Research Article

Evaluation of antibacterial activity of different *Ganoderma lucidum extracts*

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Abstract:

Bioproducts of Mushroom have multi beneficial effects for human welfare. Medicinal mushrooms are widely used as traditional medicinal ingredients for the treatment of various diseases and related health problems. Most of the medicinal extracts from mushroom are different forms of polysaccharides which strengthens the immune system with little or no side effect. Medicinal mushroom research has focused on discovery of compounds that can modulate positively or negatively the biological response of immune cells.

The antimicrobial activity of various solvent extracts (40µg/ml) of *Ganoderma lucidum* was tested against six species of bacteria: *Escherichia coli* (MTCC-443), *Staphylococcus aureus* (MTCC-737), *Klebsiella pneumoniae* (MTCC-2405), *Bacillus subtilis* (MTCC-1789) *Salmonella typhi* (MTCC-531) and *Pseudomonas aeruginosa* (MTCC-779). Acetone extract exhibited maximum antibacterial activity (31.60±0.10), while the most susceptible bacterium observed was *Klebsiella pneumoniae*.

Key Words: *Ganoderma lucidum*, extraction, bioactive compounds, antibacterial activity.

Introduction:

Ganoderma lucidum, a mushroom, is one of the most famous traditional Chinese medicinal herb. One interesting aspect of its performance is antimicrobial effect due to the extracts derived from this mushroom which contain bacteriolytic enzyme, lysozyme and acid protease (Klaus & Miomir, 2007). The mushroom attracts international attention as a valuable herb due to the wide variety of its biological activities, such as antitumor, immunomodulatory, cardiovascular, respiratory, antihepatotoxic and antinociceptive (active against pain) effect (Ha et al, 2000; Chang & Mshigeni, 2001). It's major compounds with significant pharmacological activities are ganoderic acid, triterpenes and polysaccharides. It is interesting that during the last three decades; more than 150 triterpenes (Kim & Kim, 2002; Fang & Zhong, 2002) and more than 50 carcinostatic polysaccharides have been isolated and are known to be unique compounds in this mushroom. Therefore, *G. lucidum* products with different triterpenes and polysaccharides or combinations of these two groups are most likely to result in different pharmacological activities (Leung et al, 2002). A new class of compounds with nutritional and medicinal features extractable from either the mycelium or the fruiting bodies of mushrooms have been referred to as "mushroom nutraceuticals". *G. lucidum* is rich in mushroom nutraceutical components with potential therapeutic values (Chang & Buswell, 1996).

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Material and Methods:

The wood rotting fungal species of *Ganoderma* was collected from R.D. University Campus, Jabalpur from the exposed dead trunk and roots of *Mangifera indica*. Fruiting bodies were dried and specimen deposited in Mycological Research laboratory, (Fungal Germplasm Culture Collection, Accession number is FGCC-105) R.D. University, Jabalpur. The fresh culture of *G. lucidum* was obtained by tissue culture technique (Oei, 2005).

Extraction of bioactive compounds from fruiting bodies of *G. lucidum:*

In the present study, the mushroom material was grounded to a fine powder with the help of pestle and mortar. Ten gram of mushroom powder was subjected to Soxhlet extraction using micro Kjeldahl apparatus (ASGI, India) for 10 hours using 100 ml each of the following solvents viz., ethyl alcohol, methanol, acetone and distilled water. The extracts were recovered by filtration and kept at 40^0C for further analysis (Dulger & Gonuz, 2004). All the solvent extracted fractions were subjected to *in vacuo* desiccation at 40°C in a rotary vacuum evaporator (Buchi R - 300 Rotavapor, Buchi Co. Germany) to

remove any traces of solvents and to obtain residues. The test residues were prepared as stocks using distilled water (40µg/ml) and were tested for their antibacterial activity by filter paper disc diffusion technique.

Micro-organisms tested:

From Microbial Type Culture Collection (MTCC), Chandigarh, six human pathogenic bacteria- *Escherichia coli* (MTCC-443), *Staphylococcus aureus* (MTCC-737), *Klebsiella pneumoniae* (MTCC-2405), *Bacillus subtilis* (MTCC-1789). *Salmonella typhi* (MTCC-531) and *Pseudomonas aeruginosa* (MTCC-779) were obtained (Accession numbers are depicted in brackets). Antibacterial activity was measured in terms of Inhibition Zone size (in mm) obtained after the incubation at 37ºC±2ºC for 24 hours.

Antibacterial activity:

Antibacterial activity of mushroom extract was carried out by the filter paper disc diffusion technique (Dulger & Gonuz, 2004). The bacterial colony was transferred into a tube containing 5 to 6 ml of Nutrient broth (HIMEDIA, India) and incubated at 35±2ºC. To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard was used (where turbidity was 0.5 which is equal to approximately 1 to 2×10^8 cfu/ml) of bacterial cells. One millilitre inoculum suspension of known turbidity was applied on the dried surface of prepared Muller-Hinton Agar plate (HIMEDIA, India). The inoculated plates were left for 15-20 minutes at room temperature. Each disc was 6 mm in diameter. As reference, antibiotic Gentamycin sulphate (40µg/ml) was used. All the plates were incubated in a Biological Oxygen Demand incubator (YORCO, India) at 35º±2ºC. After 24 hours each plate was examined.

Minimum inhibitory concentration (MIC) is defined as the lowest concentration which results in maintenance or reduction of inoculum viability over a period of 24 hours (Carson et al, 1995). During the course of investigation, MIC was determined for the acetone fraction of *G. lucidum* on selected test bacteria as acetone extracted fraction exhibited maximum zone of inhibition as determined by disc diffusion method (Table II).

Statistical analysis:

All experiments were performed thrice in completely randomized design (CRD) each, with three replications per treatment (antibacterial activity). The data was subjected to analysis of variance (ANOVA) using Genstat statistical software. Means of three observations were compared with Duncan's Multiple Range Test (DMRT) at P0.≤05 for determining the statistical significance.

Results:

Result of the antibacterial activity of different extracts of concentration (40µg/ml) of *G. lucidum* was determined by disc diffusion method as shown in the Table I.

It is apparent from the table that acetone extract of the strain possessed strong antibacterial activity which was most inhibitory against *K. pneumoniae* (31.60±0.10). The acetone extract was equally inhibitory against *E. coli* (27.40±0.19), *B. subtilis* (21.00±0.00) and *S. typhi* (20.60±0.14). But it was greatly reduced in case of *S. aureus* (18.00±0.20) and *P. aeruginosa* (10.20±0.14) at the same concentration. Methanolic extract of *G. lucidum* was equally inhibitory to all the bacterial strains and exhibited antibacterial effect. Maximum inhibition halos were observed against *K. pneumonia*e (21.30±0.06) and *E. coli* (20.10±0.20). The water and ethanolic extract were found to be not much effective against all the strains.

The lowest MIC values were demonstrated by acetone extract against *K. pneumoniae* (4.33±0.33), followed by *E. coli* (8.17±0.48) and *B. subtilis*.(14.00±0.46); moderate values in case of *S. aureus* (19.00±0.00) and the highest MIC values were exhibited against *P. aeruginosa* (21.30±0.34) and *S. typhi* (20.80±0.87) as depicted in Table II.

Discussion:

Many antimicrobial compounds such as terpenes, lectins, polysaccharides etc. act on the bacterial cytoplasmic membrane (Lin & Chou, 1984; Yang et al, 2002). Various extracts of *G. lucidum* have been found to be equally effective when compared with gentamycin sulphate. Dulger & Gonuz (2004) reported the antimicrobial properties of 4 different extracts of macrofungus (*Cantharellus cibarius*) against 50 important human pathogens. He observed good antimicrobial activity with ethanol and acetone extracts against most of the pathogens. Cowan (1999) reported that the most active components are generally water insoluble, hence it is expected that low polarity organic solvents would yield more active extracts. In the present study the aqueous extract exhibited least

Table I: Showing antibacterial activity of different extracts of *Ganoderma lucidum* by Filter Paper Disc Diffusion Method.

Test Bacteria	Zone of inhibition (mm) (Mean ± SD)				
	Methanol (40µg/ml)	Ethanol (40µg/ml)	Acetone (40µg/ml)	Distilled Water (40µg/ml)	Gentamycin sulphate (40µg/ml)
B. subtilis **(MTCC 1789)**	19.00 ± 0.14[a]	10.00 ± 0.27[ab]	21.00 ± 0.00[a]	8.00 ± 0.29[ab]	22.20 ± 0.14[b]
S. aureus **(MTCC 187)**	16.30 ± 0.20[b]	9.00 ± 0.18[ab]	18.00 ± 0.20[d]	8.00 ± 0.12[ab]	22.00 ± 0.18[b]
S. typhi **(MTCC 531)**	17.00 ± 0.12[b]	10.00 ± 0.26[a]	20.60 ± 0.14[a]	7.00 ± 0.18[bc]	32.00 ± 0.12[a]
E. coli **(MTCC 1591)**	20.10 ± 0.20[a]	10.60 ± 0.28[a]	27.40 ± 0.19[c]	8.30 ± 0.22[a]	31.50 ± 0.09[a]
K. pneumoniae **(MTCC 2405)**	21.30 ± 0.06[a]	11.30 ± 0.24[a]	31.60 ± 0.10[b]	9.40 ± 0.20[a]	35.50 ± 0.14[c]
P. aeruginosa **(MTCC 779)**	10.00 ± 0.26[c]	8.00 ± 0.16[b]	10.20 ± 0.14[e]	6.20 ± 0.22[c]	28 .00 ± 0.28[d]
Standard Error	0.78	0.63	0.75	0.59	0.55
LSD (P=0.05)	0.08	0.14	0.26	0.10	0.38

Table II: Showing Minimum Inhibitory Concentration (MIC) values for acetone extract.

Test Bacteria	MIC values (mg/ml)
Bacillus subtilis (MTCC 1789)	14.00±0.46[b]
Staphylococcus aureus (MTCC 187)	20.80±0.87[a]
Salmonella typhi (MTCC 531)	19.00±0.00[a]
Escherichia coli (MTCC 1591)	8.17±0.48[c]
Klebsiella pneumoniae (MTCC 2405)	4.33±0.33[d]
Pseudomonas aeruginosa (MTCC 779)	21.30±0.34[a]
Standard Error	0.85
LSD (P=0.05)	1.24

LSD=Least Significant Difference. Experiment was conducted three times. Values are Mean ± Standard Deviation. Mean value superscripted with same letter(s) along each column indicate no significant difference at P≤0.05 as determined by Duncan's Multiple Range Test (DMRT).

antibacterial activity than the organic extracts. According to Gao et al (2003), *Ganoderma lucidum* and other *Ganoderma* species more often in combination with chemotherapeutic agents have been used to treat various bacterial diseases. Its polysaccharide components were found to be the bioactive principle which play an important role in antibacterial activity. Smania et al (2007) observed maximum antibacterial activity of methyl australate, a derivative from *G. lucidum* against *E.coli* and *P. aeruginosa* followed by *S. aureus*. While least zone of inhibition was recorded for *Bacillus* species. Klaus & Miomir (2007) have studied the influence of various extracts isolated from *G. lucidum* on *E. coli*, *Bacillus* species, *S. aureus* and *Salmonella* species. The aqueous fruiting body extract showed maximum zone of inhibition against *Bacillus* species while least zone of inhibition was reported for *E. coli* and *Salmonella* species. Yoon et al (1994) investigated the bioactivity of aqueous extracts from the fruiting body of *G. lucidum* and found that the extracts also exhibited inhibitory activity towards *Bacillus* species. Extracts from *G. applanatum* (Smania et al 1999) and *G. pfeifferi* (Mothana et al 2000) have been shown to possess significant antibacterial activity against *E. coli*. Sheena et al (2003) reported that methanol extract of *G.lucidum* showed remarkable antibacterial activity against *E. coli*, *Salmonella* species and *B. subtilis*. Keypour et al (2008) investigated the antibacterial activity of a chloroform extract of *G. lucidum* from Iran. The results of disc diffusion tests showed that the chloroform extract had growth inhibitory effects on *B. subtilis* and *S. aureus*.

Smania et al (2007) observed MIC value of 2mg/ml for *E. coli* and *P. aeruginosa* while 1mg/ml in case of *S. aureus* and 0.25mg/ml for *Bacillus* species with *G. australate* extract. Significantly high MIC of

an aqueous *Ganoderma* extract against *B. subtilis* (3.5mg/ml), *Bacillus* species (3.5mg/ml) have been reported by Yoon et al (1994). Keypour (2008) recorded MIC value of 8mg/ml for *S. aureus* and *B. subtilis* with chloroform extract of *G. lucidum*. Results with present mushroom indicate the MIC values to be lower in comparison to MIC value obtained by other investigators, indicating that the acetone extract possesses more potential as an antibacterial agent at lower concentrations.

Resistance to antibiotics is emerging in a wide variety of organism and multiple drug resistant organisms pose a serious threat to the treatment of infectious diseases. Hence, mushroom derived antimicrobial substances have received considerable attention in recent years. It is apparent from the present study that mushroom extracts from *G. lucidum* could be employed to combat several diseases caused by pathogenic microorganisms.

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