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In vitro Comparative Evaluation of Antibacterial Activity of Fruiting Body and Mycelial Extracts of *Ganoderma lucidum* against Pathogenic Bacteria

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A wide variety of organisms are emerging as resistant to antibiotics, 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. In this study antagonistic effects of the methanol, acetone and water extracts of mycelia and fruiting body of *Ganoderma lucidum* were tested against seven bacterial species: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa, Enterobacter aerogenes*, *Klebsiella pneumoniae*, *and Salmonella typhimurium*. All the extracts exhibited various degree of inhibition against all test bacteria. Widest inhibitory zone (33mm) was obtained with mycelial acetone extract of *Ganoderma lucidum* against *Pseudomonas aeruginosa*. Lowest zone of inhibition (7mm) was observed with fruiting body aqueous extract against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Minimum inhibitory concentration (MIC) of acetone extract of fruiting body and mycelial extract was determined, for mycelial extract it ranged between 4 to 12mg/ml and for fruiting body extract it ranged between 15 to 35 mg/ml for test bacteria.

Key words: *Ganoderma lucidum*, Antibacterial activity, Bioactive molecules, Extraction.

Mushrooms are defined as "macrofungi" with distinctive fruiting bodies that are large enough to be seen by the naked eye and to be picked by hand. In recent years, more varieties of mushrooms have been isolated and identified, and the number of mushrooms being cultivated for food or medicinal purpose has been increasing rapidly[1]. Development of bacterial resistance to currently available antibiotics has made it necessary to search for new antibacterial agents; natural plant products are being investigated because medicinal plants have been widely used for treatment of many types of acute and chronic diseases and many plants with antimicrobial activity have been reported[2,3]. Several mushroom species belonging to the Polyporaceae family are now being regarded as the next candidate producers of valuable medicines[4]. *G.lucidum* (Curtis: Fr) Karst is a basidiomyceteous fungus used as a traditional medicine for more than 2000 years[5]. It's a popular remedy to treat many diseases like chronic hepatitis, nephritis, hypertension, hyperlipemia, arthritis, neurasthenia, insomnia, bronchitis, asthma, gastric ulcer, arteriosclerosis, leucopenia, diabetes, anorexia[6,4]. The fruiting body, mycelia and spores of *G.lucidum* contain approximately 400 different bioactive compounds, which mainly include polysaccharides, triterpenoids, fatty acids, nucleotides, protein/ peptides, sterols, vitamins, minerals etc[7,8,4,9,10]. These metabolites are reported to be responsible for the medicinal properties of

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this mushroom. Many natural products are active as anti- HIV agents[11], these compounds belong to a wide range of different structural classes viz, terpenes, polysaccharides, coumarins and flavenoids etc.[12]. Because of presence of some of these compounds (triterpenoids) in this mushroom, it has potent inhibitory activity against HIV. Some other constituents such as ganomycin, triterpenoids and aqueous extracts from *Ganoderma* species have a broad spectrum of *in-vitro* antibacterial activity against gram positive and gram negative bacteria[13, 14, 8, 15, 16, 17, 18]. This mushroom also has some antifungal compounds in it [19]. *Ganoderma lucidum* and other *Ganoderma* species are used to treat various bacterial diseases in combination with other therapeutic agents also[15]. The present study, for the first time reports the comparison of antimicrobial activity of fruiting body and mycelial extracts of *Ganoderma lucidum.*

MATERIALS AND METHODS

Fungal sample

The fruit bodies of *Ganoderma lucidum* used in this study were collected from different areas of Himachal Pradesh, and were identified on the basis of microscopic and macroscopic morphological traits with the standard description of Stamet 1993[6]. Mycelial biomass was obtained by inoculating 200ml of potato dextrose broth (Hi Media) with 6mm disc of inoculum from 7 days old plate of *G. lucidum* culture. Flasks were incubated at 28°C for 15 days and biomass was separated by filtration and was dried.

Test Organisms

In vitro antimicrobial susceptible studies were performed using seven human pathogenic bacteria (procured from MTCC Chandigarh): *Escherichia coli* (MTCC 739), *Staphylococcus aureus* (MTCC 737), *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC741*), Enterobacter aerogenes* (MTCC 111), *Klebsiella pneumoniae* (MTCC 109), *and S. typhimurium* (MTCC 98). Antibacterial activity of extracts was screened by filter paper disc diffusion method and activity was measured in terms of zone of inhibition size (mm) obtained after 24 h incubation at 37°C.

Preparation of bacterial inoculum

Test bacterial colonies were transferred in nutrient broth and were incubated at 37°C. In order to standardize the inoculums density for test, a $BaSO_4$ turbidity standard (equivalent to 0.5 Mc Farland std. = turbidity equals to 0.5 =1 to 2 x10 ^{8} cfu /ml) was used.

Preparation of fungal extract

The fruit bodies were cut into bits and dried at 40 ºC. These dried fruit bodies and dried mycelial biomass were pulverized in a blender. The extraction of the mushroom fruit bodies and mycelial biomass was carried out using three solvents (water, methanol and acetone). For water extraction, 1 litre of sterile distilled water was dispensed into conical flasks containing 100g of powdered mushroom fruit bodies and mycelial sample. These were allowed to stand for 72 h with intermittent agitation. For methanol and acetone extraction, 100g of the pulverized fruit bodies and mycelial biomass was separately soaked in 1 litre of absolute methanol and acetone in conical flasks. These were covered with aluminum foil and allowed to stand for 7 days for extraction. The mixtures were filtered using Whatman filter paper No. 1 and the filtrate was concentrated under a reduced pressure in a rotator evaporator until a semi solid substance was obtained. These were dried inside the crucible under a controlled temperature (45ºC) to obtain solid extract. The left residues were kept in refrigerator until used[20].

Antimicrobial activity of extracts

The concentration of the extracts used was 60 mg/ml and sterile distilled water was used to reconstitute the extract residues. For determination of antimicrobial activity sterile filter paper discs (6mm diameter) were soaked with the test extracts (60mg/ml) and dried at 40 ºC for 1 h. The discs were placed on bacteria seeded Muller Hinton Agar (Hi Media) plates and placed in the refrigerator for 12 h to allow the diffusion of the extracts into the growing medium. The plates were incubated for 24 h at 37ºC after which the zone of inhibition was observed and measured[20].

Minimum Inhibitory Concentration (MIC)

This study aimed in finding out the lowest concentration of acetone extract that will inhibit the growth of the test microorganisms. It was carried out by following the method described by Hirasawa *et al* (1999) [21]. Different concentrations (1- 40mg/ml) were prepared using sterile distilled water as the diluent. Filter paper disc diffusion method was used[22].

RESULTS

In this study antibacterial activity of methanol, acetone and aqueous extracts of *G.lucidum* was determined by disc diffusion method. The antimicrobial activity of samples varied according to the solvent. Results presented in Table I and Table II shows the antibacterial activity of different extracts of fruiting body and mycelial biomass respectively. Table III shows MIC value of acetone extract of fruit body and mycelial biomass. It is clear from the data presented in Table 1 and Table 2 that the acetone extract of *Ganoderma lucidum* showed maximum antibacterial activity followed by methanol and aqueous extract. Mycelial extract of the *G.lucidum* showed high antibacterial activity as compared to fruit body extract. Maximum inhibitor activity of acetone extract of mycelial biomass (Table 2) was shown against *P.aeruginosa* (33mm), followed by *E. coli* with zone size of 30mm. This extract showed equal inhibitory effect against *K. pneuminae* and *E. aerogenes* (24mm). A 14mm zone was shown for *B.subtilis* and least zone was shown against *S.aureus* (12mm) at the same concentration. In case of methanol extract, maximum activity was found against *K. pneuminae* (19mm) followed by *E. coli* (18mm). Aqueous extracts were found to be comparatively less effective against all bacterial strains. MIC of only acetone extract was determined because it exhibited maximum antagonistic activity. MIC value of mycelial biomass was found to be 4mg/ml against *P.aeruginosa* followed by *E.coli* with a value of 6 mg/ml. In case of fruit body MIC was 15 mg/ml for *P.aeruginosa* and *E.coli*.

Table 1. Antibacterial activity of various extracts of *Ganoderma lucidum* (fruiting body)

Test bacteria	Zone of inhibition (mm) Fruit body(60mg/ml)		
	Methanol	Acetone	Aqueous
S.aureus	14	10	7
E.coli	18	11	11
K.pneuminae	19	8	7
B.subtilis	17	13	9
Enterobacter	9	13	11
P.aeruginosa	11	16	9
S.typhimurium	17	19	10

Table 2. Antibacterial activity of various extracts of *Ganoderma lucidum* (mycelial biomass)

Test bacteria	Zone of inhibition (mm) Mycelial biomass(60mg/ml)		
	Methanol	Acetone	Aqueous
S.aureus	16	12	12
E.coli	22	30	10
K.pneuminae	16	24	8
B.subtilis	11	14	9
Enterobacter	15	24	12
P.aeruginosa	24	33	12
S.typhimurium	10	14	9

Table 3. Showing MIC values for acetone extract

Test Bacteria	MIC value (mg/ml)	
	Mycelial biomass	Fruit body
S.aureus	11	30
E.coli	6	15
K.pneuminae	8	25
B.subtilis	6	20
Enterobacter	12	35
P.aeruginosa	4	15
S.typhimurium	7	20

DISCUSSION

Resistant bacteria are emerging world wide as a serious threat to the outcome of common infections in community and hospitals. Therefore, novel antimicrobial agents from different biological sources are continuously sought. Rosa *et al.* (2003) detected 14 mushroom isolates with high antimicrobial activity against target micro-organisms[23]. Zjawiony (2004) observed that 75% of polypore fungi that have been tested show strong antibacterial activity[24]. *Ganoderma lucidum* was reported to be best among other *Ganoderma* species that generally exhibited high antagonistic activity against test bacteria[25]. Recently, more studies demonstrated that *G.lucidum* contain antibacterial constituents that are able to inhibit gram-positive and /or gram-negative bacteria[18, 17, 14, 15, 13]. It is evident from the results of

present investigation that *G.lucidum* extracts had inhibitory activity against both Gram positive and Gram negative bacteria. These results also affirm the claims of traditional herbalists in the south western Nigeria that *Ganoderma* species could be used as feed supplement to resist microbial infections in human being.

All the extracts were different in their antimicrobial effectiveness depending on their extractive solvent used. Our results agree favorably with the suggestions of Oloke and Kolawole (1988) that bioactive components may differ in their solubility depending on the extractive solvents used[26]. Kawagishi *et al.,* (1988), observed that some of the active phytochemicals are soluble in alcohol but insoluble in water as in case of *Agaricus blezei*[27]. 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[2], our findings of present study are consistent with these observations, the organic extracts exhibited high antimicrobial activity than aqueous extract.

Yamac and Bilgili (2006) investigated antimicrobial activity of chloroform and dichloromethane extracts of fruit body/mycelial cultures of some (20 isolates) mushrooms against 7 bacterial strains and observed good antimicrobial activity with all the extracts for all tested organisms, in their findings mycelial extract of many fungi possess high inhibitory activity against Gram negative bacteria[28], our results are in collaboration with this finding, mycelial extract had greater inhibitory effect against *E.coli* and *P. aeruginosa* in the present study. Mycelial biomass extracts exhibited high inhibitory activity as compared to fruit body extracts possibly because of varying content of bioactive molecule in fruit body and mycelia. It is essential to carry out further research regarding this difference in antagonistic activity pattern of fruit body and mycelia; unfortunately we were unable to perform this due to limited facilities.

CONCLUSION

In conclusion, this study has shown that different extracts (aqueous, methanol and acetone) have been used in vitro to inhibit the growth of some pathogenic bacteria. It can therefore be suggested that, *Ganoderma lucidum* is promising antimicrobial fungus and could be employed to combat several bacterial diseases.

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