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Antimicrobial Activity of *Ganoderma lucidum* Mycelia

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Antimicrobial activity of *Ganoderma lucidum* mycelia was studied by the agar-well diffusion method. Methanol, acetone, chloroform and aqueous extracts of mycelia were analyzed for the antimicrobial activity against different Gram positive organisms and Gram negative organisms such as *Staphylococcus aureus, Bacillus subtilis, Corynebacterium diphteriae, Escherichia coli,* Proteus *mirabilis, Klebsiella pneumoniae, Salmonella typhi* and *Pseudomonas* P_{18}. All four extracts at concentration of 100 mg/ml showed maximum inhibition. It was observed that Gram positive organisms were more susceptible than Gram negative organisms.

Key words*: Ganoderma lucidum,* extracts, anti microbial activity, inhibitory.

In recent years, varieties of mushrooms have been isolated and identified and the number of mushrooms being cultivated for food or medicinal purposes has been increasing rapidly[1]. *Ganoderma lucidum* (Family - Polyporaceae), an edible and medicinal mushroom, commonly known as *Reishi*, is highly ranked in oriental folklore. The fruiting bodies of *Ganoderma lucidum* have been regarded as a panacea for all types of diseases, perhaps due to its demonstrated efficacy as a popular remedy to treat a large number of diseases. The mushroom is a valuable herb due to its biological activities such as anti-tumor, immuno - modulatory, cardiovascular, respiratory, antihepatotoxic and antinociceptive effect. Protein bound polysaccharides and ethanolic extracts from *G. lucidum* have exhibited free radical scavenging activity[2,3]. *Ganoderma* have been investigated more as anti-tumor and antiviral agents and less so as anti-bacterial agents[4]. Organic extracts of fruiting body of *Ganoderma* species from Nigeria showed an antibiotic effect against organisms such as *Pseudomonas syringae* and *Bacillus subtilis* [5]. Antibacterial activity has been observed against Gram positive bacteria from the basidocarp extracts of *G. lucidum*[6]. *Ganoderma* contains a variety of bioactive compounds including a range of triterpenoids, and other lipids, proteins, lysozyme, polysaccharides and nucleotides. It contains elements including calcium, magnesium, potassium and germanium and different compounds such as flavonoids, alkaloids, coumarins[7, 8, 9]. Most of these compounds show various therapeutic effects. In the present work the antimicrobial activity of *G. lucidum* mycelial extracts was studied by the agar-well diffusion method.

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MATERIAL AND METHODS

Microorganism

Ganoderma lucidum ATCC 1091 was procured from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India. The culture was maintained on potato dextrose agar, 2%.

Growth medium

Potato dextrose broth was inoculated and incubated at 30°C in dark for 7 days. The mycelium layer was separated from the medium using whatmann filter paper no.1. Mycelia were dried in an oven at 60°C for 3 days.

Preparation of mycelial extracts

Dried mycelia were extracted in different solvents such as methanol, acetone, chloroform and distilled water separately using Soxhlet apparatus. The solvent was completely removed by distillation. The distillate was dried using rotary evaporator to yield a solid residue. The residue thus obtained was suspended in distilled water to obtain a concentration of 100 mg/ml of the crude extracts. The antimicrobial activity was analyzed using these extracts.

Test organisms

Gram-positive organisms namely *Staphylococcus aureus, Bacillus subtilis, Corynebacterium diphtheriae* and Gram-negative organisms namely *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Salmonella typhi* and *Pseudomonas* P_{18} were used as the test organisms. A loopful of these cultures were streaked onto 2% nutrient agar slants separately and incubated at 37°C.

Anti-microbial screening

In vitro antimicrobial activity of the mycelia extracts of *G. lucidum* was screened by agar well diffusion method. Test organisms were suspended in sterile saline to achieve optical densities corresponding 10^6 colony forming units (Cfu/ml). Under aseptic conditions 1ml of this cell suspension was inoculated in 20ml of pre - warmed 2% nutrient agar media and was poured into sterile petri - plates to yield a uniform depth of 6 mm. Wells of 6 mm diameter were bored at equal distance from each other. 10 µl of each extract of 100mg/ml concentration was dispensed into wells and the plates were incubated at 37°C for 24 h. The zone of inhibition was measured for each extract after incubation. 10 µl of standard Streptomycin solution (10µg/ml) was used as a control.

RESULTS

The preliminary antimicrobial screening indicated acetone, methanol and aqueous extracts of *G. lucidum* mycelium of concentration 100mg/ml to be effective against test organisms. The results of antimicrobial activity of mycelia are shown in the (Table 1).

The inhibition zone diameter (IZD) of different extracts against different test organisms were ranging from 7.5mm – 23.0mm. The acetone extract showed effective IZD against all the organisms. The IZD for Gram positive organisms was maximum for *Staphylococcus aureus* of 22mm, whereas it showed inhibitory effect against the Gram negative organisms namely, *Salmonella*

Table 1. Diameter of zone of inhibition [mm]

Bacterial species	Gram nature[+ / -]	Acetone extract	Methanol extract	Chloroform extract	Distilled water extract
Bacillus subtilis	+	16.5	10.5	16.0	21.0
Corynebacteriumdiphtheriae	+	21.5	12.0	18.5	23.0
Staphylococcus aureus	+	22.0	11.5	16.5	18.5
Proteus mirabilis	-	14.0	00.0	14.0	13.0
Escherichia coli	-	15.5	10.0	11.0	11.0
Klebsiellapneumoniae	-	16.0	00.0	13.5	16.5
Salmonella typhi	-	17.5	9.5	00.0	17.0
Pseudomonas P_{18}	-	12.0	00.0	8.0	00.0

typhi of 17.5mm maximally. The methanol extract showed inhibitory effect against the test strains except *Klebsiella pneumoniae, Proteus mirabilis and Pseudomonas* P_{18}. The chloroform extract was found to be effective against all the Gram positive and Gram negative organisms except *Salmonella typhi*. It showed maximum IZD of 18.5mm for *Corynebacterium diphtheriae* and 14.0mm for *Proteus mirabilis*. The distilled water extract showed inhibitory effect against all the Gram positive organisms with maximum IZD of 23mm for *Corynebacterium diphtheriae*. The extract was seen to be effective against the Gram negative organisms with maximum IZD of 17mm for *Salmonella typhi*.

DISSCUSION

The antibacterial studies conducted presently with the four extracts viz, acetone, methanol, chloroform and aqueous extract from liquid cultivated mycelium showed wide variation with respect to their effect. The extracts of concentration 100mg/ml were found to be effective against Gram positive and Gram negative bacteria. From the present investigation it was proved that the acetone extract was very efficient to control all the eight strains of bacteria. The acetone extract of fruiting bodies of *G. lucidum* has maximum antibacterial activity[10]. Aqueous extraction of the fruiting bodies and mycelia of *G. lucidum* using hot water resulted in the extraction of many proteins, lectins and polysaccharides[11-13]. The bioactivity of aqueous extracts of fruiting bodies of *G. lucidum* exhibited inhibitory activity against the Gram positive and Gram negative bacteria[14]. However, the aqueous extract of the mycelium using hot water showed effective results towards all the test strains except *Pseudomonas* P_{18}. Other reports have shown organic extracts from *Ganoderma* fruiting bodies to have antibacterial activity against some selected Gram negative bacteria[15-16]. Growth inhibitory effect against Gram positive organisms was investigated using chloroform extract of fruiting bodies[17]. However, the chloroform extract of *G. lucidum* mycelia was found to be effective against Gram positive and Gram negative organisms except *S. typhi*. The methanol extract of fruiting bodies of *G. lucidum* showed remarkable antibacterial activity against *E. coli, Salmonella* species and *B. subtilis* [18, 19]. The methanolic extracts of the mycelia and culture extracts of *G. recinaceum* and *G. lucidum* inhibited *Bacillus subtilis*. *G. recinaceum* also inhibited *S. aureus* [20]. However, during the present study it was found that the methanol extract of liquid cultivated mycelium was inhibitory for all the test strains except for *P. mirabilis, K. pneumoniae and Pseudomonas* P_{18}. Results with the mycelium extract indicated inhibitory effect using aqueous and organic extracts in comparison to the inhibitory effect obtained by other investigators, indicating the acetone extract of mycelia possesses more potential as an antibacterial agent.

The production of basidocarp takes at least 3 to 5 months, while the mycelium growth takes within one to two weeks [21]. The present investigation showed the potential for mycelial extracts of *G. lucidum* to be employed for combating several pathogenic diseases.

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