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## HPTLC FINGERPRINTING, ANTIOXIDANT POTENTIAL AND ANTIMICROBIAL EFFICACY OF INDIAN HIMALAYAN LINGZHI: *GANODERMA LUCIDUM*

Vandana Kirar<sup>1</sup>, Shubhi Mehrotra<sup>2</sup>, Prem Singh Negi<sup>3</sup>, Shoma Paul Nandi<sup>2</sup> and Kshipra Misra<sup>\*1</sup>

Defence Institute of Physiology and Allied Sciences<sup>1</sup>, DRDO, Lucknow Road, Timarpur, Delhi, India

Amity Institute of Biotechnology<sup>2</sup>, Noida, UP., India

Defence Institute of Bio-Energy Research<sup>3</sup>, DRDO, Haldwani, Uttarakhand, India

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### Correspondence to Author:

Dr. Kshipra Misra,

Scientist 'F' and HOD,  
Department of Bio-Chemical Sciences,  
Defence Institute of Physiology and  
Allied Sciences, Defence Research  
Development Organization, Min. of  
Defence, Lucknow Road, Timarpur,  
Delhi-110 054, India.


E-mail: kmisra99@yahoo.com

**ABSTRACT:** *Ganoderma lucidum* (Fr.) Karst., known as medicinal mushroom and it contains several bioactive phytochemicals such as polysaccharides, nucleosides, alkaloids, coumarin, ergosterols, ganoderic acids, lactones, mannitol, organic germanium, triterpenoids, unsaturated fatty acids, vitamins and minerals which are well known for their pharmacological properties. In the present investigation adenine, adenosine and uracil content of the aqueous and alcoholic lyophilized extract of dried *G. lucidum* powder of Indian Himalayan Region (IHR) were determined by HPTLC. Further, phytochemical analysis (total polyphenols, total flavonoids, reducing power, antioxidant potentials), antioxidant and antimicrobial efficacy of both extracts against pathogenic strains like *Vibrio cholerae*, methicillin resistant *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus* was also evaluated. The adenine, adenosine and uracil content of aqueous extract was found to be higher than the alcoholic extracts. Both extracts were identified as rich source of flavonoids, polyphenols, reducing power and antioxidants. A significant antimicrobial activity was observed in both the hydro alcoholic and aqueous extract against all the pathogenic strains tested with MIC value of 2-4 mg/ml for the hydro alcoholic extract and of 2-5 mg/ml for the aqueous extract. The present study concludes high pharmacological potential of *G. lucidum*.

**INTRODUCTION:** Mushrooms have been valued throughout the world as both food and medicine for thousands of years<sup>1</sup>. *G. lucidum* is a member from the family of Ganodermataceae<sup>2</sup> and since, 100 BC it is extensively into clinical use<sup>3</sup>.

It is a magical healing mushroom and regarded highly by the ancient people to cure many kinds of diseases. It is believed to be the king of herbal medicine. Since, it is one of the world's oldest medicinal herbs with a belief that it promotes health, the mushroom has created deep interest among researchers of modern times<sup>4</sup>.

The shiny and brilliant *Ganoderma* encompasses several bioactive components which are mainly located in the varnished fruiting body, mycelium and spores. According to the reports,

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polysaccharides and triterpenes are the two major groups present in the mushroom apart from these other phytoconstituents such as phenols, amino acids, steroids, lignin, Vitamins, mycins, nucleotides, nucleosides, coumarin and ergosterol are also present<sup>4, 5, 6, 7, 8</sup>. The phytoconstituents of *G. lucidum* helps to improve blood circulation, eliminate fatigue, enhance energy, strengthen immune system and discard toxins.

It is effective against various pharmacological activities<sup>9, 10</sup> including anti-tumor and anti-inflammatory<sup>11, 12</sup>, antimicrobial<sup>13, 14, 15</sup>, anti-aging activities<sup>16</sup> etc. Although, the crude extracts and chemical components of *G. lucidum* are well known to contribute to various biological and medicinal functions including hypoglycemic, hepatoprotective, antitumour, carcinostatic, anti-mutagenic, anti-inflammatory and anti-viral properties<sup>9, 17, 18</sup>, there is a paucity of the data regarding HPTLC fingerprinting and antimicrobial efficacy of *G. lucidum*.

Hence, the present study was undertaken with the aim to explore the HPTLC Fingerprinting, phytochemical analysis (total polyphenols, total flavonoids, reducing power, antioxidant potentials) and antimicrobial efficacy of whole body aqueous and hydro alcoholic extracts of *G. lucidum* from Indian Himalayan Region (IHR) against multi drug resistant pathogenic bacterial strains like *Vibrio cholerae*, methicillin resistant *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus*.

#### METHODS AND MATERIALS:

All the chemicals and solvents were of high analytical grade, obtained from S.D. Fine chemical, India. Adenine, adenosine, Uracil, 1,1'-diphenyl-2-picrylhydrazyl [DPPH], rutin, 3, 4, 5-trihydroxybenzoic acid [gallic acid], TPTZ [2,4,6-tripyridyl-s-triazine, 6 Hydroxy-2,5,7,8-Tetramethyl chroman-2-Carboxylic acid [trolox], were purchased from Sigma-Aldrich Chemical Co., St. Louis, USA, and. Tryptone, yeast extract and sodium chloride were obtained from Fisher Scientific, India. Agar powder was obtained from Himedia, India.

#### Collection of plant material:

*G. lucidum* was collected during the rainy season (June) from wood logs and tree stumps from the

hilly regions (at an altitude of over 4000 m) of the North-West Himalayas at different location of Pithoragarh, Uttarakhand, India where the plant grows widely under natural conditions. Plant material (Voucher specimen xxx-2011) was characterized by Dr. Mousin, an ethnobotanist at the Defence Institute of Bio Energy Research, Haldwani, India. Only the mature fruiting bodies (seen as reddish-brown open caps) were selected, removed and washed with nanopure water, dried under shade in a clean, dust free environment and milled into powder using pestle and mortar.

#### Extract preparation:

The aqueous and alcoholic extracts were prepared by soaking powdered *G. lucidum* with nanopure water and 70 % ethanol (1:5 w/v) respectively at room temperature (25±1 oC). After 24 h, the supernatant was decanted and the residue re-soaked in fresh solvents. The process was repeated four times for complete extraction. Alcoholic content of the hydro alcoholic extract was evaporated using Rota vapor at 40 °C The supernatants of aqueous and alcoholic extraction (after alcohol removal) were pooled separately, filtered through muslin cloth and centrifuged at 8000g, 4°C for 10 min.

After centrifugation the supernatants obtained from aqueous and alcoholic extraction were frozen at -20oC and then lyophilized in Heto lyophilizer (HITOSICC, Heto-Holten A/S, Denmark). Lyophilized powder of the *G. lucidum* aqueous and alcoholic extracts (yield 10.7 and 9.54% w/w respectively) were stored at -20oC in an airtight dark plastic container until further analysis<sup>19</sup>. The HPTLC fingerprinting of each batch of the extract was carried out and maintained throughout the experiment, to avoid batch-to-batch variation.

#### Assays of non enzymatic antioxidants in *G. Lucidum*:

##### Total phenol content:

Total phenol content of *G. lucidum* extracts (aqueous and alcoholic) was determined with the Folin–Ciocalteu reagent<sup>20</sup>. A mixture of 150 µl of the extract (0.2 mg/ml), 2400 µl of nanopure water and 150 µl of 0.25 N Folin–Ciocalteu reagents was prepared and allowed to react for 3 min. Then 300 µl of 1.0 N Na<sub>2</sub>CO<sub>3</sub> solution was added into the reaction mixture. After incubation for 2 h at room

temperature, the absorbance relative to that of prepared blank was measured at 725 nm using a spectrophotometer (SmartSpec 3000, BIO-RAD, CA, USA). Gallic acid was used as a reference standard and the results were expressed as mg gallic acid equivalents/g extract.

#### **Total flavonoids content:**

For Total flavonoids content a mixture of 1.0 ml of *G. lucidum* extracts (aqueous and alcoholic) (1 mg/ml), 2.0 ml of nanopure water and 0.15 ml of 5% NaNO<sub>2</sub> was prepared and allowed to react for 6 min. Then 0.15 ml of 10% AlCl<sub>3</sub> solution was added and mixed thoroughly. After 6 min, 2.0 ml of 4% NaOH solution was added and allowed to stand for another 15 min. Absorbance of the mixture was measured at 510 nm versus prepared blank. Rutin was used as standard compound for the quantification of flavonoids content. Results were expressed in mg of rutin equivalents/g extract<sup>21</sup>.

#### **In vitro antioxidant assays:**

Antioxidant activity of plant extract cannot be evaluated by only a single method due to the complex nature of phytochemicals. Therefore, in the present study antioxidant activity of *G. lucidum* extracts (aqueous and alcoholic) was evaluated by three different assays viz. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP).

#### **ABTS assay:**

ABTS assay was conducted by the standard method<sup>22</sup> with some modifications. To produce the radical cation ABTS<sup>•+</sup> 7 mmol/L ABTS diammonium salt and 2.4 mmol/L potassium persulfate were mixed in a volume ratio of 1:1, the reaction mixture was allowed to stand in the dark for 12 h at room temperature. The solution was then diluted by mixing 1 ml ABTS<sup>•+</sup> solution with 60 ml methanol to obtain an absorbance of 1.10 ± 0.02 units at 734 nm using the spectrophotometer. ABTS<sup>•+</sup> solution was freshly prepared for each assay. Then 150 µl of *G. lucidum* extracts (aqueous and alcoholic) each (0.1 mg/ml) was allowed to react with 2850 µl of the ABTS<sup>•+</sup> solution and the absorbance was measured at 734 nm after 2 h using the spectrophotometer. The standard curve was

linear between 25 and 150 ppm Trolox. Results are expressed in mg of Trolox equivalents/g extract.

#### **DPPH assay:**

The free radical scavenging activity of *G. lucidum* aqueous and hydroalcoholic extracts on DPPH radical was determined by the method as described previously<sup>23</sup> with some modifications. Stock solution of DPPH was prepared by dissolving 24 mg DPPH with 100 ml methanol and then stored at -20 °C until use. The working solution was prepared by mixing 10 ml stock solution with 45 ml methanol to obtain an absorbance of 1.10 ± 0.02 units at 515 nm using the spectrophotometer. Then 150 µl of the extracts (0.2 mg/ml) was added to 2850 µl of DPPH working solution. The reaction mixture was vortexed thoroughly and allowed to stand for 2 h in dark. Absorbance was measured at 515 nm and samples were analyzed in triplicate. The standard curve was linear between 25 and 200 ppm Trolox. Results are expressed in mg of Trolox equivalents/g extract.

#### **FRAP assay:**

The FRAP assay was carried out according to the method as described previously<sup>24</sup>. The stock solutions included 300 mM acetate buffer (3.1 g C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub>·3H<sub>2</sub>O and 16 ml C<sub>2</sub>H<sub>4</sub>O<sub>2</sub>), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-*s*-triazine) solution in 40 mM HCl and 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml FeCl<sub>3</sub>·6H<sub>2</sub>O. The temperature of the solution was raised to 37°C before use. Then 150 µl of the *G. lucidum* extracts (aqueous and alcoholic) (0.1 mg/ml) was allowed to react with 2850 µl of the FRAP reaction solution for 30 min in dark. Readings of the colored product (ferrous tripyridyltriazine complex) were measured at 593 nm using the spectrophotometer. The standard curve was linear between 25 and 150 ppm Trolox. Results are expressed in mg of Trolox equivalents/g extract.

#### **Determination of reducing potential:**

The reducing potential was determined<sup>25</sup> with some modifications. Briefly, 1.0 ml of the *G. lucidum* extracts (aqueous and alcoholic) (0.2-1.0 mg/ml) was mixed with 0.2 M phosphate buffer, pH 6.6 (2.5 ml) and 1% potassium ferricyanide (2.5

ml). The mixture was incubated in water bath at 50 °C for 20 min. Then 10% trichloroacetic acid (2.5 ml) was added and centrifuged at 3000 rpm for 10 min. The upper layer of supernatant (2.5 ml) was mixed with nanopure water (2.5 ml) and 0.1% ferric chloride solution (0.5 ml). Absorbance was measured spectrophotometrically at 700 nm.

### Identification and quantification of marker compound by HPTLC:

#### Standard stock solution preparation and Calibration:

For standard stock solution preparation 8mg of adenosine, 1 of mg adenine and 1mg of uracil were accurately weighed and transferred to a 10 ml volumetric flask containing 5ml methanol. The mixture was sonicated for 10 mins and diluted to 10 ml with methanol. Different volumes of the diluted solution (0.1, 0.5, 1.0, 5.0 and 10.0 µL) were applied in duplicate on respective plate to furnish 0.08µg to 8µg of adenosine and 0.01µg to 1µg of adenine and uracil respectively. Peak area data and the corresponding amounts were treated by linear least square regression analysis.

#### Sample solution preparation:

The lyophilized aqueous and hydroalcoholic *G. lucidum* extracts obtained from maceration (100 mg) were accurately weighed and transferred to a 5 mL volumetric flask containing 2 mL methanol. The mixture was sonicated for 15 mins and diluted to 5mL with methanol. The resulting solution was centrifuged at 3000 rpm for 10 mins and the supernatant was analyzed for Uracil, Adenine, Adenosine content. The supernatant solution (15 µL) was applied to a TLC plate followed by development in mobile phase and densitometric scanning.

#### Chromatography:

Chromatography was performed on 20x10cm aluminium backed silica gel 60 F<sub>254</sub> TLC plates. Before use, the plates were washed with methanol then dried in an oven. Sample were applied to the plates as, 5 mm bands, by spraying with Camag Linomat V sample applicator equipped with 100µl syringe (Hamilton). Ascending development of the plate, migration distance 85mm, was performed at 25±2<sup>0</sup>C with chloroform, methanol and formic acid in the ratio of 8:2:0.8 (v/v), as mobile phase in

Camag twin-trough chamber previously saturated with mobile phase for 10 min. The average development time was 25 min. After development the plate was dried and scanned at 254nm with a Camag TLC scanner III equipped with win cats software using deuterium light source; the slit was 5.00x 0.45 mm.

#### Antimicrobial efficacy studies:

**Microorganisms tested:** Antibacterial activity and the MIC value of the plant extract was evaluated *in-vitro* against gram positive bacteria- methicilin resistant *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and a gram negative bacteria *Vibrio cholerae*. The *V. cholerae* strain was obtained from National Institute of Cholera and Enteric Diseases, Kolkata, India. *S. aureus*, *B. cereus* and *B. subtilis* were obtained from NuLife Consultants and Distributors Pvt. Ltd., New Delhi. Strains of bacteria were grown and maintained at 4 °C on Luria Bertani (LB) media plates and were sub-cultured (24 h, 37 °C) prior to use. Purity of the cultures were checked regularly<sup>26</sup>.

#### Determination of antimicrobial activity and MIC of the plant extract:

The primary screening based on the susceptibility of the strains to the plant extracts was done by the agar well diffusion method<sup>27</sup>. 100 µl of standardized bacterial inoculum (0.5 McFarland turbidity equivalent to 5 x 10<sup>8</sup> cfu/ml) was spread evenly using a sterile glass spreader on media plates. The sample wells were bored on the agar plates using a sterile cork borer (8.0 mm diameter). The extracts with different concentrations (1, 2, 3, 4, 5, 6, 7 and 8 mg/ml) were loaded in the wells. 70 % ethanol and sterilized distilled water was used as negative control. For the observation of results, the plates were incubated for 24 h at 37 °C. The zone of inhibition was measured in millimeter for the evaluation of the antimicrobial activity and the MIC was determined as the minimum concentration of the extract capable of inhibiting the growth of organism on the media plate. The experiment was done thrice.

#### Statistical analysis:

Data are expressed as mean ± SE and statistical significance between experimental and control values was analyzed by ANOVA followed by

Dunnett's test using Graph Pad Prism 2.01 (Graph Pad Software Inc., La Jolla, CA). A  $P$ -value  $< 0.05$  was considered statistically significant.

## RESULTS: Assays of non-enzymatic antioxidants of *G. lucidum* extracts: Table1

TABLE 1: ANALYSIS OF TOTAL PHENOL AND TOTAL FLAVONOID CONTENT OF *G. LUCIDUM* EXTRACTS

Sample	Total Phenolic content (mg gallic acid /g of extract)	Total Flavonoid content (mg rutin/g of extract)
Aqueous extract	56.83±1.31	100.381±3.53
Hydro alcoholic extract	62.18±2.08	162.71±4.25

values are the mean  $\pm$  SEM of three observations

**In vitro antioxidant potential of *G. lucidum* extracts:** Table 2 shows the *in-vitro* antioxidant potential of aqueous and hydroalcoholic extracts of

*G. lucidum* assayed by DPPH, ABTS and FRAP assays. Both extracts exhibited potent antioxidant activity.

TABLE 2: ANTIOXIDANT ACTIVITY DETERMINATION OF *G. LUCIDUM* EXTRACTS

Sample	DPPH	ABTS (mg Trolox/g of extract)	FRAP
Aqueous extract	232.13±4.6	195.62±4.3	17.4±3.1
Hydro alcoholic extract	261.32±5.1	202.15±5.3	12.65±4.6

values are the mean  $\pm$  SEM of three observations

## Reducing potential of *G. lucidum* extracts:

**Fig. 1** depicts the reducing power of *G. lucidum* extracts in comparison to ascorbic acid. Both the extracts have shown some degree of reducing power; however, it was seen that their reducing power was lesser to ascorbic acid which is known to be a strong reducing agent.

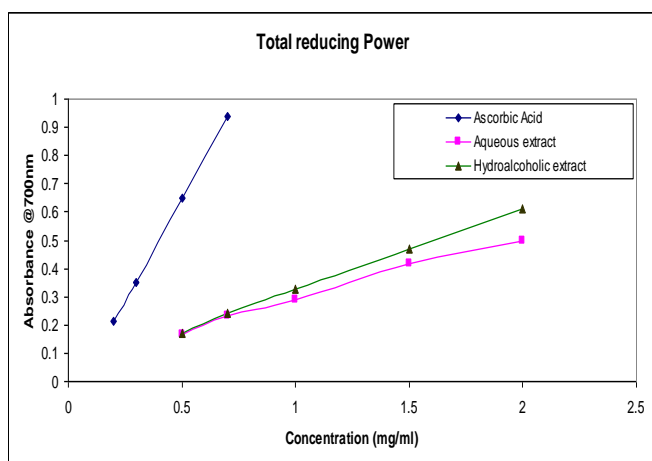


FIG.1: REDUCING POWER OF ASCORBIC ACID, AQUEOUS AND HYDRO ALCOHOLIC EXTRACT OF *G. LUCIDUM*

Similar to the antioxidant activity, the reducing power of the extract increased with the increasing amount of the extract; the equation of reducing

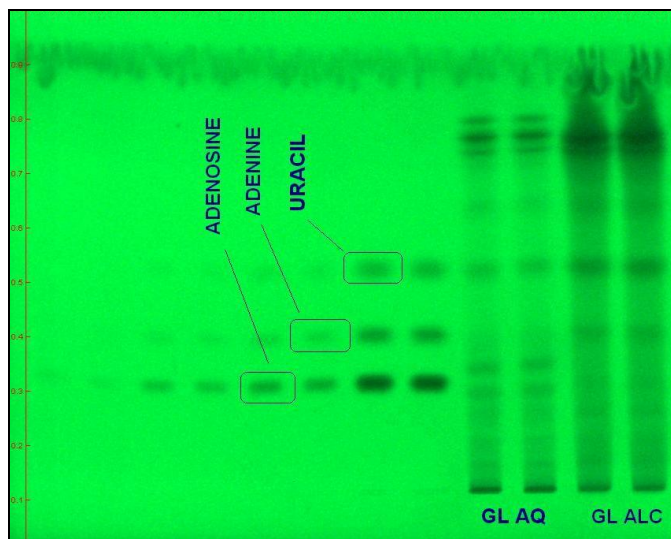
power ( $y$ ) and amount of extract ( $x$ ) was  $y = 0.4761x - 0.0301$  ( $r^2 = 0.993$ ) for aqueous extract and  $y = 0.5749x - 0.0301$  ( $r^2 = 0.998$ ) for hydro alcoholic extract, showing that the reducing ability was significant to the amount of extract. The reducing power of ascorbic acid and *G. lucidum* extracts were in the following order: ascorbic acid  $>$  hydro alcoholic extract  $>$  aqueous extract

## Identification and quantification of marker compounds by HPTLC:

Identification and quantification of marker compounds was performed on the basis of the coinjections and retention time matching with standards. Different concentrations of standard stock solution and aqueous extract of *G. lucidum* were subjected to HPTLC analysis using the mobile phase chloroform, methanol and formic acid (8:2:0.8, v/v).

**Fig. 2** shows the HPTLC fingerprints developed from adenine, adenosine and uracil standards in different concentrations, aqueous and hydroalcoholic extract of *G. lucidum*. The  $R_f$  of adenosine, adenine and uracil was found to be 0.28, 0.36 and 0.55 respectively. The correlation coefficient of 0.98 for adenosine and 0.99 for

adenine and uracil respectively was indicative of good linear dependence of peak area on concentration. The percentage of active marker compounds was calculated using peak area, and the adenosine, adenine and uracil content (mg/g of dry extract) respectively in the aqueous and hydroalcoholic extract of *G. lucidum* is shown in **Table 3**. The HPTLC densitometric scan obtained from adenine, adenosine and uracil standards along with aqueous and alcoholic extract of *G. lucidum*, the HPTLC scan of adenine adenosine and uracil standard; *G. lucidum* aqueous and alcoholic extract are shown in **Figs. 3A-D** respectively.

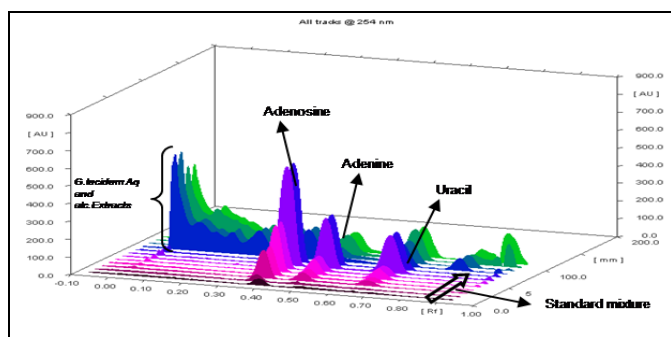


**FIG.2:** HPTLC PLATE AT  $\lambda$  254 OF ADENOSINE, ADENINE AND URACIL STANDARDS AND *GANODERMA LUCIDUM* AQUEOUS AND HYDROALCOHOLIC EXTRACTS IN DUPLICATE

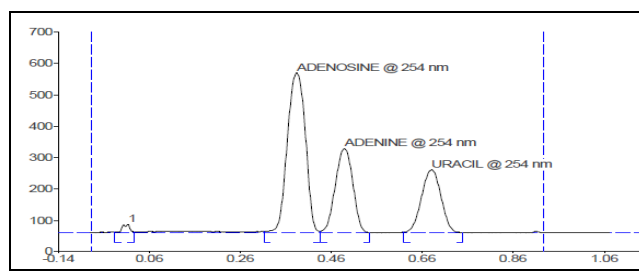
**TABLE 3:** QUANTIFICATION OF BIOACTIVE COMPONENTS IN *GANODERMA LUCIDUM* AQUEOUS EXTRACT

Bioactive component	Concentration (mg/g dry extract)	
	Aqueous	Alcoholic
Adenosine	5.07 ± 0.53	3.02 ± 0.26
Adenine	0.71 ± 0.07	0.29 ± 0.04
Uracil	1.64 ± 0.19	0.84 ± 0.05

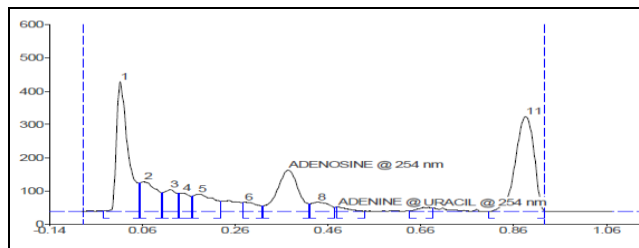
values are the mean ± SEM of three observations



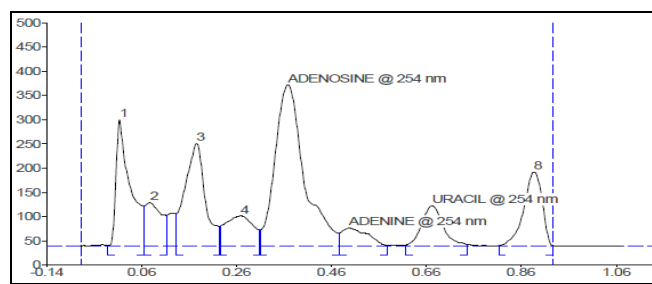
(A)



(B)



(C)



(D)

**FIG.3:** DENSITOMETRIC SCAN OF STANDARD TRACK AND *GANODERMA LUCIDUM* EXTRACTS (FIG 3A); HPTLC CHROMATOGRAMS OF ADENINE, ADENOSINE AND URACIL STANDARD (FIG 3B); HPTLC CHROMATOGRAM OF AQUEOUS EXTRACT OF *GANODERMA LUCIDUM* (FIG 3C); HPTLC CHROMATOGRAM OF ALCOHOLIC EXTRACT OF *GANODERMA LUCIDUM* (FIG 3D).

**Antimicrobial activity and MIC of the plant extract:**

**Fig. 4** shows the antibacterial activity of the aqueous and the hydro alcoholic extracts of *G. lucidum* as determined by agar cup assay. The aqueous extract possessed strong antibacterial activity which was highest against *B. cereus* and *S. aureus* strains and least against *B. subtilis* and *V. cholerae* strains. Based on the initial screening, the MIC was determined as the minimum concentration of the extract inhibiting growth of the pathogenic strain on the agar plate.

The MIC was 2 mg/ml for *G. lucidum* aqueous extract against *B. cereus* and *S. aureus* showing similar and greater sensitivity (**Fig 5**). However, *B. subtilis* was the most resistant pathogenic strain

showing a higher MIC value of 5 mg/ml for the aqueous extract. *V. cholerae* showed 3 mg/ml of MIC for the aqueous extract. For the hydro alcoholic extract, both *B. cereus* and *S. aureus* strains turned out to be sensitive to the extract by exhibiting the MIC value of 2 mg/ml. However, *V. cholerae* and *B. subtilis* strains exhibited MIC values of 3 and 4 mg/ml respectively with the hydro alcoholic extract (Fig 6).

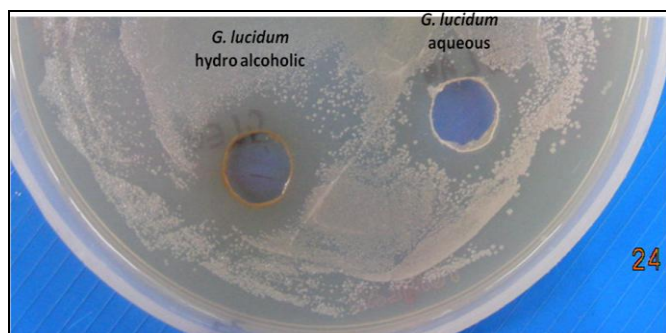


FIG. 4: ANTIBACTERIAL ACTIVITY DETERMINATION BY AGAR CUP ASSAY OF *GANODERMA LUCIDUM* (HYDRO ALCOHOLIC AND AQUEOUS) EXTRACT AGAINST *BACILLUS CEREUS* STRAIN. (ZONE OF INHIBITION OF HYDRO ALCOHOLIC =19 MM AND AQUEOUS =17 MM).

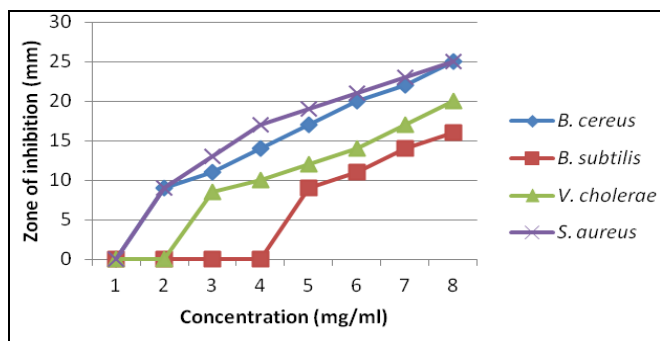


FIG. 5: MINIMUM INHIBITORY CONCENTRATION (MIC) OF *GANODERMA LUCIDUM* (AQUEOUS) EXTRACT AGAINST THE BACTERIAL STRAINS, *BACILLUS CEREUS*, *BACILLUS SUBTILIS*, *VIBRIO CHOLERAE* AND METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*.

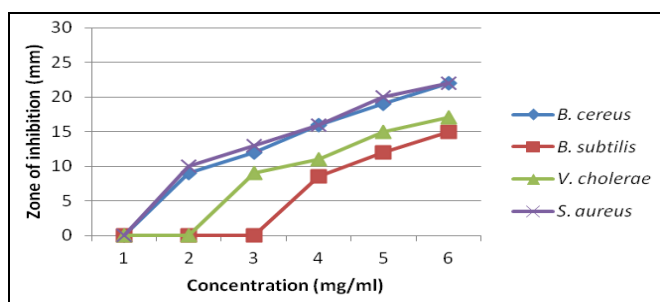


FIG.6:MINIMUM INHIBITORY CONCENTRATION (MIC) OF *GANODERMA LUCIDUM* (HYDRO ALCOHOLIC) EXTRACT AGAINST THE BACTERIAL STRAINS, *BACILLUS CEREUS*, *BACILLUS SUBTILIS*, *VIBRIO CHOLERAE* AND METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*.

**DISCUSSION:** *Ganoderma lucidum*, a basidiomycete white rot fungus, has long been prescribed to prevent and treat various human diseases, particularly in China, Japan, and Korea. Several classes of bioactive substances have been isolated and identified from *G. lucidum*, such as polysaccharides, triterpenoids, nucleosides, sterols, alkaloids and rare minerals<sup>2, 4</sup>. In the present study, HPTLC Fingerprinting, phytochemical analysis (total polyphenols, total flavonoids, reducing power, antioxidant potentials) of *G. lucidum* aqueous and hydroalcoholic lyophilized extract from Indian Himalayan Region (IHR) were evaluated. Further its antimicrobial activity was evaluated.

#### Assays of non enzymatic antioxidants in *G. lucidum*:

Aqueous and hydroalcoholic extract of *G. lucidum* was found to be rich in total phenolics and flavonoids contents. Phenolics are the major plant compounds having potential antioxidant activity. This activity is believed to be mainly due to redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides<sup>28</sup>. Results obtained in the present study revealed the presence of substantial quantity of these phenolic compounds in the GL extracts<sup>9, 11</sup>. Some of the reported pharmacological effects of GL could be attributed to the presence of these valuable constituents.

#### *In vitro* antioxidant potential of *G. lucidum*:

The results showed that the extracts of *G. lucidum* showed potent radical scavenging activity, which is in concordance with earlier reports<sup>9, 10, 14</sup>.

#### Reducing potential of *G. lucidum* extracts

The reducing power of *G. lucidum* extracts, which may be a significant reflection of the antioxidant activity, was determined using a modified iron (III) to iron (II) reduction assay. It will be significant to mention that earlier reports have demonstrated the higher antioxidant power of *G. lucidum* extracts<sup>9, 10, 14</sup>. In this study also a good antioxidant power in both the extracts was observed.

#### Identification and quantification of marker compounds by HPTLC: The extract used in the

study was characterized with analysis of marker compounds (adenine, adenosine and uracil) by HPTLC fingerprinting. Nucleosides are involved in the regulation and modulation of various physiological processes in the body, which produce function through purinergic and/or pyrimidine receptors. In addition to being precursors in nucleic acid synthesis, nucleotides were reported to enhance immune response<sup>29</sup>, influence metabolism of fatty acids contributes to iron absorption in gut and improve gastrointestinal tract repair after damage. Furthermore, nucleosides and nucleobases in *G. lucidum* were reported to be capable of inhibiting platelet aggregation and lowering the elevated serum aldolase level of experimental model mice<sup>30</sup>. It had been suggested that the increased dietary nucleoside-nucleotide mixture might be associated with decreases in the age-induced deterioration of brain morphology and certain memory tasks<sup>31</sup>.

#### **Antimicrobial activity and MIC of the plant extract:**

The antimicrobial activity exhibited marked zone of inhibition in comparison to controls, indicating the antimicrobial potential of *G. lucidum* extracts. The antibiotic resistance amongst many varieties of organisms pose a major threat to the treatment for various infectious diseases. Hence, mushroom derived antimicrobial substances have received considerable attention in recent years<sup>12, 32</sup>. Further some studies also demonstrated that *G. lucidum* contained antibacterial constituents that are able to inhibit gram-positive and, or gram-negative bacteria<sup>33</sup>.

The aqueous extract from the carpophores of *G. lucidum* inhibited 15 types of gram positive and gram-negative bacteria<sup>34</sup>. It is apparent from the present result that extracts from *G. lucidum* could be employed to combat several diseases caused by these pathogenic microorganisms. The antibiotic resistance amongst many varieties of organisms pose a major threat to the treatment for various infectious diseases.

Hence, mushroom derived antimicrobial substances have received considerable attention in recent years. A study<sup>35</sup> have shown a high MIC value of 8 mg/ml of the chloroform extract for *S. aureus* and

*B. subtilis* strains which is quite high in comparison to MIC value obtained by us indicating that the aqueous and the hydro alcoholic extracts possess more potential as an antibacterial agent at lower concentrations against the multi drug resistant pathogens.

**CONCLUSION:** *G. lucidum* is one of the most beautiful mushrooms known in traditional Chinese medicines with nucleosides with bioactive ingredients<sup>29, 36</sup>. Nucleosides (adenosine, adenine and uracil) have been identified and quantified by HPTLC in both aqueous and hydro alcoholic extracts. The present study concludes the presence of sufficient amount of the total phenol and flavonoid content in the *G. lucidum*, which is in accordance with the observed antioxidant activity and suggests high pharmacological potential of *G. lucidum*.

The process of antimicrobial activity is promoted by several natural and plant products, which are composed of active principles like flavonoids, triterpenes, alkaloids, tannins, nucleosides, polysaccharide and other bioactive molecules. These agents usually influence one or more phases of the life cycle of microorganisms. It is apparent from the present result that extracts from *G. lucidum* have marked antioxidant potential along with significant antimicrobial activity and they could be employed to combat several diseases caused by these pathogenic microorganisms. On the basis of this study it can be further concluded that the *G. lucidum* can be effectively used as an indigenous herbal preparation of Indian Himalayan Region with good antimicrobial and antioxidant properties.

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