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Antibacterial and Antibiofilm Activity of Papaya Leaf Fractions *(Carica Papaya L.)* Against *Staphylococcus Aureus* ATCC 25923

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ABSTRACT

The research on papaya leaves was aimed at determining antibacterial activity, antibiofilm activity, K+ and Ca2+ metal ion parameters, as well as the morphology of S.aureus bacteria from papaya leaf fractions and extracts. The average value of the test results for the diffusion method at 50% concentration was 20.66 ± 0.57 and the test results for the dilution method for Minimum Inhibitory Concentration at a concentration of 12.5%. Antibiofilm formation inhibitor test results at a concentration of 50 mg/ml with an average value of 72.37 ± 0.65, a concentration of 25 mg/ml 64.75 ± 0.83 and a concentration of 12.5 mg/ml with an average value of IC50 of 0.975 ± 0.012. Biofilm degradation testing at a concentration of 50 mg/ml resulted in an average value of 60.43 ± 1.11, a concentration of 25 mg/ml with an average value of 50.05 ± 0.87 and a concentration of 12.5 mg/ml with an average value -mean 35.86 ± 1.05. The average EC50 value was 26.731 ± 1.1874. Tests using AAS on K+ metal ions showed ion leakage or cell contents coming out was 1,805 mg/L, Ca2+ metal ions resulting from this leak were 3,884. Morphological observations using SEM showed that the samples experienced shrinkage and reduced size from being perfectly round to oval to S.aureus bacteria in samples with water fraction

INTRODUCTION

Infectious diseases are a very serious threat and affect society and are still an important issue for the world of health. Bacterial infections are caused by various microorganisms, one of which is caused by pathogenic bacteria. S. aureus is a bacteria that can harm humans because it can cause various infectious diseases such as pneumonia, meningitis, empyema, endocarditis, or sepsis, all of which have the same characteristics, namely inflammation, necrosis, and abscess formation (1).

S. Aureus is a gram-positive pathogenic bacterium that can attack vareious organs or body tissues and cause inflammation, necrosis and abscesses. S. Aureus is found around anatomical structures such as the skin, oral cavity and digestive system. Almost everyone has experienced S. aureus infection, which varies from severe food poisoning, mild skin infections to fatal illnesses (2).

Bacterial resistance is a growing problem throughout the world, one of which is resistance to infection. The incidence of S. aureus infections is increasing with the emergence of strains that are resistant to methicillin-resistant S. aureus (MRSA). To avoid the occurrence of resistance, alternative treatments include the development of compounds from plants that are antibacterial such as tannins, flavonoid, alkaloids, and phenols.

Papaya leaves are one of the plants that can be used as an antibacterial because they contain the enzymes papain, nicotine, myosmin, pseudocarpine, caontinin, and the alkaloid compounds carpain, papain, flavonoids, saponins, violaxatin, tannins, and caricatin (3). Flavonoid contained in papaya leavesworks as an anti-biofilm agent by preventing the expression of the icaA and icaD genes, which control how biofilms form.Biofilm is a matrix consisting of substances *Extracellular Polymeric Substances* (*EPS*) which originates from a collection of replicating bacteria that form microcolonies that adhere irreversibly to the epithelial surfaceb (4)**.** Flavonoids can damage the permeability of bacterial cells, microsomes and lysosomes due to interactions between flavonoids and *DNA* bakteri (5). The mechanism of action of antibacterial compounds causes cell wall damage and can be seen through SEM and AAS method observations. Method *SEM* observing changes in morphology and structure of bacterial cells, while the method *AAS* measuring ion leakage in the form of ions coming out of the bacterial cell membrane.

METHODS

A. Papaya Leaf Extraction

Papaya leaves are sourced from farmers in Tawangmangu, Karanganyar Regencyplant determination was carried out inLaboratory of the Faculty of Pharmacy, Setia Budi University, Surakarta. This determination is to ensure that the samples use is a genuine papaya leaf.Papaya leafsorted to select leaves that meet the criteria, then washed to remove dirt on the leaves. Next, the papaya leaves are separated from the stem, the papaya leaves are taken and then dried. The final step is smoothing toget papaya leaf powder.To make papaya leaf ethanol extract, a ratio of 1:10 g/v is used, namely weighing 1 kg of papaya leaf powder, then soaking it by adding 10L of 96% ethanol solvent, putting it in a vessel, then closing it. The extract is soaked for 3 days with occasional stirring, filtered, all the macerate is collected then the filtrate is evaporated using a rotary evaporator at 40°C until it becomes a thick extract.

B. Fraction Creation

Using the solvents N-hexane, ethyl acetate and water, the ethanol extract of papaya leaves was fractionated using the liquid-liquid extraction method. 20 grams of papaya leaf ethanol extract was dissolved in a little water, then dissolved in 50 ml of water and 50 ml of N-hexane solvent in a separating funnel which was repeated three times. The filtrate at the top is the N-hexane fraction, and the filtrate at the bottom is the water fraction. A rotary evaporator operating at 50°C was used to collect and concentrate the N-hexane fraction that had been separated from the water fraction. The remaining water fraction was then separated from the N-hexane fraction using 50 mL of ethyl acetate

through a separating funnel three times. The filtrate is the ethyl acetate fraction, while the water fraction is at the bottom. A rotary evaporator operating at 50°C was used to separate and concentrate the ethyl acetate portion of the water fraction. To make the water fraction, the remaining filtrate is fractionated with ethyl acetate and concentrated in a water bath until thickened

C. Screening Test Phytochemicals of Papaya Leaf Extract

Phytochemical screening tests were carried out to determine the bioactive components contained in papaya leaf extract, water fraction, n-hexane fraction, ethyl acetate fraction of papaya leaf extract which have antibacterial and antibiofilm activity. Phytochemical screening tests are carried out using tube tests consisting of flavonoid, tannin, saponin, terpenoid, steroid and alkaloid tests.

D. Evaluation of Papaya Leaf Extract

Organoleptic test, specific gravity test, concentration calculation air, drying shrinkage test, ash content test

E. Test the Antibacterial Activity of Papaya Leaf Extract

a. Minimum inhibitory concentration (MIC) test on S. Aureus A group of bacteria is then placed in a replicator inoculator (referred to as*replicator Steers-Folts*) calibrated (usually 0.001 mL). Then the bacterial suspension is placed on the agar surface. In this way, about 104 CFU are on the surface of the agar plate, after which the agar plate is incubated. The lowest concentration that shows colony growth below 3 is called the minimum inhibitory concentration (*MIC*).

b. Minimum Kill Concentration (KBM) Test on S. Aureus. Inoculate a clear tube containing the MIC results etched onto the media *VJA* incubated for 24–48 hours to determine the Minimum Kill Concentration (KBM). The presence or absence of black and yellow colonies around the surface of the substrate indicates the results of the solid dilution method. Minimum Kill Concentration (KBM) is the lowest concentration which indicates that bacterial colonies do not develop (6).

F. Test Activitybiofilm

a. Biofilm formation activity assay

Using media *BHI* and 96-well polystyrene round-bottom microplates, biofilm inhibition activity assays were performed. Each well received a total of 70 µl of sample in medium. The well holding sample was then given 70 µl of bacterial suspension in medium with a concentration of 1.5 x 108 CFU/ml, and the well was incubated at 370 °C for 72 hours. After incubation, the contents of the wells were discarded, the plates were washed with running water, and then the microplates were dried for 15 min by inverting them at room temperature. Each well received a total of 200 µl of 1% crystal violet solution, which was stained for 15 min. Once the hole is emptied of its contents, it is cleaned once again under waterflow. The microplate was dried for one hour at room temperature while inverting. The optical density was then measured at a wavelength of λ 595 nm after 200 µl of 96% ethanol solution was poured into each well of the plate. Each experiment was carried out three times (7).

The concentrations of the most active fraction that will be tested will be concentrations of 50%, 25% and 12.5%. IC classification$_{50}$ *S. aureus* determined from the linear regression equation between sample concentration and percent biofilm inhibition. determined from the linear regression equation between sample concentration and percent biofilm inhibition. To monitor the relationship between the average percentage of inhibition of biofilm formation with the concentration and extract fraction, the IC50 value was calculated using a linear

regression equation. The average percentage of biofilm inhibition prevented 50% of biofilm formation at each extract concentration and fraction.

b. Biofilm degradation activity assay

Created using media *BHI* with 96 wellspolystyrene roundbottom microplate. Each well received 70 µl of medium, then 70 µl of bacterial solution was added*BE*which contains 1.5 x 108 CFU/ml, to form a biofilm. After that, the microplates were incubated for 72 hours at 37°C. The well is then cleaned with running water after the contents are removed. Each well was filled with 200 µl of sample in medium, then the microplate was incubated again at a temperature of ± 37 °C for 24 hours. The same method as the activity test can be used for biofilm determination to prevent biofilm formation (7).

The control in this experiment is a negative control (bacteria + medium), namely a biofilm-forming control whose bacterial growth is not disturbed. Biofilm destruction activity assay *S. aureus* ATCC 25923 is expressed in EC metrics$_{50}$ (effective concentration), or the concentration of the test substance that causes 50% of the biofilm to be destroyed. The linear regression equation between sample concentration and percentage of biofilm destruction was used to calculate EC values$_{50}$. R-table value for the linear regression equation at the 0.95 confidence level. EC value$_{50}$negatively correlated with biofilm disrupting activity; the higher the EC value$_{50}$, the more the biofilm disrupting activity decreases*S. Aureus* ATCC 25923, which results in a 50% increase in concentration required to remove biofilm

G. Observation of the Morphology of S.Aureus Bacteria and Analysis of K-logan Ion Leakage^{+}and Ca^{2+}

The AAS method is used to analyze cell leakage. The ion leak test was carried out on tubes using 1 KBM and 2 times KBM in the dilution method. Leakage of K+ and Ca2+ ions is detected by AAS. The results of 1x KBM and 2x KBM were then adjusted to 1 mL and then diluted into a 100 mL volumetric flask. The result is measured by *AAS* with wet elimination using HNO_3. The leak test was carried out by detecting and measuring metal ions contained in the test bacteria after contact with the active fraction at concentrations 0 (control), 1 and 2. The supernatant was analyzed using *AAS Thermo Elemental Type* Solar MS. The cell solution obtained from contact with the fraction is used to measure the ion concentration (6).

Observation of Bacterial Morphology*S. aureus* By Method *SEM* (*Scanning Electron Microscope)*. The pure bacterial cell suspension was incubated for 24 hours in an incubator and shaken at a temperature of 22-25°C. The results were centrifuged, then the cell biomass was placed several times in the starting solution (2.5% glutaraldehyde in phosphate buffer and coccylate buffer). See results within hours. Then the results were centrifuged and the precipitate was washed again with phosphate buffer solution and 2% tannic acid was added for further fixation. The results were centrifuged and the precipitate was washed again with phosphate buffer and codylate buffer, vortexed and left at 10 °C. minutes and this step is repeated twice. The next step is to centrifuge the cells again and collect the cell biomass, adding 1% *osmium tetroxide,* leave at cold temperature for 1 hour. The cells were dehydrated by adding a certain amount of ethanol (50%, 70%, 80%, 95% and absolute ethanol) and then suspended in t-butanol and then frozen. The sample is then placed on a carbon tip attached to the stub. The cells were then plated with gold metal for 10 seconds using an ion sputter, which was

observed with a scanning electron analysis microscope *SEM* Jeol JSM 5310 lv (6).

RESULTS AND DISCUSSION

A. Papaya Leaf Extraction

Determination of papaya leaf plants was carried out at the UPT Laboratory of Setia Budi University. The results of the papaya leaf determination analysis based on 046/DET/UPT-LAB/15.3.2023 can be ascertained that the sample used wasdaun papaya (*Carica papaya* L). The papaya leaf samples taken in this study were 10,500 grams of old, fresh green and undamaged papaya leaves, producing 1600 grams of dried simplicia. Then a simple yield calculation was carried out and the yield was 15.2%, so it met the requirements because the yield was said to be good if the value was more than 10%. To make a fine powder, dry papaya leaves were ground with a blender and sieved using a 40 size mesh. A total of 1600 grams of papaya was ground and obtained papaya powder of 1420 with the dry shrinkage calculation that had been carried out yielding 88.7%.

The method for making extracts in this research uses the maceration or soaking method. The advantages of making extracts using the maceration method include producing a larger amount of extract, the tools used are simple, and the costs required are relatively economical. Papaya leaves were extracted using 96% ethanol solvent. Ethanol solvent is a universal solvent, ethanol solvent can dissolve almost all compounds found in simplicia, both polar compounds and non-polar compounds. The higher the concentration of ethanol solvent used, the lower the polarity level. The yield obtained was 24%, the yield was said to be good if the value was greater than 9.9%.

Table 1. Yield Results of Thick Extract

Simplicia Powder (g)	Condensed Extract (g)	Yield (%)
1100	268	24

B. Fractionation of Papaya Leaf Extract

Fraction preparation was carried out using the ECC method. The ethanol extract of papaya leaves was fractionated using a separating funnel with 3 parts of the solvent n-hexane (non-polar), ethyl acetate (semi-polar) and water (polar). The aim of the fractionation stage is to separate compounds based on the distribution or partition of an analyte between two immiscible solvents

Table 2. Rendement Fraction Frome. Gpapaya Leaf Truck

Solvent	Extract Weight (g)	Fraction Weight (g)	% Fraction Yield
Water fraction	190	78,895	41,52
Ethyl acetate	190	52,527	27,64
N-hexane	190	56,157	29,55

The yield results can be seen that the percentage of papaya leaf ethanol extract fraction has the largest weight, namely the water fraction 41,52% followed by the n-hexane fraction 32.13% and ethyl acetate 29,55%. Rendemen setiap pelarut berbeda berdasarkan tingkat kepolaran senyawa yang terkandung di dalam ekstrak pepaya, fraksi air dengan persentase terbesar melarutkan banyak senyawa polar seperti flavonoid, tanin dan saponin

C. Phytochemical Screening Test

From the results of phytochemical screeningtesting on the table data belowIt can be concluded that papaya leaves contain active compounds of flavonoids, saponins, tannins, terpenoids, steroids and alkaloids so they are effective as antibacterials and anti-biofilms.

Table 3. Results of Chemical Content Testing Using the Phytochemical Method

Metabolit Seconds	Exract	Fracion air	Fractin Ethyl	FractionN-hexane	Color
Flavonoid	+	+	+	-	Red yellow orange
Saponin	+	+	+	-	Foaming >1 minute
Tannin	+	+	-	-	Blackish green
Terpenoids	+	-	-	+	Brownish red
Steroid	+	-	+	+	Blue to green in color
Alkaloid	+	+	-	-	Mayer: white precipitate
Alkaloid	+	+	+	+	Bouch rdat brown to blackish precipitate

Information : (+) : Contains the Test Compound. (-): Does not Contain the Test Compound

D. Evaluation of Papaya Leaf Extract

a. Organoleptic test

Testing is carried out to determine the physical characteristics of the extract made, including the shape, aroma, color and taste of the extract. The organoleptic test results can be seen in table 4.

Table 4. Results of Organoleptic Observations of Papaya Leaf Powder

Organoleptic test of papaya leaf simplicia powder			
Dosage form	Aroma	Color	Feel
Powder	Typical of papaya leaves	Dark green	Bitter
Extract	Typical of papaya leaves	Blackish green	Bitter

b. Specific Gravity Test

Determining specific gravity aims to provide a limit on the mass per unit volume, which is a specific parameter for liquid extracts until they become thick extracts that can still be poured. Specific gravity is also related to the purity of the extract from contamination (Ministry of Health of the Republic of Indonesia, 2000). The results of this test obtained an extract specific gravity of 1.006 g/mL.

c. Test the Moisture Content of the Extract

The result is the average percentage of extra water contentk papaya leaves is 6.43%, menunbe in accordance with the requirements of the Indonesian Herbal Pharmacopoeia, namely no more than 10.0% (Ministry of Health of the Republic of Indonesia, 2017). Water is a medium for microbial development which can accelerate the deterioration of materials, and reduce product shelf life so that the water content is too high or > 10.0% will disrupt the stability of the extract because water can affect chemical

reactions and material conservation.The results of the water content test can be seen in table 5.

Table 5. Test Results for Water Content of Papaya Leaf Extract

Sample 1 (g)	Weight (g)	Volume air (ml)	% Water level
Replication 1	10	0,654	6,52
Replication 2	10	0,679	6,78
Replication 3	10	0,601	6,00
Rate-rate		0,644 ± 0,039	6,43% ± 0,397

d. Calculation of Extract Drying Shrinkage

The average shrinkage result was 6.6%, this result is in accordance with the determination of the water content of papaya leaf simplicia in the FHI, namely no more than 10%.

Table 6. Results of Determination of Extract Drying Loss

Cross	Initial weight (g)	Final weight (g)	Drying shrinkage (%)
Cross 1	2	1,85	7,5
Cross 2	2	1,83	8,5
Cross 3	2	1,84	8
Rate – Rate		1,84 ± 0,01	8% ± 0,5
FHI Standard			<10

e. Test the Ash Content

The total ash content test results are as stated in the table, namely 9.51%, this shows that the results are still goodadopt FHI standards, namely< 10%.

Table 7. Results of Determining Total Ash Content

Sample	Test results (%)	FHI
Extract	9,51	< 10%

E. Antibacterial test

Test the antibacterial activity of S. aureus using the Kirby Bauer diffusion method using Mueller Hinton Agar (MHA) media. The research used the disk diffusion method (Kirby Bauer) because the advantages of disk diffusion are that it is easy to do, does not require special equipment and is relatively cheap. In this diffusion test, the same concentration is used, namely 50% of each sample, namely extract, water fraction, ethyl acetate fraction and N-hexane fraction to see the most active fraction. Results from measuring the antibacterial inhibition zone *S. Aureus* are in the table below

Table 8. Bacterial Diffusion Method Test Results *S. Aureus w*ith 50% Concentration

Preparation	Replication 1	Replication 2	Replication 3	Rate-rate
Papaya leaf extract	16 mm	15 mm	16 mm	15,66 ± 0,57
Water fraction	21 mm	20 mm	21 mm	20,66 ± 0,57
Ethyl acetate fraction	18 mm	17 mm	17 mm	17,33 ± 0,57
N-hexane fraction	10 mm	11 mm	11 mm	10,6± 0,57
Control (+)	25 mm	23 mm	26 mm	24,66 ± 1,52
Control (-)	0	0	0	0 ± 0

Information : (Control +) = Ciprofloxacin Antibiotic Disk **(**Control –) = No Treatment

Results from antimicrobial testing against bacteria*S. aureus* secara difusi menunjukkan hasil bahwa fraksi air memiliki zona hambat yang paling besar dibandingkan ektrak dan fraksi lainnya untuk nilai yang paling tinggi sebesar 21 mm nilai rata-rata 20,66 ± 0,57. Pengujian statistik dengan analisis Kruskal-Wallis menunjukkan bahwa sampel uji mempunyai perbedaan secara signifikan yaitu pada fraksi air, fraksi etil asetat, N-heksan dan ekstrak dengan nilai signifikansi <0,005

The antibacterial activity test of the activated fraction, namely the water fraction from papaya leaf extract, against S. Aureus bacteria was carried out using the dilution method to find the minimum kill concentration (KBM). The respective solution concentrations are 50%, 25%, 12.5%, 6.2%, 3.1%, 1.5%, 0.7% as well as control (-), and control (+). Number of bacteria*S. aureus* turbidity used was adjusted to the Mc Farland standard of 0.5 in the medium *BE* which is then mixed with the water fraction

Table 9. Test Results of the Dilution Method for the Active Fraction of Inoculation*VJA*

Inoculation*VJA*				
NO	Concentration (%)	Replication 1	Replication 2	Replication 3
1	Control (-)	-	-	-
2	50 %	-	-	-
3	25 %	-	-	-
4	12,5 %	-	-	-
5	6,2 %	+	+	+
6	Control (+)	+	+	+

Information : (-) = no bacterial growth (+) = there is bacterial growth

The results of the antibacterial test by dilution showed that at concentrations of 50%, 25%, 12.5% there was no bacterial growth, this shows that the greater the concentration of the water fraction, the higher the antibacterial activity.*S. aureus*. The dilution method is useful for determining the minimum dose of bacteriostatic and bactericidal fractions

F. Biofilm Inhibition and Formation Test

Based on research, the most optimal optimization of absorbance and time for biofilm formation is at a wavelength of 595 and an incubation time of 72 hours. Biofilm formation

begins with bacteria attaching to a hydrophobic surface, then dividing and forming micro colonies called biofilms. The biofilm inhibition test aims to measure the ability of papaya leaves to prevent bacteria *S. Aureus*f orming a biofilm

Table 10. Percentage Results of Effectiveness of Inhibition of Biofilm Formation in Water Fraction

Water fraction (mg/ml)	Biofilm inhibition percentage			
	Replication 1	Replication 2	Replication 3	Rate-rate
50	71,68	72,98	72,46	72,37 ± 0,65
25	64,15	65,71	64,41	64,75 ± 0,83
12,5	53,50	52,72	53,24	53,15 ± 0,39
Positive control (ciprofloxacin)	74,54	73,50	74,80	74,54 ± 0,68

study, three variations of concentration were used, namely 50, 25 and 12.5 mg/ml, the greater the concentration of the water fraction used, the greater the percentage of suppression of biofilm development.

Table 11. IC Values$_{50}$Inhibition of Biofilm Formation

Sample	IC value$_{50}$inhibition of Biofilm formation (mg/ml)			
	Replication 1	Replication 2	Replication 3	Rate-rate
Water fraction	0,962	0,986	0,978	0,975 ± 0,012

The inhibition value for biofilm formation shows that the smallest IC50 value is shown by a concentration of 50 mg/ml, namely 1.022 ± 0.008 mg/ml. The smaller the IC50 value, the greater the inhibition of biofilm formation. On IC value$_{50}$ which obtained an average result of 0.975 ± 0.012 indicating that the smaller the IC value$_{50}$ the greater the inhibitory power of biofilm formation. The process of compounds inhibiting biofilm formation is by inhibiting the attachment of microbes to surfaces so that biofilm development will be disrupted. Disrupted biofilm development will affect the biofilm structure to increase defense against antimicrobials. Apart from inhibiting microbial attachment, compounds can damage the extracellular matrix (EPS) of biofilms, this will cause cell and nutrient communication pathways between microbes to be cut off so that the microbes that will form the biofilm will become lysed or die, due to the loss of nutrients that make up the biofilm. Statistical testing using Shapiro-Wilk analysis shows that the water fraction preparations with concentrations of 50%, 25% and 12.5% have significant differences with a significance value of >0.05.

The biofilm degradation test was carried out by inserting the bacterial suspension and media into a microplate so that a biofilm was formed, then the solution in the plate was discarded and then treated with water fractions with various concentrations. The results of the absorbance values obtained then calculated the percentage of biofilm degradation can be seen in table 12.

Table 12. Biofilm Degradation Percentage Results

Water fraction (mg/ml)	Absorbance of 595 water fractions			
	Replication 1	Replication 2	Replication 3	Rate-rate
50	61,24	59,16	60,89	60,43 ± 1,11
25	50,86	50,17	49,13	50,05 ± 0,87
12,5	37,02	35,64	34,94	35,86 ± 1,05
Positive control (Ciprofloxacin)	63,66	62,28	64,35	63,43 ± 1,05

From the table data above, it can be concluded that the highest percentage of biofilm degradation was at a concentration of 50 mg/ml with an average value of 60.43 ± 1.11, then the second was at a concentration of 25 mg/ml with an average value of 50.05 ± 0. 87 and the last with a concentration of 12.5 mg/ml with an average value of 35.86 ± 1.05. The EC50 value of biofilm degradation from water fractions at various concentrations was calculated using data on the percentage of biofilm destruction. By using a linear equation between the fraction concentration percentage and the biofilm degradation percentage value, the biofilm degradation percentage can be calculated.

Table 13. Biofilm Degradation Percentage Results

Sample	EC value$_{50}$inhibition of Biofilm formation (mg/ml)			
	Replication 1	Replication 2	Replication 3	Rate-rate
Water fraction	25,367	27,536	27,289	26,731 ± 1,1874

Based on the results of the biofilm degradation test using water fractions of papaya leaves, it appears to have activity in destroying or degrading biofilms on bacteria.*S.aureus*. The results show that the higher the concentration of the papaya leaf water fraction, the higher the percent of biofilm degradation. Biofilm degradation percentage was negatively correlated with EC value$_{50}$the higher the EC value$_{50}$the lower the biofilm degradation activity. Cell death, cell leakage, and destruction of the biofilm matrix are some of the mechanisms used to break down biofilms. Flavonoids, alkaloids, saponins, tannins, terpenoids and other chemical compounds found in the water fraction of papaya leaves can inhibit and eliminate biofilm because they have processes that can result in disintegration of the biofilm matrix, cell death and cell leakage. The ability of a substance to penetrate the resulting biofilm, namely the layer *Extracellular Polymeric Substance* (*EPS*) or the layer of mucus covering the bacteria correlates with the substance's ability to degrade the biofilm. This substance can destroy biofilm by removing *EPS* from existing biofilm (10). Statistical testing using Shapiro-Wilk analysis shows that the water fraction preparations with concentrations of 50%, 25% and 12.5% have significant differences with a significance value of >0.05.

G. Observation of the Morphology of S.Aureus Bacteria and Leakage of K Metal Ions^{+}and Ca^{2+}

Observation result *SEM* on bacteria *S. Aureus* differences in bacterial morphology *S. Aureus* without treatment and treatment with the water fraction from papaya leaves, there was a change in the shape of the bacterial cells, which

were initially round in image (A), after treatment the shape of the bacteria changed to oval in image (B), it was also seen in the bacterial samples without treatment that around the cells there were many biofilms formed. whereas in bacterial samples with treatment the amount of biofilm formed was reduced, changes to smaller ones occurred due to the presence ofshrinkage of the bacterial cell membrane without being accompanied by damage to the cell membrane so it can be concluded that the water fraction of papaya leaves can inhibit biofilm formation. In bacterial samples *S. Aureus* What was observed was that there were no different color gradations so it was possible that the cell walls of not all cells were intact, but the size of the bacterial cells changed with the cell shape becoming oval and shriveled, thus allowing the cell membrane to become damaged so that the contents of the cells came out. Observation using the method *AAS.*

The bacterial cell membrane functions as a selector to regulate the entry of compounds into the bacterial cell, so that depending on the permeability of the cell membrane by secondary metabolites induced by acute damage, it will damage the function of the cell membrane, in this case the cell will leak. This cellular leak causes the release of cellular components, thereby reducing the size of the bacterial cells. Damage to bacterial cells causes the bacteria to dissociate, which can cause bacterial cell death (11).

Bacterial ion leak test results *S. aureus* which had been treated with papaya leaf water fraction with a concentration of 50% showed that the presence of K ions^{+}and Ca^{2+}which is read on the device *AAS* (*Atomic Absorption Spectrophotometer*). The K ion is read^{+} which is found on the cell wall and Ca^{2+}found on the cell membrane indicates that the bacterial cell is leaking or damaged. . Release of metal $K^{+}$and $Ca^{2}$from cells can cause death of bacteria. In metal K bacteria^{+}is the main cation contained in the cytoplasm of cells and affects the stability of the permeability of bacterial cell membranes. Damage that occurs to the cytoplasmic membrane is characterized by leakage of contents from the cytoplasm, resulting in an increase in potassium metal outside the cell which indicates damage to the permeability of the bacterial membrane. *S.aureus*.

CONCLUSION

The water fraction of papaya leaf extract is the fraction that has the best effectiveness compared to other fractions as an antibacterial with an average value of 20.66 ± 0.57, as an inhibitor of biofilm formation with an average IC50 value of 0.975 ± 0.012 and biofilm degradation with an average value -average EC50 26.731 ± 1.1874, and has a cell wall damaging mechanism in S. aureus bacteria ATCC 25923 observed K+ and Ca2+ metal ions using AAS (Atomic Absorption Spectrophotometer) analysis on K+ metal without treatment of 24.00 mg/L, with air fraction treatment had a value of 1,829 mg/. Meanwhile, the Ca2+ value without treatment had a value of 2.31 mg/L, while with treatment it had a value of 6.194 mg/L.

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