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Free Radical Scavenging, Enzyme Inhibitory and Anti-*Staphylococcus aureus* Properties of the Fruiting Bodies of *Ganoderma lucidum*, Lingzhi Mushroom

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Abstract

The current study evaluated the antioxidant, *α*-amylase inhibitory and anti-*Staphylococcus aureus* activities of the fruiting bodies of *Ganoderma lucidum*. Methanolic extract of *G. lucidum* (MEGL) was investigated for its *in vitro* antioxidant activity using 1,1-diphenyl 2-picrylhydrazyl (DPPH) assay and EC_{50} value was determined. *α*-Amylase inhibitory and anti-*S. aureus* activities were carried out by conventional methods. The mushroom extract showed maximum free radical scavenging activity at a dose of 500 µg/mL with EC_{50} value of 22.1399 µg/mL. EC_{50} value for the inhibition of *α*-amylase activity was found 22.6011 µg/mL. *G. lucidum* extracts exhibited a concentration-dependent Anti-*S. aureus* activity. The vast differences of this inhibition between lower and higher doses were assessed significantly ($p < 0.05$). This study shows that the fruiting body of *G. lucidum* has moderate antioxidant, *α*-amylase inhibitory, and anti-*S. aureus* activities. Thus this mushroom may be the alternative plant source for the antioxidant, anti-diabetic and antibacterial agents and further *in vivo* studies and isolation of bioactive compounds are required.

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Keywords

Ganoderma lucidum, Mushroom, Antioxidant Activity,
Enzyme Inhibitory Activity, Anti-*Staphylococcus aureus* Activities

1. Introduction

Medicinal mushrooms have a very long history of traditional uses and are attracting much attention in the nutraceuticals and pharmaceuticals field. Mushrooms have become impressive as functional foods for their physiologically beneficial bioactive compounds [1]. A number of secondary metabolites were discovered from the fungi kingdom with high therapeutic values like antioxidant, anti-diabetic, antimicrobial, anti-inflammatory, and anticancer properties [2] [3] [4] [5] [6]. Important bioactive components reported from the various edible and medicinal mushrooms are polysaccharides, proteins, fatty acids, sterols, polyphenolic compounds, vitamins, and minerals [7] [8] [9] [10]. Several studies revealed that these active components of mushrooms have a favorable impact on human health through the modulating of the immune system, and by their antioxidant and anti-inflammatory activities [1] [2] [3]. Thus mushrooms can be used as an alternative for prevention as well as treatment of various diseases of mankind.

Ganoderma lucidum, is a wood degrading basidiomycete belonging to the family of Ganodermaceae of Polyporales. It is commonly named "Lingzhi" in Chiana and "Reishi" in Japan [11]. Ganoderma is one of the most common medicinal mushrooms used traditionally for the longevity and promotion of human health [12] [13] [14]. It is considered a popular folk medicine for the treatment of general disorders, arthritis, hepatitis, bronchitis, dermatological disorders and to boost up the immune functions by indigenous peoples worldwide [15]. Several bioactive compounds have been isolated from the *G. lucidum* among them polysaccharides, triterpenes, peptidoglycans, steroids, and nucleotides are the important physiologically active constituents [16]. It has been known that *G. lucidum* has exhibited diverse pharmacological effects which are antioxidative [17], antiobesity [18], anticancer [19], antihepatic damage [20], neuroprotective [21], cardioprotective [22], antidiabetic [23], antiproliferative, and cytotoxicity activities [24].

Recently, multi-drug resistant bacterial strains represent a serious healthcare problem in the treatment of infectious diseases [25]. On the other hand, diabetes is a rapidly growing disease and considered one of the leading causes of morbidity and mortality worldwide [26]. Moreover, free radicals which are highly reactive to DNA, protein, membrane lipids [27], play a role in a number of human diseases including cancers, diabetes, atherosclerosis, and various types of neurodegenerative disorders [28]. So, it is necessary to search the new bioactive agents for the effective treatment of these diseases. In this case, medicinal mushrooms have triggered a significant research interest to develop novel compounds for the treatment of various diseases.

Fruiting body is the main medicinal part of Ganoderma mushrooms but spore

and mycelium also retain their medicinal values. In this study, we evaluated the free radical scavenging, *α*-amylase inhibitory, and anti-*S. aureus* properties of the methanolic extract of the fruiting bodies of *G. lucidum* in *in vitro* model.

2. Materials and Methods

Collection of *Ganoderma lucidum*

The fruiting bodies of *G. lucidum* were collected from Mushroom Strengthening Center, Savar, Dhaka, Bangladesh. The *G. lucidum* was appropriately authenticated by a scientific officer at the National Mushroom Development and Extension Centre, Department of Agriculture Extension, Ministry of Agriculture, Sobhanbag, Savar, Dhaka, Bangladesh (Voucher number: NMDEC/2016/234).

Preparation of extract

The fruiting bodies were air-dried, milled by grinding using mortar and pestle. Subsequently, the coarse powder (300 gm) of *G. lucidum* was exposed to successive solvent extraction by soaking for 48 h using a solvent of 100% methanol. Filtered contents were distilled, evaporated, air-dried, and stored in air-tight plastic containers at 4˚C until use [29].

Assay of free radical scavenging properties

The free radical scavenging activity of MEGL was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, a method reported by Villano [30]. Herein, 200 µL of MEGL extract was incubated with 3.8 mL DPPH solution in the dark at room temperature for 1 h. In this reaction, DPPH was reduced into yellow-colored and the intensity of color was read at 517 nm. Similarly, a control reaction was carried out without the MEGL. The DPPH radical scavenging activity of MEGL was expressed as the percentage of inhibition and value was calculated by the following formula:

$$\text{Inhibition}(\%) = (A_o - A_1)/A_o \times 100 .$$

A_o = Absorbance of control Butylated hydroxytoluene (BHT).

A_1 = Absorbance of the test sample (MEGL).

Assay of enzyme inhibitory activity

In vitro enzyme inhibitory activity of MEGL was examined by a slight modification of the Bernfeld method [31]. Acarbose as the positive control obtained by dissolving 50 mg in 50 ml of phosphate buffer and diluted to get the appropriate concentration of 31.25 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, and 500 µg/mL using a buffer. At the same time, MEGL was dissolved in a buffer to get the aforementioned concentrations.

In this experiment, extract and enzyme (*α*-amylase, Hi media Rm638) were mixed with 1:2 ratio. In brief, 100 µL of MEGL was taken to react with 200 µL of *α*-amylase and 100 µL of 2 mM of phosphate buffer (pH-6.9). After 30 minutes of incubation, 100 µL of 1% starch solution was added and kept this reaction mixture for 10 minutes.

Consequently, after 10 minutes, 500 µL of dinitrosalicylic acid (DNS) reagent was added to test and control and kept in a boiling water bath for 5 minutes. Finally, absorbance was recorded at 540 nm using UV-vis spectrophotometer. The

percentage of inhibition of *α*-amylase enzyme was estimated using the following equation:

$$\text{Inhibition } (\%) = \{(\text{control} - \text{test})/\text{control}\} \times 100 .$$

Here, Control = Absorbance of Acarbose.

Test = Absorbance of MEGL.

Anti-*Staphylococcus aureus* activity

Anti-*Staphylococcus aureus* activity was investigated by the disc diffusion method [32]. *Staphylococcus aureus* (*S. aureus*), gram-positive bacteria obtained from the department of Biochemistry and Molecular Biology, Rajshahi University, Bangladesh. The strain was retained by periodic subculture on nutrient agar and well-kept-up at 4˚C prior to use. In this experiment, Whatman No. 1 paper was used to make discs of 6 mm in diameter. The discs were then sterilized by autoclaving at 121˚C for 20 minutes after which they were kept to stand for cooling. Stock solutions of MEGL were prepared by dissolving solid crude extract in DMSO. From this stock solution, nine (09) different concentrations of MEGL were prepared. These were 5, 10, 15, 20, 25, 30, 60, 120, and 240 mg/mL which finally yielded disc potencies of 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.6, 1.2, and 2.4 mg/disc respectively. Each disc was allowed to absorb the solution and kept for further analysis. Each paper disc was capable of absorbing 0.01 mL. Percentage (%) of zone of inhibition (ZoI) of MEGL against *S. aureus* was calculated using the following formula:

$$\%\ \text{Zone of inhibition} = \{(\text{ZoI}(\text{mm})\text{of control} - \text{ZoI}(\text{mm})\text{of sample})/\text{ZoI}(\text{mm})\text{of control}\} \times 100 .$$

3. Results

Free radical scavenging properties

Several concentrations of samples, ranging from 31.25 - 500 µg/ml of BHT and MEGL were tested for their antioxidant activities. It was observed that the test compound showed different antioxidant activity in different concentrations. From the given Figure 1, it was observed that the highest free radical scavenging activity was found at a higher concentration of BHT and MEGL. At 500 µg/ml, the BHT and MEGL showed approximate 95% and 94% of free radical scavenging activity respectively (shown in Figure 1).

Table 1 exhibits the effective concentrations of BHT and MEGL. It indicates that the EC_{50} values of standard and *G. lucidum* extract, and predicted EC_{80}, EC_{90}, EC_{95}, and EC_{99} values of BHT and mushroom extract, which calculated from each EC_{50} value.

In Table 1, the EC_{50} value of BHT was 16.2637 µg/mL while 22.1399 µg/mL was found for MEGL ($p < 0.05$). The predicted EC_{90} values, which were calculated from EC_{50} value, of BHT and MEGL were 1610.1063 µg/mL and 2191.8501 µg/mL correspondingly.

***α*-Amylase inhibitory activity**

In this study, the *α*-amylase inhibitory activity of the methanolic extract of *G.*

lucidum was investigated. Figure 2 illustrates the concentration-dependent of percent (%) of inhibition of *α*-amylase activity by methanolic extract of *G. lucidum*. The indicative results were found at a high concentration of MEGL. Acarbose (*α*-glucosidase inhibitor) was used as a standard.

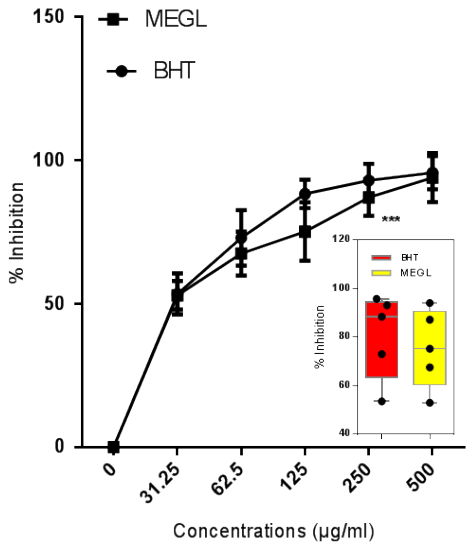

Figure 1. Free radical scavenging activity of methanolic extract of *Ganoderma lucidum* (MEGL) and Butylated hydroxytoluene (BHT). "***" indicates the p-value of <0.001.

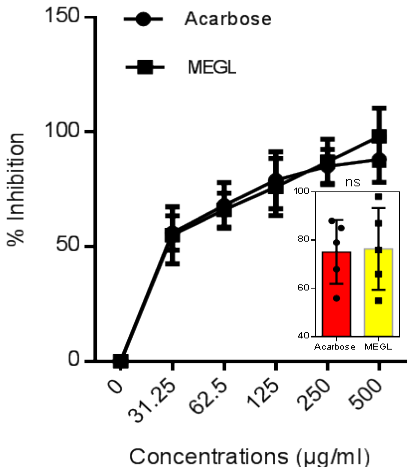

Figure 2. Doses-dependent amylase inhibitory activity of methanolic extract of *Ganoderma lucidum* (MEGL). Error bars were calculated at 95% confidence interval (CI).

Table 1. Effective concentrations of BHT and MEGL in µg/mL.

	EC_{50}	EC_{70}	EC_{80}	EC_{90}	EC_{95}	EC_{99}
BHT	16.2637	37.94863	65.0548	146.3733	309.0103	1610.1063
MEGL	22.1399*	51.65977	88.5596	199.2591	420.6581	2191.8501

Legends: BHT-Butylated hydroxytoluene, MEGL-Methanolic extract of *Ganoderma lucidum*. EC-Effective concentration. *p < 0.05.

At low concentration, 31.25 µg/mL acarbose showed around 9% more *α*-amylase inhibitory activity than that of MEGL, and their activities were steadily raised up and continued to increase about 88% and 81% for acarbose and MEGL respectively at 500 µg/mL. Non-significant column mean difference of percent inhibition was found in this experiment.

EC_{50} values of Acarbose and MEGL are shown in Table 2 and indicate the relative activity of acarbose and MEGL.

Anti-*Staphylococcus aureus* activity

The antibacterial activities of MEGL on *S. aureus* are shown in Figure 3. The range of 5 - 240 mg/ml MEGL was used to examine the inhibitory activity and 30 - 240 mg/ml were found to have the paramount inhibitory activity on *S. aureus*. We observed that the anti-*Staphylococcus aureus* activity of MEGL shows the different zone of inhibition in different concentrations. In case of very low concentration eg. 5 and 10 mg/ml of MEGL, no zone of inhibition was observed. The zone of inhibition gradually increased from 15 to 240 mg/ml.

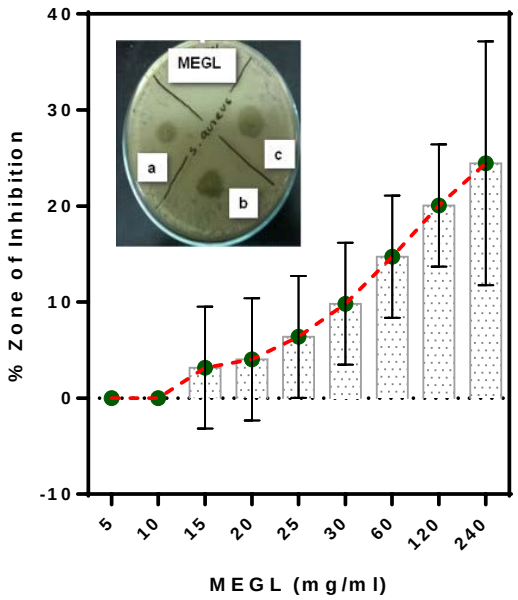

Figure 3. Anti-*Staphylococcus aureus* activity of methanolic extract of *Ganoderma. lucidum*. The activity was observed after 48 hrs of microbe inoculation. Error bars with 95% confidence interval (CI).

Table 2. Effective concentrations of acarbose and MEGL in µg/mL.

	EC_{50}	EC_{70}	EC_{80}	EC_{90}	EC_{95}	EC_{99}
Acarbose	14.4544	52.7259	90.4044	203.4099	429.4209	2237.509
MEGL	22.6011*	33.7269	57.8176	130.0896	274.6336	1430.986

Legends: MEGL-Methanolic extract of *Ganoderma lucidum*. EC-Effective concentration, *p < 0.05. The calculated EC_{50} values of acarbose and MEGL were 14.4544 µg/mL and 22.6011 respectively. On the other hand, EC of 70, 80, 90, 95, and 99 values were computed from respective EC_{50} values.

4. Discussion

In this study, DPPH reduction assay was used to investigate the free radical scavenging potentiality of mushroom extract, against positive control BHT. This DPPH reduction was dose-dependent action, and MEGL exhibited more significant free radical scavenging activity at high concentration (500 μg/mL). According to previous reports, polysaccharides and polysaccharide complexes isolated from various parts of mushrooms may attribute to the antioxidant activities of mushrooms. Antioxidant capacities of fungal polysaccharides may relate to the number of terminal hydroxyl groups and low molecular weight polysaccharides usually have higher antioxidant potentiality [33]. Several studies reported the radical scavenging potentiality of polysaccharides isolated from *G. lucidum* [34] [35] [36] [37]. According to Saltarelli *et al.,* the ethanolic extract of *G. lucidum* exhibited a signal-free radical scavenging activity. These findings show slightly higher DPPH radical scavenging activity which may be attributed to a strong proton donor [38]. Kang *et al.* reported the antioxidant potentiality of polysaccharide (EPS) of *G. lucidum* as H_2O_2 and DPPH scavenging activity. Isolated polysaccharides also showed strong reducing power and cellular protective effect on yeast cells from UV damage [39]. Wong *et al.* also reported the inhibitory effect of hot water extract *G. lucidum* on free radical generation in mice. This radical inhibitory effect is probably associated with the inhibition of lipid peroxidation and free radical scavenging capability of hot water extract of *G. lucidum* [40].

Diabetes mellitus is a chronic metabolic disorder which is associated with various types of health complications [41]. Several mechanisms are involved with the antidiabetic effects of herbal medicine like increases insulin secretion, act on pancreatic beta cells, or modify glucose absorption from the intestine. Mammalian α-amylase enzyme digests the starch into simple glucose in the intestine and facilities their absorption into blood. Thus, inhibition of the α-amylase enzyme delays the glucose absorption and consequently reduces the postprandial blood glucose level [42]. Here, we investigated the α-amylase inhibitory activity of MEGL and result showed moderate inhibition of α-amylase enzyme compared to acarbose as standard. Ganoderic acids of *G. lucidum* may play an important role in the antidiabetic activities of this mushroom [43]. According to Islam S. *et al.* edible mushrooms showed in vitro and in vivo antidiabetic activity [44]. The fruiting body of *G. lucidum* has been known a natural remedy for the treatment of diabetes, cancer, hepatitis, and others [45] [46].

Anti-*S. aureus* activity of *G. lucidum* was investigated in this study with different concentrations (5 - 240 mg/mL). The mean zone of inhibition of methanolic extract was found at higher doses in the application. At 240 mg/mL dose of mushroom, approximate 25% of mean zone of inhibition was observed. This result consistent with previous studies where, aqueous and organic extracts (methanol, hexane, dichloromethane, ethyl acetate) of *G. lucidum* showed antimicrobial activity against a variety of bacterial strains such as *S. aureus, Escherichia*

coli, *Pseudomonas aeruginosa, and Enterobacter aerogenes* [47]. A study carried out by Heleno *et al.* and reported that methanolic extract of *G.lucidum* showed higher antibacterial activity against *S. aureus* and *Bacillus cereus* than streptomycin and ampicillin [48]. In 2015, Islam *et al.* have been demonstrated that *G. lucidum* showed potent antibacterial activity [49]. Kamara *et al.* also reported the antimicrobial activities of aqueous extract of *G. lucidum* against *Pseudomonas aeruginosa, Proteus vulgaris*, *Enterococcus faecalis, Salmonella typhimurium, Klebsiella pneumoniae* , *Streptococcus mutans*, and the *Bacillus subtilis* [50]. Although, the mechanisms of antimicrobial activities of *G. lucidum* are not clear but a mixture of compounds (polysaccharides, phenolic compounds, triterpenoids, glycosides, and tannins) may responsible for the antibacterial activities of this mushrooms [47].

In a summary, *G. lucidum* mushroom showed moderate radical scavenging, hypoglycemic and antibacterial activities. Thus, *G. lucidum* represents a high potential for searching new bioactive compounds for the treatment of various diseases.

5. Conclusion

This study indicates that the methanolic extract of *G. lucidum* has antioxidant, *α*-amylase inhibitory, and anti-*S. aureus* effects in *in vitro* model. Further *in vivo* studies are necessary to assess the above stated biological activities *of G. lucidum* which will be great benefits in treating oxidative stress, diabetes, and bacterial infections as a whole.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

BHT: Butylated hydroxytoluene

CI: Confidence Interval

DPPH: 1, 1-diphenyl-2-picrylhydrazyl

EC: Effective concentration

***G. lucidum*:** *Ganoderma lucidum*

MEGL: Methanolic extract of *Ganoderma lucidum*

***S. aureus*:** *Staphylococcus aureus*

ZoI: Zone of inhibition