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Evaluation of phytochemical, antioxidant, cytotoxicity and *in vitro* antibacterial activity of aqueous extract of *Ganoderma lucidum* cultivated in Bangladeshi habitat

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ABSTRACT

Ayurvedic treatment is one of the most ancient treatment systems against various diseases in Indian subcontinent and the western countries have also begun to take this alternative natural therapy as their major treatment system. The medicine of ayurvedic treatment came from various plants or plant parts. *Ganoderma lucidum* is one of fungus that has been extensively used as various therapeutic agents and contains approximately 400 different bioactive compounds which have been reported to have a number of pharmacological effects. But the biochemical composition of *G. lucidium* can be changed from its native origin. The biochemical composition, antioxidant activity, cytotoxic activity and antibacterial activity of the *G. lucidium* cultivated in Bangladesh were detected in this experiment. The phytochemical screening of aqueous extract of *G. lucidium* was determined using standard methods, the antioxidant activity was evaluated by DPPH free radical scavenging assay, the cytotoxic effect was conducted by brine shrimp lethality assay and the antibacterial activity was done using disc diffusion assay. The primary phytochemical screening of *G. lucidium* revealed that the extracts contain proteins, carbohydrates, glycosides, saponins, terpenoids, phenolic compounds. The result indicated that *G. lucidium* has good antioxidant activity and showed IC_{50} value as 89.05±3.59 µg/mL. The aqueous extract of *G. lucidium* showed medium cytotoxic effect and LC_{50} value was calculated as 142.49±5.31 µg/mL. About 40% and 60% of used bacterial strains were shown intermediate and resistance respectively after overnight incubation where Amoxicillin was used as control antibiotics. Hence, *G. lucidium* contains major therapeutic and antibacterial agents for potential pharmaceutical applications.

Keywords: Antibacterial, Antioxidant, Cytotoxic effect, *Ganoderma lucidum*, Phytochemical, Pharmacological effects.

1. INTRODUCTION

Millions of people in rural areas of the worlds are largely depend on herbal medicines for their health needs. About 80% of world's population and 65% population of developing countries use medicinal plants as their basic health care [1]. From thousands of years, various important medicinal reagents and many fascinating number of newly medicines have been generated from natural resources i.e. various plants that have been used as traditional medicines against many diseases or disorders [2]. The poor people of the developing countries use these traditional medicines not only for their preliminary health care but also use as only curing agents [3].

A well-known fungus, *Ganoderma lucidum* belonging to the family of Ganodermataceae is one of the most potential resources for isolating many important medicinal reagents using in traditional treatment system. It is found in both tropical and temperate regions including Africa, Asia, Europe, North and South America. This fungus is famous as powerful medicinal plants due to its various excellent properties of medicine which are associated with health [4]. *G. lucidum* is most commonly called as "Reishi," "Ling Zhi," "Mannentake" etc and in traditional Japanese medicines it is used to promote health and longevity [5]. It can relieve fatigue, curb high blood pressure, keep cholesterol in cheek, tame inflammation, support the immune system and built stamina [6].

In Chinese Traditional Medicine, the compounds of *G. lucidum* are considered as one of the most important medicinal agents which have been used for over four thousand years to treat various illnesses or disorder like cancer, diabetes, stress and coronary disorders, allergies and microbial infections [7-10]. In our country, it is rare to investigate the biological principles of *G. lucidum*. So, it is important to evaluate the phytochemical composition, anti-oxidative, cytotoxic and antimicrobial activities of G. lucidum that are very close related to health.

Phytochemicals are the chemical constituents of plants that are helping to fight against predators or pathogens [11, 12]. They are responsible for the culinary and fragment uses of aromatic plants and are used as traditional medicines from ancient time in all over the world [13]. Phytochemical screening is one of the most common methods to evaluate the chemical composition of plants that are linked to treatment of various diseases.

In general, antioxidant substances as like as phenolic acids, polyphenols and flavonoids can scavenge free radical particles such as peroxide, hydro peroxide of lipid hydroxyl and thus inhibit the oxidative mechanisms [14]. The antioxidants have availed vast interest in the sector of biochemical and biomedical research for last 40 years. Recently, it is proved that antioxidants are playing most important role in fighting against ageing and many other diseases that are associated with oxidative stress due to free radicals [15, 16]. Free radicals are the principal substances to any biological activities and are generated by the general use of oxygen through different parts of the body. We can affect by these free radicals daily from many different environmental resources such as tobacco, drugs, alcohol, smoke, barbecued food, pesticides, harmful chemicals and pollutants in the air etc. [17]. These free radicals are responsible for occurring mutation, breaking proteins and lipids by binding with biological macromolecules of cells in healthy human during electron pairing [18, 19]. Antioxidants are the most important compounds in living organism that have a great capability to save the body from mutation and damage caused by free radicals inducing oxidation [20, 21]. Antioxidants properties of medicinal plants have a great interest as therapeutic agents in diminishing such mutations and damages induced by free radicals [22].

Brine shrimp lethality assay is one of the simplest and available bioassay to evaluate the cytotoxicity in plants that is regarded as the preliminary screening methods of effect of bioactive compounds [23, 24]. This assay is considered as a rapid and easy way for detecting the bioactivity of plant extracts that have a connection with human health and diseases [25, 26]. In developing countries, the infectious diseases, caused by various pathogenic microbes become a burning issue for public health and environment due to increasing the microbial resistance on various antimicrobial drugs [27, 28]. The antimicrobial compounds isolated from plants have represented a wide range of activity against infectious diseases that are caused by microbial agents such as virus, bacteria, yeast, fungi etc. [29, 30]. The bioactive compounds of plant show multiple biological properties with abundant applications in foods, drinks, cosmetics, perfumes, sanitary and pharmaceutical industries and play an important preventive role against different diseases or disorders [31, 32]. Due to these numerous functions of plant's derivates, the present investigation was set up to evaluate the phytochemicals, antioxidant, cytotoxic and antibacterial properties in *G. lucidum*.

2. MATERIALS AND METHODS

2.1 Collection of sample

G. lucidium for current study was collected from a mushroom farm situated at Rangpur district Bangladesh and identified by the taxonomist of Botany department, University of Rajshahi - 6205, Bangladesh.

2.2 Extract Preparation

The collected samples were washed properly with distilled water to remove dirty materials and were allowed to dry for seven to ten days. The dried materials were ground into fine powder using a grinding machine (Jaipan, India). These powder samples were kept in air tight poly bag for further use. About 200 gm of powdered materials were taken in 1L conical flasks and allowed for soaking the materials with 500 mL of de-ionized water. The conical flasks with sample materials were sealed and kept on orbital shaker (Digital rotator, Taiwan) for continuous shaking at 180 rpm for 24 hours. Sonication was also done with help of an ultrasound sonicator machine (Soniprep 150, U.K) for breaking the cell walls completely. The conical flasks were again kept on orbital shaker for 6 hours and the mixtures were filtered through vacuum pump filtration system using Whatman No.1 filter paper. The filtrated samples were kept into fridge dryer for evaporating the solvent and after 16-24 hours, the solvents were completely evaporated and the extracts became ready for experiment.

2.3 Chemicals and Reagents

1,1-diphenyl-2-picrylhydrazyl, (DPPH, Sigma chemical company, USA), Ethanol and Methanol (Sigma chemical company, USA), Ascorbic Acid (Merck, Germany), De-ionized water, Sodium Chloride (NaCl), Benedicts reagents, Biuret reagents, Wagner's reagents, Sulfuric Acid (H_2SO_4), Copper Sulphate ($CuSO_4$) solution, Sodium Hydroxide (NaOH) solution, Acetic anhydride solution, Hydrochloric Acid (HCl), 5% Ferric Chloride ($FeCl_3$) solution, Standard Antibiotic (Amoxicillin), Nutrient medium **(**Luria broth, LB and Luria agar, LA Medium).

2.4 Ethical clearance

This research work was certified by the Institutional Animal, Medical Ethics, Bio-safety and Bio-security Committee (IAMEBBC) for Experimentations on Animal, Human, Microbes and Living Natural Sources, memo no: 57/320/IAMEBBC/IBSc, Institute of Biological Sciences, University of Rajshahi-6205, Bangladesh.

2.5. Phytochemical Screening

The bioactive constituents of medicinal plants are widely used for remedying various human diseases and have a prominent role in healing illness. The phytochemical screening of various plants is a great important work in biochemical and pharmaceutical industries for the formulation of the new medicine against various dangerous diseases [33, 34]. In these experiment, the aqueous extracts of *G. lucidum* was determined for the qualitative estimation of main active constituents like proteins, fats, carbohydrates, alkaloids, flavonoids, tannins, saponins, glycosides etc. The phytochemical analysis of the experimental extract was done followed by standard methods with slide modification described by various scientists.

2.5.1. Test for Proteins

2 mL of selected plant extract was heated with 1 drop of $CuSO_4$ (2%) solution. 1 mL of ethanol (95%) were added into this solution followed by excess of KOH Pellets. A pink color was appeared near the ethanolic layers and that was the indication of presence of proteins [3].

2.5.2 Test for Carbohydrates

2 mL of Benedict's reagent was added into 1mL of aqueous extract solution and mixed well in a test tube. The test tube was heated on a boiling water bath for 2-3 minutes. A significant color formation was the indication of carbohydrates presence [7].

2.5.3 Test for Fats

100 mg of experimental extract was kept on a filter paper and covered with another filter paper. This extract was pressed between filter paper and heated at 60°-70° C. Translucency of the filter paper that persists due to warming indicated the presence of fats [35].

2.5.4 Test for Saponins

About 3 mL of water solution of selected plant extract was taken in a test tube and shaken vigorously. A frothing condition was formed and the test tube was warmed for 2-3 minutes. The frothing situation remained after warming was the preliminary conformation for saponins presence [36].

2.5.5 Test for Phytosterol

200 mg of plant extract was taken into 2 mL of acetic anhydride solution in a test tube and mixed well. A few drops of concentrated H_2SO_4 were added with care at the side of the test tube. A characteristic color change formation was the indication of phytosterols presence [37].

2.5.6 Test for Tannins

100 mg of plant extract were taken into 3 mL of de-ionized water and mixed very well in a test tube. A few drops of 5% $FeCl_3$ solution was added into the test tube. The blackish blue color formation was ensuring presence of tannin [38].

2.5.7 Test for Flavonoids

A few amount of plant extract was dissolved into 2 mL of water in a test tube. Slowly added a few drops of concentrated H_2SO_4 at the side of the test tube with care. A yellowish color formation was formed which was the primary indication of flavonoid presence [39].

2.5.8 Test for Terpenoids

A little amount of aqueous solution of plant extract was taken in a test tube were added. Few drops of concentrated H_2SO_4 into test tube with care, shaken vigorously and kept to stand for 4-5 minutes. A yellow color formation at lower layer ensured the presence of terpenoids [40].

2.5.9 Test for Phlobatannins

5 mL of water solution of plant sample was taken in a test tube, added few drops of diluted HCl were added and the test tube was allowed to stand for few minutes. The reddish color precipitation was the confirm indicator for phlobatannin presence [41].

2.5.10 Test for Glycosides

200 mg of plant extract was dissolved in 3 mL of de-ionized water into a test tube, few drops of aqueous NaOH solution were added. A yellow color formation indicated the presence of glycosides [42].

2.5.11 Test for Phenolic compounds

About 5 mL of aqueous solution of experimental extract was taken in a test tube, added a small amount of 5% $FeCl_3$ solution and the kept test tube for 2 minutes. A blackish green color formation was the indication of phenolic compound presence [43].

2.5.12 Test for Alkaloids

A small amount of plant extract was taken in a test tubes, added few drops of diluted H_2SO_4 at the side of test tube and the solution was treated with few amount of Wagner's reagent. A brownish-black color was observed which confirm indicator of alkaloid presence [44].

2.6 Antioxidant activity Assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a free radical that produces a violet color solution in alcohol and this color is reduced due to the persistence of antioxidant molecule [45]. DPPH was used to evaluate the free radical scavenging activity of plant extract [46, 47]. The DPPH free radical scavenging activity of extracts was evaluated by using the method which was described by Brand Williams et al. [48] with a small modification. In this experiment, 1.0 mL of methanolic solution of *G. lucidium* and Ascorbic Acid solution (standard) at different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/mL) were taken in different test tubes. Afterward 1.5 mL of methanolic solution of DPPH (1 mg/25 mL) was added into each of these test tubes and allowed them to incubation for 30 minutes in dark place at room temperature to complete the reaction. The absorbance of reaction mixture was measured at 517 nm using a spectrophotometer (GENESYS 10S UV-Vis, USA) against blank. Ascorbic acid was used as standard to see the level of antioxidant activity of plant extract. The inhibition percentage (%) of plant extract was calculated using following equation,

$$I\ \% = \{(A_o - A_1) \div A_o\} \times 100\%$$

(Where, A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard). The 50% inhibition concentration of the extract, IC_{50} was calculated using regression line developed from plotting a graph of scavenging percentage against the different concentrations of the extract [49].

2.7 Brine shrimp lethality test

Brine shrimp lethality assay is one of the most important bioassay which is capable of determining a wide range of bioactivity present in plant extracts [50]. It has been used with great success as a primary study of cytotoxicity as well as an important tool for the isolation of biologically active compounds from

plant extracts [51, 52]. This assay was carried out to detect the cytotoxicity of plant extracts using brine shrimps (*Artemia salina*). These shrimps were hatched in a 1L beaker filled with NaCl solution at the concentration of 38 gm/L. In different 10 test tubes, 20 nauplii were taken for each by the help of a glass capillary. Plant extract was added to these 10 test tubes at 10 different concentrations (20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 µg/mL) and incubated these test tubes at room temperature for 24 hours with suitable aeration system. After passing 24 hours, the shrimps were counted as live or dead and LC_{50} (50% lethal concentration) value was evaluated by using regression line developed from a graph plotting mortality percentage against different concentration.

2.8 Antibacterial activity test

The antibacterial activity of aqueous extracts of *G. lucidium* species was determined using the disc diffusion method and this method was conducted to detect the bacterial susceptibility to aqueous extracts [53, 54]. There were 10 bacterial strains were used for this experiment such as *Bacillus subtilis, E. coli, Acinetobacter sp., Staphylococcus aureus, Pseudomonas sp., Acetobacter cloacae, Bravibacillus bravis, Salmonella typhi, RVM (Rhizobium for vigna mungo)* and *RCA (Rhizobium for cicer arietinum),* and all the bacterial cultures were provided by Microbiology Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi-6205, Bangladesh. The stock solution, Luria broth (LB), Luria agar (LA) Medium and culture plates were prepared as per standard protocol. These bacterial strains (100 µL) were inoculated on the surface of solid agar medium in different 10 petridishes. The aqueous extracts from *G. lucidium* at three different concentrations (50, 100 and 200 µL/disc) were impregnated from 1mg/mL stock solution on paper disc. The agar plates with the bacterial cultures were incubated at 37°C for 24 hours. The inhibition activity of bacterial strains was calculated by measuring the diameter (mm) of clear zone around each disc. The disc of Amoxicillin (10µL/disc) was used as control.

2.9 Statistical analysis

All the statistical analyses were accomplished in triplicates and all data are expressed as mean ± SD. The tests of significance were performed by free software named SPSS-16 using one way ANOVA followed by Dunnett Post hoc test compare with control. The significant test were set up at 5% level, 1% level and 0.1% level where P* = 0.05, P** = 0.01 and P*** = 0.001 respectively. The statistical and graphical presentations of data were completed with free software named Microsoft Excel 2007.

RESULTS

3.1. Phytochemical Screening

About 80% of world's population including in both developed and developing countries use the active chemical compounds of various plants as their traditional therapies and it is reported that these medicinal compounds reduce the more side effects than synthetic drugs [7, 55]. In this current research work, the aqueous extract of *G. lucidium* was subjected to qualitative analysis to determine the active compounds like proteins, fats, carbohydrates, saponins, phytosterol, tannins, flavonoids, terpenoids, phlobatannins, glycosides, phenolic compounds, and alkaloids. Phytochemical analysis of aqueous extract of *G. lucidium* indicated that carbohydrates, saponins, glycosides were present at high level, proteins, tannins, terpenoids, phlobatannins, phenolic compounds were present at moderate level and fats, alkaloids were present at low level but phytosterol and flavonoids were not detected which is shown in table 1. The sign (+ + +), (+ +), (+) are expressed for high, moderate and low level of phytochemical presence respectively but the sign (-) is expressed for absence or not detected.

3.2. Antioxidant activity assay

The widely used DPPH free radical scavenging assay for determining the antioxidant activity is worked based on the reduction reaction of DPPH molecule [56, 57]. Higher concentration of sample react more free radicals that were generated by DPPH and decrease the absorbance as well as color and increase the IC_{50} value and the lower concentration of sample work in reverse way [58]. The changes of absorbance of reaction mixture were measured at 517 nm using a spectrophotometer (GENESYS 10S UV-Vis, USA) against blank (Only methanol). The aqueous extract of *G. lucidium* showed significant antioxidant activity and the IC_{50} value was measured as 89.05 ± 3.59 µg/mL where the standard ascorbic acid showed the IC_{50} value as 33.14 ± 2.87 µg/mL. The DPPH free radical scavenging activity of aqueous extract of *G. lucidium* and standard ascorbic acid were shown in Figure 1.

3.3. Brine shrimp lethality test

Brine shrimp lethality test is one of the most common biological assays to detect the cytotoxicity of phytochemical compounds of plants and which is considered as proved method as a preliminary

Table 1. Phytochemical screening of aqueous extract of *G. lucidium*

Sl. No.	Name of Compounds	Results
01	Proteins	+ +
02	Fats	+
03	Carbohydrates	+ + +
04	Saponins	+ + +
05	Phytosterols	-
06	Tannins	+ +
07	Flavonoids	-
08	Terpenoids	+ +
09	Phlobatannins	+ +
10	Glycosides	+ + +
11	Phenolic Compounds	+ +
12	Alkaloids	+

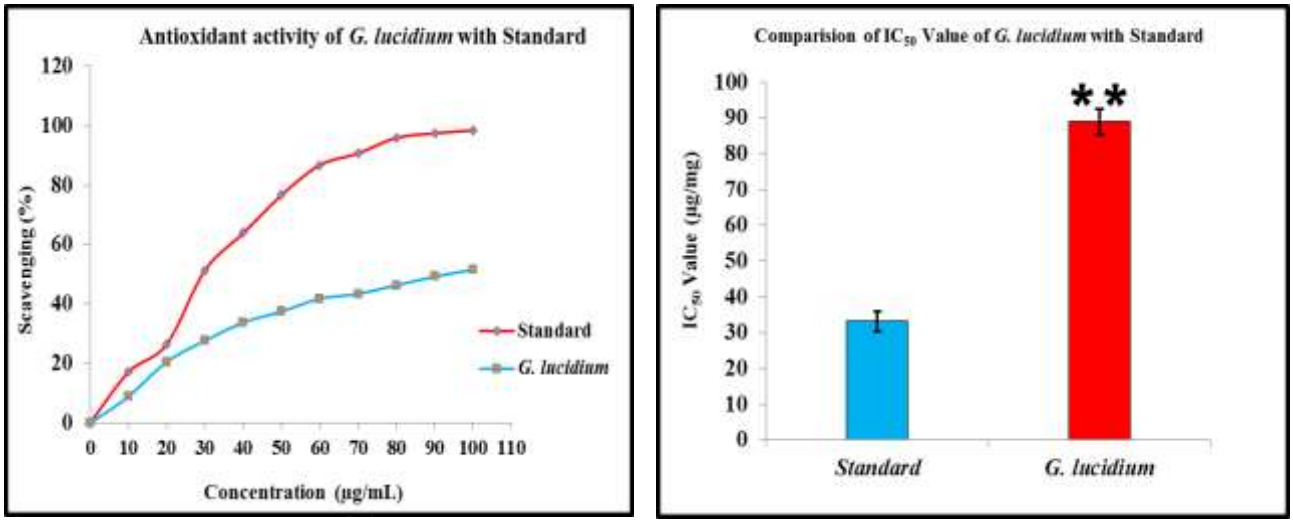

Figure 1. Antioxidant activity of aqueous extract of *G. lucidium* with standard ascorbic acid (A) Scavenging % of aqueous extract of *G. lucidium* with standard ascorbic acid. (B) Comparison of IC_{50} value between aqueous extract of *G. lucidium* sample and standard ascorbic acid. All data are expressed as mean ± SD (n = 3) for all tested dosages. Significant differences of values are compared to values of standard and sample which marked as (*p<0.05, **p<0.01, and***p<0.001).

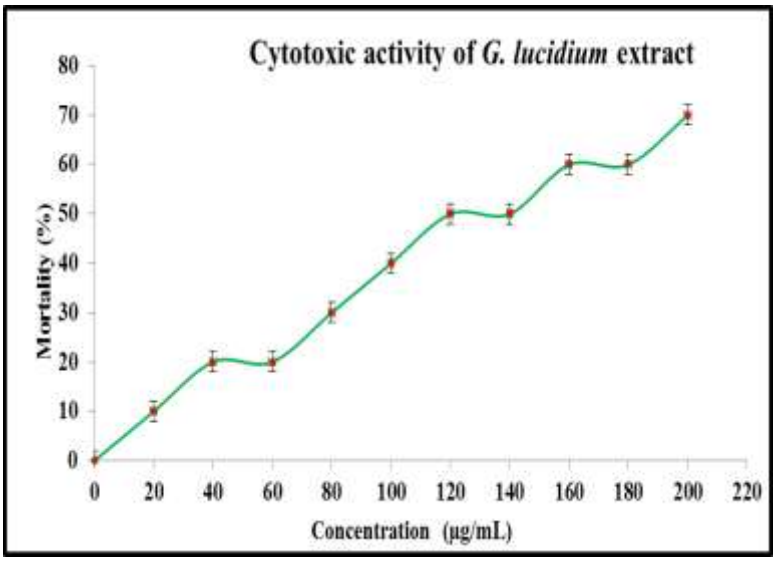

Figure 2. Cytotoxic activity test of aqueous extract of *G. lucidium*. Each value is expressed as mean ± SD (n=3) and significance was set at P<0.05 (*), P <0.01 (**) and P <0.001 (***).

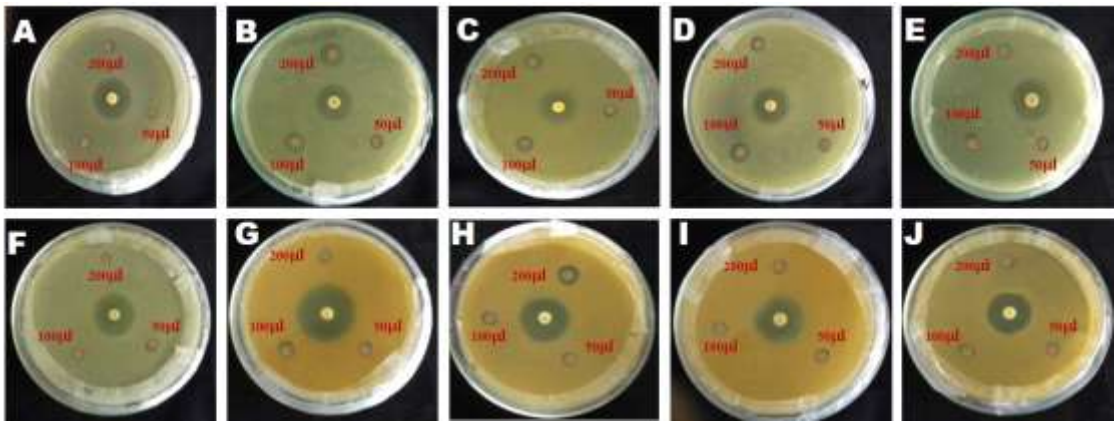

Figure 3. The antibacterial activity of aqueous extract of *G. lucidum* against 10 Bacterial strains. All the bacterial strains are indicated as **A**. *Bacillus subtilis* **B**. *Escherichia coli (E. coli)* **C**. *Acinetobacter sp.* **D**. *Staphylococcus aureus* **E.** *Pseudomonas sp.* **F.** *Acetobacter cloacae* **G.** *Bravibacillus bravis* **H.** *Salmonella typhi* **I.** *RVM* **J.** *RCA*. Three concentrations (50, 100 and 200 µL/disc) of experimental extract were used where antibiotic Amoxicillin was used as positive control.

Table 2. Antibacterial activity of aqueous extract of *G. lucidum* at different concentrations along with standard antibiotic Amoxicillin. (Zone in diameter: ≤ 10 mm = Resistant; 10-15mm = Intermediate response; ≥ 15 mm = Susceptible).

Bacteria	Diameter of zone of inhibition (mm) at different concentration (µL/disc)			Antibiotic (Amoxicillin)	Results
	50	100	200		
Bacillus subtilis	-	6.2 ± 0.4	6.3 ± 0.2	22.5 ± 0.5	Resistant
E. coli	6.2 ± 0.3	11.2 ± 0.3	11.4 ± 0.4	18.8 ± 0.3	Intermediate
Acinetobacter sp.	-	10.1 ± 0.4	10.3 ± 0.2	17.3 ± 0.5	Intermediate
Staphylococcus aureus	-	10.3 ± 0.5	6.1 ± 0.4	18.5 ± 0.4	Intermediate
Pseudomonas sp.	-	6.2 ± 0.3	6.1 ± 0.3	23.1 ± 0.4	Resistant
Acetobacter cloacae	-	6.5 ± 0.2	-	23.2 ± 0.5	Resistant
Bravibacillus vravis	-	6.4 ± 0.3	-	28.3 ± 0.4	Resistant
Salmonella typhi	-	6.1 ± 0.4	10.5 ± 0.3	25.2 ± 0.3	Intermediate
RVM	-	6.3 ± 0.3	-	23.4 ± 0.5	Resistant
RCA	-	6.2 ± 0.2	-	23.5 ± 0.5	Resistant

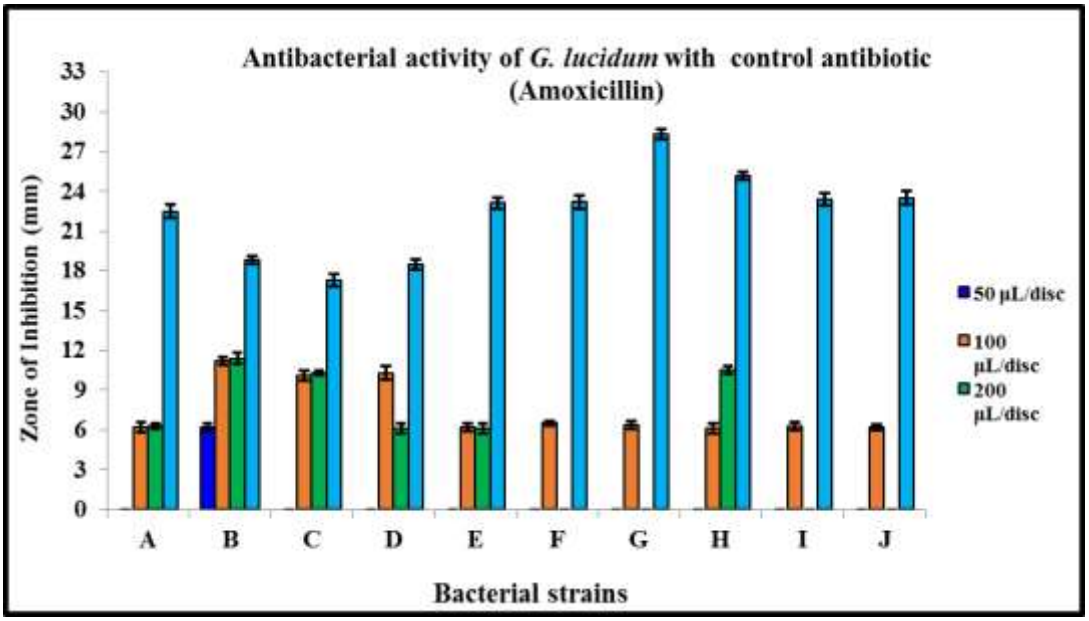

Figure 4. Graphical presentation of Bacterial susceptibility to the aqueous extract of *G. lucidum* with standard antibiotic (Amoxicillin). The ten bacterial strains are indicated as **A**. *Bacillus subtilis* **B**. *E. coli* **C**. *Acinetobacter sp.* **D**. *Staphylococcus aureus* **E.** *Pseudomonas sp.* **F.** *Acetobacter cloacae* **G.** *Bravibacillus bravis* **H.** *Salmonella typhi* **I.** *RVM* **J.** *RCA*. All data are expressed as mean ± SD (n = 3) for all tested dosages. Significant differences of values are compared to values of standard and sample which marked as (*p<0.05, **p<0.01, and***p<0.001).

toxicity testing system of plant extracts [24, 25, 59]. In our current investigation, cytotoxicity of aqueous extract of *G. lucidium* was evaluated using the method described by Meyer et al. [60] with little modification. The aqueous extract of *G. lucidium* showed moderate cytotoxic effect against brine shrimp and the 50% lethal concentration, LC_{50} value of experimental extract was calculated as 142.49 ± 5.31 µg/mL that is shown in Figure 2.

3.4. Antibacterial activity test

The disc diffusion assay was conducted to evaluate the bacterial sensitivity to aqueous extract of *G. lucidium*. The antibacterial activity was assessed by measuring the zone of inhibition on the plates in millimeter (mm). The bacterial susceptibility to the aqueous extract of *G. lucidium* revealed non-significance inhibition of bacterial growth in comparison to control antibiotic (Amoxicillin). Among these bacterial strains, no one shows significant sensitivity to aqueous extract of *G. lucidium* at any concentration. About 40 % of used bacterial species show intermediate response and 60 % show resistance. The results of antibacterial activity of aqueous extract of *G. lucidium* are not remarkable and the results were summarized in Table 2 and Figure 3, 4.

4. DISCUSSION

Medicinal plants are good sources for the treatment of numerous diseases or disorder and in many countries these natural resources were used in their treatment system from ancient time. Recently, the plants have become a principal target of investigation for searching a novel biologically active compound to develop many natural antibiotics that have been used for different infectious diseases [1, 61].

Phytochemicals are environment friendly, locally renewable and are regarded as an important therapeutic agents for immune stimulating activities [62, 63]. The phytochemical screening test is the most useful tools to detect the biologically active compounds into the plant's extracts [39]. The present study reported that the aqueous extract of *G. lucidum* contains high level of carbohydrates, saponins and glycosides but phytosterols and flavonoids were not detected (Table 1). Generally, free radicals are dangerous chemical compounds that are originated from various biochemical reactions in the body of living organisms and have a great impact on creating many diseases like cancer, heart diseases etc. The antioxidant compounds are able to scavenge these free radicals by oxidative reaction [64, 65]. DPPH free radical scavenging assay was used to evaluate the antioxidant activity of the experimental extract and the results showed the extract have good ability to scavenge the DPPH free radicals. The aqueous extract of *G. lucidum* represented an excellent IC_{50} value as 89.05 ± 3.59 µg/mL where the standard ascorbic acid showed the IC_{50} value as 33.14 ± 2.87 µg/mL (Figure 1).

Cytotoxic effects of plant extracts represent the medicinal and pharmacological activities of plants and it is evaluated by a widely used, easy, cheapest and available bioassay called brine shrimp lethality assay [59, 66]. In the current study, the brine shrimp lethality assay was set up to determine the cytotoxicity of *G. lucidum* extract. This assay reported that the aqueous extract of *G. lucidum* has moderate cytotoxic effect and the LC_{50} value was calculated as 142.49 ± 5.31 µg/mL that is shown in Figure 2. Though the pharmaceutical companies have generated a huge number of antibiotics, the number of microorganism's resistance to these drugs have raised up. Due to this resistance problem of microbes, it is crying need to look for new antimicrobial agents from nature and it is proved that the plant extracts as well as phytochemicals of plant can be a significant therapeutic agents in various microbial infectious disease treatments [67-70]. The disc diffusion assay was carried out in the current investigation to look for the antibacterial properties of aqueous extract of *G. lucidum* and this extract showed partial antibacterial activity (Figure 3 & 4 and Table 2) when compared with standard antibiotics (Amoxicillin). The present study showed that, the aqueous extract of *G. lucidum* contain many essential biochemical compounds and have important biological properties like antioxidant activity, cytotoxic activity and antibacterial activity which become helpful to develop many vital drugs or treatment agents.

5. CONCLUSION

The *G. lucidum* is a pharmaceutically important fungus that contains numerous phytonutrients such as proteins, carbohydrates, glycosides, phenolic compounds and tannins would be the main responsible for antibacterial properties. The detected biologically active compounds in *G. lucidum* have a broad spectrum of adjuvant biomedical and biochemical virtues that prevent the different physiological disorders and diseases. The cytotoxic effects of *G. lucidum* indicated the presence of biologically active compounds which have the

ability to fight against various diseases. The antioxidant properties of *G. lucidum* can decrease the rate of mutation as well as block the development of tumors by scavenging free radicals formed by oxidative reaction. Although the *G. lucidum* extract has great biological activities, it has a partial antibacterial activity. So, our current investigation suggests that, the aqueous extract of *G. lucidum* have excellent biomedical and pharmaceutical properties and will become a good natural resource to develop many drugs for numerous life threatening diseases and disorders.

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Conflicts of Interest

There are no conflicts of interest.

References

1. Kamaraj C, Rahuman AA, Siva C, Iyappan M, Kirthi AV (2012). Evaluation of antibacterial activity of selected medicinal plant extracts from south India against human pathogens. *Asian Pacific Journal of Tropical Disease*; 2:S296-S301.
2. Abraham J, Thomas TD (2012). Antibacterial activity of medicinal plant *Cyclea peltata* (Lam) Hooks & Thoms. *Asian Pacific Journal of Tropical Disease*; 2:S280-S284.
3. Shah P, Modi H, Shukla M, Lahiri SK (2014). Preliminary phytochemical analysis and antibacterial activity of *Ganoderma lucidum* collected from Dang District of Gujarat, India. *Int J Curr Microbiol App Sci*; 3:246-255.
4. Wasson RG (1968). Soma: Divine mushroom of immortality. Harcourt Brace Jovanovich New York.
5. Wachtel-Galor S, Yuen J, Buswell JA, Benzie IF (2011). *Ganoderma lucidum* (Lingzhi).
6. Yang F-C, Ke Y-F, Kuo S-S (2000). Effect of fatty acids on the mycelial growth and polysaccharide formation by Ganoderma lucidum in shake flask cultures. *Enzyme and microbial technology*; 27:295-301.
7. Shamaki B, Geidam Y, Abdulrahma F, Ogbe A, Sandabe U (2012). Evaluation of phytochemical constituents and in vitro antibacterial activity of organic solvent fractions of *Ganoderma lucidum* methanolic extract. *International Journal of Medicinal Plant Researc*h; 1:026-031.
8. Thyagarajan A, Zhu J, Sliva D (2007). Combined effect of green tea and *Ganoderma lucidum* on invasive behavior of breast cancer cells. *International journal of oncology*; 30:963-969.
9. Wasser SP, Weis A (1997). Medicinal mushrooms *Ganoderma lucidum*,(Curtis, Karst).
10. Moradali M-F, Mostafavi H, Hejaroude G-A, Tehrani AS, Abbasi M, Ghods S (2006). Investigation of potential antibacterial properties of methanol extracts from fungus *Ganoderma applanatum*. *Chemotherapy*; 52:241-244.
11. Jabeen A, Rani S, Ibrahim M, Mohammad. as pharmaceutical sciences History 10:12.
12. Heneman K, Zidenberg-Cherr S (2008). Nutrition and health info sheet: Phytochemicals.
13. Dorman H, Deans S (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of applied microbiology*; 88:308-316.
14. Frankel EN, Huang SW, Aeschbach R, Prior E (1996). Antioxidant activity of rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil in water emulsion. *Journal of Agricultural and Food Chemistry;* 44:131-135.
15. Hertog MG, Feskens EJ, Kromhout D, Hollman P, Katan M (1993). Dietary antioxidant flavonoids and risk of coronary heart disease. The Zutphen Elderly Study *The Lancet*; 342:1007-1011.
16. Halliwell B, Gutteridge JM (1985). Free radicals in biology and medicine. Pergamon.
17. Tiwari AK (2001) Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidants therapy *Current science*:1179-1187.
18. Hayet E et al. (2008) Antimicrobial, antioxidant, and antiviral activities of *Retama raetam* (Forssk.) Webb flowers

growing in Tunisia. *World Journal of Microbiology and Biotechnology* 24:2933-2940.

19. Riaz T, Abbasi MA, Rehman A, Shahzadi U, Qureshi MZ, Ajaib M (2012). *Dicliptera bupleuroides*: an imperative source for protection from oxidative stress. *Journal of the Chemical Society of Pakistan*; 34:326-332.
20. Jothy SL, Zuraini Z, Sasidharan S (2011). Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitiory activities of *Cassia fistula* seeds extract. *Journal of Medicinal Plants Research*; 5:1941-1947.
21. Ozsoy N, Can A, Yanardag R, Akev N (2008). Antioxidant activity of *Smilax excelsa* L. leaf extracts. *Food Chemistry*; 110:571-583.
22. McClements D, Decker E (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science*; 65:1270-1282.
23. Kibiti CM, Afolayan AJ (2016). Antifungal activity and brine shrimp toxicity assessment of *Bulbine abyssinica* used in the folk medicine in the Eastern Cape Province, South Africa. *Bangladesh Journal of Pharmacology*; 11:469-477.
24. Sarah QS, Anny FC, Misbahuddin M (2017). Brine shrimp lethality assay. *Bangladesh Journal of Pharmacology*; 12:5-2017.
25. Krishnaraju AV, Rao TV, Sundararaju D, Vanisree M, Tsay H-S, Subbaraju GV (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *Int J Appl Sci Eng*; 3:125-134.
26. McLaughlin JL, Chang C-j, Smith DL (1993). Simple bench-top bioassays (brine shrimp and potato discs) for the discovery of plant antitumor compounds: review of recent progress. In. ACS Publications.
27. Sökmen M et al. (2004). In vitro antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of *Origanum acutidens*. *Journal of agricultural and food chemistry*; 52:3309-3312.
28. Chopra I (2007). The increasing use of silver based products as antimicrobial agents: a useful development, a cause for concern. *Journal of antimicrobial Chemotherapy*; 59:587-590.
29. Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International journal of food microbiology*; 94:223-253.
30. Saeed A, Sultana B, Anwar F, Mushtaq M, Alkharfy KM, Gilani A-H (2014). Antioxidant and antimutagenic potential of seeds and pods of green cardamom (*Elettaria cardamomum*). *International Journal of Pharmacology*; 10:461-469.
31. Saleem A, Ahotupa M, Pihlaja K (2001). Total phenolics concentration and antioxidant potential of extracts of medicinal plants of Pakistan. Zeitschrift für Naturforschung C; 56:973-978.
32. Cai Y, Luo Q, Sun M, Corke H (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life sciences*; 74:2157-2184.
33. Nostro A, Germano M, D'angelo V, Marino A, Cannatelli M (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in applied microbiology*; 30:379-384.
34. Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Ghaffar R (2013). Phytochemical analysis of plants occurring in local area of Mardan. *Biochem Anal Biochem*; 2:1-4.
35. Obasi Nnamdi L, Egbuonu A, Ukoha P, Ejikeme P (2010). Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* (fabaceae or mimosaceae) pods. *Afr J Pure Appl Chem*; 4:206-212.
36. Oyekunle M, Aiyelaagbe O, Fafunso M (2006). Evaluation of the antimicrobial activity of saponins extract of *Sorghum bicolor* L. Moench. *African journal of Biotechnology*; 5.
37. Khanna VG, Kannabiran K (2007). Larvicidal effect of *Hemidesmus indicus, Gymnema sylvestre, and Eclipta prostrata*, against Culex qinquifaciatus mosquito larvae. *African Journal of Biotechnology*; 6.
38. Parekh J, Chanda S (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African Journal of Biomedical Research; 10.
39. Sofowara A (1993). Screening plants for bioactive agents pp 42ñ44, 221ñ229,

246ñ249, 304ñ306, 331ñ332, 391ñ393, Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria.

40. De S, Dey Y, Ghosh A (2010). Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphaphallus paeoniifolius* (Araceae). *Int J Pharm Biol Res*; 1:150-157.
41. Edeoga H, Okwu D, Mbaebie B (2005). Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology*; 4:685-688.
42. Trease GE, Evans WC Pharmacognosy (1989). Bailliere Tindall, London:45-50.
43. Yadav R, Agarwala M (2011). Phytochemical analysis of some medicinal plants. *Journal of phytology*; 3.
44. Firdouse S, Alam P (2011). Phytochemical investigation of extract of *Amorphophallus campanulatus* tubers. *International Journal of Phytomedicine*; 3:32-35.
45. Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TCd, Coube CS, Leitão SG (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy research*; 15:127-130.
46. Desmarchelier C, Mongelli, Coussio J,Ciccia G (1997). Evaluation of *invitro* antioxidant activity in extracts of *Uncaria tomentosa*. *Phytotherapy Research*; 11:254-256.
47. Choi HY, Jhun EJ, Lim BO, Chung IM, Kyung SH, Park DK (2000). Application of flow injection-chemiluminescence to the study of radical scavenging activity in plants. *Phytotherapy Research*; 14:250-253.
48. Brand-Williams W, Cuvelier M-E, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. LWT-*Food science and Technology*; 28:25-30.
49. Ajila C, Naidu K, Bhat S, Rao UP (2007). Bioactive compounds and antioxidant potential of mango peel extract. *Food chemistry*; 105:982-988.
50. Pisutthanan S, Plianbangchang P, Pisutthanan N, Ruanruay S, Muanrit O (2004). Brine shrimp lethality activity of Thai medicinal plants in the family Meliaceae. *Naresuan University Journal*; 12:13-18.
51. Sam TW (1993). Toxicity testing using the brine shrimp: Artemia salina Bioactive natural products, detection, isolation and structural determination:441-456.
52. Ramachandran S, Vamsikrishna M, Gowthami K, Heera B, Dhanaraju M (2011). Assessment of cytotoxic activity of Agave cantula using brine shrimp (*Artemia salina*) lethality bioassay. *Asian J Sci Res*; 4:90-94.
53. Naz R, Ayub, Hafsa, Nawaz, Sajid Islam, Zia Ul Yasmin, Tayyaba Bano, Asghari, Wakeel, Abdul, Zia, Saqib, Roberts, Thomas H (2017). Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. *BMC complementary and alternative medicine*; 17:302.
54. Chang C-C, Yang M-H, Wen H-M, Chern J-C (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis*; 10.
55. Iwu MW, Duncan AR, Okunji CO (1999). New antimicrobials of plant origin Perspectives on new crops and new uses. ASHS Press, Alexandria, VA:457-462.
56. Kaur N, Dhuna V, Kamboj SS, Agrewala, Singh J (2006). A novel antiproliferative and antifungal lectin from *Amaranthus viridis* L seeds. Protein & peptide letters; 13:897-905.
57. Schulz V, Hänsel R, Blumenthal M, Tyler VE (2004). Medicinal plants, phytomedicines, and phytotherapy. In: *Rational phytotherapy*. Springer, pp 1-42.
58. Obi R, Iroagba I, Ojiako O (2006). Virucidal potential of some edible Nigerian vegetables. *African Journal of Biotechnology*; 5.
59. Oberlies NH, Rogers LL, Martin JM, McLaughlin JL (1998). Cytotoxic and insecticidal constituents of the unripe fruit of *Persea americana*. *Journal of Natural Products*; 61:781-785.
60. Meyer B, Ferrigni N, Putnam J, Jacobsen L, Nichols Dj, McLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta medica*; 45:31-34.
61. Roberts LM (2004). Australian Ganoderma: identification, growth & antibacterial properties. Environment and Biotechnology Centre, School of Engineering and Science, Swinburne University of Technology.
62. Anjorin S, Salako E (2009). The status of pesticidal plants and materials identification in Nigeria. *Nigerian Journal of Plant Protection*; 23:25-32.
63. Rotimi M, Moens M (2002). The use of leaf extracts of some herbs in the control of

Meloidogyne incognita. In: 30th Annual Conference Nigerian Society for Plant Protection, University of Agriculture, Abeokuta.

64. Al-Dabbas MM, Suganuma T, Kitahara K, Hou D-X, Fujii M (2006). Cytotoxic, antioxidant and antibacterial activities of *Varthemia iphionoides* Boiss. Extracts. *Journal of ethnopharmacology*; 108:287-293.
65. Li, Peiyuan, Huo, Lini Su, Wei Lu, Rumei Deng, Chaocheng Liu, Liangquan Deng, Yongkun Guo, Nana Lu, Chengsheng He, Chunling (2011). Free radical-scavenging capacity, antioxidant activity and phenolic content of *Pouzolzia zeylanica*. *Journal of the Serbian Chemical Society;* 76:709-717.
66. Carballo JL, García-Grávalos MD, Pérez P, Hernández-Inda ZL (2002). A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. *BMC biotechnology*; 2:17.
67. Almagboul A, Bashir A, Salih AKM, Farouk A, Khalid S (1988). Antimicrobial activity of certain Sudanese plants used in folkloric medicine. Screening for antibacterial activity (V). *Fitoterapia*; 59:57-62.
68. Artizzu N, Bonsignore L, Cottiglia F, Loy G (1996). Studies on the diuretic and antimicrobial activity of *Cynodon dactylon* essential oil. *Fitoterapia*; 67:174-176.
69. Izzo, Di Carlo, G Biscardi, De Fusco, R Mascolo, F Fasulo (1995). Biological screening of Italian medicinal plants for antibacterial activity. *Phytotherapy research*; 9:281-286.
70. Nascimento GG, Locatelli J, Freitas PC, Silva GL (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian journal of microbiology*; 31:247-256.