



The Potential of Parijoto Fruit Extract Fraction (*Medinilla speciosa* Blume) as Antibiofilm against *Staphylococcus aureus* Bacteria

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ABSTRACT

Parijoto fruit extract (*Medinilla speciosa* Blume) contains flavonoids, saponins, tannins, and terpenoids and has antibacterial potential in inhibiting the growth of *Staphylococcus aureus* bacteria. *S. aureus* bacteria are bacteria that are capable of forming biofilms. This research aims to determine the potential of extracts and fractions (water, ethyl acetate, and n-hexane) in inhibiting the formation and degradation of *S. aureus* biofilms. Parijoto fruit was extracted using the maceration method with 96% ethanol solvent. The extract was fractionated using a liquid-liquid partition technique with water, ethyl acetate, and n-hexane as solvents. The antibiofilm activity test against the *S. aureus* bacteria ATCC 25923 was carried out by treatment inhibiting biofilm formation and biofilm degradation. The method is to read absorbance using a microplate reader with a wavelength of 595 nm. Optical density results to calculate IC₅₀ and EC₅₀ values. The results showed that the extract and fractions (water, ethyl acetate, and n-hexane) had the potential to inhibit the formation and degradation of *S. aureus* bacterial biofilms. The IC₅₀ of biofilm inhibition in the extract, water fraction, ethyl acetate, and n-hexane against *S. aureus* bacteria was 2.51; 1.03; 1.10; 1.52 mg/ml. The EC₅₀ value of the biofilm degradation test on the extract, water fraction, ethyl acetate, and n-hexane against *S. aureus* bacteria was 1.03; 1.17; 1.37; 1.88 mg/ml. The most active fraction of parijoto fruit (*Medinilla speciosa* Blume) in inhibiting and degrading *S. aureus* bacterial biofilms is the water fraction.

1. Introduction

Infectious diseases are a problem in the health sector and continue to grow all the time. Infectious diseases are caused by viruses, bacteria, fungi, and other microorganisms. Antibacterials are drugs used to treat bacterial infections. Treatment of infectious diseases using antibacterials can result in a decrease in the effect of the drug due to bacterial resistance. One of the causes of bacteria being resistant to antibiotics is because the bacteria form biofilms.¹⁻³ Biofilm is a collection of microorganisms that live in an extracellular polymer matrix produced by bacteria and are permanently attached. Bacteria that form biofilms can defend themselves from host body disturbances and are able to survive in poor environmental

conditions such as extreme pH, extreme temperatures, and low oxygen content. *S. aureus* bacteria cause infectious diseases and can form biofilms. Biofilm formation by *S. aureus* depends on the presence of the *ica* gene, namely the operon gene consisting of *ica* A, B, C, and D, which can produce polysaccharide adhesin or polysaccharide intracellular adhesin (PIA).⁴⁻⁷

One of the plants that have antibacterial activity is the parijoto plant (*Medinilla speciosa* Blume). Parijoto is a plant that lives around Mount Muria, Kudus Regency, Central Java. Empirically, people who consume parijoto fruit during pregnancy will have more handsome or beautiful babies. In Syaima's (2015) research, the antibacterial activity of the active

fraction obtained from parijoto fruit ethyl acetate extract with a concentration of 50 mg/ml resulted in the most active fraction having an inhibitory power against *S. aureus* of 18.5 mm and against *E. coli* of 14.7 mm.⁸⁻⁹ Parijoto fruit contains saponins, flavonoids, tannins and terpenoids. This compound has activity as an antibiofilm. Research from Winarsih et al. (2019) explained that flavonoid compounds can disrupt biofilm activity by interfering with the intercellular adhesion genes *ica A* and *ica D*. Tannin compounds have the potential to inhibit biofilm growth by inhibiting genes that influence the formation of polysaccharide intercellular adhesion (PIA) which plays an important role in cell aggregation in biofilm formation. Saponins, in inhibiting biofilms, can react with bacterial cell membranes and prevent initial attachment to the cell surface. Terpenoids inhibit biofilm activity, namely by inhibiting biofilm formation, disrupting communication between cells, reducing the biofilm that has been formed, and killing bacteria in the biofilm. Based on this description, this research aims to determine the activity of parijoto fruit in inhibiting the growth and degrading *S. aureus* bacterial biofilms.¹⁰⁻¹⁴

2. Methods

Tools and materials

The tools used in this research were autoclave, micropipette, rotary evaporator, microscope, oven, pycnometer, hotplate, Laminar Air Flow (LAF), 96 well polystyrene flat-bottom microtiterplate, I Mark-Biorad Microplate Reader.

The material used in this research was parijoto fruit from Colo, Dawe, Kudus, Central Java. The solvents used in the extraction process were 96% ethanol (Nofrills), ethyl acetate, n-hexane and water. The test bacteria used were *S. aureus* ATCC 25923 from the collection of the Setia Budi University microbiology laboratory. The media from oxzoid are nutrient agar, mannitol salt agar, liquid brain heart infusion (BHI), and nutrient broth. Other test materials were tetracycline (positive control), dimethyl sulfoxide (DMSO), Mg powder, HCL, FeCl₃, crystal

violet, Lugol, safranin, H₂O₂ liquid, glutardialdehyde (Merck), and phosphate buffer.

Parijoto fruit extraction process

Parijoto fruit is washed using running water until clean, drained, sliced thinly to speed up the drying process, and dried in an oven at 40°C. Parijoto fruit simplicia was extracted using the maceration method with 96% ethanol solvent (1:10). The macerate was filtered using flannel cloth and remaciated. The filtrate obtained was then concentrated with a rotary evaporator.¹⁵

Characterization of simplicial

Characterization of simplicia includes powder water content, powder drying loss, and specific gravity of the diluted extract. How it works is as follows:

Powder water content. 20 grams of simplicia was put into a round flask, and then 100 ml of toluene solvent was added. The water content was measured using a Sterling Bidwell. The water obtained is then calculated using a concentration formula.

Shrink drying powder. Put 1 gram of sample into the cup and spread the sample evenly to form a layer. Place the cup containing the sample into the oven and heat at 105°C for 30 minutes. Then put the cup in a desiccator and let the cup cool. Finally, record the fixed weight of the cup and the sample obtained.

Determination of the specific gravity of the extract. The pycnometer is filled with distilled water, and the temperature is adjusted to reach 25°C, then the pycnometer containing the distilled water is weighed. The aquadest in the pycnometer was removed and dried. The pycnometer is filled with liquid extract with a concentration of 5%. Set the pycnometer temperature to 25°C, then the pycnometer containing the extract is weighed.

Fractionation of parijoto fruit ethanol extract

20 grams of parijoto fruit ethanol extract was dissolved in 10 mL of ethanol until completely dissolved and 100 mL of distilled water was added, then put into a 250 ml separating funnel. 100 mL of

n-hexane solvent was put into a separating funnel and shaken to mix. The separating funnel was left standing until it separated into two parts, namely the water fraction and the n-hexane fraction. The n-hexane fraction was removed from the separating funnel, while the water fraction was partitioned again using 100 mL of ethyl acetate solvent. The separating funnel was shaken to mix and allowed to stand until it separated into two parts, namely the ethyl acetate fraction and the water fraction. The fraction obtained was then concentrated using a rotary evaporator with a temperature of 40°C.

Phytochemical screening

The chemical compounds that will be tested are flavonoids, saponins, tannins, alkaloids, glycosides, steroids, and terpenoids. The test procedure is as follows:

Flavonoid test. The extract and fractions were dissolved in boiling water, then the filtrate obtained was added with Mg powder and concentrated HCl, then shaken to mix. Flavonoids are indicated by the formation of an orange, orange, or red color.

Tannin test. The extract and fraction were dissolved in hot water, then FeCl₃ was added 1% 3 drops. The formation of a blue or blue-black color means that the extract contains tannin compounds.

Saponin test. The extract and fraction were dissolved in hot water; after dissolving, the tube was shaken vigorously for 10 seconds until foam formed, and then 1 drop of HCl 2N was added. The presence of saponin is indicated by foam, which does not disappear.

Alkaloid test. Extracts and fractions were dissolved in HCl 2N. The solution was divided into 3 test tubes. The first tube is used as a blank, the second tube is filled with Dragendorff's reagent, and the third tube is filled with Mayer's reagent. The presence of alkaloids is indicated by the second tube forming an orange precipitate and the third tube forming a white precipitate.

Glycosides. The extract and fraction were dissolved in ethanol and then evaporated. The solution was dissolved with acetic anhydride, then concentrated sulfuric acid was added. A blue or green color change indicates the presence of glycosides.

Steroids and terpenoids. The extract and fraction were dissolved in chloroform, then acetic anhydride and acetic acid were added. The presence of steroids shows a blue-green color change, and the presence of terpenoids shows a brownish color between the surfaces.

Identify *S. aureus* ATCC 25923 bacteria

Gram staining method, namely *S. aureus* bacteria: Take 1 dose, then scratch it on the surface of the glass object and fix it over a spirit fire. A total of 1 drop of crystal violet was placed on the surface of the glass object and left for 1 minute. The glass object was rinsed using running water until the crystal violet color faded. The slide was dripped with 1 drop of Lugol's solution and left for 1 minute. The slide was rinsed with running water until the lugol color disappeared. The object glass was rinsed using alcohol until the color faded and rinsed with running water. The dry object glass is then added with 1 drop of safranin on the object glass and left for 45 seconds. The glass object was washed and dried over a spirit fire. The preparations were observed using a microscope to observe the color of the *S. Aureus* ATCC 25923 bacteria.

Differential media method using selective media mannitol salt agar. The differential media test was carried out by taking 1 loop of bacteria and streaking it on MSA media, then incubating it at 37°C for 24 hours.

Biochemical methods There are two types of bacterial identification, namely catalase and coagulase tests. The catalase test is carried out by dropping H₂O₂ liquid on a glass object, adding one cycle of bacterial inoculum, and then observing. The catalase test is positive in the presence of gas bubbles (O₂) above the glass object. The coagulase test is done by taking 1

dose of bacteria, then planting it in 1 ml of nutrient broth and incubating it at 37°C for 24 hours to form a bacterial suspension. The bacterial suspension was added to 1 ml of blood plasma and then incubated at 37°C for 24 hours. A positive result in the coagulase test shows the occurrence of white lumps in the tube containing the bacterial suspension.

Preparation of bacterial suspension

Preparation of bacterial suspension *S. aureus* ATCC 25923 by bacterial means *S. aureus* ATCC 25923 which had been planted on slanted nutrient agar media was taken 1 cycle, then put into a tube containing 9 ml of sterilized BHI media. The turbidity of the bacterial suspension was adjusted to a McFarland turbidity of 0.5 (1.5×10^8 CFU/mL).

Optimization of biofilm formation time

A total of 200 µl of bacterial suspension was added to each well microplate then optimize the incubation time. The time variations used are 24, 48, 72, and 96 hours. The microplate was rinsed using running water, then 200 µl of 1% crystal violet solution was to each well and left for 15 minutes. The microplate was rinsed with running water, 200 µl of 96% ethanol solution was added to the microplate, and the absorbance was read using an instrument Mark-Biorad Microplate Reader with a wavelength of 595 nm. The formation time with the largest absorbance value was used as a reference for incubation of biofilm formation *S. aureus* ATCC 25923.

Biofilm inhibition assay

The biofilm formation inhibitory activity test was done using a microplate round bottom polystyrene 96 wells, and the media used was BHI. Parijoto fruit fraction of 70 µl was put into each well with a concentration series of 20; 10; 5; 2.5; 1.25; 0.625; 0.3125; and 0.156 mg/ml. 70 µl of media was taken and added to each well, then 70 µl of bacterial suspension was added into the well containing the media and incubated at 37°C for 96 hours. The wells were removed, and the plate was washed with running water and then dried for 15 minutes by turning it upside down microplate at room temperature.

200 µl of 1% crystal violet solution was added to each well for a staining time of 15 minutes. The solution in the well is discarded and the well is rinsed again with running water. Microplate dried by turning it upside down at room temperature for one hour, then given 200 µl of 96% ethanol solution to each well on the plate. Observations were made with microplate reader with a wavelength of 595 nm and each test was replicated 3 times.

% biofilm inhibition =

$$\frac{\text{Negative Control Absorbance} - \text{Sample Control Absorbance}}{\text{Negative Control Absorbance}} \times 100 \%$$

Obtaining the average percent inhibition of biofilm formation from each fraction concentration was then followed by determining the IC₅₀ value by using a linear regression line equation between the percent inhibition of biofilm formation and the concentration of the fractions to see the relationship between the concentration and the percent inhibition of the biofilm in inhibiting 50% of the biofilm.

Biofilm degradation assay

100 µl of media was put into each well, and 100 µl of bacterial suspension was added to the well containing the media. The microplate was then incubated at 37°C for 96 hours. The solution in the well is then discarded and the well is washed using running water. The parijoto fruit fraction was 200 µl with 20 concentration variations; 10; 5; 2.5; 1.25; 0.625; 0.3125; and 0.156 mg/ml were added to each well, wells containing only media and bacterial suspension were used as negative controls, then incubated at 37°C for 24 hours. The solution in the wells was discarded, and the plate was washed with running water and then dried for 15 minutes by turning it upside down microplate at room temperature.

200 µl of 1% crystal violet solution was added to each well for a staining time of 15 minutes. The solution in the well is discarded, and the well is rinsed again with running water. The microplate was dried by turning it upside down at room temperature for one hour, then given 200 µl of 96% ethanol solution to

each well on the plate. Observations were made with a microplate reader with a wavelength of 595 nm, and each test was replicated 3 times.

% biofilm degradation =

$$\frac{\text{Negative Control Absorbance} - \text{Sample Control Absorbance}}{\text{Negative Control Absorbance}} \times 100 \%$$

The absorbance results obtained were then calculated as the percent biofilm degradation value. Then, the EC₅₀ value will be determined by using a linear regression line equation between percent biofilm degradation and fraction concentration.

3. Results and Discussion

Characterization of simplicial

The water content parameter is the minimum limit or range of the amount of water content contained in the simplicia. Water content test results with tools sterling bidwell obtained an average result of 6.17% ± 0.76. This is in accordance with the maximum limit of water content, namely 10%.¹⁴ The lower water content in simplicia can reduce the growth of bacteria, fungi, or other microorganisms.²⁶ The lower the water content in the simplicia, the longer the storage period for the simplicia.²⁷

Drying loss is a parameter that determines the maximum limit of the amount of compounds lost in the drying process. The average drying shrinkage was 6.33% ± 1.53. According to the Herbal Pharmacopoeia (2017), the requirement for drying loss of simplicia in the form of fruit is that it should not be more than 10%, such as for dewas crown fruit, fennel fruit and separantu fruit.

Specific gravity is the weight of the extract compared to the weight of distilled water with the same volume and temperature.²⁸ The average specific gravity of the extract obtained was 1.022 ± 0.02 g/ml. The specific gravity value obtained provides an overview of the chemical content dissolved in the extract.

Extraction and fractionation results

The thick extract obtained during the maceration process was 91.43 grams with an extract yield percentage of 22.86%. The yield of parijoto fruit water fraction obtained was 11.55%, the ethyl acetate fraction obtained was 49.56% and the n-hexane fraction obtained was 27.35%. The ethyl acetate fraction had the highest yield, then the n-hexane fraction, and the water fraction had the lowest. The yield results for each fraction are different because each solvent has a different ability to extract the compounds contained in the ethanol extract of parijoto fruit. Research from Legawati *et al.* (2020) showed that the fractionation of 70% ethanol extract of parijoto fruit resulted in a yield of n-hexane fraction of 51.92%, ethyl acetate fraction of 42.17%, and ethanol fraction of 41.18% (16).

Phytochemical screening

Phytochemical screening aims to determine the compounds contained in the ethanol extract, water fraction, ethyl acetate fraction and n-hexane fraction of parijoto fruit (*Medinilla speciosa* Blume) which has the potential to act as an antibiofilm. The results of phytochemical screening can be seen in Table 1.

Table 1. Phytochemical screening results.

Secondary metabolites	Extract	Water fraction	Ethyl acetate fraction	n-hexane fraction
Flavonoid	+	+	+	-
Saponin	+	+	+	-
Tannin	+	+	+	+
Alkaloid	-	-	-	-
Glycosides	-	-	-	-
Steroid	-	-	-	-
Terpenoids	+	+	+	+

The results of phytochemical screening showed that the ethanol extract, water fraction and ethyl acetate fraction of parijoto fruit contained flavonoid, saponin, tannin and terpenoid compounds. Meanwhile, the n-hexane fraction contains tannin and terpenoid compounds.

Identify *S. aureus* ATCC 25923 bacteria

The identification results can be seen in Figure 1. Results of the bacterial Gram staining test *S. aureus* (Figure 1. A) show bacteria that are round and Gram-positive. The results of Gram staining produce a purple color. The visible purple color is the result of staining from crystal violet and Lugol's solution.³⁰

Identification with selective media (Figure 1. B) to identify the type of microorganism that grows to form certain colonies. Mannitol salt Agar (MSA) is a selective medium used to identify bacterial types of *Staphylococcus*. The results of bacterial identification using differential media showed that *S. aureus* bacteria planted on MSA media formed yellow

colonies, and there was a yellow color change in the media. The yellow zone on media is caused by fermentation mannitol, that is, resulting in a change in the phenol red on agar media. From red, it changes to yellow.²² There are two biochemical methods for bacterial identification (Figure 1. C), namely the catalase test and the coagulase test. Catalase test results on *S. aureus* ATCC 25923 bacteria showed positive results, as evidenced by the presence of gas bubbles on the slide. *Staphylococcus* bacteria form the enzyme catalase, which can hydrolyze hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen gas (31). Coagulase test results on *S. aureus* ATCC 25923 bacteria showed positive results because there were white lumps in the tube. *S. aureus* bacteria is a bacteria that can produce the enzyme coagulase. The coagulase enzyme binds to prothrombin, both of which become enzymatically active and convert fibrinogen into fibrin. The final result is plasma coagulation in the form of fibrin clots or clots.

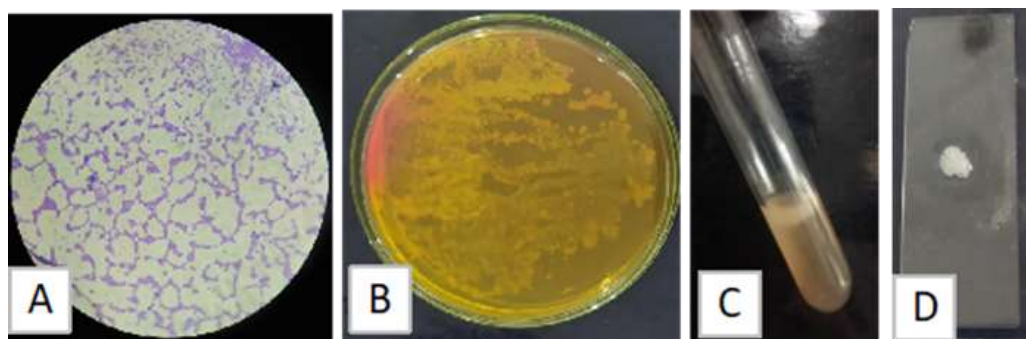


Figure 1. Results of identification *S. aureus* bacteria with Gram Staining (A), Selective Medium (B), and biochemical tests of coagulase (C) and catalase (D).

Optimization of biofilm formation time

Optimization of biofilm formation time is used to determine the optimum time for *S. aureus* bacteria to form biofilms. The optimization time variants used are 24 hours, 48 hours, 72 hours, and 96 hours. The turbidity of the bacterial suspension before optimizing the biofilm formation time was adjusted to a McFarland turbidity of 0.5 (1.5×10^8 CFU/mL).

Results of optimization of bacterial biofilm formation time *S. aureus* ATCC 29523 can be seen in Table 2. The largest absorbance value was shown by an incubation time of 96 hours. The results of optimizing the time for biofilm formation were used as a reference for the incubation period in bacterial biofilm inhibition and degradation tests *S. aureus* ATCC 29523.

Table 2. Optimization results for biofilm formation time.

	Absorbance			
	24 hours	48 hours	72 hours	96 hours
Average	0,398 ± 0,06	0,546 ± 0,10	1,002 ± 0,11	1,184 ± 0,16

The results of optimizing the time for biofilm formation showed that at varying times, there were different levels of turbidity in the bacterial suspension. The level of turbidity of the bacterial suspension is proportional to the bacterial growth phase that occurs during the incubation time. The bacterial growth phase consists of 4 phases, namely the lag phase, log phase, stationary phase, and death phase. The research results showed that the most optimal time for biofilm formation was 96 hours during the stationary phase. Research by Fitri K. et al. (2015) showed that when the bacteria reached the stationary phase, the turbidity did not decrease after incubation for four to six days. Biofilm formation begins with bacteria attaching to a hydrophobic surface, then dividing and forming a biofilm. Factors that influence the attachment of bacterial cells in biofilm formation include surface properties, film conditions, hydrodynamics, characteristics of the liquid medium, and the surface condition of the bacterial cells.

Biofilm inhibition assay

The results show that the greater the concentration of parijoto fruit extract or fraction, the higher the

percentage of inhibition of bacterial biofilm formation *S. aureus*. The greater the concentration of the test sample, the greater the secondary metabolite content contained in the extract or fraction, so the greater its potential to inhibit biofilm formation. The positive control in this study was the antibiotic tetracycline 0.03mg/ml. Tetracycline is a class of antibiotics that can inhibit the growth of *S. aureus* bacterial biofilms.

Data on the percent inhibition of biofilm formation is used to determine IC₅₀ values. The analysis results use a linear equation between the percent inhibition of biofilm formation and the fraction concentration. IC₅₀ value results can be seen in Table 3. IC₅₀ The smallest is shown by the water fraction, namely 1.026 ± 0.204 mg/ml. The smaller the IC₅₀ value, the greater the inhibitory power of biofilm formation. Inhibition of biofilm formation with IC₅₀ value smallest to largest is shown by the water fraction, then the ethyl acetate fraction, the n-hexane fraction and the ethanol extract of parijoto fruit.

Table 3. IC₅₀ values biofilm inhibition.

Sample	IC ₅₀ value biofilm inhibitors (mg/ml)			Average IC ₅₀
	Replication 1	Replication 2	Replication 3	
Extract	1,689	3,129	3,274	2,698 ± 0,876
Ethyl acetate fraction	1,073	1,112	1,123	1,103 ± 0,026
Water fraction	1,096	1,186	0,796	1,026 ± 0,204
n-hexane fraction	1,355	1,522	1,699	1,525 ± 0,172

The mechanism of action of a secondary metabolite contained in plants provides an anti-biofilm effect by inhibiting the process of attachment (adhesion) of bacteria to the surface of objects, disrupting the

regulation of whose sensing and reducing the growth of extracellular polymers (EPS). 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.

Biofilm degradation assay

Biofilm degradation activity shows the power to destroy biofilm that has been formed from the test sample. The results of the percent biofilm degradation are used to determine the EC₅₀ value of biofilm degradation from ethanol extract, water fraction, ethyl acetate fraction, and n-hexane fraction. The positive control in this study was the antibiotic tetracycline at a dose of 0.03 mg/ml.

The positive control on biofilm degradation showed an average percentage of 65.33%. The ethyl acetate fraction had the highest value of percent biofilm degradation with a value of 89.59%, followed by the water fraction with a value of 87.16%, the extract with a value of 85.36%, and the n-hexane fraction with a value of 84.36%. Calculation of percent biofilm degradation using a linear equation between the percent concentration of the extract or fraction and the percentage value of biofilm degradation.

The EC₅₀ value results shown in Table 4 show that the extract has the lowest value, followed by the water fraction, ethyl acetate fraction, and EC₅₀ value. The highest is the n-hexane fraction. The smaller the EC₅₀ value obtained, the greater the fraction's activity in degrading bacterial biofilms *S. aureus*.

Table 4. EC₅₀ values Biofilm degradation.

Sample	EC ₅₀ value biofilm degradation (mg/ml)			Average EC ₅₀
	Replication 1	Replication 2	Replication 3	
Extract	1,115	1,040	1,056	1,070 ± 0,039
Ethyl acetate fraction	1,258	1,387	1,480	1,375 ± 0,112
Water fraction	1,245	1,295	1,016	1,185 ± 0,149
n-hexane fraction	1,958	1,974	1,736	1,889 ± 0,133

Parijoto fruit contains flavonoids, saponins, tannins, and terpenoids based on the results of the phytochemical screening that was carried out. Flavonoid compounds have activity in destroying biofilms. The mechanism of flavonoids in destroying biofilm is by means of the flavonoid structure; namely, the hydroxyl group binds to the proteins contained in the biofilm and forms complex compounds, resulting in the biofilm being denatured. Tannin compounds damage bacterial biofilms by binding iron ions, which are needed by bacteria to maintain the biofilm matrix, resulting in a decrease in bacterial viscosity and reduced binding of the biofilm matrix. Tannins can also affect EPS by reducing the amount of EPS in

bacterial biofilms. The mechanism by which saponins damage biofilms is by affecting the extracellular polymer matrix in the bacterial biofilm matrix so that the polymer substance is reduced and changes the integrity of the bacterial cell membrane, causing instability in the bacterial cell wall. Terpenoid compounds in degrading biofilms can reduce the biofilm that has been formed and kill bacteria in the biofilm.¹⁴

4. Conclusion

Water fraction, ethyl acetate and n-hexane ethanol extract of parijoto fruit (*Medinilla speciosa* Blume) can inhibit the formation and degradation of bacterial biofilms *S. aureus* ATCC 25923. The most active

fraction of parijoto fruit (*Medinilla speciosa* Blume) as an antibiofilm against bacteria *S. aureus* ATCC 25923 is a water fraction with an IC₅₀ value biofilm inhibition of 1.026 ± 0.204 mg/ml and EC₅₀ value Biofilm degradation was 1.185 ± 0.149 mg/ml.

5. References

- Lestari DRS, Soegianto L, Hermanu LS. Antibacterial and antibiofilm potential of bintaro flower ethanol extract (*Cerbera odollam*) to *Staphylococcus aureus* ATCC 6538. *J Pharm Sci.* 2017; 4(1): 30–35.
- Oktasila D, Nurhamidah, Handayani D. Antibacterial activity test of kalamansi orange leaves (*Citrofortunella microcarpa*) against bacteria *Staphylococcus aureus* and *Escherichia coli*. *J Chem Educ Sci.* 2019; 3(2): 158–69.
- Fitria A. Bactericidal and antibiofilm activity of stems *Jatropha multifida* L. against *Staphylococcus aureus* and MRSA. *J Math Nat Sci.* 2018; 18: 42–55.
- Jamal M. Bacterial biofilm and associated infections. *J Chin Med Assoc.* 2018; 81(1): 7–11.
- Kining E, Falah S, Nurhidayat N. The in vitro antibiofilm activity of water leaf extract of papaya (*Carica papaya* L.) against *Pseudomonas aeruginosa*. *J Cur Biochem.* 2015; 2(3): 150–63.
- Cramton SE. The intercellular adhesion (ica) locus is present in *Staphylococcus aureus* and is required for biofilm formation. *Infect Immun.* 1999; 67(10): 5427–33.
- Liu M, Wu X, Li J, Liu L, Zhang R, Shao D, et al. The specific anti-biofilm effect of gallic acid on *Staphylococcus aureus* by regulating the expression of the ica operon. *Food Control.* 2017; 73: 613–8.
- Hanum A, Prihastanti E, Jumari. Ethnobotany of utilization, role, and philosophical meaning of parijoto (*Medinilla*, spp) on Mount Muria in Kudus Regency, Central Java. American Institute of Physics (AIP). 2017.
- Syaima. Isolation of antibacterial active fractions from ethyl acetate extract of parijoto fruit (*Medinilla speciosa* Blume), I [Thesis]. Jakarta: UIN Syarif Hidayatullah. 2015.
- Niswah L, Antibacterial activity test of parijoto fruit extract (*Medinilla speciosa* Blume) using disc diffusion method. Thesis. UIN Syarif Hidayatullah. Jakarta. 2014.
- Lee JH. Anti-biofilm activities of quercetin and tannic acid against *Staphylococcus aureus*. *Biofouling.* 2013; 29(5): 491–9.
- Dong S. Antibacterial activity and mechanism of action saponins from *Chenopodium quinoa* Willd. husks against foodborne pathogenic bacteria. *Ind Crops Prod.* 2020; 149: 112350.
- Kanaan H. Screening for antibacterial and antibiofilm activities in *Astragalus angulosus*. *J Intercult Ethnopharmacol.* 2017; 6(1): 50–57.
- Khameneh B. Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrob Resist Infect Control.* 2019; 8: 1–28.
- Legawati HE, Kunarto B, Sani EY. Fractionation of parijoto fruit extract (*Medinilla speciosa* L.) and stability of anthocyanins at various heating times. Repository Universitas Semarang. 2020.
- Dewi AK. Isolation, identification and sensitivity test *Staphylococcus aureus* against Amoxicillin from milk samples of Ettawa crossbreed goats (PE) suffering from mastitis in the Girimulyo Region, Kulonprogo, Yogyakarta. *J Vet Sci.* 2013; 31(2): 138–50.
- Rosidah I. Standardization of 70% ethanol extract of chayote fruit (*Sechium edule* (Jacq.) Sw.). *Farmasains.* 2020; 7(1): 13–20.
- Amanto BS, Siswanti, Atmaja A. Drying kinetics of temu giring (*Curcuma heyneana* Valeton & van Zijp) using a cabinet dryer with pre-blanching treatment. *J Agric Prod Tech.* 2015; 8(2): p.107.

19. Kristian J. The effect of extraction time on the yield and quality of white jasmine flower oil using the solvent extraction method. *Teknotan J.* 2016; 10(2): 34–43.
20. Suryadini H. Standard parameter test and phytochemical screening on sterile kalakai leaves (*Stenochlaena palustris* (Burm.f.) Bedd.) using multistage extraction. *Farmasyifa Pharm Sci J.* 2019; 2(1): 40–51.
21. Rachma A, Sarjono PR, Aminin ALN. Isolation of thermophilic bacteria in gedongsongo hot springs using minimal enrichment media YT (Yeast Tryptone) and genotypic and phenotypic identification. *J Chem Sci App.* 2009; 12(3): 66–71.
22. Toelle NN, Lenda V. Identification and characteristics *Staphylococcus Sp.* and *Streptococcus Sp.* of ovarian infections in commercial laying hens. *J Anim Sci.* 2014; 1(7): 32–37.
23. Jiwintarum Y, Srigede L, Rahmawati A. Differences in coagulase test results using human plasma citrate 3.8%, plasma citrate sheep 3.8%, and plasma citrate rabbit 3.8% on bacteria *Staphylococcus aureus*. *Prima Health J.* 2015; 9(2): 1560–1.
24. Fitri KSA, Agung MUK, Meika J. Antibacterial screening of extracellular products of marine bacterial exosymbionts in macroalgae against Biofilms *Staphylococcus aureus* ATCC 25923. *Indonesian Aquatics J.* 2015; 6(2): 128–39.
25. Costerton J, Stewart PS. Battling biofilms. *American: Sci Am.* 2001.
26. Dewi M. Antibiotic Activity on Biofilms *Staphylococcus aureus* Aceh cow preputium isolate. *J Vet Sci.* 2020; 38(1): 1.
27. Hamzah H. Effect of saponins on inhibition of planktonic and mono-species biofilms *Candida albicans* ATCC 10231 in the middle phase, maturation and degradation. *Majalah Farmaseutika.* 2021; 17(2): 198–205.
28. Winarsih S, Khasanah U, Alfatah AH. Antibiofilm activity of ethyl acetate fraction of putri malu leaf extract (*Mimosa pudica*) In Methicillin-Resistant Bacteria *Staphylococcus aureus* (MRSA) in vitro. *Majalah Kesehatan.* 2019; 6: 76–85.