than synthetic antioxidant BHA. Lin et al. found that methanolic extracts from other medicinal mushrooms were extremely effective in inhibiting the lipid peroxidation [6.41% for *Ganoderma lucidum* [Ling-chih], 2.62% for *Ganoderma lucidum* and 2.30% for *Ganoderma tsugae* [Sung-shan-ling-chih] at 0.6 mg/mL] [34]. In addition, The total triterpenes that were obtained *G. lucidum* showed significant DPPH scavenging activity. 100 µg/mL of the total triterpenes showed a maximum of 81.81% activity. The IC_{50} value of triterpenes was 41.45±5.2 µg/mL. The total triterpenes efficiently scavenged $ABTS^{+}$ radicals generated by the reaction of 2,2'-azinobis (3- ethylbenzothiazolin-6-sulphonic acid) (ABTS) andammonium per sulphate (IC_{50} 30±5.4µg/mL) [35].

These results revealed that *Ganoderma* species are free radical inhibitors or scavengers, acting possibly as primary antioxidants. Their methanolic extracts might react with free radicals, particularly of the peroxy radicals, which are the major propagator of the autoxidation chain of fat, thereby terminating the chain reaction [8].

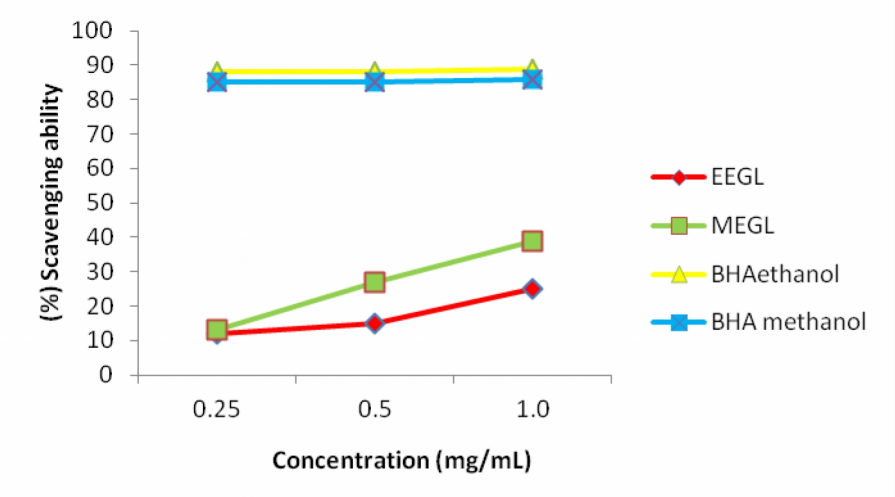

Fig. 1. DPPH radical scavenging activity of the mushroom extracts.
EEGL: Ethanol extract of G.lucidum; MEGL: Methanol extract of G.lucidum; BHA [Butylated hydroxyanisole] : Synthetic antioxidant

3.3.2 Determination of bioactive component contents

The amounts of phenolic compounds, total flavanoid, β-carotene, and lycopene in the mushroom extracts were determined. Phenolic compounds in the extracts were determined spectrofotometrically according to the Folin–Ciocalteu procedure and calculated as gallic acid equivalents. The amounts of β-carotene and lycopene found in the two mushroom extracts were very low, which emphasises the idea that phenolic compounds could make a significant contribution to the mushrooms antioxidant activity. The phenolic compounds of the ethanol and methanol extracts of *G. lucidum* were found in highly amounts, whereas β-carotene and lycopene were only found in trace amounts in the mushroom extracts [Table 2]. The phenolic compounds and flavonoid contents values do not significantly differ for ethanolic and methanolic extracts.

As compared to the contents of phenolic compounds in methanolic extracts from specialty mushrooms [7.61-16.28 mg/g], commercial mushrooms [6.27-15.65 mg/g] and ear mushrooms [3.20-8.72 mg/g], the highest contents of phenolic compounds in *Ganoderma* [46.21 mg/g] might be the key components accounting for their better results found in antioxidant activity, and scavenging and chelating abilities [8]. Yegenoglu et al. reported that the total phenolic contents of the ethanol and water extracts of *G. lucidum* were significantly higher than those of *F. trogii*. Ascorbic acid was found in moderate amounts, whereas β-carotene and lycopene were only found in trace amounts in the mushroom extracts Ascorbic acid was found in small amounts in the mushroom extracts. β-Carotene and lycopene were not found in the mushrooms water extracts because of their fat-soluble nature. The highest ascorbic acid (11.75±0.05mg=g), b-carotene (0.465±0.004mg=g), and lycopene (0.067±0.001mg=g) contents were found in ethanolic extract of *G. lucidum* [36].

In our study, a higher amount of was determined in the methanolic extract of *G.lucidum*. According to our results, *G.lucidum* could find use an antioxidant source.

3.3.3 Metal chelating activity on ferrous ions [Fe^{2+}]

Transition metals are recognized to be involved in both initiation and propagation of lipid peroxidation. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen peroxide and lipid peroxides to reactive free radicals via the Fenton reaction [$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$] [37]. Fe^{2+} ions are the most powerful pro-oxidants among the various species of metal ions [23]. The chelating activity of the extract was determined by the ferrozine assay. Ferrozine can quantitatively form complexes with Fe^{2+}. Measurement of the rate of color reduction therefore enables estimation of the chelating activity of the coexisting chelator [38]. The complex formation is disrupted with the result that the purple colour of the complex is decreased. Effects of the extracts on inhibition of ferrylbipyridyl formation are presented in Table 3. showed that the methanol extract [IC_{50}=14.11±0.01mg/mL] ferrous iron chelating ability was higher than the ethanol extract [IC_{50}=50.33±0.07 mg/mL]. Chelating effects of methanolic extracts from *G. lucidum* on ferrous ion increased with increased concentrations. At 0.25 mg/mL, 0.5 mg /mL and 1.0 mg/mL chelating effects were respectively 11%, 13%, 17%. It was found that methanolic extract of *G. tsugae* showed a strong chelating activity on Fe^{2+} ion. This indicates that the chelating activity of methanolic extract of *G.tsugae* on a metal ion may play an important role in its antioxidant activity [39]. Mau and Lin [8] found that the methanolic extracts of *G.lucidum* chelated 55.5-67.7% ferrous ions at 2.4 mg /mL. However, Mau and Lin used the methods of Shimada et al. [34] to determine the chelating effect instead of the method Decker and Welch [22]. Yegenoglu *et al.*indicated that significant differences in chelating activity were observed among the extracts. The ethanol extracts showed higher chelating activity than the water extracts. The ethanolic extract of *G. lucidum* (IC_{50}= 2.69±0.67 mg=mL) interfered with formation of the ferrous–ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. ethanolic extract of *G. lucidum* can be observed as a potent iron chelating source for further investigation [36]. In addition, it was reported that the two purified polysaccharides GLP_{L1} and GLP_{L2} (*Ganoderma lucidum* polysaccharide) were compared to EDTA for their Fe^{2+}- chelating capacity. GLP_{L1} showed moderate Fe^{2+}- chelating activity at concentrations above 3.0 mg/mL but at a level much lower than that of EDTA, while GLP_{L2} only showed weak chelating activity less than 22% even at a high concentration [40].

Table 3. Antioxidant activities of the mushroom extracts[a]

Material	DPPH IC_{50} (mg/mL)	Metal chelating IC_{50} (mg/mL)	Total antioxidant (% inhibition) (0.25 mg/mL)[c]
EEGL	7.03±0.07	50.33±0.07	8.37
MEGL	3.82±0.04	14.11±0.01	41.88
** $BHA_{ethanol}$	0.32±0.04	NS[b]	NS[b]
**$BHA_{methanol}$	0.30±0.01	NS[b]	NS[b]
***$BHT_{ethanol}$	NS[b]	NS[b]	10.59
***$BHT_{methanol}$	NS[b]	NS[b]	34.9

EEGL: Ethanol extract of G. lucidum, MEGL: Methanol extract of G. lucidum; BHA [Butylated hydroxyanisole]: Synthetic antioxidant; BHT [Butylated hydroxytoluene]: Synthetic antioxidant; [a] Values represent averages ± standard deviations for triplicate experiments. Values in the same column with different superscript upper case letters are significantly (p < 0.05) different;
[b] Not studied; [c.]The highest total antioxidant value in all concentration.

3.3.4 Total antioxidant activity determination in linoleic acid emulsion by ferric thiocyanate method

The ferric thiocyanate method measures the amount of peroxide produced during the initial stages of oxidation which are the primary products of oxidation. Total antioxidant activity of methanolic extract, ethanolic extract and standard compounds [BHT] was determined by the ferric thiocyanate method in the linoleic acid system. Methanol and ethanol extracts had effective antioxidant activity. The effects of various concentrations of ethanolic and methanolic extracts [from 250, 500 and 1000 µg/mL] on lipid peroxidation of linoleic acid emulsion are shown in Fig. 2 and Table 3. were found to be for ethanolic extracts 8.37,6.15 and 3.69% respectively and for methanolic exracts 41.88, 37.35 and 34.55% respectively. On the other hand, BHT exhibited 34.9, 36.99 and 40.13% inhibition on peroxidation of linoleic acid emulsion, respectively at the 250,500 and 1000 µg/mL for methanolic extracts. The results clearly showed that methanolic extracts had stronger total antioxidant activity than ethanolic extracts at the same concentration. Huang found that methanolic extracts from the medicinal mushroom, *Agaricus blazei* [Brazilian mushrooms], showed a high antioxidant activity [26.0% of lipid peroxidation] at 1.0 mg/mL [9]. It was reported that methanolic extracts from ear mushrooms, including black, red, jin, snow and silver ears, showed low to moderate antioxidant activities [57.7-71.5% of lipid peroxidation] at 1.0 mg/mL [34]. Lin found that methanolic extracts from other medicinal mushrooms were extremely effective in inhibiting the lipid peroxidation [6.41% for *Ganoderma lucidum*, 2.62% for *Ganoderma lucidum* and 2.30% for *Ganoderma tsugae* at 0.6 mg mL^{-1}] [34,41]. Mau *et al*. found that using the conjugated diene method, the methanolic extracts from mature and baby Ling chih forms of s *Ganoderma tsugae* showed moderate antioxidant activities (49.3% and 46.4%) at 0.5 mg mL^{-1} and high antioxidant activities (96.8% and 93.6%, respectively) at 20 mg mL^{-1}. On the other hand, the methanolic extract from mycelia showed a low antioxidant activity of 10.4–19.3% at 0.5–20 mg ml^{-1}, whereas no antioxidant activity was found in the methanolic extract from filtrate. However, the antioxidant activities were 99.9% at 0.1 mg ml^{-1} for BHA, 95.1% at 1 mg ml^{-1} for α-tocopherol and 59.3% at 20 mg ml^{-1} for ascorbic acid [42].

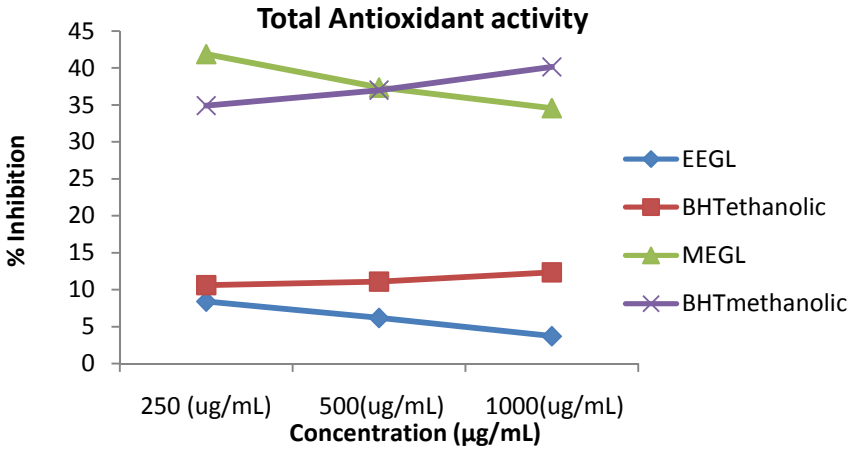

Fig. 2. Antioxidant activity of different concentrations of ethanolic and methanolic extracts of *G. lucidum.*

EEGL: Ethanol extract of G. lucidum, MEGL: Methanol extract of G.lucidum, BHT [Butylated hydroxytoluene] : Synthetic antioxidant

4. CONCLUSIONS

It is clearly indicated that the methanolic extracts were more effective than the ethanolic extracts in antioxidant activity using the scavenging ability on DPPH radicals and metal chelating activity whereas the ethanolic extracts were more quantity β- carotene, lycopene, phenolic compounds and flavanoid contents. It can be concluded from the present investigation that the ethanolic and the methanolic extracts of the edible mushroom *G. lucidum* show biopharmaceutical potentiality. However, whether such extracts will act as effective therapeutic agents remains to be investigated and the identification of the bioactive compounds and the study of mechanisms of actions are necessary prior to application.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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