ANTIBACTERIAL ACTIVITY OF BUNGUR *(Langerstroemia speciosa (L) PERS)* LEAF EXTRACT AGAINST BACTERIA *Staphylococcus aureus ATCC 29213* BY DISC DIFFUSION METHOD AND DILUTION METHOD

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Abstract

Infection is one of the diseases that is still the biggest health problem worldwide, including in Indonesia. The most common bacteria causing nosocomial infections is Staphylococcus aureus. One of the plants suspected of having antibacterial activity is Bungur *(Lagerstroemia speciosa (L) Pers)* Plant, Bungur plant has secondary metabolites such as Alkaloids, Flavonoids, Saponins, Steroids, Terpenoids, and Tannins. The extraction process is done by maceration method with the solvent used is 96% ethanol. The antibacterial activity test of Bungur leaf extract using disc diffusion and dilution methods aims to determine whether Bungur leaf extract can inhibit and kill the growth of *Staphylococcus aureus ATCC 29213*. The concentrations used in this test are 50%, 75%,100%, positive control used was Ciprofloxacin and the negative control used was the result of standardized bacterial dilution with mc Farland 0.5. The results of the diffusion method test showed that Bungur leaf was able to inhibit bacterial growth in the presence of an inhibition zone at a concentration of 75% (8.11 mm) but the inhibition zone given was still in the weak category and Bungur leaf extract had no killing power against *Staphylococcus aureus ATCC* 29213 in the method. dilution in the absence of MBC values. it is necessary to develop research related to the antibacterial potential of the extract of Bungur leaf by testing the effect of the extract of Bungur leaf in inhibiting and killing the growth of the bacteria *Staphylococcus aureus ATCC 29213* with an increased concentration.

Keywords: Antibacterial, Bungur leaf extract, Staphylococcus Aureus ATCC 29213.

Introduction

Infection is one of the diseases that is still the biggest health problem worldwide, including in Indonesia. The infection itself can be sourced from the hospital environment which is called a nosocomial infection. The percentage of nosocomial infections in hospitals worldwide reaches

9% (variation 3-21%). Nosocomial infections are experienced by more than 1.4 million hospitalized patients. The number of nosocomial infections in Indonesia is quite high, namely 6-16% with an average of 9.8% in 2010. As many as 90% of nosocomial infections are caused by bacteria, while mycobacteria, viruses, fungi or protozoa are rare causes of infection. nosocomial. The most common bacteria causing nosocomial infections is *Staphylococcus aureus* (Agung *et al.,* 2021).

Data from the Centers for Disease Control and Prevention states that in Europe it is estimated that around 35,000 people die each year due to infections caused by multiresistant bacteria (Care *et al.,* 2020). The main cause of resistance is the widespread and irrational use of antibiotics, causing bacteria not to die completely but some are still surviving. Several antibiotic-resistant bacteria have been found throughout the world, including*: Penicillin-Resistant Pneumococci, Multiresistant Mycobacterium tuberculosis* and *Methicillin-Resistant Staphylococcus aureus* (Husniah & Gunata, 2020). Along with the increasing incidence of bacterial infections, the World Health Organization (WHO) has recommended starting to look for new antibiotics or other alternative treatments, both as main drugs and as adjuvants, for example by doing treatment using traditional plant medicines that have antibacterial properties (Rahmadeni *et al.,* 2019). One of the plants suspected of having antibacterial activity is Bungur (*Lagerstroemia speciosa* (L) Pers). According to research conducted by (AL-SNAFI, 2019) Bungur plants have antibacterial activity based on secondary metabolites found in these plants such as Alkaloids, Flavonoids, Saponins, Steroids, Terpenoids, and Tannins.

Materials and Methods

a. Materials

The materials used were Bungur Leaf Extract obtained from the city of Amuntai (South Kalimantan) as a test material or sample, *Staphylococcus aureus ATCC 29213*, ethanol 96%, Ciprofloxacin as an antibiotic used, aqua pro injection, NaCl 0.9 %, mc Farland 0,5 and the media used, *Nutrient Broth* (NB) and *Mueller Hinton Agar* (MHA).

b. Methods

This type of true experimental research uses Posttest Only Control Group Design with the aim of measuring the effect of the test group with the control group (Agung et al., 2021).

1) Sample preparation

Bungur leaves obtained from Amuntai City (South Kalimantan) then carried out a simplicia management process, namely the collection of raw materials, wet sorting, washing, chopping, drying, dry sorting, and storage.

2) Extraction

Bungur leaf extract was obtained by maceration method which was done by soaking the simplicia of Bungur leaf with 96% ethanol as solvent. During the maceration process, the solvent used must be changed 3x24 hours. Bungur leaf extract that has been thickened using a rotary evaporator at a temperature of 50°C to obtain a thick extract (Wahid and Safwan, 2018). The thick extract from the leaves of Bungur was dried using a freeze drying device to ensure that there was no ethanol content in the extract. After that it is weighed and then put into a closed container and stored.

3) Tool Sterilization

Equipment used during the testing process such as measuring cups, petri dishes, test tubes, etc, must first be sterilized using an autoclave at a temperature of 121°C for 15 minutes at a pressure of 2 atm.

4) Media Creation

a) *Mueller Hinton Agar* (MHA)

Weigh 38 grams of MHA media in 1 L of aquadest then heat and wait until the media is completely dissolved and then put it in an erlenmayer. After that, sterilize in an autoclave at a temperature of 121°C with a pressure of 2 atm for 15 minutes, after that, put it in a 20 ml petri dish (Sapara & Waworuntu, 2016).

b) *Nutrient Broth* (NB)

Weigh 8 grams of NB media in 1 L of aquadest then heated until completely dissolved then put it in an erlenmayer then sterilize in an autoclave at 121°C for 15 minutes with a pressure of 2 atm and put into a test tube as much as 1 ml (Adha & Ibrahim, 2021).

5) Bungur Leaf Extract Antibacterial Activity Testing

a) Disc Diffusion Method

Negative Control Making

Making negative control results from dilution of bacteria with 0.9% NaCl, then spread using L rods as much as 1 ml on a petri dish containing MHA media, incubating for 18-24 hours in an incubator at 37°C.

Positive Control Making

The positive control used was Ciprofloxacin Antibiotics. Weigh 75 mg of ciprofloxacin then dissolve it into 100 ml of aqua pro injection as a stock solution. Then take 1 ml of the stock solution and drop it on the disc, after that place it on the MHA media that has been given the test bacteria. incubation for 18 - 24 hours in an incubator at 37°C.

Antibacterial Activity Screening using disc diffusion method

Prepare a petri dish that already contains MHA media then each plate is labeled positive control, negative control, 50%, 75%, and 100%. Put 1 ml of the results of the standardized bacterial dilution with mc Farland 0.5 into the media so that it is spread using L rods. Wait 30 minutes and then insert each disc on each labeled label. then Incubate for 18 - 24 hours at 37°C. After incubation, observe the inhibition on each label and measure using a caliper.

b) MIC and MBC Using the Dilution Method

Determination of Minimum Inhibitory Concentration (MIC) Value

Prepare test tubes, add 1 ml of bacterial suspension and NB media in each tube marked with 50%, 75%, 100%, positive and negative controls. Take 1 ml of the filtered extract and put it in a test tube by adjusting the concentration of the extracted extract. Incubate at 37°C for 18-24 hours, then observe, if it causes turbidity, it indicates the growth of test bacteria, while for a clear tube, it shows no growth of test bacteria. Tubes that have a low concentration with a clear result can be assigned a MIC value.

Determination of Minimum Bactericida Concentration (MBC) Value

Prepare a petri dish that already contains MHA media, then the results of the MIC test which has the lowest concentration value that can inhibit the growth of Staphylococcus aureus ATCC 29213 bacteria are taken as much as 1 ml and put into a petri dish containing MHA media then spread using L rods and let stand for 30 minutes . Incubate for 18 - 24 hours at 37°C. After that, observe the results, if it does not show the growth of the test bacteria marked with clear media, it can be set as the value of the MBC.

Results

a. Testing the Antibacterial Activity of Bungur (*Langerstroemia speciosa* (L) Pers) Leaf Extract *against Staphylococcus aureus ATCC 29213*

1) Screening Using Disc Diffusion Method

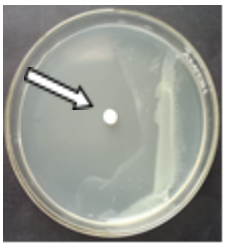
The results of observations using the Disc Diffusion method by looking at the Inhibitory Zone Diameter of Bungur (*Langerstroemia speciosa* (L) Pers) leaf extract against *Staphylococcus aureus ATCC 29213* with 3 replications.

Table 1. Result of Inhibition Zone of Bungur (Langerstroemia speciosa (L) Pers) Leaf Extract Against Staphylococcus aureus ATCC 29213

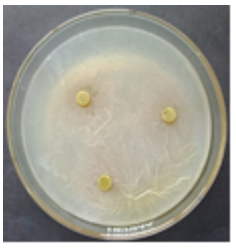
Concentration	**mm**				**SD**	Bacterial Inhibitory Strength
	I	II	III	Average		
50 %	0	0	0	0	0	Nonsusceptible
75%	0	10,43	13,92	8,11	5,12	Resistent
100%	9,98	10,05	10,46	10,16	0,18	Resistent
Control (+)		44,37		44,37	0	Susceptible
Control (-)		0		0	0	Nonsusceptible

Based on Table 1, it shows that the extract of Bungur leaf has a diameter inhibition zone against Staphylococcus aureus ATCC of 8.11 mm. These results can be continued to see the MIC and MBC with Dilution method. The picture of the results of the Antibacterial Screening Test for Bungur (Langerstroemia speciosa (L) Pers) Leaf Extract against Staphylococcus aureus ATCC 29213 using the disc diffusion method:

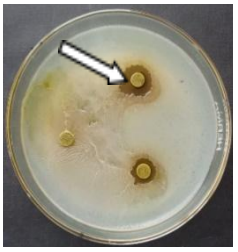
a. Control (-)



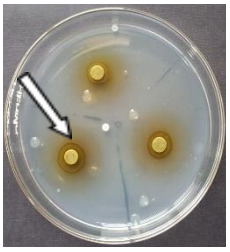
b. Control (+)



c. Bungur leaf extract concentration 50%



d. Bungur leaf extract concentration 75%



e. Bungur leaf extract concentration 100%

Description :

a. Control (-) : (0 mm)
b. Control (+) : (44,37 mm)
c. Bungur leaf extract concentration 50% : (0 mm)
d. Bungur leaf extract concentration 75% : (8,11 mm)
e. Bungur leaf extract concentration 100% : (10,16 mm)
⇒ ***:*** *Obstacles zone*

2) Test of Antibacterial Activity Based on Concentration of MIC and MBC Using the Dilution Method

The results of observations using the dilution method by looking at the values of the MIC and MBC Bungur (*Langerstroemia Speciosa* (L) Pers) leaf extract has antibacterial activity against *Staphylococcus aureus ATCC 29213* with 3 replications.

Table 2. Results of MIC Value of Bungur (Langerstroemia speciosa (L) Pers) Leaf Extract Against Staphylococcus aureus ATCC 29213

NO	Concentration MIC	After Testing MIC			Description
		I	II	III	
1	75% (0,75 g/ml)				Clear (MIC)
2	100 % (1 g/ml)				Clear

Table 2 shows that Bungur (*Langerstroemia speciosa* (L) Pers) leaf extract has antibacterial activity against *Staphylococcus aureus ATCC 29213* with a MIC value (0.75 g/ml).

Table 3. Results of MBC Value of Bungur (Langerstroemia speciosa (L) Pers) Leaf Extract Against Staphylococcus aureus ATCC 29213

NO	Concentration	After Testing MBC			Description
		I	II	III	
1	75%				Can't kill
2	100 %				Can't kill

Based on Table 3, it shows that Bungur (*Langerstroemia speciosa* (L) Pers) leaf extract has no killing power against *Staphylococcus aureus ATCC 29213 bacteria*.

Discussion

In this study using 3 different concentrations, namely 50%, 75%, 100%. Based on the results of the disc diffusion test, it was obtained at a concentration of 50% with an average value of

the inhibition zone diameter of 0, at a concentration of 75% with an average value of the inhibition zone of 8 ,11 mm, and at a concentration of 100% with an average inhibition zone of 10.16 mm. The results above indicate that there are differences in each treatment, according to (CLSI, 2021) the inhibition zone of 75% concentration is included in the category of Resistant with an inhibition zone of 8.11 mm and a concentration of 100% is included in the category of Resistant with an inhibition zone of 10.16 mm. Based on the results of the average value of the inhibition zone above, it can be concluded that the higher the concentration of the extract of the leaf extract tested, the larger the diameter of the inhibition zone that will form around the paper disc. This is in accordance with research conducted (Sapara & Waworuntu, 2016) on antibacterial effectiveness, which in this study stated that the activity of an antibacterial agent could increase if the antibacterial concentration was increased beyond the previous minimum inhibitory concentration. Meanwhile, according to (Adha & Ibrahim, 2021) The high concentration of an extract can affect the size of the inhibition zone. The higher the concentration of the extract, the larger the size of the inhibition zone produced. This is because if more extracts from a plant are used at the time of making the concentration, the content of secondary metabolites will also increase. Secondary metabolites are compounds that have the ability as antibacterial because of their role in inhibiting bacterial growth (Septiani *et al*., 2017). The formation of the inhibition zone is due to the presence of secondary metabolites that have antibacterial activity (Egra et al., 2019). According to (AL-SNAFI, 2019) Bungur leaves have secondary metabolites such as Alkaloids, Flavonoids, Saponins, Steroids, Terpenoids, and Tannins.

The next test uses the dilution method which in this test will see the clarity in the test tube. This method is used, because this method is the most appropriate for determining the value of MIC (*Minimum Inhibitory Concentration*) and the value of MBC (*Minimum Bactericida Concentration*), the media used in this test is NB media. The test this time only used 2 concentrations of 75% and 100%. The reason that underlies the use of 2 concentrations is that at the time of testing using the diffusion method, the 50% concentration did not provide an inhibition zone. Based on the test results for 18-24 hours giving clear results on media with a concentration of 75% and 100% then the process of spreading on MHA media is carried out to determine whether the tested extract can kill bacterial growth. The method used is solid dilution with Standard Plate Count (SPC). SPC testing aims to determine the

number of bacteria present in a medium by counting bacterial colonies that grow on the agar using a colony counter. The provisions for the number of colonies ranged from 30-300 CFU (Colony Forming Unit)/mL from the bacterial dilution. In addition, the purpose of bacterial dilution is to control the proliferation of bacteria used in the test. The greater the concentration of the bacterial suspension used, the higher the bacterial growth so that its sensitivity to antibacterial compounds will be weaker (Rahmitasari et al., 2020).

Bungur leaves have secondary metabolites such as Alkaloids, Flavonoids, Saponins, Steroids, Terpenoids, and Tannins which have antibacterial effects with different working mechanisms. Alkaloids are compounds that contain basic groups that can interact with amino acid compounds that make up bacterial cell walls and bacterial DNA. The mechanism of action of alkaloid compounds as antibacterial is by inhibiting the peptidoglycan constituent components of the bacterial cell wall so that the bacterial cell wall does not undergo a perfect formation. bacteria (Ahmad et al., 2019). Alkaloids can also inhibit the DNA synthesis process by disrupting the work of the topoisomerase enzyme so that the DNA replication process is inhibited. The inhibition of the DNA replication process causes bacteria to be unable to divide. This can affect the metabolism of bacteria because the DNA synthesis process is disrupted (Adha & Ibrahim, 2021).

Flavonoid compounds have a role as antibacterial with the mechanism of action of inhibiting cell membrane function and bacterial energy metabolism. When inhibiting cell membrane function, flavonoids form a complex compound with extracellular proteins that can damage the bacterial cell membrane, followed by the release of the bacterial intracellular compound. Flavonoids can also inhibit energy metabolism by inhibiting the use of oxygen by bacteria. Energy is needed by bacteria for macromolecular biosynthesis, so that if the metabolism of a bacterium is inhibited, the bacterial molecule cannot develop into a complex molecule. The mechanism of action of flavonoids in inhibiting nucleic acid synthesis is through the B ring on flavonoids which has an important role in the interclassification process or the formation of hydrogen bonds by accumulating nucleic acid bases that inhibit DNA and RNA synthesis. In addition, in flavonoid compounds there are also phenolic compounds that can interfere with bacterial growth. Phenol itself is an alcohol that has acidic properties so that it can denature proteins and damage bacterial cell membranes (Sapara & Waworuntu, 2016).

The next compound is saponins. The mechanism of action of saponins as antibacterial is by causing leakage of proteins and enzymes from the bacterial cell. Saponins also have active substances that can increase the permeability of a membrane so that hemolysis occurs in cells. If the saponins have interacted with bacterial cells, the bacteria will break or lyse (Sapara & Waworuntu, 2016). The next compound is steroids. The mechanism of action of steroids as antibacterial is by inhibiting the growth of bacteria that are directly related to lipid membranes and sensitivity to steroid components which result in leakage of bacterial liposomes. Steroids can also interact with cell phospholipid membranes which are permeable to lipophilic compounds, resulting in decreased membrane integrity and cell membrane morphology turning brittle and lysis (Sapara & Waworuntu, 2016).

The next compound is terpenoids. The mechanism of action of terpenoids is to react with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming a strong polymer bond so that porin damage occurs. Damage to porins results in a lack of permeability of the bacterial cell wall which causes bacterial cells to lack nutrients, so that bacterial growth is inhibited and dies (Rahmitasari et al., 2020). The last compound is tannins. The mechanism of action of tannins as antibacterial is by causing bacterial cells to lyse. This happens because tannins have a target on the polypeptide wall of the bacterial cell wall so that the formation of the cell wall becomes less perfect and then the bacterial cell will die. Tannins also have the ability to inactivate bacterial enzymes and interfere with the passage of proteins in the inner layer of cells (Sapara & Waworuntu, 2016).

There are several factors that can affect the success of a study that can be seen in terms of the quality of the extract which has two factors, namely biological and chemical factors. For example, biological factors such as plant species, plant origin location, harvest time, storage of raw materials, age, and plant parts used in research, while chemical factors include external and internal factors. External factors consist of size, filter, content of heavy metals and pesticides, as well as the extraction method used and internal factors consist of type, composition, and content of active compounds (Brigitta et al., 2021). There are also factors that can affect the activity of a bacterium according to (Mahdiyah et al., 2020) such as environmental conditions such as temperature, pH, nutrient sources, the presence of oxygen and salinity. According to (Zamilah et al., 2020) the growth of bacteria on agar media can also be influenced by several factors such as nutrient content, extract manufacturing process,

and storage. According to (Egra et al., 2019) Factors that can affect bacterial activity are the type of bacteria that are inhibited, the content of antibacterial compounds, the concentration of the extract and the diffuse power of an extract. In addition, differences in the structure of the cell wall of a bacterium can also determine the activity, penetration, bonding of antibacterial compounds. The thicker the agar medium in the petri dish, the smaller the inhibition zone that will be given because the diffusion speed is lower and the pH value of the agar medium can also affect because some bacteria can only work well in acidic conditions and some in alkaline conditions.

The study of the antibacterial activity test of Bungur Leaf extract affected the growth of *Staphylococcus aureus ATCC 29213* bacteria with the formation of an inhibition zone on disc diffusion with inhibiting power in the Resistant category, while the dilution test did not give results that could kill bacterial growth. The inhibition of the growth of a bacterium can be caused by the presence of secondary metabolites in the leaves of Bungur. Therefore, it is necessary to develop research related to the antibacterial potential of Bungur leaf extract by testing the effect of Bungur leaf extract in inhibiting and killing the growth of *Staphylococcus aureus ATCC 29213* bacteria with increased concentrations.

Conclusion

Based on the results of the antibacterial activity test of Bungur (*Langerstroemia speciosa* (L) Pers) leaf extract against *Staphylococcus aureus ATCC 29213*, that Bungur leaf extract has antibacterial activity marked by the formation of an inhibition zone at a concentration of 75%. However, the inhibitory power given is not strong or weak with the value of the inhibition zone given is 8.11 mm. The MIC test was carried out from the lowest concentration that could inhibit *Staphylococcus aureus ATCC 29213*, which was a concentration of 75%. Meanwhile, for the Killing Power (MBC) test, Bungur leaf extract did not have a MBC value because when it was spread on solid media it was still overgrown with bacteria.

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