Comparative study on the antibacterial activity of *Ganoderma lucidum* with some commercial antibiotics

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ABSTRACT

In the present study, the extracts of *Ganoderma lucidum* were prepared using methanol, ethanol and water and the antimicrobial activity of that extracts have been tested against human pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella typhi*, *Yersinia enterocolitica* and methicillin resistant *Staphylococcus aureus* using hole plate diffusion method. Commercial antibiotics also tested against these organisms for comparison. The extracts are found to be potent against the organisms tested and out of the three solvents used, methanol is found to be most effective.

Keywords: *Ganoderma lucidum*, antibacterial activity, commercial antibiotics.

INTRODUCTION

Most of the currently used drugs are expensive or not readily available. A major setback for their continued usage is the development of resistance. Thus, there is an urgent need for new inexpensive drugs that will be able to act for a longer period before resistance sets in. Prolonged administration of costly drugs leads to drastic side effect, while a drug derived from a plant or microbial source may reduce the production cost and free from side effect. So many clinically useful drugs have been obtained through the screening of natural products. Over 1,800 species of fungi are considered to have medicinal properties (Chang, 1995). The importance of fungi in medicine was recognized long back with the discovery of penicillin. Mushrooms are macrofungi; some of them have long been used as garnishes or for tonics in folk medicine (Jang and Birmingham, 1992). There have been a number of reviews published on the bioactive substances of mushrooms (Jong et al., 1991, Mizuno, 1995a, Mizuno et al., 1995c, Borchers et al., 1999, McAfee and Taylor, 1999, Ooi and liu, 1999).

In recent years there has been a growing interest to evaluate mushrooms that possessing the antibacterial activity against various infective agents. The fruiting bodies and cultural mycelia of mushrooms are prescribed to treat chronic hepatopathy, hyper tension, neoplasia, neurasthenia, bronchitis, arthritis, in several countries, especially in China. With particular focus on *Ganoderma* species, it is apparent that most of the available data on active extracts and compounds relates to the pharmacological effects. *Ganoderma lucidum*
otherwise known as king mushroom or Reishi and commonly known as ‘lacquered dark’ with glossy exterior medicinal mushroom, is being widely used in the countries of pacific region as a herbal medicine for the treatment of various diseases like cardiovascular diseases, anti-inflammatory, Human leucocyte, cancer management, longevity, vitality, Hepatitis ‘B’, diabetes, hypertension and so on (Shiao et al., 1994).

Earlier investigations showed that extracts of the fruiting bodies of *G. lucidum* occurring in South India possessed profound antioxidant, antitumour, anti-inflammatory and antinociceptive properties (Sheena et al., 2003, Jones and Janardhanan, 2000). The present study is focused to screen the antibacterial activity of *Ganoderma lucidum* against many pathogens like MRSA, *Listeria monocytogenes*, *Salmonella typhi*, *Yersinia enterocolitica*.

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major hospital associated as well as a community associated pathogen causing a wide range of diseases, including endocarditis, osteomyelitis, toxic-shock syndrome, pneumonia, food poisoning and cabuncles (Oliveira et al., 2002 and Chambers, 2005). Nosocomial MRSA isolates are mostly multi-drug resistant. In fact, many strains of MRSA exhibit resistance to both lactams and aminoglycosides (Thornsberry, 1998). However, due to development of methicillin resistance amongst *Staphylococcus aureus* isolates, treatment of these infections has become problematic (Vidhani et al., 2001).

*Listeria monocytogenes* causes meningitis and septicaemia mainly in neonates, pregnant women, and the elderly and immunosuppressed persons. Listeriosis during pregnancy may lead to abortion and still birth. Enteric fever (typhoid) with bacteremia caused by *Salmonella typhi*, the organisms multiply in reticulo endothelial cells, invasion of the intestine causes inflammation and ulceration, epistaxis, intestinal haemorrhage and perforation, toxæmia and renal failure may occur in untreated late typhoid (often fatal). *Yersinia enterocolitica* is pathogenic for many animal species and occasionally humans, causing mesenteric adenitis, chronic diarrhoea and severe septicemia.

The present investigation was made to evaluate the antibacterial activity of the methanol, ethanol and water extracts of *Ganoderma lucidum* against these pathogenic bacterial strains.

**MATERIAL AND METHODS**

**Cultures and microbes:** All the chemicals including all commercial antibiotics used in the present study were purchased from Hi-media, Mumbai. The microorganisms used for the study were *Listeria monocytogenes* (MTCC 1143), *Staphylococcus aureus* (MTCC 96), *Salmonella typhi* (MTCC 733), *Yersinia enterocolitica* (MTCC 859) obtained from the Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India. The *Staphylococcus aureus* strains were isolated from different clinical samples received from diabetic patients and five MRSA strains were chosen and tested. The isolates were
identified and confirmed by conventional methods viz., Gram's stain, catalase test, mannitol fermentation test and coagulase test (Colle et al., 1996). Methicillin resistance was detected by Oxacillin Resistant Screening Agar (ORSA). After incubation, the formation of blue colour colonies was the indication of the presence of methicillin resistant *S. aureus.*

**Preparation of the extract:** The sporocarps of *Ganoderma lucidum* were cut into small pieces, dried at 40-50ÚC for 48h and powdered. Twenty five grams of the powdered material was loaded in soxhlet apparatus and extracted in 125ml of different solvents. Then the extract obtained was evaporated to dryness at 40-50ÚC using a rotary vacuum evaporator. For different solvents, different temperature was set in mandle of soxhlet apparatus. In the present study, methanol (40-50ÚC), ethanol (40-50ÚC), and water (100ÚC) were used as solvent. The dried extracts were dissolved in DMSO at a concentration of 50mg/ml and employed for the experiments.

The Muller Hinton Agar plates were prepared and sterilized. After solidification, each bacterial culture was biogramed on individual plates. The wells were made on the plates in the diameter of 8 mm using aseptic cork borer. Ethanol, methanol and aqueous extracts of *Glucidum* (2,500µg/well) were added into individual wells (50µl). Plates were kept for 24-48 hours at 37ÚC to observe the growth by zone formation and its diameter was detected and recorded.

**Antibiogram study of commercial antibiotics against selective pathogen:** The commercial antibiotics like ampicillin, streptomycin, tetracycline, chloramphenicol were prepared with known concentration (5,000µg/ml). The solution of different antibiotics were added into individual wells (2,4,6µl) separately and the plates were kept in refrigerator for 1 hour for the diffusion of the antibiotics. Then the plates were incubated for 24hrs at 37ÚC and zones of inhibition were measured after the incubation period. This zone of inhibition was compared with inhibition zone of the extracts of *Glucidum.*

**RESULTS AND DISCUSSION**

In the present investigation, antimicrobial activity of crude extract of *Glucidum* against 5 microbial species showed considerable antimicrobial activity. No growth of bacteria was observed in the zone of inhibition after 48 hours. Effects of crude extract of *Glucidum* on different organisms are well depicted in the Table.

The methanol, ethanol and aqueous extracts of *Glucidum* were found to be potent as an inhibitory agent against *Staphylococcus aureus.* The growth was considerably affected with the formation of inhibitory zone of 21,16 and 11 mm respectively. The methanol, ethanol and aqueous extracts of *Glucidum* proved its inhibitory activity against *Listeria monocytogenes* with a zonation of 13,15 and 10mm respectively. *Glucidum* methanol, ethanol and aqueous extracts also showed remarkable antimicrobial activity against *Salmonella typhi* (20,11 and
Table – Antibacterial activity of crude extract of *Glucidum* and commercial antibiotics against selective bacterial strains. The values indicated are the average of triplicates and ± indicates the S.D.

<table>
<thead>
<tr>
<th>Type</th>
<th>Test Bacterial strains</th>
<th>Antibacterial activity of crude extract of <em>Glucidum</em> (2,500 µg/well)</th>
<th>Inhibition zone of commercial antibiotics (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Zone formation (in mm)</td>
<td>Methanol extract</td>
</tr>
<tr>
<td>Gram-positive</td>
<td><em>Staphylococcus aureus</em></td>
<td>21±1</td>
<td>16±1</td>
</tr>
<tr>
<td></td>
<td><em>Listeria monocytogenes</em></td>
<td>12.6±6±1</td>
<td>14.6±6±1</td>
</tr>
<tr>
<td>Gram-negative</td>
<td><em>Salmonella typhi</em></td>
<td>21±1</td>
<td>11.3±3±1</td>
</tr>
<tr>
<td></td>
<td><em>Yersinia enterocolitica</em></td>
<td>18.3±3±1</td>
<td>13.6±6±1</td>
</tr>
<tr>
<td>Clinical sample</td>
<td>MRSA</td>
<td>18.3±3±1</td>
<td>14±1</td>
</tr>
</tbody>
</table>
13mm zones). *Yersinia enterocolitica* (18, 15 and 12mm zones) and against MRSA with an inhibition zone of 18, 15 and 13mm respectively.

Comparison of the inhibition zones of *Glucidum* with those of standard antibiotics, (Table) indicated that the extracts also possessed good antibacterial activity. When compare with commercial antibiotics (30 µg), the extracts of *Glucidum* (2,500 µg) gives more or less equal diameter of inhibition zone. When compared to the commercial antibiotics, the quantum of extracts used is very high. Since it is crude one such a quantum of extract is needed for action against the microbes. If the crude extract is purified and the active compound is isolated then the exact quantity needed may be identified and it could be compared effectively with the commercial antibiotics.

Among the three extracts used in the present study, the methanol extract showed higher antibacterial activity than that of ethanol and aqueous extract. Vlachos *et al.*, (1996) had the opinion that, the most effective solvent for extracting antibacterial compounds from the selected seaweeds is methanol. More or less the similar results were obtained from methanolic extract of Zulu medicinal plants (Kelmanson *et al.*, 2000), seeds of *Syzygium jambolanum* (Chandrasekaran and Venkatesalu, 2004a), *Rhizophora mucronata*, *Rhizophora lamarkii*, *Bruguiera cylindrica* and the bark of *Cassia siamea* (Chandrasekaran and Venkatesalu, 2004b). Sahin *et al.*, (2003) reported that the methanol extracts of *Saturaja hortensis* showed stronger and broader spectrum of antimicrobial activity than the hexane extract. Habsah *et al.*, (2000) reported that the dichloromethane and methanol extracts of *Alpinia rafflesiana*, *A.nutans*, *A.mutura* and *A.malaccensis* showed the most broad spectrum activity against MRSA.

From the present findings, it is concluded that methanol extracts of *Glucidum* have more antibacterial activity than the ethanol and aqueous extracts.

**REFERENCES**


