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Exploitation Antibiofilm Substances Endophytic Bacteria of Renggak Fruit (*Amomum dealbatum*) Against *Staphylococcus aureus*, Cause of Nosocomial Infections

Eksploitasi Substansi Antibiofilm oleh Bakteri Endofit Buah Renggak (Amomum dealbatum) terhadap Staphylococcus aureus Penyebab Infeksi Nosokomial

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Abstrak

Infeksi nosokomial dapat disebabkan oleh mikroorganisme pembentuk biofilm. Kemampuan membentuk biofilm mengakibatkan infeksi yang persisten, berulang, dan 100-1000 kali lebih resisten terhadap antibiotik. Salah satu bakteri pembentuk biofilm adalah *Staphylococcus aureus*. Tanaman renggak (*Amomum dealbatum Roxb*.) yang mengandung alkaloid, steroid, flavonoid, terpenoid, tanin, dan saponin diketahui berperan dalam menghambat pembentukan biofilm bakteri. Penelitian ini menggunakan rancangan percobaan eksperimental in vitro yang bertujuan untuk menganalisis aktivitas antibiofilm bakteri endofit renggak terhadap *Staphylococcus aureus* menggunakan metode *tissue culture plate*. Hasil penelitian diperoleh lima isolat bakteri endofit, meliputi BI1, BI2, BI3, BI4, dan BI5. Persentase aktivitas penghambatan biofilm oleh supernatan bebas sel bakteri endofit adalah masing-masing 35%, 0%, 37%, 44%, dan 39%, dengan kontrol positif sebesar 42%. Sementara itu, aktivitas penghancuran biofilm masing-masing adalah 39%, 42%, 41%, 38%, dan 36%, dengan kontrol positif sebesar 56%. Hasil uji statistik *One Way* ANOVA didapatkan hasil signifikansi 0,000 (p<0,005), yang artinya terdapat perbedaan yang bermakna signifikan antar kelompok perlakuan dalam uji antibiofilm. Kesimpulannya adalah supernatan bebas sel bakteri endofit buah renggak memiliki aktivitas penghambatan dan penghancuran terhadap biofilm bakteri *Staphylococcus aureus*.

Kata kunci: antibiofilm; bakteri endofit; buah renggak; *Staphylococcus aureus*

Abstract

Nosocomial infections can be caused by biofilm-forming microorganisms. The ability to form biofilms results in persistent, recurrent, and 100-1000 times more resistant to antibiotics. One of the biofilm-forming bacteria is *Staphylococcus aureus*. The renggak plant (*Amomum dealbatum Roxb*.) which contains alkaloids, steroids, flavonoids, terpenoids, tannins, and saponins is known to play a role in inhibiting bacterial biofilm formation. This study used an in vitro experimental design aimed to analyzing the antibiofilm activity of endophytic bacteria against *Staphylococcus aureus* using the plate tissue culture method. The results obtained five endophytic bacterial isolates, including BI1, BI2, BI3, BI4, and BI5. The percentage of biofilm inhibitory activity by cell-free supernatants of endophytic bacteria was 35%, 0%, 37%, 44%, and 39%, respectively, with a positive control was 42%. Meanwhile, the biofilm revealing activity was 39%, 42%, 41%, 38%, and 36%, respectively, with a positive control was 56%. The results of the *One Way* ANOVA statistical test obtained a significance result of 0.000 (p<0.005), which means there was a significant difference between the treatment groups in the antibiofilm test. The conclusion is that the cell-free supernatant of endophytic bacteria from renggak fruit has inhibitory and degradation activity against *Staphylococcus aureus* bacterial biofilms.

Keywords: antibiofilm; endophytic bacteria; renggak fruit; *Staphylococcus aureus*

Introduction

Infectious diseases remain one of the greatest challenges faced by all countries, with the highest proportion occurring in developing countries (Bloom & Cadarette, 2019; WHO, 2015). The rapid rate of infection transmission makes infectious diseases a major cause of high morbidity and mortality in hospitals, known as nosocomial infections (Sheykhsaran *et al.,* 2022). Nosocomial infections are infections acquired by patients during hospitalization (Khan *et al.,* 2017). Nearly 1.5 million deaths worldwide are caused by nosocomial infections (Sumarningsih *et al.,* 2020). In developing countries, an estimated 40% of hospital patients suffer from nosocomial infections (Chairani *et al.,* n.d.).

One treatment for nosocomial infections is the administration of antibiotics. Rational use of antibiotics can increase treatment success rates and reduce the incidence of antibiotic resistance. Approximately 40%-62% of antibiotics are used inappropriately, leading to the emergence of multidrug-resistant organisms (MDROs) (Ji & Ye, 2024). The death rate from antibiotic resistance reaches approximately 700,000 people per year (Kementerian Kesehatan RI, 2016). Therefore, nosocomial infections pose a threat to public health due to the increase in MDROs.

Antibiotic resistance is significantly linked to bacterial biofilm formation (Baiq Isti Hijriani, 2021). *Staphylococcus aureus* biofilms increase antibiotic resistance through three main mechanisms: (1) The EPS (extracellular) matrix inhibits antibiotic penetration, (2) Persister (metabolically inactive) cells survive high drug concentrations, and (3) Horizontal gene transfer facilitates the spread of resistance genes within the dense biofilm structure. Bacterial biofilm formation in the body is known to be involved in various bacterial infections and is highly detrimental, complicating treatment.

Biofilms are aggregates of interactive bacteria that adhere to solid surfaces or adhere to each other, encased in an exopolysaccharide matrix. Several cases of bacterial infections have shown an increasing prevalence of biofilm formation (Cavallo *et al.,* 2024).

One bacterium capable of forming biofilms is *Staphylococcus aureus*, which is commonly found in the human environment and can form complex biofilm structures. This ability to form biofilms is a virulence factor for *Staphylococcus aureus*, making it resistant to antibiotics and the body's immune cells. Biofilm formation can be found in 80% of infections and is a serious problem because it makes the bacteria 1,000 times more resistant to antibiotics (Sun *et al.,* 2013).

The use of medicinal plants is currently being developed by the community, becoming an alternative in treating bacterial infections that are already resistant to antibiotics (Cavallo *et al.,* 2024). One such plant with medicinal properties is the renggak plant (*Amomum dealbatum Roxb*.), a native plant of Lombok Island and belonging to the *Zingiberaceae* family.

The renggak plant is known to contain secondary metabolites, such as alkaloids, steroids, flavonoids, terpenoids, tannins, and saponins (Rizky *et al.,* 2023). Extraction of secondary metabolites can be achieved by extracting plant parts, which is considered less effective due to the high biomass requirement and time-consuming process. One efficient method is to utilize endophytic bacteria, which can produce the required number of secondary metabolites in a shorter time than extraction (Ayu *et al.,* 2021).

Endophytic bacteria are microorganisms that interact with host plants without harming them (Ali *et al.,* 2024; Soesanto, 2023). These bacteria can produce compounds to protect tissues from pathogenic microorganisms, while the plant provides nutrients for the endophytic bacteria to survive (Astriani & Dwijayanti, 2022). Endophytic bacteria isolated from the renggak plant are likely capable of producing substances with antibiofilm properties.

This study aims to determine the potential antibiofilm substances of endophytic bacteria from renggak fruit (*Amomum dealbatum Roxb*.) against *Staphylococcus aureus*, a cause of nosocomial infections.

Material dan Metode

This study used a purely in vitro experimental design in the laboratory by measuring the Optical

Density (OD) value using a microplate reader. The population in this study was the renggak plant (*Amomum dealbatum Roxb*.) from Central Lombok Regency, West Nusa Tenggara. The sample in this study was the endophytic bacteria of the renggak fruit. The object of this study was *Staphylococcus aureus* bacteria.

Preparation of Tools and Materials

The tools used in this study included laboratory glassware, analytical balance, autoclave, hotplate, magnetic stirrer, filter paper, loop, Petri dish, microscope, incubator, microplate reader, spectrophotometer, aluminum foil, and laminar air flow meter. The materials used in this study included nutrient agar (NA), nutrient broth (NB), trypticase soy broth (TSB), distilled water, renggak plants, absolute ethanol, sodium hypochlorite (NaOCl), a biofilm-forming *Staphylococcus aureus* isolate, NaCl, Gram stain kit, H_2O_2, rabbit plasma, glucose, vancomycin, acetic acid, and phosphate buffer saline (PBS).

Isolation of endophytic microorganisms from renggak fruit

Renggak fruit samples were washed with clean water, then surface sterilized with 70% ethanol for 1 minute, 2% sodium hypochlorite (NaOCl) for 1 minute, and rinsed again with 70% ethanol for 30 seconds. The renggak fruit was then dried with sterile filter paper, cut, and spread on Nutrient Agar (NA) media, incubated for 24 hours at 37°C. Colonies that grew around the samples indicated the presence of endophytic microorganisms. Endophytic bacterial colonies grown on NA media were examined macroscopically and microscopically. The endophytic bacterial colonies were then purified into NA media and incubated at 37°C for 24 hours(Astriani & Dwijayanti, 2022).

Preparation of cell-free supernatant of endophytic bacteria

Endophytic bacterial isolate cultures in NA media were taken using a loop and then added to 10 mL of NB media with the aim of producing secondary metabolites from endophytic bacteria. After that, it was incubated for 2-3 days at 37°C under stationary conditions, then homogenized using a vortex. The resulting metabolites were centrifuged at 13,000 rpm for 15 minutes. The supernatant obtained from the centrifugation was then filtered using a 0.22 µm diameter microfilter to separate any remaining bacterial cells in the supernatant (Purwaningsih & Wulandari, 2021).

Preparation of *Staphylococcus aureus* bacterial suspension

A test tube containing 3 mL of Tryptone Soya Broth (TSB) medium was mixed with 60 µL of 5% glucose, followed by a loop of *Staphylococcus aureus* colonies. The mixture was then homogenized and incubated at 37°C for 24 hours. Afterward, another 2 mL of TSB and 40 µL of 1% glucose were added, homogenized again, and incubated at 37°C for 24 hours. The optical density (OD) was measured using a spectrophotometer at a wavelength of 620 nm(Carneiro *et al.,* 2019).

***Staphylococcus aureus* biofilm growth test**

The test was conducted using a microplate by pipetting 200 µL of *Staphylococcus aureus* bacterial suspension into each well for the test group. Meanwhile, the control group was filled with 200 µL of TSB media that had been added with 1% glucose. The microplate was then covered with aluminum foil and incubated at 37°C for 24 hours. After incubation, the microplate was washed with Phosphate Buffer Saline (PBS) solution 3 times, then each well was added with 200 µL of 1% crystal violet solution and incubated at room temperature for 15 minutes. The microplate was washed again with PBS 3 times, after which 200 µL of 96% ethanol solution was added to each well, and incubated again at room temperature for 15 minutes. Next, biofilm growth was read using a microplate reader with a wavelength of 620 nm(Besan *et al.,* 2023).

***Staphylococcus aureus* biofilm inhibition test by endophytic bacterial cell-free supernatant**

This test was conducted in vitro and aseptically, and was performed with 4 repetitions. The treatment groups were divided into positive, negative, media, and test groups. In the test group, wells were filled with 100 µL of *Staphylococcus aureus* bacterial suspension and 200 µL of endophytic bacterial supernatant. The positive control group contained 100 µL of *Staphylococcus aureus* bacterial suspension and 200 µL of 0.1% tetracycline antibiotic. The negative control group contained 200 µL of *Staphylococcus aureus* bacterial suspension, and the media group contained 200 µL of TSB medium supplemented with 1% glucose. The microplates were then covered with aluminum foil and incubated at 37°C for 24 hours. After incubation, the microplate was washed with Phosphate Buffer Saline (PBS) solution three times, then each well was added with 200 µL of 1% crystal violet solution and incubated at room temperature for 15 minutes. The microplate was washed again with PBS three times, after which 200 µL of 96% ethanol solution was added to each well, and incubated again at room temperature for 15 minutes. Next, biofilm growth was read using a microplate reader with a wavelength of 620 nm (Besan *et al.,* 2023).

Staphylococcus aureus **biofilm destruction test by endophytic bacterial cell-free supernatant**

This test was conducted similarly to the biofilm growth inhibition test, except that the endophytic bacterial supernatant was added to the formed biofilm, and was performed with 4 repetitions. Each well was filled with 200 µL of *Staphylococcus aureus* bacterial suspension, then incubated at 37°C for 72 hours to promote biofilm formation. After incubation, the microplate contents were removed, washed three times with PBS, and dried. Next, 200 µL of the endophytic bacterial supernatant was added to each well for the test group. 200 µL of 1% tetracycline antibiotic was added to the positive control well, 200 µL of *Staphylococcus aureus* bacterial suspension was added to the negative control well, and 200 µL of TSB and 1% glucose were added to the media control well. Afterward, the microplate was covered with aluminum foil and incubated again at 37°C for 60 minutes. After incubation, the microplate was washed with PBS and dried at room temperature. Next, the treated biofilm was stained by adding 200 µL of 1% crystal violet solution to each well and incubated at room temperature for 15 minutes. The microplate was washed again with PBS three times, after which 200 µL of 96% ethanol solution was added to each well and incubated again at room temperature for 15 minutes. Next, the biofilm growth was read using a microplate reader with a wavelength of 620 nm (Besan *et al.,* 2023).

Data Analysis

The obtained data were tested using SPSS. Normality was tested using the Shapiro-Wilk test and homogeneity using the Levene test. If the data were normally distributed and homogeneous, a one-way ANOVA test was performed. A significance value <0.05 indicated a significant difference between treatment groups, indicating that the endophytic bacteria of renggak fruit have antibiofilm activity against *Staphylococcus aureus.*

Results and Discussion

Isolation of Endophytic Bacteria from Renggak Fruit

This study yielded bacteria isolated from the endophytes of renggak fruit that could grow and develop well on Nutrient Agar (NA) media. Five bacterial isolates were isolated. Observations were made by observing the macroscopic morphology of endophytic bacterial colonies by observing characteristics such as shape, edge, elevation and color, and microscopic observations with bacterial gram staining, as well as biochemical tests to determine the species of endophytic bacteria. The results of macroscopic and microscopic observations of endophytic bacteria are listed in Tables 1 and 2.

Table 1. Results of Macroscopic Observations of Endophytic Bacteria in Renggak Fruit

Isolate	Shape	Edge	Elevation	Color
BI1	Round	Flat, Smooth	Convex	White
BI2	Round	Irreguler	Convex	White
BI3	Irreguler	Flat, Smooth	Convex	White
BI4	Round	Flat, Smooth	Convex	White
BI5	Round	Flat, Smooth	Convex	White

Table 2. Results of Microscopis Observations of Endophytic Bacteria in Renggak Fruit

Isolate	Shape	Arrangement	Color	Characteristic
BI1	Bacil	Chain	Purple	Gram+
BI2	Bacil	Spread	Purple	Gram+
BI3	Bacil	Spread	Purple	Gram+
BI4	Short bacil	Spread	Red	Gram-
BI5	Cocobasil	Spread	Red	Gram-

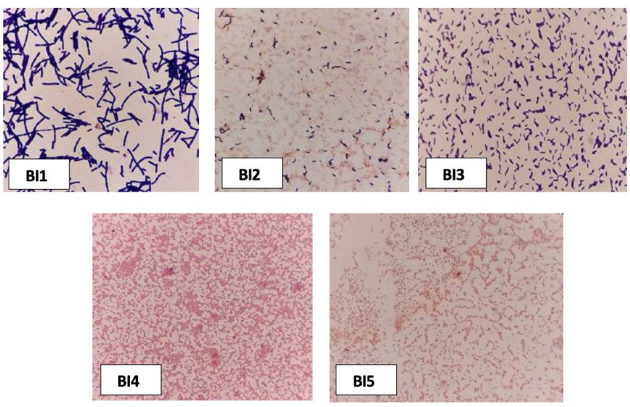

Figure 1. Microscopic examination of endophytic bacteria (Amomum dealbatum) with bacterial gram staining

Five endophytic bacterial isolates were successfully isolated from the renggak fruit: BI1, BI2, BI3, BI4, and BI5. These were then identified based on macroscopic and microscopic, as shown in Tables 1, and 2. Based on observations, the endophytic bacterial isolates included three isolates are gram-positive bacteria, and two isolates are gram-negative bacteria (Figure 1).

Endophytes are naturally part of healthy plants. Endophytes are defined as microbes that live within plant tissue without causing any negative effects. Endophytes generally come from the fungi and bacteria groups. Approximately 300,000 plant species are known to host endophytes (Strobel *et al.,* 2004) in a mutualistic symbiotic relationship (Aly *et al.,* 2011).

Through advances in biotechnology, endophytic microbes are now being utilized as a means of producing antibiotics for medicinal and pharmaceutical purposes, and as a means of transgenic resistance genes. Bioactive compounds from endophytic microbes are used to inhibit the growth of pathogens that are detrimental to human life.

Inhibitory activity of *Staphylococcus aureus* biofilm formation by cell-free supernatant of endophytic bacteria

The biofilm inhibition test aimed to determine the activity of cell-free supernatants from endophytic bacteria from renggak fruit in inhibiting *Staphylococcus aureus* biofilm formation. Biofilm formation in this study was measured quantitatively using the tissue culture plate method (Figure 2).

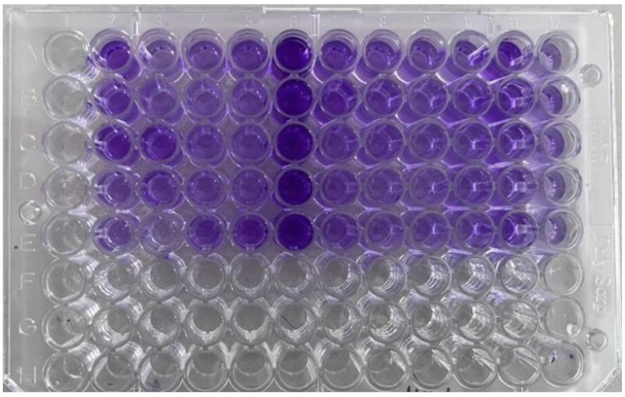

Figure 2. Inhibition test of *Staphylococcus aureus* bacterial biofilm growth by cell-free supernatant of endophytic bacteria of renggak fruit, tissue culture plate method, after staining with crystal violet

The graph shown in Picture 3 represents data generated from calculating the percentage of biofilm inhibition based on optical density (OD) values. The OD value indicates the thickness of the biofilm formed; a higher OD value indicates a thicker biofilm. The average percentage of biofilm inhibition shown in Figure 3 increased with increasing antibiofilm dosage. This contrasts with the OD value, where higher antibiofilm dosages led to a decrease in absorbance.

ANOVA

OD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.004	5	.001	30.825	.000
Within Groups	.000	18	.000		
Total	.005	23			

Table 3. One Way ANOVA statistical analysis of biofilm inhibition

The results showed that the cell-free supernatant of endophytic bacteria from the fruit did not have significant inhibitory activity against *Staphylococcus aureus* bacterial biofilms. One-Way ANOVA statistical analysis showed a significance value of 0.000 ($p<0.05$), as can be seen in Table 3.

This study used cell-free supernatants from endophytic bacteria from renggak fruit. The treatment group with the highest biofilm inhibition rate was BI4 isolate, with an inhibition rate of 42%. The antibiosis mechanism of endophytic bacteria against pathogenic fungi is related to the ability of endophytic bacterial isolates to produce degradative enzymes such as chitinase, protease, and cellulase, as well as other compounds related to the induction of host plant resistance (Hallmann *et al.,* 1997).

Research conducted by (Nufus 2020) stated that secondary metabolites from renggak fruit extracted with ethanol solvent include alkaloids, flavonoids, terpenoids, saponins, and steroids. In crude extracts of renggak fruit peel using ethanol solvent, phytochemical screening results showed the presence of flavonoids and tannins (Cahyani & Hidayati, 2024). The detected flavonoids have various health benefits such as antioxidant, anti-inflammatory, and anticancer properties. The detected tannins also have potential as antioxidants, as well as antibacterial and antiviral activity. Previous research by (Rizky *et al.,* 2023) showed that renggak fruit extract can inhibit the growth of S. aureus bacteria. The biofilm-inhibiting ability of a compound is related to its penetration capacity into the formed biofilm, namely its ability to penetrate the Extracellular Polymeric Substance (EPS) layer, or the slime layer that envelops the bacteria.

The results of calculating the percentage of inhibition of biofilm formation showed that cell-free supernatant from endophytic bacteria from renggak fruit was able to increase the inhibition of biofilm formation. The process of biofilm formation on solid surfaces occurs in two stages. The first stage involves cell growth and the formation of Extracellular Polymeric Substances (EPS), resulting in the accumulation of biofilm cells. The second stage involves detachment or reattachment. Biofilm growth can be prevented by inhibiting or even killing cells, so they don't multiply, and preventing the formation of EPS. When the biofilm development stage is not inhibited, the biofilm that forms increase and forms a three-dimensional structure containing enveloped cells in several groups that are connected to each other (Renner LD, 2011). The positive control used in this study was 1% tetracycline antibiotic. Tetracycline is a class of antibiotics that can inhibit the growth of *Staphylococcus aureus* bacterial biofilms (Dewi *et al.,* 2020). Tetracycline has inhibitory activity against quorum sensing during the biofilm formation process (Deryabin, 2018). The principle of quorum sensing is that when single bacterial cells release autoinducers into the environment, their concentration is too low to be detected because it is diluted in the environment. The autoinducer concentration increases when the bacterial population is large enough and can reach a threshold concentration when bacterial cells can detect it again and subsequently activate the expression of target genes simultaneously.

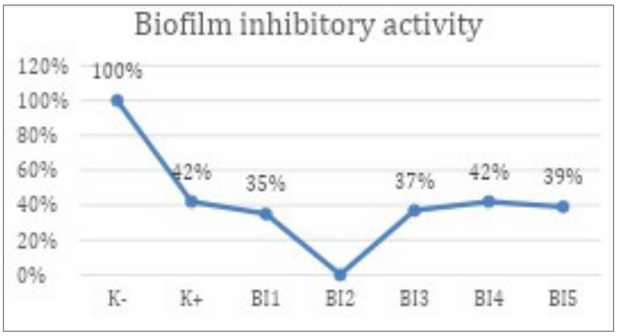

Figure 3. Graph of percentage inhibition of *Staphylococcus aureus* biofilm formation by endophytic bacterial cell-free supernatant

The results of the percentage inhibition of biofilm formation showed that tetracycline had an average inhibition of biofilm formation of 42%, which is close to the average inhibition of biofilm formation in BI4, which was 42%. Based on these percentage results, tetracycline has almost the same effectiveness against the BI4 isolate. The dose of tetracycline used as a positive control was 1%, indicating that tetracycline has the potential to inhibit biofilm formation in *Staphylococcus aureus* bacteria. This finding also aligns with research by (Dewi *et al.,* 2020) which stated that tetracycline can inhibit biofilm growth and has the highest inhibitory effect compared to the antibiotics fosfomycin and oxytetracycline.

The percentage of bacterial biofilm inhibition by BI2 endophytic bacteria was 0%, the cell-free supernatant of BI2 endophytic bacteria was not able to inhibit *Staphylococcus aureus* bacterial biofilm, which can be caused by several factors, including the complex biofilm structure which provides strong physical and biochemical protection, making it difficult for BI2 endophytic bacteria to penetrate and eradicate the cells inside.

Degradation activity of *Staphylococcus aureus* biofilms by cell-free supernatants of endophytic

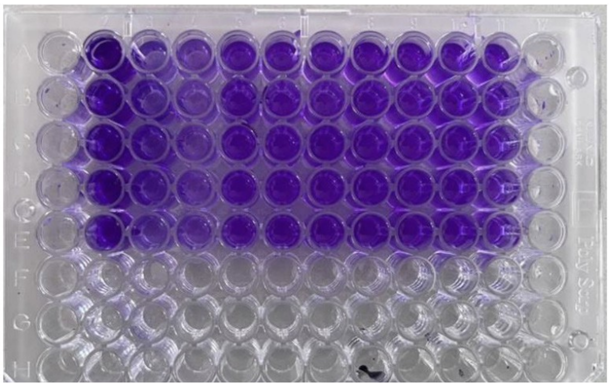

Figure 4. Destruction test of *Staphylococcus aureus* bacterial biofilm by cell-free supernatant of endophytic bacteria of renggak fruit, tissue culture plate method, after staining with crystal violet

Table 4. One-Way ANOVA Statistical Analysis of Biofilm Destruction

ANOVA

OD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	5	.000	4.023	.013
Within Groups	.002	18	.000		
Total	.004	23			

bacteria. The biofilm degradation test was conducted by adding a bacterial suspension and media to a microplate until a biofilm formed (Figure 4).

The solution was then discarded and washed, followed by treatment with cell-free supernatants of endophytic bacteria from renggak fruit. Figure 5 shows that the highest biofilm degradation percentages were found in the BI2, and BI3 isolate treatment groups, at 42% and 41%, respectively. The positive control in this study was 1% tetracycline. The positive control for biofilm degradation showed an average percentage of 56%.

The results showed that the cell-free supernatant of endophytic bacteria from renggak fruit had significant biofilm-destroying activity against *Staphylococcus aureus* bacteria. The One-Way ANOVA statistical analysis showed a significance value of 0.013 ($p<0.05$), as seen in Table 4.

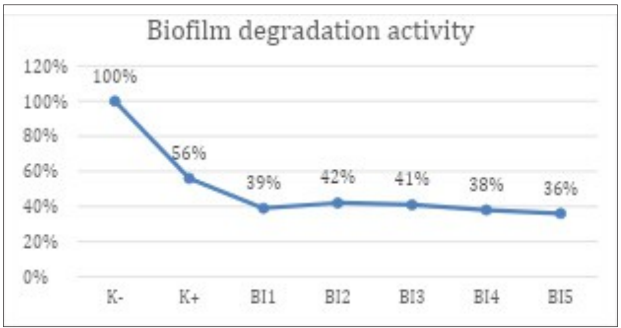

Figure 5. Graph of Percentage degradation of *Staphylococcus aureus* biofilm formation by endophytic bacterial cell-free supernatant

Based on the results of the biofilm degradation test using cell-free supernatants of endophytic bacteria from renggak fruit, it appears to have activity in destroying or degrading *Staphylococcus aureus* biofilms. However, BI2 and BI3 isolates were significantly more effective than BI1, BI4, and BI5.

Several mechanisms for destroying biofilms include biofilm matrix degradation, cell death, and cell leakage. The secondary metabolites of endophytic bacteria in the renggak fruit include saponins, tannins, and flavonoids. These compounds inhibit and destroy biofilms because they have mechanisms that can cause biofilm matrix degradation, cell death, and cell leakage. The biofilm degradation ability of a compound is related to its penetration ability into the formed biofilm, namely its ability to penetrate the Extracellular Polymeric Substance (EPS) layer or the mucus layer that covers the bacteria. In addition, the compound's ability to degrade biofilms is to remove EPS in the formed biofilm (Singh *et al.,* 2025). The mechanism of saponins in destroying biofilms is by affecting the extracellular polymer matrix present in the bacterial biofilm matrix, reducing the polymer substance and altering the integrity of the bacterial cell membrane, causing instability in the bacterial cell wall. Terpenoid compounds in degrading biofilms can reduce the formed biofilm and kill bacteria within the biofilm (Cosmo Andrade *et al.,* 2019). Tannin compounds have the effect of cell death and cell leakage in biofilms, in addition to tannins also have a bactericidal effect. Flavonoid compounds have the effect of inhibiting adhesin molecules that are essential in biofilm formation. Tannins and flavonoids work by binding one of the bacterial adhesin proteins used as bacterial surface receptors, resulting in reduced bacterial adhesion and inhibition of protein synthesis for cell wall formation (Deryabin, 2018; Dewi *et al.,* 2020; Sun *et al.,* 2013).

Conclusion

Five endophytic bacteria were isolated from renggak fruit, namely BI1, BI2, BI3, BI4, and BI5. The cell-free supernatant of endophytic bacteria from *Amomum dealbatum* fruit has activity in inhibiting the formation and degradation of *Staphylococcus aureus* bacterial biofilm with a significance value of 0.000 and 0.013,respectively ($p<0.005$). The cell-free supernatant from endophytic bacteria from *Amomum dealbatum* fruit that had the highest biofilm inhibitory activity was the cell-free supernatant from bacterial isolate BI4, and isolates BI2 and BI3 had the highest percentage of biofilm degradation.

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