Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(3): 42-46
Received: 17-06-2015
Accepted: 25-07-2015

Nazia Hoque
Senior Lecturer, Department of Pharmacy, East West University Plot no. A/2, Main Road, Jahurul Islam City, Aftabnagar, Dhaka-1212, Bangladesh.

Abdullah-Al-Faysal
Lecturer, Department of Pharmacy, East West University Plot no. A/2, Main Road, Jahurul Islam City, Aftabnagar, Dhaka-1212, Bangladesh.

Iftekhar Ahmed
Lecturer, Department of Pharmacy, East West University Plot no. A/2, Main Road, Jahurul Islam City, Aftabnagar, Dhaka-1212, Bangladesh.

Md. Rafi-Uz-Zaman Akanda
East West University, Plot no. A/2, Main Road, Jahurul Islam City, Aftabnagar, Dhaka-1212, Bangladesh.

Nargis Sultana Chowdhury
Assistant Professor, Department of Pharmacy, Manarat International University (MIU) Plot # 1, Block # B, Section # 1, Zoo Road, Mirpur # 1, Dhaka-1216, Bangladesh

Correspondence:
Nazia Hoque
Senior Lecturer, Department of Pharmacy, East West University Plot no. A/2, Main Road, Jahurul Islam City, Aftabnagar, Dhaka-1212, Bangladesh.

In vitro antioxidant, antimicrobial and cytotoxic activities of the various extracts of *Ganoderma lucidum* available in Bangladesh

Nazia Hoque, Abdullah-Al-Faysal, Iftekhar Ahmed, Md. Rafi-Uz-Zaman Akanda, Nargis Sultana Chowdhury

Abstract
The present study was designed to investigate antioxidant, antimicrobial and cytotoxic potential of pet ether, chloroform and methanol extracts of *Ganoderma lucidum* available in Bangladesh. Antioxidant activity of the extracts was evaluated using DPPH radical scavenging assay and determination of total phenolic content. Antibacterial and cytotoxic activities were studied using disc diffusion method and brine shrimp lethality bioassay respectively. Results showed that the methanol extract had highest antioxidant activity (IC_{50} value for DPPH was 47.58 µg/ml and total phenolic content was 167.9±3.252 in mg/g, Gallic acid equivalents) compared to the pet ether and chloroform extract. In antibacterial study, all the extracts showed mild to moderate activity with zone of inhibition ranging from 7 mm to 21 mm. In brine shrimp lethality bioassay, the LC_{50} values for pet ether, chloroform and methanol extracts were 941.88 µg/ml, 367.28 µg/ml and 295.8 µg/ml respectively which revealed weak cytotoxic potential of the extracts.

Keywords: *Ganoderma lucidum*, Antioxidant, DPPH, Total Phenolic Content, Cytotoxic, Antimicrobial.

1. Introduction
Ganoderma lucidum (Reishi, Lingzhi) that belongs to Ganodermataceae is an edible mushroom that has been used for centuries in Traditional Chinese Medicine for its health promoting properties [1]. It is widely grown on a commercial scale and commonly purchased for its medicinal and spiritual properties in China, Korea, Japan and other Asian countries [2]. The fruiting body, mycelia and spores of *G. lucidum* contain approximately 400 different bioactive compounds, which mainly include triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, proteins and trace elements which are responsible for its anti-inflammatory, analgesic, antibacterial, antiviral (including anti-HIV), immunomodulatory, antiatherosclerotic, chemopreventive, antitumor, radioprotective, sleep promoting, hypolipidemic and anti-ulcer properties [3-10]. Reishi has now become recognized as an alternative adjuvant in the treatment of leukemia, carcinoma, hepatitis and diabetes, raising the possibility that it could be effective in preventing oxidative damage and resulting diseases [3, 9]. The antioxidants in human diets are of great interest as possible protective agents against oxidative stress and decrease the adverse effects of reactive species on normal physiological functions in humans [11]. *G. lucidum* is rich in "mushroom nutraceutical" which is a new class of compounds with medicinal values collected from the mycelium or the fruiting bodies of it [12].

A large number of research works on the phytochemistry, pharmacology and several other aspects of *G. lucidum* has been conducted but there has been no report on phytochemical screening and *in vitro* bioactivities of different extracts of *G. lucidum* collected from Bangladesh. So the present investigations were carried out to study the phytoconstituents and *in vitro* antioxidant, cytotoxic and antimicrobial activities of the different extracts of *G. lucidum* available in Bangladesh.

2. Materials and methods
2.1 Chemicals and solvents: DPPH (2, 2-Diphenyl-1-picrylhydrazyl) was obtained from Sigma-Aldrich co. USA. Folin-Ciocalteu reagent, ascorbic acid and sodium carbonate were purchased from Merck, Germany. All the other chemicals used, including the solvents were of analytical grades.

2.2 Plant material and extraction: Dried powder of the fruiting body and mycelia of *G. lucidum* was purchased from the National Mushroom Development and Extension Centre, Savar, Dhaka, Bangladesh on 13th April, 2013. 200 gm powdered plant materials were submerged into pet ether, chloroform and methanol using 1 liter of each solvent in an air-tight flat bottom container for seven days with occasional shaking and stirring. The major portion of the extractable compounds of the plant materials were dissolved in different solvents which were collected and then evaporated with rotary evaporator (IKA, Germany) at low temperature (40-50 °C) and reduced pressure. The dried extracts were stored at 4 °C until used.

The yield percentage (w/w) of *G. lucidum* in different solvents is shown in Table 1.

Table 1: Represents the yield % of *G. lucidum* in different extracts

Sl. No	Solvent used	Yield %
1	Pet ether	1.8
2	Chloroform	2.4
3	methanol	5.1

2.3 Phytochemical screening: The freshly prepared extracts were qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts were performed using the following reagents and chemicals: alkaloids with Wagner's and Hager's reagent; terpenoids with modified Salkowski test; carbohydrates with Molisch's test, tannins with 0.1% ferric chloride; flavonoids with the use of concentrated hydrochloric acid; saponins with ability to produce stable foam and steroids with concentrated sulfuric acid. These were identified by characteristic color changes using standard procedures [13].

2.4 Tests for antioxidant activity:

2.4.1 DPPH radical scavenging activity:

The free-radical scavenging activity of *G. lucidum* extracts was measured by decrease in the absorbance of methanol solution of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) [14]. Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\%\ \text{free radical scavenging activity} = \frac{A_o - A}{A_o} \times 100$$

A_o = Absorbance of control and A = Absorbance of sample

Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} was calculated. Ascorbic acid, a potential antioxidant was used as positive control.

2.4.2 Determination of total phenolic content

The total phenolic contents of the extracts were determined by using Folin-Ciocalteu reagent [15] and Gallic acid (Merck, Germany) as standard. 10% Folin-Ciocalteu reagent was used to oxidize the extracts which was neutralized with 700 mM sodium carbonate solution. After 60 minutes, absorbances were taken at 765 nm. The total phenolic contents were determined from a standard curve prepared with Gallic acid.

2.5 Antibacterial assay

The antibacterial assay was carried out by the disc diffusion method [16] against 5 Gram-positive and 7 Gram-negative bacterial strains. 100µL of suspension of each microorganism containing ~100-150 CFU/mL was spread over the nutrient agar (Himedia, India). Dried and sterilized filter paper discs (6 mm diameter), impregnated with 500 and 1000 µg of different extracts were placed gently in the agar plates. Standard disc (Himedia, India) of Kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. After incubation at 37 °C for 24 hours, the antimicrobial activity of the test samples were determined by measuring the diameter of zone of inhibition expressed in mm.

2.6 Cytotoxic activity:

Brine shrimp lethality bioassay was used following the procedure [17] for evaluating cytotoxic activity using concentrations of 1.56 – 400 µg/ml for each extract. Different concentrations of Vincristine sulfate were taken as positive control. The percentage of lethality of brine shrimp nauplii was calculated for each concentration of the extracts.

2.7 Statistical analysis

Statistical comparisons were performed using Microsoft Excel, 2007. Mean values ± S.D. were calculated for the parameters where applicable.

3. Results

3.1 Phytochemical screening: Phytochemical analysis revealed the presence of alkaloids, terpenoids, carbohydrates, tannins, flavonoids and steroids in all extracts of *G. lucidum.* The results also showed the presence of saponin in methanol extract (Table 2).

Table 2: Result of chemical group tests of the extracts of *G. lucidum*

Plant extract	Carbohydrates	Alkaloids	Terpenoids	Tannins	Flavonoids	Saponins	Steroids
PEGL	++	++	+++	+	+	-	+
CEGL	++	++	+++	+	+	-	+
MEGL	+++	++	+++	+	+	+	+

PEGL: Pet ether extract of *G. lucidum*, CEGL: Chloroform extract of *G. lucidum*, ME: Methanol extract of *G. lucidum*

(+): Present; (-): Absent.

Symbol (+++) indicates presence in high concentration, Symbol (++) indicates presence in moderate concentration and (+) indicates low concentration of the respective constituents.

3.2 DPPH radical scavenging activity: From the analysis of Figure 1, it can be concluded that the scavenging effect of the extracts increases with the concentration. Methanol extract of *G. lucidum* (MEGL) showed the highest radical scavenging activity whereas pet ether extract (PEGL) showed the lowest activity. The IC_{50} values for the extracts and the standard ascorbic acid is shown in Table 3.

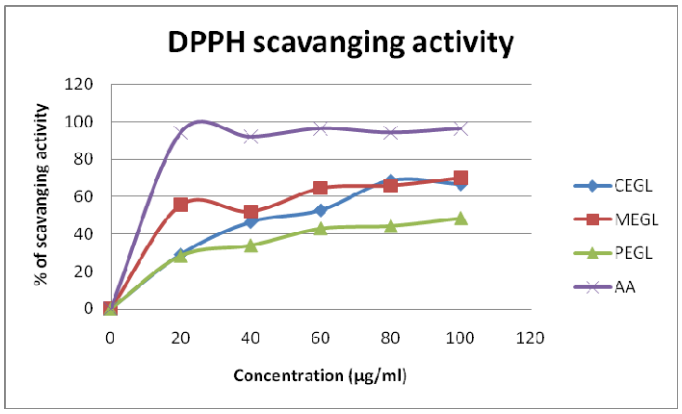

Fig 1: DPPH scavenging activity of various extracts of *G. lucidum*

PEGL: Pet ether extract of *G. lucidum,* CEGL: Chloroform extract of *G. lucidum*, MEGL: Methanol extract of *G. lucidum,* AA: Ascorbic acid

Table 3: IC_{50} value for DPPH radical scavenging activity of various extarcts and ascorbic acid

Extract/ standard	IC_{50} (µg/ml)
PEGL	89.55
CEGL	59.26
MEGL	47.58
AA	8.34

3.3 Total phenolic content

Among the three extracts, the methanol extract (MEGL) showed the highest amount of phenolic compounds followed by the chloroform extract (CEGL) and the pet ether extract (PEGL) (Table 4).

Table 4: Total phenolic content of different extracts of *G. lucidum*

Extracts	Total phenolic content (in mg/g, Gallic acid equivalents)
PEGL	61.7±1.272
CEGL	106.6±3.111
MEGL	167.9±3.252

Values are represented as mean± SD with duplicate estimation.

3.4 Antibacterial assay

The pet-ether soluble fraction displayed zone of inhibition ranging from 8 mm to 12 mm with highest antibacterial activity against *Escherichia coli (*12±0.5 at 1000 µg/disc). This fraction showed very mild activities against other strains. Both the chloroform and methanol extracts showed mild to moderate activities against all strains of bacteria. The chloroform extract showed zone of inhibition ranging from 7 mm to 15 mm with highest antibacterial activity against *E. coli (*15±0.29 at 1000 µg/disc). Among the three extracts, the methanol extract exhibited the maximum antimicrobial activity against all the experimental bacterial strains (zone of inhibition ranging from 7 mm to 21 mm) with highest activity against *E. coli* (21±0.5 at 1000 µg/disc). From the result (Table 5), it is obvious that this plants extracts can be highly useful in the treatment of diseases caused by *E. coli*.

Table 5: Zone of inhibition of pet ether (PEGL), chloroform (CEGL) and methanol (MEGL) extract of *G. lucidum* and positive control Kanamycin

Serial	Name of the test organisms	PEGL		CEGL		MEGL		Kanamycin
		500 µg/disc	1000 µg/disc	500 µg/disc	1000 µg/disc	500 µg/disc	1000 µg/disc	30 µg/disc
Gram-positive bacteria		**Zone of inhibition (mm)**						
1	*Sarcina lutea*	-	8±0.5	-	10±0.29	10±0.29	13±0.76	30±0.76
2	*Bacillus megaterium*	-	8±0.29	7±0.5	12±0.5	11±0.29	12±0.5	30
3	*Bacillus subtilis* ATCC 6059	-	9±0.29	7.5±0.5	12±0.29	12±0.29	15±0.76	30
4	*Staphylococcus aureus* ATCC25923	-	11±0.5	8±0.5	10±0.58	9±0.5	11±0.29	34
5	*Bacillus cereus* ATCC 14579	-	10±0.29	8±0.29	8.5±0.29	10±0.58	14±0.76	30±0.76
Gram-negative bacteria								
1	*Pseudomonas aeruginosa* ATCC 27853	7±0.29	11±0.29	-	8±0.5	8±0.5	9±0.5	28
2	*Salmonella typhi* ATCC 13311	8±0.5	11.5±0.29	7±0.5	10±0.29	8±0.5	11±0.29	17.5
3	*Escherichia coli* ATCC 25922	8±0.58	12±0.5	12±0.76	15±0.29	17±0.58	21±0.5	30
4	*Vibrio parahemolyticus*	-	-	8±0.5	10±0.29	10±0.58	14±0.76	20
5	*Vibrio mimicus* ATCC 33653	-	-	7.5±0.5	11±0.5	8±0.5	8±0.58	13.5
6	*Shigella boydii* ATCC13147	-	-	-	8±0.58	7±0.76	10±0.29	23
7	*Shigella dysenteriae* ATCC 26131	-	-	-	8±0.5	8±0.5	11±0.29	24

Values are expressed as mean±SD (n=3). '-'Indicates no zone of inhibition.

3.5 Cytotoxic activity

In brine shrimp lethality bioassay, the lowest LC_{50} value (295.8µg/ml) was revealed by the methanol extract (MEGL) and the highest LC_{50} value (941.88µg/ml) was demonstrated by the pet ether soluble fraction (PEGL) where the standard Vincristine sulphate (VS) showed LC_{50} value of 0.451 µg/ml. The extracts showed very negligible cytotoxic activity compared to the standard (Table 6).

Table 6: Effect of various extracts of *G. lucidum* on shrimp nauplii.

Concentration	Log C	% mortality			LC_{50} (µg/ml)		
(µg/ml)		PEGL	CEGL	MEGL	PEGL	CEGL	MEGL
400	2.60206	50	55	60			
200	2.30103	40	40	45			
100	2	30	40	40			
50	1.69897	20	30	30			
25	1.39794	15	25	20	941.889	367.282	295.801
12.5	1.09691	10	20	15			
6.25	0.79588	0	10	10			
3.125	0.49485	0	0	0			
1.5625	0.19382	0	0	0			
0	0	0	0	0			

PEGL: Pet ether extract of *G. lucidum,* CEGL: Chloroform extract of *G. lucidum*, MEGL: Methanol extract of *G. lucidum.*

4. Discussion

Phytochemical tests showed the existence of terpenoids in high concentration in all of the extracts with low to moderate concentration of alkaloids, carbohydrates, tannins, flavonoids, and steroids. Presence of these phytocompounds can be correlated to the biological activities of *G. lucidum* found in this research.

In recent years, there has been increasing interest in the involvement of reactive oxygen species (ROS) in several pathological incidences because the oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease [18]. As a result, antioxidants with free radical scavenging activities may have great contribution in the prevention and treatment of such diseases. Preliminary phytochemical screening of the extracts of *G. lucidum* showed the presence of flavonoids and tannins. Polyphenolic compounds, like flavonoids, tannins and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity. Therefore, these compounds present in the plant extract may be responsible for the antioxidant action in the tested models. From our study, a positive correlation was seen between total phenolic content and total antioxidant activity (DPPH radical scavenging activity) of *G. lucidum*. The variation in total phenolic content can be expected for the plant extracts due to the presence of other constituents. From the result, a straight relationship can be drawn between total phenolic content and DPPH scavenging activity, since higher the total phenolic content, lower the IC_{50} values of the respective extracts. All of the extracts showed highly potential antioxidant activities as compared to the activity of the standards.

In the present study, almost all of the extracts of *G. lucidum* (at different concentrations) exhibited low to moderate antimicrobial activity against various strains of Gram-positive and Gram-negative bacteria. The ability of the crude extracts of *G. lucidum* to inhibit the growth of bacteria is an indication of its antimicrobial potential which may be employed in the management of microbial infections. Methanol extract showed the maximum potential antimicrobial activity which was also found consistent with other literature findings [19].

Various extracts of *G. lucidum* produced concentration dependent increment in percent mortality of brine shrimp nauplii. All the extracts of *G. lucidum* showed weak cytotoxic activity compared to the positive control Vincristin sulphate which is supported by the previous studies [20]. The results also support the use of *G. lucidum* as a nontoxic vegetable by mass people.

G. lucidum contains different triterpenes and polysaccharides which may be responsible for different pharmacological activities including its antioxidant and antimicrobial action [21-23].

This study is suggestive that *G. lucidum* can be used as antioxidant and antibacterial agent in the development of new drugs. Further work is under progress to identify the bioactive principles and elucidate their mechanism of action of specific bioactivities.

5. Acknowledgement: The authors are grateful to Dr. Chowdhury Faiz Hossain, Professor and Chairperson, Department of Pharmacy, East West University, Dhaka, Bangladesh for providing proper facilities to conduct the research works.

6. References

1. Wang Y, Khoo K, Chen S, Lin C, Wong C, Lin C. Studies on the immuno-modulating and antitumor activities of *Ganoderma lucidum* (reishi) polysaccharides: functional and proteomic analyses of a fucose-containing glycoprotein fraction responsible for the activities. Bioorg Med Chem 2002; 10(4):1057-1062.
2. Lowy B, Wasson RG. Soma, divine mushroom of immortality. Mycologia 1969; 61(4):849.
3. McKenna DJ, Jones K, Hughes K. *Botanical Medicines*. New York: Haworth Herbal Press, 2002.
4. Gao Y, Zhou S, Chen G, Dai X, Ye J. A phase I/II study of a *Ganoderma lucidum* (Curt: Fr.) P. Karst. extract (ganopofy) in patients with advanced cancer. Int J Med Mushrooms. 2002; 4(3):8.
5. Govindarajan R, Vijayakumar M, Singh M, Rao CHV, Shirwaikar A, Rawat AKS *et al*. Antiulcer and antimicrobial activity of *Anogeissus latifolia*. J Ethnopharmacol 2006; 106(1):57-61.
6. Wasser SP, Weis AL. Medicinal mushrooms. *Ganoderma lucidum*, (Curtis: Fr.), P. Karst; Nevo, E., Eds.; Peledfus Publ House: Haifa, Israel, 1997, 39.
7. Eo S, Kim Y, Lee C, Han S. Antiviral activities of various water and methanol soluble substances isolated from *Ganoderma lucidum*. J Ethnopharmacol. 1999; 68(1-3):129-136.
8. Van der Hem LG, Van der Vliet JA, Bocken CF, Kino K, Hoitsma AJ, Tax WJ. Ling Zhi-8: studies of a new immunomodulating agent. Transplantation 1995; 60(5):438-43.
9. Wasser SP. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol 2002; 60(3):258-274.
10. Chang S, Buswell JA. *Ganoderma lucidum* (Curt: Fr.) P.

Karst. (Aphyllophoromycetideae)-a mushrooming medicinal mushroom. Int J Med Mushrooms. 1999; 1(2):139-146.
11. Celık G. *In vitro* antimicrobial and antioxidant properties of *Ganoderma lucidum* extracts grown in Turkey. Eur J Med Plants. 2014; 4(6):709-722.
12. Chang ST, Buswell JA. Mushroom nutriceuticals. World J Microbiol Biotechnol. 1996; 12(5):473-476.
13. Ghani a. Medicinal plants of bangladesh with chemical constituents and uses. Asiatic Society of Bangladesh 2nd edition, 2003.
14. Apu AS, Liza MS, Jamaluddin A, Howlader MA, Saha RK, Rizwan F *et al*. Phytochemical screening and in vitro bioactivities of the extracts of aerial part of *Boerhavia diffusa* Linn.. Asian Pac J Trop Biomed. 2012; 2(9):673-678.
15. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-ciocalteu reagent. Nat Protoc 2007; 2(4):875-877.
16. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol. 1966; 45(4):493-496.
17. Meyer B, Ferrigni N, Putnam J, Jacobsen L, Nichols D McLaughlin J. Brine Shrimp: a convenient general bioassay for active plant constituents. Planta Med 1982; 45(5):31-34.
18. Liao K, Yin M. Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems: Importance of the partition coefficient. J agric food chem. 2000; 48(6):2266-2270.
19. Kamra A, Bhatt AB. Evaluation of antimicrobial and antioxidant activity of *Ganoderma lucidum* extracts against human pathogenic bacteria. Int J Pharm Pharm Sci. 2012; 4(2):359-362.
20. Sherwani SK, Khan RU, Bhatti MI, Rao TA, Bokhari TZ, Sualeh M *et al*. Diuretic activity and cytotoxic study of various extracts of *Ganoderma lucidum*. World Appl Sci J. 2013; 26(7):964-967.
21. Leung SWS, Yeung KY, Ricky YLS, Man YK. Lingzhi (Ganoderma) research the past, present and future perspectives in Ganoderma: Genetics, Chemistry, Pharmacology and Therapeutics. Lin ZB (Ed), Beijing medical University Press, Beijing, 2002, 1-9.
22. Gao Y, Tang W, Gao H, Chan E, Lan J, Li X *et al*. Antimicrobial activity of the medicinal mushroom Ganoderma. Food Revs Int 2005; 21(2):211-229.
23. Sun T, Tang J, Powers JR. Antioxidant activity and quality of asparagus affected by microwave-circulated water combination and conventional sterilization. Food Chem 2007; 100(2):813-819.