

Effect of the Mycelial Culture of *Ganoderma lucidum* on Human Pathogenic Bacteria

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Abstract

The mycelial culture of *Ganoderma lucidum* found in Nigeria was tested on some human pathogenic bacteria using dual and Weller techniques. The mycelial culture was active against all the test organisms: *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The bacterium, *Escherichia coli* showed the highest susceptibility to the mushroom mycelia at 10.6 mm on the first day of incubation which increased to 15.2 mm on the fourth day using the dual method while *Pseudomonas aeruginosa* showed the least susceptibility of 5.7 mm on the fourth day. In the Weller method the growth of the test organisms were completely antagonized by the mycelia of *Ganoderma lucidum* as total inhibition was recorded from all the plates. These could be an indication that *Ganoderma lucidum* can be used in the treatment of diseases caused by these organisms as well as preventive measure against them.

Keywords: Bacteria, *Ganoderma lucidum*, Mycelial culture, Prevention, Preatment

1. Introduction

Ganoderma lucidum known as lingzhi in Chinese, reishi in Japanese, yeonghi in Korea is a mushroom in the group commonly known as polypores in the family *Ganodermataceae* and they have a worldwide distribution in both tropical and temperate region. (Ofodile, 2005). The genus *Ganoderma* fruit bodies are sessile or stipitate, with shinny upper surface having district context. They may be yellow, black, white and reddish to purple (Ofodile & Bikomo, 2008).

Ganoderma species, especially *Ganoderma lucidum* is highly ranked medicinal mushroom in oriental traditional medicine which has been widely used for the therapy of chronic diseases of various etiologies. (Carmen & Xin 2004). *Ganoderma lucidum* extract has been reported active against *Bacillus subtilis* and *Pseudomonas syringes* which are plant pathogen (Ofodile *et al.*, 2005). Also the antimicrobial activity of *Ganoderma lucidum* extract from Nigeria has been reported by (Ofodile *et al.*, 2005; Ofodile and Bikomo, 2008). *Escherichia coli* is known to cause gastro-enteritis, *Staphylococcus aureus* also causes food poisoning, vomiting and loss of fluid, *Klebsiella pneumoniae* causes lung abscess, bacteremia and *Pseudomonas aeruginosa* causes lung infection, urinary tract infection and septicemia. (Geo *et al.*, 2005; Willey *et al.*, 2008).

Mycelial culture of *Ganoderma lucidum* is the primary stage in the cultivation of the mushroom and its effect against human pathogen seems not to have been reported in Nigeria. This communication reports the effect of mycelial culture of *Ganoderma lucidum* as bioactive agent against *Escherichia coli* *Staphylococcus aureus*, *Klebsiella Pneumoniae* and *Pseudomonas aeruginosa*.

2. Materials and methods

2.1 Mushroom sample

Ganoderma lucidum sample was collected from National Research Institute Vom, Plateau State Nigeria. The mushroom was identified by its ornamental truncate spores which are oblong with one tapering end and other characteristics. (Zoberi, 1978; Smith & Sivasthamparan, 2003; Ofodile, 2006).

2.2 Bacteria used in the assay

The test organisms *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *pseudomonas aeruginosa* used in this study were obtained from microbiology laboratory, Lagos university teaching hospital, Idi-Araba Lagos. Each of the bacterium was maintained in sterile nutrient agar slant at 4°C.

2.3 Preparation of mushroom sample

Mycelial culture of *Ganoderma lucidum* was obtained by rinsing the *Ganoderma lucidum* sample collected with sterile water for 2-3 minutes, sterilized by wiping with 70% alcohol under aseptic condition and then rinsed with water again. With the aid of sterile scapel, the basidiocarp of the mushroom sample was cut to expose the interior tissues, pieces of the tissue were excised and placed on PDA plates already prepared and plates were then incubated at 25°C for 5 days. From the developing mycelia, mycelial tissues were transferred using inoculating needle to fresh PDA plates and into slant tubes and stored in the refrigerator at 4°C.

2.4 The bioassay

2.4.1 Inoculation of the bacteria isolates with *Ganoderma lucidum* using dual method

In this method the mycelia culture of *Ganoderma lucidum* was evaluated for its ability to grow and lyse the colonies of the bacteria species (Utkheda & Rahe, 1980).

Fresh PDA plates were prepared and kept in a lamina flow in order to maintain sterility. Using a sterile cork borer of 6mm diameter, two wells were made at opposite edge of the fresh PDA plates, using another sterile cork borer a disk culture of 7days old culture of *Ganoderma lucidum* was taken and placed into one of the wells previously made on the fresh PDA plate. Another sterile cork borer of 6mm diameter was used to take a disk culture of 24hrs old culture of bacteria isolate which has been adjusted to a concentration of 10^6 and inoculated it into the other well at the opposite edge of the plate already inoculated with *Ganoderma lucidum* culture. Each of the bacteria isolate and antagonist were placed at the opposite sides on the same plate at the same time (Utkheda & Rahe, 1980). This was done in triplicate for all the bacteria isolates and all the plates were incubated at 25°C and checked daily to measure the zone of inhibition for four consecutive days.

2.4.2 Interaction between the bacteria species and the mycelial culture of *Ganoderma lucidum*

Mycelial culture of the mushroom was tested against the four bacteria species on agar media using the method of Weller *et al.* (1985). In this method *Ganoderma lucidum* was evaluated for its ability to grow and lyse the colonies of bacteria. Fresh PDA medium was prepared, sterilized and poured into sterile plates. On each of the freshly prepared PDA plates two wells were made opposite to each other using 6mm diameter cork borer. A culture disk of 7days old *Ganoderma lucidum* was placed in one of the well made on the plate using sterile cork borer. The plates were then incubated at 25°C for 48hrs. After 48hrs of incubation a culture disk of 24hrs old culture of the bacteria isolates which have been adjusted to a concentration of 10^6 was taken and placed in the other well in the plate already inoculated with mycelia of *Ganoderma lucidum* for 48hrs plates were then incubated at 25°C and were checked to measure and record zone of inhibition for four consecutive days.

3. Results

The result of testing mycelial culture of *Ganoderma lucidum* against bacteria pathogen *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are presented in tables 1 and 2.

The result showed that the mycelia growth of *Ganoderma lucidum* was very active against all the test organisms. Table 1 shows that the zone of inhibition by mycelia of *Ganoderma lucidum* on the test organisms increased daily. *Escherichia coli* showed the highest susceptibility of 10.6 mm on the first day which increased to 14.9 mm on the fourth day and *Pseudomonas aeruginosa* was the least susceptible with zone of inhibition of 5.7 mm on the fourth day.

Table 2 shows the total inhibition of all the test organisms as the organisms were totally suppressed by the 7day -old culture of mycelia of *Ganoderma lucidum*. None of the pathogenic bacteria grew when the mushroom was introduced into the media 48 hours before the pathogens were inoculated.

4. Discussion

This activity could be because when the polypore was inoculated into the medium 48hrs before the introduction of the bacteria pathogens the mycelia of the mushroom secreted some metabolites which totally suppressed the growth of the test organisms (Ofodile *et al.*, 2005).

The results above suggest that *Ganoderma lucidum* contain substances that are bactericidal during its growth stages and can be used to control human bacteria pathogen which cause various diseases (Gao *et al.*, 2002). Fang and Zhong (2002) reported the production of the antibacterial metabolite-ganoderic acid from Submerged Fermentation of *Ganoderma lucidum*. In the antimicrobial screening of different solvent extracts of *Ganoderma lucidum* on some species of bacteria reported by Ofodile and Bikomo (2008), some bioactive components such as phenolics, terpenoids and alkaloids were implicated which could also be the reason for the activity of its mycelial culture. Triterpenoid in *Ganoderma lucidum* has also been reported as the major antimicrobial constituents (Yihuai *et al.* 2003; Ofodile, 2006). The activity of the extracts from the fruiting body and mycelia of *Ganoderma lucidum* against some clinical bacteria in Asia have also been reported by some workers (Yoon *et al.*, 1994; Char *et al.*, 1997; Yihuai *et al.*, 2003). Therefore, there are secondary metabolites released by *Ganoderma lucidum* which could be responsible for its antibacterial activity. Patrick *et al.* (1998) Ryan and Ray (2004) reported that *Pseudomonas aeruginosa* is notorious for resistance against synthetic antibiotics, therefore results of this work implies that the mycelia of *G. lucidum* can be used to combat such stubborn organism.

This antibacterial activity suggest that the mycelial culture of *Ganoderma lucidum* isolated from dead wood in Nigeria does indeed contain certain bioactive elements or ingredients that might be beneficial for the prevention and treatment of varieties of ailments including important diseases such as gastro-enteritis, bronchitis, respiratory tract infection, food poisoning, urinary tract infection, and lung infection.

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Table 1. The mean zone of inhibition of mycelia of *Ganoderma lucidum* on bacteria pathogens using dual method

TEST ORGANISMS	Mean zone of inhibition (mm) per day			
	1	2	3	4
<i>Escherichia coli</i>	10.6	12.5	13.9	15.2
<i>Staphylococcus aureus</i>	10.5	12.6	13.4	14.9
<i>Klebsiella pneumoniae</i>	5.7	5.9	6.5	6.6
<i>Pseudomonas aeruginosa</i>	4.5	4.7	5.2	5.7

Table 2. The mean zone of inhibition of mycelia of *Ganoderma lucidum* on bacteria pathogens using Weller method

TEST ORGANISMS	Mean zone of inhibition (mm) per day			
	1	2	3	4
<i>Escherichia coli</i>	Ti	Ti	Ti	Ti
<i>Staphylococcus aureus</i>	Ti	Ti	Ti	Ti
<i>Klebsiella pneumoniae</i>	Ti	Ti	Ti	Ti
<i>Pseudomonas aeruginosa</i>	Ti	Ti	Ti	Ti

Ti- means total inhibition