ANTIMICROBIAL ACTIVITY OF *GANODERMA LUCIDIUM* FRUITING BODY EXTRACT FROM HIMACHAL PRADESH

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***Abstract*: In this paper the antimicrobial activity of methanolic and aqueous extracts obtained from the fruiting body of *Ganoderma lucidium* a medicinal mushroom were investigated. Over the past century it has been discovered and develop a number of synthetic antimicrobial agents, but toxicity and drug resistance are still the major hindrance to gaining successful therapeutic outcomes. The antimicrobial activity of methanolic and aqueous extracts was tested against five bacteria namely, *E.coli, Staphylococcus aureus, Streptococcus pyogenes, Burkholderia sp and Pseudomonas aerogenosa* collected from wound sample. The inhibition zone diameter of methanolic extracts against different test organisms were ranging from 5mm – 23.0mm. Where, antimicrobial activity of aqueous extract of fruiting body of *Ganoderma lucidium* the inhibition zone diameter of test organism range from 6mm-21mm.Both extracts showed effective inhibition zone diameter against all the organisms. The Inhibition Zone was maximum for Gram positive organisms *Staphylococcus aureus*, was resistance to antibiotic Cetotaxime, polyxin, Amoxycillin, Novobiocin and Amoxyclav. Further studies were carried out for compound characterization and Pharmacological studies.**

***Keywords*: *Ganoderma lucidium*, antimicrobial activity, methanolic, Inhibition zone, Burkholderia**

Introduction

Ganoderma lucidum, a cosmopolitan mushroom species, is a polypore rack mushroom that changes its color during growth until maturity from orange-white to bright red. There are both historical and contemporary research that supported the use of *G. lucidum* in various conditions including chronic inflammation and cancer. Its potent anti-oxidant and liver protective properties help slow the aging process, thus it is known as the "mushroom of immortality". antimicrobial effect due to the extracts derived from this mushroom which contain bacteriolytic enzyme, lysozyme and acid protease was one interesting aspect of its performance is (Klaus & Miomir, 2007)

For thousands of years nature is a very good source of many medical compounds. With an enormous variety of chemical structures Macromycetes are rich sources of biologically active compounds. Many different studies are carried about antimicrobial activity of different types of fungi extracts from India (Sheena *et al*.,2003, Quereshi *et al*., 2010) and China (Gao *et al*., 2005). Mushrooms are also considered as functional foods because they elicit their positive effect on human being in several ways (Akyush *et al.*,2010). Mostly bacterial and fungal in recent years, for several infectious diseases many possible sources of natural antibiotics have been used.

Antibacterial, antifungal, antiparasitic and antiviral agents is the third widespread therapeutic effect reported in mushrooms antimicrobial activity (Kettering *et al*.,2005, Ngai *et al*.,2003). Mushroom have been a major focus of investigations for novel biologically active compounds from natural resources and in recent years pharmaceutical companies have spent a lot of time developing these natural products to produce more affordable and cost-effective remedies (Farnsworth.1994). In the present work the antimicrobial activity of G. lucidum fruiting body extracts with ten antibiotics was studied by the agar-well diffusion method.

MATERIALS AND METHODS

The fruiting body powder of Ganoderma lucidium was collected from Daxen Agrotech India Pvt Ltd, Himachal Pradesh. Fresh fruiting bodies of Ganoderma lucidium were kept for further analysis.

Preparation of Extract

Air dried powder of *Ganoderma lucidium* fruiting body powder was extracted by using soxhlet apparatus .10 g of fruiting powder was taken in a paper cone and placed in soxhlet apparatus.100 ml of solvent (methanol and water) was taken in the round bottom flask attached to this set up. Solvents get vapourized and rises up to the condenses back into the liquid and falls into the sample in the cone and extract certain compounds falls into the round bottom flask. Methanol extract of *Ganoderma lucidium* fruiting body powder was golden brown colour and aqueous extract was dark brown colour.

Test Organisms

Gram positive bacteria namely *Staphylococcus aureus*, *Streptococcus pyogenes* gram negative bacteria *E.coli*, *Pseudomonas aerogenosa*, *Burkholderia sp* , were used as the test organism. On 2% nutrient agar slants of loopful of these cultures were streaked and incubated at 37°C.

Preparation of Inoculums

Wound infections samples were collected from the patients with complaints of non-healing wound by using a sterile swab, taking care to avoid contamination of the specimen with commensals from the skin, and were immediately transported to the laboratory. They were studied for identification of isolates by Gram stains and culture growth on nutrient, blood and MacConkey agar. Colonies from nutrient agar were used for biochemical tests and antibiotic sensitivity. On isolation of Gram-positive cocci, catalase, and coagulase tests were done. Gram negative bacilli were distinguished using biochemical tests IMViC (indole, methyl red, Voges-Proskauer, citrate utilization), oxidase and triple sugar iron (TSI) agar tests. After confirmation of the organism, to a 250ml containing sterilized nutrient broth (Hi media) loopfull of organisms from mother culture were transferred. On a rotary shaker the flasks were incubated for 24hr at 37°C. For antimicrobial test the broth cultures were subjected to standard plate count method to enumerate the population and the dilution having 1x10 CFU/ml. culture growth was tested for in vitro antibiotic susceptibility testing performed by disc diffusion method (modified Kirby Bauer method) on Muller Hinton agar. Antibiotics such as Amikacin, Neomycin, Cetotaxime, polymyxin, Norfloxacin, Amoxycillin, Novobiocin, Amoxyclav, Ceflaxin and Amphotericin was used as positive control and DMSO as negative control.

Samples for wound infections were collected from the patients with complaints of discharge, pain, swelling, foul smelling, delayed and non healing wound by using a sterile swab, taking care to avoid contamination of the specimen with commensals from the skin, and were immediately transported to the laboratory. They were studied for identifi cation of isolates by Gram stains and culture growth on nutrient, blood and MacConkey agar. Colonies from nutrient agar were used for biochemical tests and antibiotic sensitivity. On isolation of Gram positive cocci, catalase, and coagulase tests were done.

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Antibacterial Assay Screening by Agar Well Diffusion method

In-vitro antimicrobial activity of the fruiting body extract of Ganoderma lucidium was screened by agar well diffusion method. Muller Hinton Agar (Hi media) plates were swabbed with cotton swabs with 24hr old-broth culture of bacteria. About 2cm apart wells of 6mm diameter were made in each of these plates using sterile cork borer. At a concentration of 2mg/ml stock solution of each extract was prepared. Different concentration of about 100µl with the help of sterile syringe 25%, 50%, and 75% of solvent extract and allowed to diffuse at room temperature for 24hrs.The plates were incubated for 24hrs at 37°C, control experiments comprising inoculums without extracts were setup. The diameter of the inhibition zone(mm) was measured. The experiment was repeated thrice triplicates were maintained, the readings and the average values were recorded.

Table :1

Antimicrobial activity of methanolic fruiting body extract of *Ganoderma lucidium*

Bacterial Strain	Diameter of Zone of Inhibition (mm)			Control DMSO
	25 µl	50 µl	75 µl	
E. coli	9	11	16	-
Staphylococcus aureus	5	17	23	-
Streptococcus pyogene	13	16	17	-
Burkholderia sp	7	9	17	-
Pseudomonas aerogenosa	9	12	18	-

Table :2

Antimicrobial activity of aqueous fruiting body extract of *Ganoderma lucidium*

Bacterial Strain	Diameter of Zone of Inhibition(mm)			Control DMSO
	25 µl	50 µl	75 µl	
E. coli	7	8	13	-
Staphylococcus aureus	9	10	21	-
Streptococcus pyogene	11	12	13	-
Burkholderia sp	6	8	9	-
Pseudomonas aerogenos	11	12	16	-

Table :3

Antimicrobial activity of antibiotics against Microorganism

Antibiotics	Diameter of Zone of inhibition (mm)				
	E.coli	*Staphylococcus aureus*	*Streptococcus pyogene*	*Burkholderia sp*	*Pseudomonas aerogenosa*
Amikacin	11	2	10	10	11
Neomycin	8	6	7	9	-
Cetotaxime	12	-	12	12	5
Polymyxin	4	-	-	-	7
Norfloxocin	9	12	11	8	15
Amoxycillin	11	-	-	10	-
Novobiocin	10	-	9	6	11
Amoxyclav	6	-	5	-	-
Cefelaxin	-	11	7	-	-
Dicloxacillin	14	12	14	13	-

Result

The preliminary antimicrobial screening indicated methanol and aqueous extracts of *G. lucidum* fruiting body of concentration 75mg/ml to be effective against test organisms. The results of antimicrobial activity of methanolic extract of fruiting body of *Ganoderma lucidium* are shown in the (Table 1). The inhibition zone diameter of methanolic extracts against different test organisms were ranging from 5mm – 23.0mm. The results of antimicrobial activity of aqueous extract of fruiting body of *Ganoderma lucidium* are shown in the (Table 2), here the inhibition zone diameter of test organism range from 6mm-21mm.Both extracts showed effective inhibition zone diameter against all the organisms. The Inhibition Zone for Gram positive organisms was maximum for *Staphylococcus aureus*, whereas it showed inhibitory effect against the Gram-negative organisms. The results of antimicrobial activity of antibiotics against microorganism are shown in (Table 3). *E.coli* was resistant to antibiotic Cefelaxin, *Staphylococcus* was resistance to antibiotic Cetotaxime, polyxin, Amoxycillin, Novobiocin and Amoxyclav. Streptococcus was resistance to polymyxin and Amoxycillin. *Burkholderia* resistance to polymyxin, Amoxyclav and Cefelaxin. *Pseudomonas* was resistance to Neomycin, Amoxycillin, Amoxyclav, Cefelaxin and Dicloxacillin.

Discussion

Methanol and aqueous extract of fruiting body of *Ganoderma lucidium* showed wide variation with respect to their effect. Dulger & Gonuz (2004) reported the antimicrobial properties of 4 different extracts of macrofungus (*Cantharellus cibarius*) against 50 important human pathogens**.** The concentration of 75mg/ml extracts were found to be effective against Gram positive and Gram-negative bacteria. From the present investigation it was proved that the methanol extract was very efficient to control all the five strains of bacteria. The methanol extract of fruiting bodies of *G. lucidum* has maximum antibacterial activity. The bioactivity of aqueous extracts of fruiting bodies of *G. lucidum* exhibited inhibitory activity against the Gram positive and Gram-negative bacteria. Other reports have shown organic extracts from Ganoderma fruiting bodies to have antibacterial activity against some selected Gram-negative bacteria. However, the methanolic and aqueous extract of the fruiting body showed effective results towards all the test strains (*E. coli, Staphylococcus Sp, Streptococcus Sp, Burkholderia Sp and Pseudomonas sp).* Mostly Gram-negative bacteria are sensitive to antibiotics when compared with Gram positive bacteria.

Conclusion

In conclusion the methanolic and aqueous extracts of fruiting body of *Ganoderma lucidium* showed antimicrobial activity against Gram positive and Gram-negative bacteria. The present finding was carried out for characterization of bioactive compounds, clinical trial and pharmacological studies.

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References

1. Klaus A, Miomir N: Influence of the extracts isolated from *Ganoderma lucidum* mushroom on some microorganisms. Proceedings of National Science, Matica Srpska Novi Sad, 2007; 113: 219-226

2. Sheena, N., Ajith, T. A., Mathew, A.T., Janardhanan, K.K., Antibacterial activity of three macrofungi, *Ganoderma lucidum*, *Navesporus floccosa* and *Phellinus rimosus* occuring in South India. Pharm. Biol., 2003, 41, 564-567.

3. Quereshi, S., Pandey, A.K., Sandhu, S.S. Evaluation of antibaterial activity of different *Ganoderma lucidum* extracts. J. Sci. Res., 2010, 3, 9- 13.

4. Gao, Y., Tang, W., Gao, H., Chan, E., Lan, J., Li, X., Zhou, S., Antimicrobial activity of medical mushrooms Ganoderma. Food Rev. Int., 2005, 21, 211- 222.

5. Akyuz, M., A.N. Onganer, P. Erecevit and S. Kirbag. Antimicrobial activity of some edible mushrooms in the eastern and southeast Anatolia region of Turkey. GU J. Sci., 2010; 23(2): 125-130

6.Kettering M, Valdivia C, sterner O, Anke H, Thines E and Heptemerones AG (2005). Seven novel diterpenoids from Coprinus heptemerus: producing organism, fermentation, isolation and biological activities. Journal of Antibiotics.,2005, 58; 390-6.

7. Ngai P.H and Ng T.B Lectin, a novel and potent antifungal protein from shitake mushroom with inhibitory effects on activity of human immunodeficiency virus-1 reverse transcriptase and proliferation of leukemia cells. Life Science.,2003, 73; 63–74.

8. Farnsworth NR (1994). Ethnopharmacology and drug development, In Ethno botany and the search for new drugs Ciba Foundation Symposium.,1994., Vol. 185 (ed.G.T.prance): pp. 42-59.

9. Dulger B, Gonuz A: Antimicrobial activity of certain plants used in Turkish traditional medicine. Asian Journal of Plant Science, 2004; 3: 104-107.