

Anti infective agent

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RESEARCH ARTICLE



The Potential of *Ganoderma Lucidum* as Antimicrobial Agent for Multi-drug-Resistant *Mycobacterium Tuberculosis*



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Abstract: Background: The problem of bacterial resistance of *Mycobacterium tuberculosis* should be solved by seeking for alternative substances that potentially inhibit the growth or kill the bacteria. *Ganoderma lucidum* is one type of fungus which is potential to be an antimicrobial agent. This study aimed to determine the potential of *G. lucidum* on inhibiting the growth of multidrug-resistant bacteria of *M. tuberculosis in vitro*.

Methods: This study used a solid dilution method to test the extract of *G. lucidum* as an antibacterial agent.

Results and Conclusion: Results showed that all strains of multidrug-resistant tuberculosis (MDR-Tb) gave similar responses to *G. lucidum* extract at various concentrations. The bacteria did not grow on the medium containing *G. lucidum* extract at the smallest concentration of 12.5%, as well as concentrations of 25% and 50%. *Ganoderma lucidum* can be used as one of the alternatives for MDR-Tb drugs in the future.

Keywords: Antibacterial agent, *Ganoderma lucidum*, *Mycobacterium tuberculosis*, resistance, MDR-Tb, antimicrobial agents.

1. INTRODUCTION

Tuberculosis infection still becomes an issue on the health of people in various countries around the world. Multidrug-resistant tuberculosis (MDR-Tb) is one of the health problems which cannot be perfectly solved. In 2015, it was estimated that there were about 480,000 new cases of MDR-Tb, and 100,000 people with tuberculosis resistant to rifampicin, in which the sufferers will become MDR-Tb in the later time. Data from drug resistance surveillance reported that 3.9% of new cases and 21% of cases with previous medication history are predicted to be resistant to rifampicin or MDR-Tb in 2015. Multidrug-resistant tuberculosis has caused the deaths of approximately 250,000 cases occurring in Asia [1].

Multidrug-resistant tuberculosis is caused by *M. tuberculosis* that gives no response to first-line antituberculosis drugs, i.e., isoniazid and rifampicin. Both drugs are the two most powerful anti-tuberculosis drugs. Patients who are resistant to rifampicin or MDR-Tb require treatment with second-line medication regimens. The drugs classified as second-line antituberculosis drugs are more complex and have worse side effects than the first-line drugs [1].

The problem of resistance to *M. tuberculosis* can be solved by seeking for other substances that potentially inhibit the growth or kill the bacteria. Fungi are one of the plants widely used as a medicinal ingredient. Some countries are

reported to use fungi as medicinal ingredients such Japan, China, and Korea [2]. These countries have used fungi to cure some infectious diseases, metabolic and malignant disorders [3]. Some types of fungi commonly used for medicine are *G. lucidum*, *Coprinellus micaceus*, and *Lentinula edodes*. It is reported that certain parts of these fungi are proven to inhibit the growth of several types of microbes and function as antioxidants [4].

In Japan, *G. lucidum* is popularly known as Reishi, while in China, it is called by the name of Lingzhi. *Ganoderma lucidum* contains several types of bioactive substances, including terpenoids, steroids, phenols, glycoproteins, and polysaccharides, especially beta glucans [5-8]. These substances are reported to have a potential as antimicrobial, antiviral, immunomodulatory, antioxidant, anticancer, and antidiabetic agents [4, 9-12].

The potential of *G. lucidum* as an antibacterial agent has been reported in previous studies, as this fungus can inhibit the growth of several types of bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Salmonella typhi* and *Pseudomonas aeruginosa in vitro* [13]. *Ganoderma lucidum* may also inhibit the replication of *M. tuberculosis* in lung and spleen tissues of rats [14]. However, the strength of *G. lucidum* against *M. tuberculosis* resistant to antituberculosis drugs is not yet known. In some previous studies, it is reported that *G. lucidum* has a great potential as an antimicrobial agent; nevertheless, its use as anti *M. tuberculosis* has not been deeply investigated. This potential indicates that *G. lucidum* may give hope for the treatment MDR-Tb infections in the future.

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This study aims to determine the potential of *G. lucidum* on the growth of drug-resistant bacteria of *M. tuberculosis* *in vitro*.

2. METHODS

This study had received approval from the Ethics Committee of the Faculty of Medicine, Sultan Agung Islamic University, Semarang.

2.1. Materials

This study used the extract of *G. lucidum* (Lingzhi) produced by CV. Herba Nusantara, in which the product was Herbalus. Dimethyl sulfoxide (DMSO) of 5% was utilized as a solvent, whereas the bacteria used were multidrug-resistant *M. tuberculosis*. These bacteria were resistant to all first-line antituberculosis drugs and were obtained from the Health Laboratory of Central Java Province in Semarang, Indonesia. Lowenstein Jensen (LJ) was used as the bacterial culture.

2.2. Test of Anti-Tuberculosis Materials

This study was conducted using MDR *M. tuberculosis* isolates. The testing of antituberculosis drug resistance was performed at the Health Laboratory of Central Java, while the antimicrobial test was carried out at the Microbiology Laboratory of the Faculty of Medicine, Gadjah Mada University, Yogyakarta. The tests were performed on 4 isolates of MDR-Tb, *i.e.*, isolate 1103, 1377, 1378 and 1382. The preparation of the testing media is shown in Fig. (1).

2.3. Dilution of Solutions

The extract of *G. lucidum* 100% was taken for 15 mg and then dissolved into 15 ml of dimethyl sulfoxide (DMSO) 5%. The extract was diluted to various concentrations in series, *i.e.*, 12.5%, 25% and 50%, and each was placed on tubes 1, 2 and 3. Tube 4 is a positive control containing 1 ml of aquadest and 1 ml of bacterial suspension of MDR-Tb. Tube 5 is a media control containing 1 ml of aquadest and 1 ml of middle brook double strength (MBDS). Tube 6 is a solvent control containing 900 ul of aquadest, 100 ul of DMSO 100% and 1

ml of bacterial suspension of MDR-Tb. Tube 7 is an antibiotic control, *i.e.*, Rifampicin of 40 ug/ml. The concentration of antibiotics was made by adding 8 ul of Rifampicin concentration of 10,000 ug/ml, and 992 ul of Aquadest, and 1 ml of abacterial suspension of MDR-Tb.

2.4. Inoculation of Bacterial Suspension

The MDR-Tb bacterial suspension of 10^6 CFU/ml was inoculated at each concentration (Tube 1-3): positive control, solvent control and antibiotic control, for as much as 1 ml. The final concentration of bacteria was 5×10^5 CFU/ml, and the final concentration of the compound was 50%, 25%, and 12.5%. The suspension was incubated at 37° C for 10 days. Furthermore, MDR-Tb bacteria were inoculated in each tube containing LJ medium and incubated at 37°C for 3-4 weeks. Observations of bacterial growth were conducted after the inoculation. The growth of bacteria was observed every week for 10 weeks. The results of observation are shown in Fig. (2). In this study, the testing of each strain was carried out in the triple and obtained consistent results.

3. RESULT AND DISCUSSION

The results of *G. lucidum* test as an inhibitor of MDR-Tb bacterial growth can be seen in Table 1.

Table 1. Results of extract test of *G. lucidum* on bacterial growth of MDR- Tb.

Samples	Concentration (%)			K (+)	K (P)	K (A)	K (M)
	12.5	25	50				
1103	-	-	-	+	+	+	-
1377	-	-	-	+	+	+	-
1378	-	-	-	+	+	+	-
1382	-	-	-	+	+	+	-

K(+)-positive control, K(P)-solvent control, K(A)-antibiotic control, K(M)-media control.

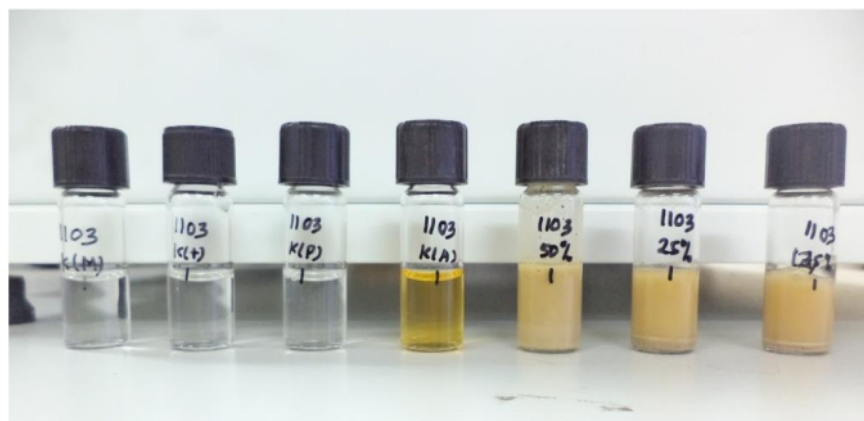


Fig. (1). Media for testing *Ganoderma lucidum* extract as an inhibitor of bacterial growth of MDR-Tb. Strain number 1103K(M) = media control; K(+)= positive control; K(P) = solvent control; K(A) = control of antibiotics; 50%, 25%, 12.5% = concentration of *G. lucidum* extract.

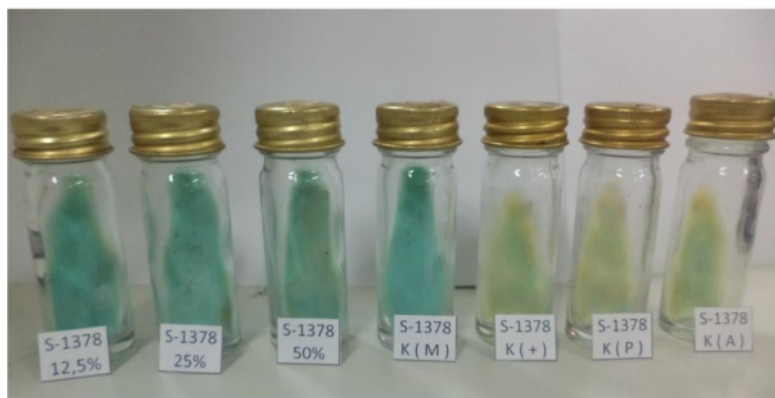


Fig. (2). Results of observation of MDR-Tb bacterial growth of strain number 1378 in various media after 10 weeks. In tubes with media containing *G. lucidum* extract at concentrations of 12.5%, 25%, 50%, and tube K(M) (media control), no visible bacterial growth was reported. Meanwhile, in Tube K(+), K(P), and K(A), there was the visible growth of bacteria.

The present study shows that *G. lucidum* extract could inhibit the growth of MDR-Tb bacteria. Several previous studies also reported that *G. lucidum* has a potential as antibacterial agents. Extract of *G. lucidum* is antibacterial in Gram-positive and Gram-negative bacteria. *Ganoderma lucidum* extracted with methanol actively fight against *S. aureus*, *Bacillus cereus*, and *P. aeruginosa* strains. The level of resistance of each bacteria against *G. lucidum* is different. Of the three bacteria, *P. aeruginosa* is the most resistant bacteria to *G. lucidum* [15]. The results of this study indicated that MDR-Tb bacteria did not grow in cultures containing *G. lucidum* with various concentrations. The lowest inhibitory power of *G. lucidum* was at a concentration of 12.5%. This indicates that *G. lucidum* is a powerful anti-tuberculosis substance that can inhibit the growth of MDR -Tb.

The results of this study are congruent with previous studies which showed that the inhibitory power of *G. lucidum* on *S. aureus* and *B. cereus* bacteria was 0.0125-0.75 mg/ml. Meanwhile, at a concentration of 0.035-1.5 mg/ml, *G. lucidum* has the potential to be bactericidal [15]. Some bacteria which show high sensitivity to Ethylacetate of *G. lucidum* are *Corynebacterium pyogene* and *B. subtilis* isolates. Meanwhile, *P. aeruginosa* and *Proteus mirabilis* showed lower sensitivity. The highest sensitivity was shown by *K. pneumoniae* isolate with minimum bactericidal concentration (MBC) below 12.5 mg/ml. *Ganoderma lucidum* may inhibit and interfere with the growth of *E. coli*, *S. aureus*, *K. pneumoniae*, *B. subtilis*, *S. typhi* and *P. aeruginosa*. *Ganoderma lucidum* extracted with acetone showed a maximum inhibitory ability to the growth of *K. Pneumonia* [13]. Miselia *G. lucidum* is very effective in inhibiting the growth of *E. Coli* [16]. In some cases, polysaccharides in certain *G. lucidum* can kill bacteria [12]. Other studies reported that *G. lucidum* also has the potential as an antibacterial agent for acid-resistant bacteria. *Mycobacterium tuberculosis* is acid-resistant bacteria. The results of the study on mice showed that the administration of *G. lucidum* extracts in the mice before the *M. tuberculosis* infection, can protect the mice from infection. *Ganoderma lucidum* extract can inhibit the growth of MTB bacteria by preventing the process of lengthened replication of the bacteria [14].

Ganoderma lucidum has a strong potential as an antibacterial agent since it contains some substances that can disrupt the growth of bacteria and even kill them. One of the substances that can act as an inhibitor of bacterial growth is Ethylacetate [17]. In addition to Terpenoid, there are other bioactive substances in *G. lucidum* [18]. These substances can also act as an antibacterial agent. In a study of bacterial culture with Terpenoid media, it was reported the growth of *B. cereus* and *S. aureus* were inhibited [19]. Terpenoids can interfere with the survival of pathogenic bacteria by destroying the membrane permeability of the bacteria. This process applies both to Gram-positive and Gram-negative bacteria [20-21].

CONCLUSION

This study proved that *G. lucidum* extract could inhibit the growth of MDR-Tb isolates at the smallest concentration (12.5%). These findings indicate the potential of *G. lucidum* as one of the alternative to anti-MDR-Tb drugs in the future. As a follow-up of this study, it is necessary to test the potential of *G. lucidum* extract as anti MDR-Tb drugs *in vivo*.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study had received approval from the Ethics Committee of the Faculty of Medicine, Sultan Agung Islamic University, Semarang.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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