

ANTIBACTERIAL ACTIVITY OF BUNGUR BARK EXTRACT (*Lagerstroemia Speciosa* (L.) *Pers*) AGAINST *Staphylococcus Aureus* ATCC 29213 By DIFFUSION AND DILLUTION METHODS.

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Abstract

Staphylococcus aureus is one of the multidrug resistance pathogenic bacteria which is emphasized by supervision world health organization because it has characteristics that easily avoid the target of antibiotic activity and resistant to various antibiotics. It narrows the choice of treatment. Bungur bark is a plants that has potential as an antibacterial, it is necessary to explore its activities as a new alternative medicine. This study aims to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Bungur bark extract against *S. aureus* ATCC 29213. This type of research is *true experimental with post test only with control goup design* with 5 treatment groups namely 25%, 50%, 75%, 100%, ciprofloxacin as a positive control and negative control without treatment using diffusion and dilution methods with determination of MIC and MBC. The results showed that Bungur bark had inhibition zone against *S. aureus* ATCC 29213 at a concentrations of 25%, 50%, 75% and 100% is 7.23 mm, 11.73 mm, 17.51 mm and 21.09 mm with MIC values at 100% concentration. As for the dilution method, there was no MBC againts *S. aureus* ATCC 29213. The results showed that MIC of Bungur bark was at a concentration of 100% . As for the MBC test, Bungur bark did not show any killing power against *S. aureus* ATCC 29213.

Key words : Antibacterial, *S. aureus* ATCC 29213, *Lagerstroemia* bark.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a normal skin flora but is pathogenic in some hosts (Nuryah et al., 2019). About 20% of the human population are long-term carriers of *S. aureus*, but most people have no clinical signs. *S. aureus* can spread through objects that are around such as faucets, cell phones, pens, notebooks, suitcases, watches and personal facilities can also be a medium for the spread of *S. aureus*. *S. aureus* is one of the multidrug resistant and pathogenic bacteria that is emphasized by the world health organization because it has characteristics that easily avoid the target of antibiotic activity and easily resistant to various antibiotics, it narrows the choice of treatment. (Putri et al., 2018). The success of *S. aureus* as a bacterial pathogen is largely due to its good ability to adapt with environment due to presence of new DNA by horizontal gene transfer (HGT). Through HGT, *S. aureus* can colonize, evade immune factors, produce toxins (enterotoxins, exfoliative toxins, leukocidins and so on) and can

grow so rapidly, that it has become one of the greatest microbial challenges since the introduction of antibiotics (Monaco et al., 2017).

In the past century, one of the strains of *S. aureus*, namely MSSA (Methicillin Sensitive *Staphylococcus aureus*) was rampant in the middle of the last century in hospitals causing infection and death in newborns, patients and some hospital staff in the UK, USA, and Canada. The case became the first identified pandemic *S. aureus* clone. The MSSA strain is penicillin-resistant, easily transmissible, and hypervirulent (Monaco et al., 2017). In 2003, it was reported that there was an increase in *S. aureus* superinfection with outbreaks of severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1). Some of these cases resulted in necrotizing pneumonia induced by *Panton-Valentine leukocidin* (PVL), a toxin secreted by MSSA. MSSA has also been found in some patients infected with COVID-19. A case-cohort study found that patients with COVID-19 pneumonia had a higher risk of developing ICU bloodstream infections (ICU-BSIs) than critically ill patients without COVID-19 infection (Chaudhry et al., 2021)..

Therefore, it is necessary to explore related plant compounds that have antibacterial activity as an alternative treatment. One of the plants suspected of having antibacterial is the Bungur plant (*Lagerstroemia speciosa*). Based on a pharmacological review research conducted by (Al-Snafi, 2019) the pharmacological effects of bungur include antibacteriasl, anticancer, antioxidant, antidiabetic, antiobesity, hypolipidemic, anti-inflammatory, analgesic, gastrointestinal, diuretic, thrombolytic, cardiovascular, central nervous, xanthine oxidase inhibition, inhibition of production of TNF α hepatoprotective and nephroprotective effects (Al-Snafi, 2019).

Bungur (*Lagerstroemia speciosa*) is a tree that can grow to a height of 25m. The leaves are elongated with alternating layout, short petiole measuring 10-20 cm x 5-7.5 cm with a round brown stem. The flowers are compound bordered, regularly shaped, have a color varies from pink to purple which is quite attractive with a width of 5.0-7.5 cm with wavy edges up to 3.5 cm, stamens with purple filaments and golden yellow anthers. The pistil is simple with a purple pattern up to 5 cm long, the pistil is dark green and the ovary is superior, the fruit is hard woody, measuring about 2.5 cm which when young is very hard green, but brown when mature and only the outer layer of the dried fruit remains, the seeds have wings and the roots ride round and stringy (J & Shah, 2020). Bungur plants are usually used by the community for reforestation, shade, road decoration, markers of the rainy season, as firewood, huts and livestock fences. However, bungur plants are mostly used only as ornamental plants in urban areas because these plants have attractive flowers, lush crowns and are easy to plant (Rahmah et al., 2021). The abundant diversity of natural resources opens up opportunities to research and identify candidates for new and useful herbal medicines. Therefore, this study was conducted to identify the antibacterial activity of the extract of Bungur Bark against *S. aureus* ATCC 29213.

2. Materials

The materials used in the study as a sample was a Bungur Bark (*Lagerstroemia speciosa*), *S. aureus* ATCC 29213 for the test of bacteria. Supporting materials are ciprofloxacin, etanol 96%, media MHA (*Mueller Hinton Agar*), NB (*Nutrient broth*), NA (*Nutrient Agar*), aquadest, Aqua pro injeksi, 0,5 McFarland, Nacl 0,9%, %, BaCl 1.175%, H₂SO₄ 1% and culture of *Staphylococcus aureus* ATCC 29213.

3. Methods

3.1 Sample Processing

The manufacture of simplicia is made through several processes from raw material collection, wet sorting, washing, draining, chopping (shaping), drying, dry sorting, packing and storage. Bungur bark that has been collected is cleaned of foreign objects that are still attached and then washed thoroughly, drained and then cut into small pieces in order to expand the surface contact so that the drying process can take place optimally. The bark of bungur is dried in the sun while being covered with a black cloth on top. The dried bark pieces are then sorted dry and then packaged and stored. Then the bungur bark was ground into powder to increase the surface area so that simplicia will be easier to extract. The larger the surface area in contact with the solvent, the easier it will be for the solvent to enter and filter the phytochemical compounds in the simplicia (Kurniawan et al., 2019).

3.2 Extraction by Maserati

Bungur bark extract was prepared using the maceration method. The simplicia powder from the Bungur bark was macerated by immersing the simplicia in 96% ethanol with solvent replacement Every 24 hours. During the maceration process, the filter fluid will penetrate the cell wall and enter the cell containing the active substance. Then the active substance will leave the cell wall through the diffusion process. The macerated extract was then accommodated and evaporated to separate the extract and the solvent. Evaporation of the extract was carried out using a rotary evaporator at a temperature of 50°C. while the extract thickening process was carried out on a water bath at a temperature of 50°C (Kurniawan et al., 2019).

3.3 Antibacterial Activity Test

3.3.1 Sterilization of Tools and materials

The tools and media to be used in the antibacterial activity test were sterilized using an autoclave at 121°C for 15 minutes at a pressure of 2 atm. While the ose and tweezers are sterilized by heating them over spiritus (Noval et al., 2019).

3.3.2 Media Preparation

In this study, the media used were MHA (Mueller Hinton Agar), NB (Nutrient Broth) and NA (Nutrient Agar) media.

3.3.2.1 NA Media Preparation

NA was weighed as much as 5.6 g, then dissolved in 200 mL of distilled water using an erlenmeyer and then homogenized on a hotplate until homogeneous. The homogenized media was then sterilized in an autoclave at 121°C at a pressure of 2 atm for 15 minutes. The media then poured into a sterile plate of 20ml for each plate and kept at room temperature until compacted (Pehino & Suoth, 2021).

3.3.2.2 MHA Media Preparation

The preparation was carried out by weighing 4.5 g of MHA then dissolved in 120mL of distilled water using an erlenmeyer and then homogenized on a hotplate until homogeneous. Furthermore, the media was sterilized using an autoclave at a temperature of 121°C with a pressure of 2 atm for 20 minutes. The homogeneous MHA media was poured into sterile petri dishes as much as 20 ml for each plate and then allowed to stand at room temperature until solidified. (Utomo et al., 2018).

3.3.2.3 NB Media Preparation

The preparation of media by dissolving 2 g of NB into 250mL aquadest in a erlenmeyer and then heating it on a hotplate. The media was sterilized using an autoclave at 121°C for 15 minutes (Utomo et al., 2018).

3.3.3 Bacteria Rejuvenation

Rejuvenation is carried out on culture media that have been made and sterilized. Take 1 ose of *S. aureus* ATCC 29213 bacteria culture, then streak it into a new agar medium and then incubate for 18-24 hours in an incubator at 37°C. Colonies formed after the incubation process will cause a change in the media from clear to cloudy. This indicates the presence of growth and the bacteria are ready to be used for further testing (Utomo et al., 2018).

3.3.4 Preparation of Bacterial Suspension

The preparation of bacterial suspension was carried out by balancing the solution with a standard solution of 0.5 McFarland. Standard solution of turbidity 0.5 McFarland was prepared by mixing 9.95 mL of 1% H₂SO₄ and 0.05 mL of 1% BaCl₂ solution. Then shaken until a cloudy solution is formed. This turbidity is used as a standard for the turbidity of the test bacterial suspension and is equivalent to a bacterial density of 10⁸ CFU/mL. The suspension was made by inoculating bacteria aged 18-24 hours into a tube containing 0.5 ml of 0.9% NaCl solution. If the turbidity is not equal, then add 0.9% NaCl until the same turbidity is obtained with a standard turbidity of 0.5 McFarland which is equivalent to a bacterial concentration of 1.5 x 10⁸ CFU/mL (Pehino & Suoth, 2021).

3.3.5 Preparation of Bungur Bark Extract Test Solution

The concentration of Bungur bark extract used were 25%, 50%, 75% and 100%. The stock solution was made by weighing 0.25gr, 0.50gr, 0.75gr and 1gram of the extract and then dissolved into 10ml of aqua pro injection so that the concentration of the extract of the bark of the bungur bark was obtained in accordance with the desired concentration (Bawondes et al., 2021).

3.3.6 Antibacterial Activity Test

3.3.6.1 Antibacterial Activity Test by Diffusion Method

Take 1 ml of bacterial suspension which has been equivalent to 0.5 McFarland, then spread it using L rod on MHA media. Take a sample solution (bark extract) as much as 1 ml for each concentration and then drop the sample on a paper disc. After the solution is absorbed, place the paper disc on the test medium and incubate the media at 37°C for 18-24 hours.

The clear zone formed around the paper disc indicates that the sample has activity in inhibiting bacterial growth. The negative control was not treated, while the positive control used ciprofloxacin. Observe the inhibition zone in each sample, then measure the diameter of the inhibition zone formed using a caliper (Handayani et al., 2020).

3.3.6.2 Antibacterial Activity Test by Dillution Method

Dilution testing was carried out using liquid dilution and solid dilution methods. Testing is done by first sterilizing the tools and materials to be used. Take a bacterial culture that is 18-24 hours old, dilute it until the turbidity is equivalent to 0.5 McFarland. Take 2 oses of bacteria then put them in 250ml of prepared NB media. Take the test tubes then put 1 ml of extract and 1 ml of media which already contains bacteria into each tube for the test group, 1 ml ciprofloxacin and 1 ml of media for positive control and 1 ml of media for negative control then homogenized using a vortex. Incubate the tube in the incubator for 18-24 hours at 37°C. Observe the level of turbidity in the tubes that have been incubated. The tube with the clearest level of turbidity at the lowest concentration is the MIC value. After the liquid dilution test is complete, it is followed by solid dilution using the pour plate method, which is to take 1 ml of the dilution test results and then spread it over a media, flatten using an L rod. Incubate the media at 37°C for 18-24 hours (Vensia Magdalena & Kusnadi, 2015). The incubation results were then observed

to determine the MBC value of Bungur bark extract by looking at the presence or absence of bacterial growth using a colony counter. If the colony does not exceed 30-300 then it can be stated as MBC (Arinda et al., 2019).

4. Results and Discussion

Antimicrobial Activity Test Results

Table. 1 Result of Antibacterial Activity by Disc Diffusion Method

Inhibition Zone (mm)						
Replication	Concentration				Control	
	25%	50%	75%	100%	Ciprofloxacin (+)	-
R1	10.02	6.59	8.23	15.88	44.37	0
R2	7.83	10.26	18.88	28.09		
R3	3.86	18.35	25.44	19.31		
Total	21.71	35.2	52.55	63.28	44.37	0
Average	7.23	11.73	17.51	21.09	44.37	0
SD	2.54	4.912	7.09	5.14	0	0
Category	Resistant	Resistant	Intermediate	Susceptible	Susceptible	-
Description :				Breakpoints :		
R1 = Replication 1				Susceptible : ≥ 20		
R2 = Replication 2				Intermediate : 15-19		
R3 = Replication 3				Resistant : ≤ 14		

Based on the MIC test of the Bungur bark extract against *S. aureus* ATCC 29213 using disc diffusion method, the diameter of the inhibition zones at a concentration of 25% was 10.02mm, 7.83mm, and 3.86mm. At a concentration of 50% of 6.59mm, 10.26mm, and 18.35mm. At a concentration of 75% obtained diameters of 8.23mm, 18.88mm and 25.44mm. Meanwhile, the results at 100% concentration were 15.88mm, 28.09mm, and 19.31mm. The average diameter obtained at each concentration was 7.23 mm, 11.73 mm, 17.51 mm and 21.09 mm. If the result was classified based on the category of inhibition, then the concentration of 25% and 50% fall into the resistant category with an inhibition zone of 7.23 mm and 11.73 mm, a concentration of 75% Intermediate with an inhibition zone of 17.51 mm and a concentration of 100% susceptible with an inhibition zone of 21.09 mm. This indicates that there is a difference in the inhibition of the concentration of the Bungur bark extract on the growth of *S. aureus* ATCC 29213. The results showed that there were differences in the inhibition zones at each concentration of the Bungur bark extract where there was an increase diameter of the inhibition zone as the concentration used increased.

Table. 2 Result of Antibacterial Activity by Dillution Method

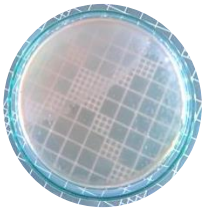
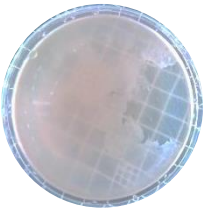
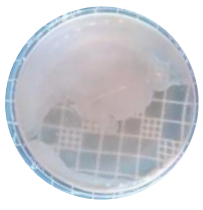
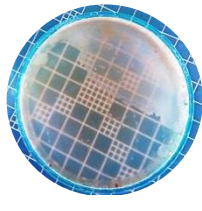
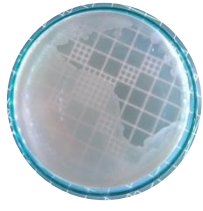
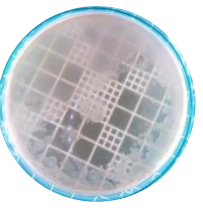
Antibacterial Activity Test by Broth Dillution Method				
Concentration	R1	R2	R3	Description
75%				-
100%				+ (MBC)

Description :

- = Turbid

+ = Clear

Table. 3 Result test Antibacterial Activity by Dillution Method

Antibacterial Activity Test by Solid Dillution Method				
Concentration	R1	R2	R3	Description
75%				Colony $\geq$ 300 CFU
100%				Colony $\geq$ 300 CFU

Description :

R1 = Replication 1

R2 = Replication 2

R3 = Replication 3

The testing of antibacterial activity using the dilution method was aimed at determining the MIC and MBC of the extract of Bungur Bark. The lowest concentration of antibiotics in the tube that showed clear culture results was the MIC value of an extract. Based on the research that has been carried out and the data that has been obtained, it is known that the extract of the bark of the bungur stem has no killing power against the bacteria *S. aureus* ATCC 29213. This refers to the number of colonies formed after the media is incubated, which exceeds 30-300 CFU (Colony). Forming Unit)/mL (Rahmitasari et al., 2020).

The antibacterial activity of Bungur bark extract is due to the content of secondary metabolites of flavonoids, alkaloids, tannins and saponins. (Riyanti et al., 2020). The mechanism of alkaloids as antibacterial is by destroying the peptidoglycan component in bacterial cells, so that the cell wall layer cannot be fully formed and causes the growth of these cells to be inhibited or die. Another mechanism of the antibacterial activity of alkaloids is by inhibiting bacterial cell topoisomerase (Erlina et al., 2021). Flavonoid have two mechanisms as antibacterial. The mechanisms is damaging bacterial cell membranes and inhibiting bacterial cell protein synthesis. The mechanism of action of flavonoids in damaging bacterial cell membranes is to form complex compounds with extracellular proteins so that bacterial cell membranes will be damaged, bacterial cell damage is followed by uncontrolled entry of water into bacterial cells that causes bacterial cell swelling and finally ruptures. Another mechanism of flavonoid compounds is to inhibit bacterial cell protein synthesis by forming complex hydrogen bonds and bacterial cell proteins so the structure of cell walls and cytoplasmic membranes of bacteria containing proteins becomes unstable and loses their biological activity. This affects the permeability function of the bacterial cell wall and causes growth disorders or bacterial cell death (Rahmitasari et al., 2020). Tannin have antibacterial activity by forming hydrogen bonds with proteins present in bacterial cells. The proteins are denatured and bacterial cell metabolism will be disrupted. In addition, tannins can also form bonds with phospholipids in cell membranes and cause membrane leak age so as to prevent the entry of nutrients needed for bacterial growth. While saponins have an antibacterial mechanism by lowering the surface tension so that the permeability of the bacterial cell membrane increases and causes cell leakage and the intracellular compounds will come out. When saponins interact with bacterial cells, the bacteria will break down or lysis (Rahmitasari et al., 2020).

S. aureus ATCC 29213 is a gram-positive bacteria that cell wall structures mostly consists of peptidoglycan (90%) and polysaccharide (teichoic acid). Teichoic acid is soluble in water and functions as a positive ion transporter. This indicates that the cell wall of *S. aureus* is polar. The polar nature of the cell wall will cause secondary metabolites that have polar properties to more easily penetrate the peptidoglycan layer which is also polar on the bacterial cell wall, causing lysis or inhibition of cell growth (Owu & Jayanti, 2020). Several things that can affect the antibacterial activity of an extract are the quality of the extract used, the time of harvest, storage, the part of the plant used, the type of compound, the composition of the compound, the content of the active compound, the diffusion power of the extract and the type of bacteria inhibited (Brigitta et al., 2021). The factors that can affect the growth of bacteria are the thickness of the media in each dish, nutrients or materials contained in the media, environmental conditions for bacterial growth, pH, temperature and nutrients. (Mahdiyah et al., 2020). While the formation of the inhibition zone or compound activity is influenced by the amount and antibacterial compound used, the solubility of the antibacterial into the media, the diffusion coefficient, and the effectiveness of the antibacterial used. (Zamilah et al., 2020). The structure of the cell wall

of a bacteria also determines the penetration, binding and activity of antibacterial compounds. (Pehino & Suoth, 2021).

5. Conclusion

Based on the results of the research on the Activity of Bungur Bark Extract (*Lagerstroemia speciosa* (L) Pers) against *S. aureus* ATCC 29213, it can be concluded that the MIC value of Bungur Bark Extract is at 100% concentration with an inhibition zone of 21.09mm. Meanwhile, in the MBC test, the extract of Bungur bark was not found to have any killing power against *S. aureus* ATCC 29213.

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