Antibacterial Activity of Clove Medicinal Plants (*Bischofia javanica* Blume) Against Cell Damage of *Staphylococcus aureus* and *Salmonella enterica*

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Abstract. This study aimed to isolate secondary metabolites and to test the antibacterial activity of cingkam bark extracts. The antibacterial activity test was carried out using the disc diffusion method. Determination of the minimum inhibitory concentration (MIC) in vitro by micro dilution method against pathogenic bacteria, namely *Salmonella enterica* ATCC 14028 and *Staphylococcus aureus* ATCC 25923. The results of the research that have been carried out are known to have antibacterial activity against *Salmonella enterica* and *Staphylococcus aureus*. The extraction that produced the strongest antibacterial activity was the extraction technique with methanol as a solvent on *Salmonella enterica* bacteria. In *Staphyloccus aureus*, the extraction technique with ethyl acetate solvent produces the strongest antibacterial activity. The MIC concentration value produced the same response to the two test bacteria, namely 6.25 mg/mL. Cingkam bark extracts has antibacterial activity against *Salmonella enterica* and *Staphylococcus aureus*.

Keywords: antibacterial, cingkam, *Salmonella enterica*, *Staphylococcus aureus*.

1 Introduction

Bacteria are microorganisms that live cosmopolitanly so they can be found everywhere in the environment, air, soil, water, and a large number of them live on human skin and digestive tract [1]. The gut microbiome consists of a bacterial community mostly in the distal intestine, where nearly 100 trillion microorganisms are present [2]. Intestinal bacteria are important components of the microbiota ecosystem in the human gut and play important roles in human health, such as supplying essential nutrients, synthesizing vitamin K, aiding cellulose digestion, and promoting angiogenesis and enteric nerve function. However, they are also potentially dangerous due to changes in their composition when the gut ecosystem undergoes abnormal changes due to antibiotic use, disease, stress, aging, poor eating habits, and lifestyle [3]. Various kinds of pathogenic bacteria that colonize the human digestive system can cause many chronic diseases, such as peptic ulcer disease, gastric lymphoma, obesity, cancer [4].

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Staphylococcus aureus and *Salmonella enterica* are pathogenic bacteria that cause infections in the digestive tract in humans. Diseases caused by bacteria in the digestive system are second only to respiratory diseases. In 2019, based on data from the Ministry of Health of the Republic of Indonesia (Kemenkes RI) reported that infectious diseases such as pneumonia and the digestive tract system are the main problems that cause death.

The use of antibiotics in overcoming diseases caused by bacteria is a must, but the number of resistance and death caused by them continues to increase in line with the use of antibiotics [5]. Antibiotic-resistant bacteria pose a global health threat. Traditional antibiotics can lose their effectiveness, and the development of effective new antibacterials has become a priority in recent years. Plants are an invaluable source of antimicrobial compounds with broad therapeutic potential. The increasing incidence of drug-resistant pathogens raises an urgent need to identify and isolate novel bioactive compounds from medicinal plants using modern standardized analytical procedures.

Traditional medicine has long used plants to prevent or cure infectious diseases. Plants are rich in various secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antibacterial properties. Various studies have shown that phytochemical secondary metabolites from plants are a valuable source of bioactive compounds with strong antimicrobial activity [6].

Bischofia javanica Blume plant. or known as cingkam (Karo) is included in the *Euphorbiace* family, traditionally it has been widely used by people in various regions to treat various diseases. The cingkam plant (*B. javanica* Blume) has been used traditionally to treat inflammatory diseases such as tonsillitis, cancer, inflammation, tuberculosis, diarrhea, sore throat, burns and ulcers across Asia [7]. The content of bioactive compounds contained in this plant is the main key to the practice of traditional medicine among many tribes.

Based on empirical experience, cingkam has long been used as traditional medicine for generations. However, pharmacologic information, especially antibacterial from shingles, is still very little and the observed variables are also very limited, such as the zone of inhibition and the description of morphological damage to pathogenic bacterial cells as a result of the extract. Based on the background of the problem, the title of this research is the Antibacterial Activity of Cingkam (*Bischofia javanica Blume*) Against Cell Damage of *Staphylococcus aureus* and *Salmonella enterica*.

2. Methods

2.1 Types of research

This research is a descriptive and experimental study consisting of 2 stages, namely: 1) determination of the diameter of the extract inhibition zone by agar diffusion method, testing the antibacterial activity of the active extract by determining the minimum inhibitory concentration (MIC).

2.2. Research Tools and Materials

The tools used in this research are laboratory glassware, autoclave, vortex, stirer, laminar air flow, metal assay cylinder, micropipette, tips, centrifuge, Bunsen, ose needle, balance.

The materials used in this study were divided into three parts, namely: 1) The plant material used was cingkam bark (*B. javanica* Blume). 2) The chemicals used in this study were: aquades, nutrient agar (Merck®), mueller hinton agar (Merck®), mueller hinton broth (Merck®), n-hexane (Merck®), methanol (Merck®), ethyl acetate (Merck®), alcohol

(Merck®), acetone (Merck®), spirit and filter paper (Merck®). 3) Test bacterial culture material. Pure bacterial cultures used were *Staphylococcus aureus* ATCC 25923 Gram's (+) and *Salmonella enterica* ATCC 14028 Gram's (-).

2.3. Cingkam Bark Sampling (*B. javanica* Blume)

Bark samples were collected from Ketangkangan Village, Sibolangit District, Deli Serdang Regency. North Sumatra. The bark of the cingkam (*B. javanica* Blume) was put in ziplok plastic, then taken to the microbiology laboratory, Universitas Negeri Medan.

2.4. Extract Making

The bark was rinsed using running water, first scraped the outer surface and sliced into 2 cm size and then dried in a shady place. After drying then mashed using a blender and filtered. The extraction methods used are infusion, decoctation and maceration. Infusion is done by boiling 10 grams of fresh bark as much as 10 grams using 100 ml of water at a temperature of 90-98ºC for 15 minutes then filtered. Decoction is made by boiling 10 grams of bark using 100 ml of water at a temperature of 90-98ºC for 30 minutes and then filtered [8]. Three types of solvents used in maceration are methanol, n-hexane and ethyl acetate. Filter using two layers of muslin cloth, centrifuged at 9000 rpm for 10 minutes. Filter using whatman paper no. 41 to get the filtrate. The filtrate was evaporated using a rotary evaporator at a temperature of 40ºC. The extracts were weighed (except for infusion and decoction), and stored in small bottles and put in the refrigerator at 4ºC.

2.5. Antibacterial Activity Test

Testing the antibacterial activity of each extract obtained by the agar well diffusion method (Agar Well Plate Diffusion Assay Method) using a sterile metal buffer (Salihovic, et al., 2018). Pour 10 ml of Mueller hinton agar into a sterile petri dish and allow it to solidify as the first layer. Then, on the surface of the layer, 0.1 ml of the tested bacterial inoculum suspension was poured and 20 ml of MHA media as the second layer was then homogenized. Then the metal backers are placed and arranged on the surface of the media and arranged in such a way that the observation areas do not overlap each other. After that, the metal backing is slowly removed using sterile tweezers from the surface of the solidified agar medium, so that it eventually forms wells (holes). The test extract solutions with various concentrations and blanks (a mixture of dimethylsulfoxidan and ethanol) were each added to the wells that had been provided in the amount of 0.1 ml. The petri dish was immediately closed and allowed to stand for 30 minutes, then incubated at 35±2 ºC for 24 hours. Observations were made by measuring the clear zone in the form of a circle around the well using a caliper. Data were taken from five treatments, so that the diameter of the growth inhibition was known in millimeters (mm).

2.6. Minimum Inhibitory Concentration (MIC)

The concentration of the test to determine the MIC was obtained from the results of the test of the diameter of the inhibition zone which had been carried out by showing a significant increase in the diameter of the antibacterial inhibition zone. The preparation of the test bacterial inoculum was carried out with the same procedure as testing the diameter of the extract inhibition zone.

3. Results and Discussion

3.1 Antibacterial activity of cingkam stem extract (*Bischofia javanica* Blume) against *Salmonella enterica* bacteria

The results of the antibacterial activity test of cingkam stem extract (*B. javanica* Blume) using the maceration extraction method with methanol, ethylacetate and hexane solvents at a concentration of 100 mg/ml against *Salmonella enterica* bacteria can be seen in **Figure 1**.

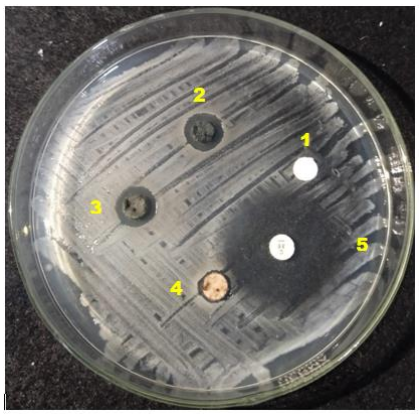

Fig. 1. Test results of antibacterial activity of cingkam bark extract against *Salmonella enterica* bacteria. 1. DMSO; 2. methanol; 3. ethyl acetate; 4. hexane; 5. Chloramphenicol

The results obtained showed that the antibacterial activity of the cingkam stem bark extract had a clear zone extracted with methanol, ethyl acetate and n-hexane as solvents. Of the three solvents used, successively the widest zones of inhibition were found in ethylacetate, methanol and hexane as shown in **Figure 2**. The antibacterial activity of cingkam bark extract against Salmonella enterica bacteria on average with methanol solvent with a clear zone diameter of 13.1 mm, 15.3 mm ethyl acetate and 2.7 mm hexane. Based on the wide diameter of the inhibition zone on extraction with ethyl acetate solvent can be categorized into the category of medium spectrum.

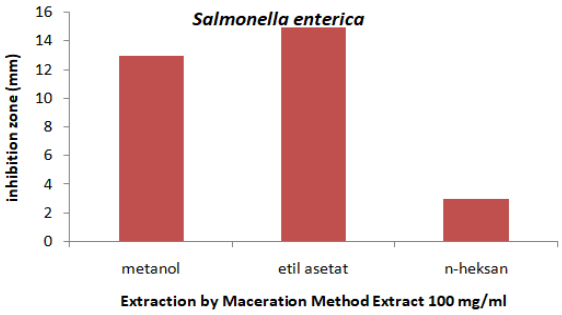

Fig. 2. The results of measuring the diameter of the inhibition zone of cingkam bark extract (*Bischofia javanica* Blume) from several solvents against *Salmonella enterica* bacteria

3.2. Antibacterial activity test of cingkam stem extract (*Bischofia javanica* Blume) against *Staphylococcus aureus*

The results of the antibacterial activity test of cingkam stem extract (*B. javanica* Blume) using the maceration extraction method with methanol, ethyl acetate and hexane solvents at a concentration of 100 mg/ml against *S. enterica* bacteria can be seen in **Figure 3**. The antibacterial activity of cingkam stem bark extract against S. *aureus* bacteria aureus with methanol solvent average inhibition zone diameter of 10.9 mm, ethyl acetate 13.3 mm and hexane did not show any antibacterial activity. These results indicate that most likely the compounds contained in the bark of the cingkam stem extracted with hexane solvent have been removed. These results are similar to the research reported by [9] Masfria, et al., (2019) which stated that hexane extract of cingkam stem bark containing triterpenoids and steroids did not provide activity against *E. coli*, *S. aureus* and *C. albicans*. Based on the extent of the clear zone in the extraction with ethylacetate solvent, it can be categorized into a medium spectrum category.

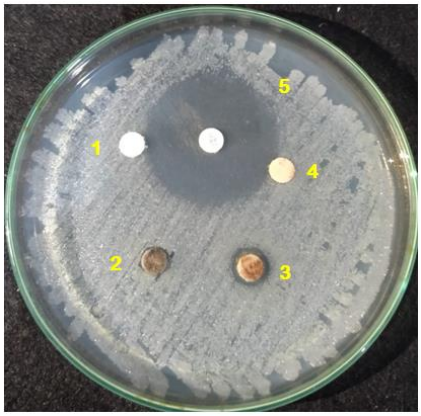

Fig. 3. Test results of antibacterial activity of cingkam bark extract on *Staphylococcus aureus* bacteria. 1. DMSO; 2. methanol; 3. ethyl acetate; 4. hexane; 5. chloramphenicol.

The results obtained showed that the antibacterial activity of the cingkam stem bark extract had a clear zone extracted with methanol, ethyl acetate and hexane as solvents that did not show antibacterial activity as shown in **Figure 4**. Of the three solvents used, the widest inhibition zones were found in Figure 4. ethyl acetate and methanol solvents.

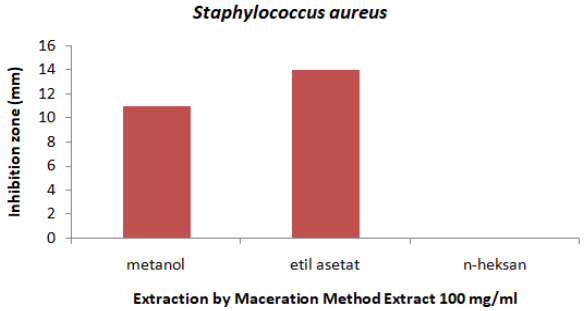

Fig. 4. The results of measuring the diameter of the inhibition zone of cingkam bark extract (*Bischofia javanica* Blume) from several solvents against *Staphylococcus aureus* bacteria

3.3. Minimum Inhibitory Concentration (MIC)

Based on the results of the MIC test of ethylacetate extract from the bark of cingkam stems against *S. enterica* and *S. aureus* bacteria, it can be seen in Table 1. The MIC concentration value produced the same response to the two test bacteria, namely 6.25 mg/mL.

Table 1. Results of MIC values for ethylacetate extract on *S. enterica* and *S. aureus* bacteria

Treatment	Extract Concentration (%)	Test Bacteria		MIC	
		S. enterica	*S. aureus*	*S. enterica*	*S. aureus*
Ciprofloxacin	10% control (-)	-	-		
Extract	100%	-	-		
	50%	-	-		
	25%	-	-		
	12,5%	-	-		
	6,25%	-	-	6.25	6.25
	3,12%	+	+		
	1,56%	+	+		
	0,78%	+	+		
	0,39%	+	+		
	0,19%	+	+		
Test Bacteria	Test Bacteria control (+)	+	+		

The purpose of determining the value of the minimum inhibitory concentration is to determine the minimum concentration needed to inhibit the growth of bacteria known as bacteriostatic. At high concentrations, extracts of antibacterial compounds that are bacteriostatic can turn into bacteriocides. The MIC values were different for each plant extract containing antimicrobial compounds. This is as reported by [10] using alang-alang extract (*Imperata cylindrica*) has a MIC of 7% against *E. coli* and 8% against *S. aureus*. Furthermore, [11] on extracts of bitter melon (*Momordica charantia*) against *E. coli* bacteria has a MIC value of 70%.

4 Conclusion

The cingkam bark extract has antibacterial activity against *Salmonella enterica* and *Staphylococcus aureus*. Extraction using ethyl acetate solvent showed the diameter of the widest zone of inhibition against *S. enterica* and *S. aureus*. The MIC concentration value produced the same response to the two test bacteria, namely 6.25 mg/mL

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