

Antimicrobial activity of aqueous extract of spore powder of *Ganoderma lucidum* - an in vitro study

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Abstract

Introduction: Bacteremia has become the scourge of modern medical care. With increasing resistance to antibiotics, the search is always on to discover newer molecules with antibacterial activity. Mushrooms have always been a source of antibiotics. *Ganoderma lucidum*, is one such mushroom with known medicinal properties. **Objectives:** Varying concentrations of aqueous extract of spore powder of *Ganoderma lucidum* was tested in vitro for its antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* and *Klebsiella pneumoniae*. **Methods:** Antibacterial activity of *Ganoderma lucidum* was tested against the above mentioned organisms by serial broth dilution method and was expressed by minimum inhibitory concentration (MIC). **Results:** The MIC value for - *S. aureus* was 125 mcg /ml, *E. coli* was 125 mcg /ml., *E. faecalis* less than 02 mcg /ml. and *K. pneumoniae* 62.5 mcg / ml. **Conclusion:** Aqueous extract of *Ganoderma lucidum* exhibited antibacterial activity against the tested organisms.

Key-words: *Ganoderma lucidum*, aqueous extract, spore powder, antimicrobial, serial broth dilution, MIC.

Introduction

Bacteremia is a scourge of modern medical care. With increasing number of individuals having undergone some type of prosthetic implants, this bacteremia has the potential to become life threatening. Compounding this problem is the increasing drug resistance developed by pathogenic bacteria especially in nosocomial infections. The search is therefore forever on to create new molecules to overcome this problem. A faster and cheaper alternative would be to investigate naturally occurring substances, in the plant, animal and fungal kingdom, which demonstrate antimicrobial properties.

E- ISSN
0976 – 1799

*Journal of
International
Oral Health*

*Oral and Maxillofacial
Surgery*

Original Research

Mushrooms have been mentioned in ancient ayurvedic texts, very sporadically and broadly as Chatraka and Bhuchatras. Botanical terms and Latin names have been prevalent only in the last two centuries. *Ganoderma lucidum*, is one such Basidiomycetes fungus belonging to the family Polyporaceae (1). It has been in use for thousands of years due to its varied medicinal properties in Traditional Chinese Medicine. The first mention of its healing properties was in an ancient Chinese medicine text "Shen Nung Bao Tsao Zing" (2)

It is known to have many biologically active components like triterpenes (3,4) polysaccharides (5,6), ganoderic acids (4), etc. giving it, its antimicrobial (7,8,9,10,11,12), antiviral (13,14,15,16), immunomodulatory (14), antioxidant (17), antitumour (18) and anticancer (19) properties. It has been rightly termed as a therapeutic fungal bio factory (20) It is a prime example of an ancient remedy being of great relevance to the modern era.

S. aureus, *E.coli*, *E.faecalis* and *K.pneumoniae* are microorganisms involved in human infections which show increasing drug resistance.

This preliminary study was aimed at determining the Minimum Inhibitory Concentration (MIC) of the aqueous extract of the powdered spores of *Ganoderma lucidum* on these bacteria by serial broth dilution method

Subjects and Methods:

A standard procedure for performing the MIC test was followed (21). The standard strains of the above organisms used in this study were *S. aureus* – ATCC9144, *E.coli* – ATCC25922, *E.faecalis* – ATCC35550 and *K.pneumoniae* – ATCC11298. Brain Heart Infusion broth (BHI), aqueous extract of powdered spores of *Ganoderma lucidum*,

sterile MIC tubes and micropipettes were the other armamentarium used.

Procedure: Revival of the organisms – The respective bacterial strains from the stock were revived by plating on blood agar medium. After overnight incubation at 37⁰ C, isolated colonies were selected and the identities of the organisms were confirmed. Isolated colonies were transferred to sterile BHI broth and once again incubated overnight. The growth concentration was adjusted to 10⁵ organisms / ml by using 0.5 McFarland's turbidity standard.

To prepare the stock, 10 milligrams of the powdered spores of *Ganoderma lucidum* was added to 1 ml of sterile saline in a sterile vial. Two hundred µl of the BHI broth was added in each of ten MIC tubes per bacterial strain. (Total 40 MIC tubes for 4 bacterial strains.) In the first MIC tube containing 200 µl broth, 200 µl of stock was added. After mixing well, 200 µl was transferred to the second MIC tube. This was continued till the last (10th) tube. From the last tube 200 µl final solution was discarded. By following this serial dilution, the concentration of the aqueous extract was achieved as the following – 500, 250, 125, 62.5, 31.25, 16, 8, 4, 2 mcg /ml. respectively.

To each of the ten such prepared MIC tubes with varying concentrations, 200 µl of the earlier prepared strain of *S. aureus* was added such that the final volume per tube was 400 µl. The procedure was repeated for *E.coli*, *E.faecalis* and *K. pneumoniae*. The tubes were then incubated for 24 hours at 35⁰ C.

After the incubation, the MIC values were determined by visual inspection of the tubes. With each batch of tests, positive and negative controls were put up. Positive control containing broth plus bacterial strain showed turbidity and negative control

containing broth only appeared clear. In each series of tubes, the last tube with clear supernatant was considered to be without any growth and taken as MIC value.

Turbidity in the MIC tube indicated growth of the bacteria implying that the bacteria were resistant to the aqueous extract of powdered spores of *Ganoderma lucidum*.

Results:

Results as shown in Table 1 showed - the MIC value for *S. aureus* as 125 mcg /ml, for *E.coli* 125 mcg / ml., *E.faecalis* less than 2 mcg /ml. and for *K. pneumoniae* the MIC was 62.5 mcg /ml.

Table 1 – MIC of tested organisms

Organism	Concentration of aqueous extract of powdered spores of <i>Ganoderma lucidum</i> in mcg /ml.								
	500	250	125	62.5	31.25	16	08	04	02
<i>S.aureus</i>	S	S	S	R	R	R	R	R	R
<i>E.coli</i>	S	S	S	R	R	R	R	R	R
<i>E.faecalis</i>	S	S	S	S	S	S	S	S	S
<i>Klebs.pneu</i>	S	S	S	S	R	R	R	R	R

S = Sensitive

R = Resistant

Discussion:

Only compounds from microscopic fungi are on the market as antibiotics till now. The antibacterial activity of the spores of *Ganoderma lucidum* against Gram positive and Gram negative bacteria by serial broth dilution method was carried out by Yoon et

al (7). Other studies demonstrated that *Ganoderma lucidum* contained antibacterial components that were able to inhibit Gram positive and Gram negative bacteria (8,9,10,11). The antibacterial activity of *Ganoderma lucidum* in vivo is enhanced by its immunomodulatory properties (14), because of its polysaccharides (5,6), triterpenes (3,) and ganoderic acid (4) components.

In our study, we tested an aqueous extract of the powdered spores of *Ganoderma lucidum* against the following pathogens *S.aureus*, *E.coli*, *E.faecalis* and *K.pneumoniae*. All these four bacteria were sensitive.

Staphylococci are widespread in nature, their major habitat being the skin and mucous membranes of mammals and birds (22). They may be found in the mouth, mammary glands, intestinal, genitourinary and upper respiratory tracts of these hosts (22).. *S. aureus* prefers the anterior nares as a habitat (23).

S. aureus is an important cause of community acquired sepsis and is leading nosocomial pathogen (22). Disease manifestations can be broadly classified into toxin-mediated diseases (such as food poisoning, scalded skin syndrome and Toxic Shock Syndrome (TSS), infection of the skin and soft tissues (furuncles or boils, cellulitis and impetigo), infections of deep sites (such as bone and joints and the heart valve, spleen and liver – almost any organ can be involved) and infection of the lung and urinary tract (22) . An important complication of *S.aureus* bacteremia is dissemination of the organism to one or more distant sites (22). The incidence of *Staphylococcus* species in refractory periodontitis and chronic periodontitis has been found to be nearly 30% (24) . The microorganisms that cause acute suppurative

salivary gland infections are usually bacteria and the most commonly reported are *S.aureus* and *Haemophilae* (25) . *S.aureus* is one of the abnormal bacteria isolated from the mouths of medically compromised patients, such as those who have undergone - Irradiation for tumours of head and neck , Cytotoxic treatment of leukemias, Cytotoxic therapy for other tumours , Cerebrovascular accidents or strokes (25).

We found that *S.aureus* was sensitive to the aqueous extract of powdered spores of *Ganoderma lucidum* at MIC - 125 mcg / ml. It's use as a preventive, therapeutic or curative agent in the infections, caused by this organism such as refractory periodontitis, suppurative salivary gland disease, and patients undergoing cancer treatment has to be clinically determined to corroborate with these laboratory findings.

Escherichia coli is nearly an ubiquitous constituent of the bowel flora of healthy individuals. However certain strains may cause extra-intestinal and intestinal infections in healthy and immunocompromised individuals (26) . Urinary tract infections, bacteremias, meningitis and diarrheal disease are the most frequent clinical syndromes caused primarily by limited number of pathogenic clones of *E.coli*. (27). *E.coli* are one of the abnormal bacteria isolated from the mouths of medically compromised patients such as those undergoing irradiation for tumours of head and neck , cytotoxic treatment of leukemias and cytotoxic therapy for other tumours (25) . We found that *E.coli* was sensitive to the aqueous extract of powdered spores of *Ganoderma lucidum* with a MIC of 125 mcg / ml. *Ganoderma lucidum* would probably be useful in the above conditions where these organisms are

implicated. Clinical studies would have to be undertaken to corroborate these findings.

Enterococcus faecalis are catalase negative Gram positive cocci that inhabit the gastrointestinal tract, oral cavity and the vagina in humans as normal commensals (29) . They are increasingly associated with nosocomial and opportunistic infections in humans. They can cause a variety of diseases – infecting the urinary tract, bloodstream, endocardium, abdomen, biliary tract, burn wounds and indwelling foreign devices (30). Upto 90% Enterococcal infections in humans are caused by *E.faecalis* (29). *E.faecalis* was the most commonly recovered bacteria from samples of microbial flora found in canals after failed endodontic therapy (31). A high prevalence of *E.faecalis* (34.9%) was found in subgingival biofilm and saliva samples from patients with chronic periodontitis (32) suggesting that periodontal infection may favour colonization of the species. In our study, *E.faecalis* was highly sensitive to the aqueous extract of *Ganoderma lucidum* exhibiting a MIC less than 2 mcg / ml. *Ganoderma lucidum* spore powder used locally could be of use as a endodontic irrigant and as a local application in periodontitis. This would have to be clinically tested in these conditions and could prove to be useful.

Klebsiella pneumoniae strains are distributed widely in the environment and contribute to the biochemical and geochemical process (33). However, strains of *K.pneumoniae* also cause human infections, ranging from asymptomatic colonization of the intestinal, urinary and respiratory tracts to diarrhoea, oral ulcerations in HIV patients (34) to fatal pneumonia, septicaemia and meningitis (33). Spontaneous Bacterial peritonitis is also seen in patients with chronic underlying

liver disease and ascites. This is usually caused by bacteria such as *E. coli* and *K.pneumoniae*. (35). Salivary gland infections with mixtures of facultative and aerobic gram negative bacteria appear to be associated with patients who are medically compromised and have these bacteria present in their oral microflora. The microorganisms found in these mixed infections are – combinations of *E.coli*, *Pseudomonas*, *Serratia*, and *Klebsiella* species (25). In our study, *K.pneumoniae* exhibited a MIC of 62.5 mcg /ml and therefore could be tested clinically in these conditions especially salivary gland infections, to corroborate our findings.

Many studies have demonstrated that *G. lucidum* contains antibacterial constituents that are able to inhibit gram-positive and / or gram-negative bacteria (8, 9, 10, 11, 36, 37, 38). The aqueous extract from the carpophores of *G. lucidum* inhibited 15 types of gram positive and gram negative bacteria (7). Further studies indicate that the antimicrobial combinations of *G. lucidum* extract with four antibiotics (ampicillin, cefazolin, oxytetracycline, and chloramphenicol) resulted in additive effects in most instances and synergism in two instances when combined with cefazolin against *Bacillus subtilis* and *Klebsiella oxytoca* (7). Among seven components separated from the ether fraction of *G. lucidum* extract by silica gel column chromatography, P3 was the most potent with a minimum inhibitory concentration of 200 mg/ml. It appears that some 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 and *H. Pylori* (9). Thus, it is possible that the antibacterial activity of *Ganoderma* species may be

beneficial for those patients with chronic infection (e.g. chronic bronchitis) and those with *H. pylori*-positive peptic ulcer diseases, though clinical studies are required to confirm this (9, 39).

The mechanisms for the antibacterial and antiviral activity of *Ganoderma* species are largely undefined. Gao et al (9) suggest that multiple mechanisms may be involved. *Ganoderma* species constituents (e.g.: polysaccharides and triterpenoids) may inhibit viral replication of HSV, HBV, HIV, and other types of viruses by interfering with their adsorption, virus–hepatocyte fusion and endocytosis, viral integration, assembly, and release. Immunomodulating effects of *G. lucidum* are considered to play a role in antimicrobial activity, (9, 40). Activation of immune effector cells (e.g., T cells, macrophages, and natural killer cells) by both pathogen infection and *G. lucidum* administration caused an enhanced production of cytokines, radicals, and NO, facilitating the killing of viruses and bacteria. For example, activation of Kupffer cells by *G. lucidum* polysaccharides and triterpenoids within the liver facilitate the killing of HBV. In addition, a study on mice indicated that a proteoglycan with a carbohydrate protein ratio of 11.5: 1 isolated from *G. lucidum* stimulated the proliferation of spleen lymphocytes, resulting in a three-to-four fold increase in the percentage of B cells. These B cells were enlarged, expressed CD71 and CD25 on the cell surface, and showed an increase in the production of immunoglobulins. Therefore, *Ganoderma* species may stimulate B cells in vivo, producing immunoglobulins, which can neutralize HBV (9, 40). Furthermore, the immunosuppressive activity of *G. lucidum* constituents may decrease tissue and cellular damage following infection. Further studies are required to identify the

molecular targets of *G. lucidum* constituents for viruses and bacteria.

Herbal medicines often contain multiple active substances with individual constituents possibly contributing to the bioactivity observed in vitro and in vivo. Therefore, multiple important molecules might be the targets of an herbal medicine. The identification of these targets may provide molecular evidence of the pharmacological activity and toxicity of herbs (41).

Ganoderma lucidum may play a role of an adjunct in the management of infectious diseases. In our experience of using this herb clinically over two decades and observing the excellent clinical response, we decided to test the herb in vitro first and then to take up a clinical study to see whether the results drawn from this study can be corroborated in vivo. This clinical study will be undertaken shortly.

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