Effect of ethanol extract of *Ganoderma lucidum* from Tasikmalaya against the growth of Salmonella sp bacteria and some Pathogenic Fungi using the Kirby-Bauer method in vitro

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ABSTRACT

Ganoderma lucidum (lingzhi) can be used as traditional medicine. This mushroom has many properties, one of which is as an antimicrobial. Antimicrobial functions as antibacterial, antiviral, and antifungal. Lingzhi contains organic alkaloid compounds, flavonoids, and triterpenoids. One of the bacteria that often cause disease in Indonesia is Salmonella sp and several dermatophyte diseases caused by infection with several pathogenic fungi such as Malassezia furfur, Trichophyton rubrum, and Trichophyton mentagrophytes . This study aimed to determine the inhibitory power of Lingzhi from Tasikmalaya ethanol extract against Pathogenic Microorganism. The method used is tested against the anti-microbial used by using the diffusion method using disc paper or Kirby Baure. The analysis showed that the 70% ethanol extract of Lingzhi mushrooms had antibacterial activity against salmonella sp as evidenced by the presence of an average inhibition zone at a concentration of 50% (16,57mm), 60% (18,1mm); 70% (18,55mm), 80% (19.17mm), 90% (21,2mm), and 100% (21,8mm). Inhibitory ability against several pathogenic fungi was able to inhibit at a concentration of 100% with a diameter of Trichophyton rubrum, Trichophyton mentagrophytes and Malassezia furfur with an average diameter of inhibition of 12.9 mm, 9.4 mm, and 6 mm.

Keywords: *Ganoderma lucidum, Inhibition Test, Salmonella sp, anti-fungal*

INTRODUCING

Ganoderma lucidum can be used as traditional medicine. This mushroom has many properties, one of which is as an antimicrobial. Antimicrobial functions as antibacterial, antiviral, and antifungal. *Ganoderma lucidum* contains organic alkaloid compounds, flavonoids, and triterpenoids. One of the bacteria that often cause disease in Indonesia is *Salmonella* sp. This genus of bacteria is one of the causes of typhoid fever or typhoid, typhoid fever is still a very important health problem in various developing countries including Indonesia. One of the natural ingredients that can be used as medicine is mushrooms. This organism lives in a certain place, at a certain time, and is often found in wood. The mushroom belongs to the *Ganoderma lucidum* species, which has many types with textured body characteristics such as wood, hard, and shaped like a fan. In the Tasikmalaya area, it grows quite a lot. This mushroom is often found in hot and humid areas in the Asian region. The mushroom grows in the mountains of dense forests with very high humidity. *Ganoderma lucidum* has many properties, one of which is antibacterial and antimicrobial. One of the bacteria that often cause disease in Indonesia is *Salmonella*. This genus of bacteria is one of the causes of typhoid fever or typhus. Typhoid fever is an acute infectious disease. Typhoid fever is still a very important health problem in various developing countries, including Indonesia. One of the highest mortality rates in foodborne diseases is caused by bacteria, one of which is *Salmonella*., which causes 216,000 deaths every year. The symptoms are high fever, nausea, vomiting, dizziness, and abdominal pain (Putri, et al, 2019).

Lingzhi mushroom (*Ganoderma lucidium*) has antitumor, anticancer, anti-inflammatory, antiallergic, antioxidant, antibacterial, hepatoprotective activity, lowers blood sugar levels, lowers cholesterol and blood pressure, inhibits platelet aggregation, inhibits histamine release, and is anti-HIV (Sanodiya et al., 2005). is a member of the Basidiomycotina that lives on tree trunks, has a hard body with an uneven surface and wavy edges. Lingzhi mushroom is known to contain organic compounds, such as polysaccharides, adenosine, ganoderic acid, protein, oleic acid, vitamins, triterpenoids, organic germanium (GeO), ascorbic acid, and riboflavin. This mushroom can be profitable because of its medical potential (Ratnaningtyas, 2012).

Several studies related to the ability of lingzhi mushrooms as antibacterial, including research by Handrianto (2017) on gram-negative bacteria stated that the *Ganoderma lucidum* had antibacterial activity against *Escherichia coli*. *Ganoderma lucidum* mushroom extract at concentrations of 60% (13.65 mm), 80% (14.9 mm) and 100% (16.5 mm). Research conducted on other gram-negative bacteria by Sudarwati and Fernanda (2021) stated that the *Ganoderma lucidum* has antibacterial activity against using the Bioautography Test method which produces triterpenoid soluble compounds as an antibacterial of medium category *Escherichia coli* and *Bacillus subtillis*. The research conducted by Singh et al (2014) on In vitro Evaluation of Antimicrobial Activity of *Ganoderma lucidum* stated that lingzhi

mushroom extract using methanol as a solvent had antibacterial activity. This may be due to the polar nature of methanol which binds to the more polar active antimicrobial compounds of *Ganoderma lucidum*, namely alkalides, coumarins, and flavonoids.

Research related to the antibacterial activity test of wild *Ganoderma lucidum* on the growth of salmonella bacteria has not been widely studied, so in this study to determine the effect of an ethanol extract of wild *Ganoderma lucidum* on inhibiting the growth of *Salmonella* sp bacteria.

METHOD

1. Matrials

The tools used in this study were autoclave (Portable M300), light microscope (Olympus CX23), evaporator (IKA RV 10), dry sterilizer (Corona ZTP8A-7 (IR Ray), incubator (Memmert UNB 4000, analytical balance (Excellent Analytical). Balance AB HZK-2104, oven (T100-200), stir bar, mattress thread, petri dish, beaker, Erlenmeyer, measuring cup, spirit flask, round loop, hot plate, micropipette, tip, spatula, test tube, gauze, umbrella paper. The materials used are Muller Hinton Agar (oxoid), Physiological NaCl, 1% BaCl2, 1% H2SO4, ciprofloxacin, *Ganoderma lucidum* from Tasikmalaya Singaparna region, culture *Salmonella* sp, *Malassezia furfur*, *Trichophyton rubrum,* and *Trichophyton mentagrophytes*.

The research on the inhibition test of *G.lucidum* ethanol extract on several microorganism was experimental, with samples were taken from pure cultures that had been cultured on Muller Hinton Agar media and given ethanol extract of *G.lucidum* then incubated at 37ºC for 24 hours. . The culture results were seen and the size of the inhibition zone formed was observed. The concentration used for this study was the concentration of 50%, 60%, 70%, 80%, 90%, 100%, with the diffusion method or Kirby-Bauer with samples using MHA media,

2. Procedure

1. Preparation of Wild *Ganoderma lucidum* extract.
   a. The powdered *G.lucidum* that has been pulverized is weighed 100 grams and then put into a beaker with 1000 ml 96% ethanol solvent. Soak for 3 times 24 hours, stirring occasionally, then filtered.
   b. The filtered filtrate is evaporated to evaporate the ethanol solvent that is still mixed so that a pure extract is obtained at a temperature of 70º C so that a concentrated extract is obtained.
2. Antibacterial Testing of *Ganoderma lucidum* against *Salmonella* sp
   a. Standard 0.5 Mc Farland 1%
   9.5 sulfuric acids (H2SO4) solution is put into a sterile tube and add 1% BaCl2 as much as 0.5 ml. Then shake until you get 0.5 Mc Farland turbidity
   b. Making Microorganism Suspensions
   The Microorganism culture was taken using a sterile loop needle and then put in a physiological NaCl solution, shake until it reaches 0.5 Mc Farland turbidity. The suspension is homogenized just before inoculation on a petri dish to prevent deposition.

3. Making Variations of Clove Flower Ethanol Extract Solution The ethanol extract of *G.lucidum* in this study was made in concentrations of 50%, 60%, 70%, 80%, 90% and 10% by calculating w/v (g/10ml). That concentration was made by weighing the extracts 5, 6, 7, 8, 9, 10 grams, then dissolved each with sterile distilled water to a volume of 10 mL.
4. Activity Test of *G.lucidum* Extract
   a. 100µL of *Salmonella* suspension was poured into Muller Hinton Agar medium.
   b. Add 20µ *G.lucidum* ethanol extract to each media that has been placed on discs with various concentrations of 50%, 60%, 70%, 80%, 90%, 100%. And sterile distilled water was used as a negative control.
   c. The media was incubated at temperature 37^{o} C for 1 x 24 hours. The results are seen in the inhibition zone around the disc.
   5. Data Analysis

The research design used a completely randomized design (CRD) with the type of treatment in the form of several concentrations of *G.lucidum* extract, in each treatment 4 repetitions were carried out so that a total of 24 experimental units were obtained. The antibacterial activity was observed from the clear zone around the paper disc. The data obtained were then analyzed statistically using a One-way Analysis of Variance with a validity level of 95%, the level of significance between treatments then analyzed using the Duncan test or DMRT.

RESULTS AND DISCUSSION

Based on research results with a sample of 24 tests, at a concentration of 50% to concentration 100% contained zone of inhibition (mm), where he zone is marked as a no the growth of bacteria around the discs that each have different in diameter, where is the *Salmonella* sp, average inhibition zone at a concentration of 50% (16,57mm), 60% (18,1mm); 70% (18,55mm), 80% (19.17mm), 90% (21,2mm), and 100% (21,8mm) with the positive control (Ciprofloxacin) equal to 30,32 mm. One-way or oneway analysis of variance data one-way ANOVA shows the difference which was very evident between the treatments several concentrations of *G.lucidum* extract to the inhibition zone diameter *Salmonella* sp, where p = 0.000 or p-value <0.05. This matter shows that the concentration variation of the ethanol extract of wild *G.lucidum* has an effect against the growth of *Salmonella* sp, from these results, can be carried out a further test using the Duncan test. The Duncan test results were mean the different diameter of resistivity of every concentration. The result of *G.lucidum* extract treatment almost the entire concentration showed different effectiveness. The test results are presented in Table 1 and Figure 1.

Based on the results of further tests on Table 1, shows that the ability of every concentration *G.lucidum* extract against *Salmonella* sp produces growth distinct zone of inhibition, however, several concentrations are it's inhibitory ability is not significantly different namely between the concentrations 60% with 70% and 90% with 100%, at a

positive control showed the maximum inhibition with inhibition of 30,32 mm.

Based on the results of the inhibition test of the ethanolic extract of the fungus against the fungal pathogens, including *Trichophyton rubrum, Trichophyton mentagrophytes*, and *Malassezia furfur*. Lingzhi mushroom ethanol extract contains antifungal compounds through phytochemical tests. Pathogenic fungi that have been rejuvenated from pure culture on MHA media. The hyphae and colonies grown were dissolved with 3 ml of physiological NaCl to be used as a suspension, then the turbidity was measured according to the standard Mc Farland 0.5. This antifungal test was tested using the Kirby Bauer method or disc diffusion because there are advantages such as easier clear zone measurement. The results of the inhibition test showed that at a concentration of 100% the extract was able to produce the highest inhibitory power. Inhibitory diameter of the fungus Trichophyton rubrum with an average diameter of inhibition of 12.9 mm, Trichophyton mentagrophytes with an average diameter of inhibition of 9.4 mm, and Malassezia furfur with an average diameter of inhibition of 6 mm. The ability of the resistance is presented in Figure 2.

The zone of inhibition that is formed is due to the presence of compounds active capable of influencing bacterial growth so that it forms the clear zone around the disc. Test the active compound can be done using screening phytochemical test where positive results can be seen in the presence change color of extract after addition of certain solvents. Result Phytochemical test screening on the ethanol extract *G.lucidum* shows these flavonoid compounds, alkaloid, and triterpenoid as presented in table 2. The results of the phytochemical screening test of the ethanol extract of Wild *G.lucidum* were shown in Table 2.

Table 1. The average diameter of the inhibition zone of ethanol extract *G.lucidum* against *Salmonella* sp after Duncan's test.

Treatment (s)	The average diameter of inhibition zone (mm)	Category
50%	$16,57^{a} \pm 0,82$	Intermediate
60%	$18,1^{b} \pm 0,60$	Intermediate
70%	$18,55^{bc} \pm 0,81$	Intermediate
80%	$19,17^{c} \pm 0,53$	Intermediate
90%	$21,20^{d} \pm 0,52$	Sensitive
100%	$21,80^{d} \pm 0,40$	Sensitive
Control (+) Ciprofloxacin	$30,32^{e} \pm 0,09$	Sensitive
Control (-)	0	

Note(s): The number followed by the same alphabet was identical based on the Duncan test
- Susceptible response sensitive (> 21 mm), intermediate (16 - 20 mm), resistant (< 15 mm). (CLSI2018)

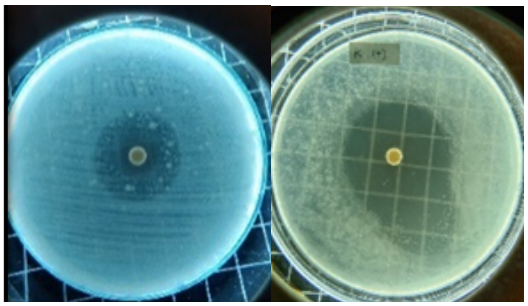

Figure 1. The clear zone shows the inhibitory activity of extract ethanol G.lucidum against Salmonella sp (a. concentration 100%, b. positive control: Ciprofloxacin)

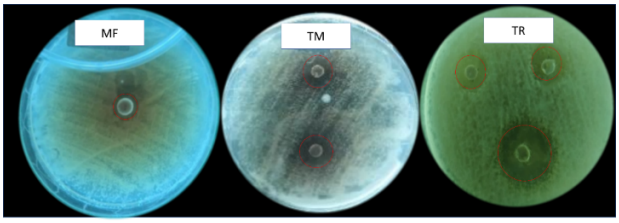

Figure 2. The clear zone shows the inhibitory activity of extract ethanol G.lucidum against Malassezia furfur (MF), Trichophyton mentagrophytes (TM) and Trichophyton rubrum (TR) from concentration 100%.

Table 2. Extract ethanol G.lucidum phytochemical screening test results

Phytochemical Test	Results
Flavonoid	Positive
Alkaloid	Positive
Triterpenoid	Positive

Alkaloids are secondary metabolites that have one or more nitrogen and have antibacterial properties by interfering with the peptidoglycan constituent components of bacterial cells so that the cell wall layer is not fully formed and causes cell death (Prahastuti *et al*, 2001). Flavonoids have antibacterial properties with the mechanism of inhibiting nucleic acid synthesis, cell membrane function, and energy metabolism by inhibiting the use of oxygen by bacteria, namely by preventing the formation of energy in the cytoplasmic membrane and then inhibiting the motility or movement of bacteria that play a role in antimicrobial activity and extracellular proteins (Qinghu Wang et al. 2016).

Triterpenoids have bacteriostatic properties that can inhibit bacterial growth by interfering with the process of forming cell membranes or microbial cell walls (Widiyati, 2006). According to Surahmaida, from the results of the chromatogram, the active compound of *Ganoderma lucidum* whose composition is larger, namely 2,7-Diphenylindole, is a heterocyclic compound containing an indole ring. Heterocyclic compounds are an important group of compounds that form almost all organic compounds. Heterocyclic compounds play an important role as the basic structure in the fields of pharmaceuticals (drugs), agrochemicals, and other important biological activities such as antimicrobials, natural pesticides (herbicides, fungicides, and insecticides), antivirals, and others. Natural compounds that have an indole ring are classified as alkaloids. Indole is an aromatic heterocyclic compound with a bicyclic structure. This component is a precursor in the pharmaceutical field, namely drugs (Kumar and Singh, 2013). These indole alkaloids have pharmacological effects such as analgesic, antiallergic, anticonvulsant, antifungal, antihistamine, anti-inflammatory, anticancer, antihypertensive, cardiovascular, antioxidant (Raju *et al*., 2015), as well as Gram-positive and Gram-negative antibacterial (Cinchana *et al*., 2015). 2011).

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CONCLUSION

of Lingzhi mushrooms had antibacterial activity against salmonella sp as evidenced by the presence of an average inhibition zone at a

concentration of 50% (16,57mm), 60% (18,1mm); 70% (18,55mm), 80% (19.17mm), 90% (21,2mm), and 100% (21,8mm). Inhibitory ability against several pathogenic fungi was able to inhibit at a concentration of 100% with a diameter of *Trichophyton rubrum, Trichophyton mentagrophytes* and *Malassezia furfur* with an average diameter of inhibition of 12.9 mm, 9.4 mm, and 6 mm.

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