# Antibiofilm Potential of Pisang Batu Extract (*Musa balbisiana* Colla) for *Staphylococcus aureus* Bacteria

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**Abstract.** Phytochemical compounds present in Pisang Batu (*Musa balbisiana* Colla) can inhibit the growth of bacteria on the surface of a film. This study aims to determine the effect of the anti-biofilm capacity of Pisang Batu extract ( *Musa balbisiana* Colla) on *Staphylococcus aureus* bacteria and to determine significant differences in the antibiofilm capacity of Pisang Batu extract (*Musa balbisiana* Colla) against *Staphylococcus aureus bacteria*. This study used an experimental method consisting of 6 treatments with 4 replications. The treatment group was a concentration of 25%, 50%, 75%, 100%, positive control (Ciprofloxacin ), and negative control (CMC 1%). Measurement of biofilm inhibition was obtained from the reading of the Optical Density Value on the tool spectrophotometer which then calculated the percentage inhibition biofilm. Data were analyzed using the One Way Anova Test and then Duncan's Post Hoc test was carried out. The results showed that Pisang Batu extract had an effect on the antibiofilm effect of Pisang Batu extract (*Musa balbisiana* Colla) on Staphylococcus aureus bacteria where at a concentration of 100% an inhibition percentage value of 39.8% was obtained, a concentration of 75% obtained an inhibition percentage value of 58.03%, 50% concentration obtained inhibition percentage value of 28.11%, 25% concentration obtained inhibition percentage value of 18.06%, control positive (Ciprofloxacin ) obtained an inhibition percentage value of 95.19% and negative control (CMC 1%) obtained an inhibition percentage value of 0%. Then after further testing, a significant difference was obtained for all treatment groups with the best concentration obtained at a concentration of 75%.

## 1 Introduction

Bacteria is one of the infected microorganisms that are the main causes of illness and death in hospitals and other health care facilities around the world, especially in Indonesia. Infection occurs when pathogenic microorganisms enter the body and cause illness or even death [1]. There are several types of bacteria that cause infection, including *Staphylococcus aureus*. *Staphylococcus aureus* is an opportunistic microorganism that can result in severe infections [2].

Antibiotics are usually given as gifts to treat diseases caused by bacteria. Currently, there is a problem with resistance bacteria to a number of antibiotics that have been widely used for many years. Developmental resistance is caused by various types of mutation genes that bacteria make in order to protect themselves from a molecule that is harmful to them. In addition, pathogens can also develop resistance to antibiotics communally and in biofilms [3].

A biofilm is made up of living organisms attached solidly to the surface of moist and shrouded biotic or abiotic conditions by matrix polysaccharides [3]. Biofilms on bacteria can cause resistance to antibacterial reactivity, as they can exchange genetic material, differ in composition, and a variety of bacteria proteins that can react in a manner different against agents bactericidal [4]. Currently, biofilms are the main mediator of disease infection. The cause of infection is 80% caused by biofilm formation. This because biofilms can increase tolerance to disinfectants and antimicrobials, leading to resistance to disease infection. Giving antibiotics in a general way will only kill planktonic bacteria, while bacteria in biofilms will remain live [5]. Also explained that biofilms can increase resistance 1000 times compared to bacteria in a planktonic form [6].

*Staphylococcus aureus* is the most common organism that forms biofilms. Colonization of these organisms as biofilms allows production of exotoxins. The organisms continue to grow temporarily protected by biofilm structure and defense mechanisms. Besides that, changing phenotype of planktonic bacteria becomes biofilm shape improves production of a toxic substance. Regarding factors of virulence, S. aureus has diverse biological characteristics, including damage to membrane epithelium and deactivation of peptides that inhibit the growth of bacteria [7]. There are already a lot of resistant bacteria to antibiotics so it is necessary to develop invention of antibiotics is needed, especially antibiotics that have antibiofilm properties. Stone banana has its own potency as a plant medicine, but only one is from the section of the hump. Based on research discovery obtained results from screening phytochemicals show that Pisang Batu contain flavonoids, saponins, tannins and quinones, which are

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expected to be an alternative agent possible antibiofilm prevent cell attachment, inhibit growth or degrade biofilms [8]. Pisang Batu plants have an effective inhibitory effect on bacteria *Staphylococcus aureus* [9].

Pisang Batu (*Musa balbisiana* Colla) has characteristic morphology like banana plant on generally, but there is some difference. Generally, Pisang Batu as having sufficient fruit, large size, seed-sized fruits, and a large number of meaty fruits, brown brownish stems and thicker leaves than banana leaves [10]. It has been found in a study that the extract from banana weevils has a very strong antibacterial activity more than other banana plant parts [11]. The sap from Pisang Batu contains many phytochemicals like flavonoids, tannins, and saponins that are antibacterial [12].

## 2 Material and method

Study This was carried out at the laboratory of Microbiology and Biotechnology at the Universitas Negeri Gorontalo. Study This started from the stage of preparation until drafting report results research, that is July 2021 – October 2022.

Tools used in study This is micropipette, oven, beaker, spatula, Erlen Meyer, incubator, blender, filter small, autoclave, hotplate, spectrophotometer, cuvette, laminar air flow, measuring cup, bluetip, 2 ml microtube, rotary evaporator, hotplate, tube reaction, shelf tube reaction and balance analytics. Materials used in study this is Pisang Batu (*Musa balbisiana* Colla), culture pure bacteria *Staphylococcus aureus*, Brain Heart Infusion Broth (BHIB) medium, distilled water, 96% alcohol, 1% CMC, antibiotics Ciprofloxacin, aluminum foil, tissue, label paper, paper filter, and crystal violet.

The test was carried out using microtubes, suspension bacteria, extract from Pisang Batu, and BHIB medium were combined in microtubes. BHIB medium 60 µL, suspension bacteria 70 µL, and extract 70 µL with variation concentrations of 25%, 50%, 75%, and 100% v/v, positive control (Ciprofloxacin) and negative control (CMC 1%). Afterward, suspension was incubated at 37oC for 48 hours. After incubation, the microtube is washed 3 times with running water. A solution of Crystal violet 1% was added to each microtube and incubated for 15 minutes at room temperature. After incubating , the microtube washed with running water 3 times. Afterward, 200 µL of 96% ethanol solution were added to every microtube and incubated for 15 minutes at room temperature. Using a Spectrometer with long 595nm waves, Optical Dencity (OD) can be measured. For antibiofilm, the following formula was used [13]:

$$\%\ PPS = \frac{OD\ kn - OD\ se}{OD\ kn}\ x\ 100\% \quad (1)$$

Besides that, screening phytochemicals is as follows:

### 2.1 Saponin test

10 ml of hot water is added with extract 1 ml then cooled, then shaken for 10 seconds. Will form froth (foam) as high as 1 centimeter and stay for 10 minutes. Then added 2N HCL. When foam disappear after addition of 2N HCL, then tested positive for saponins.

### 2.2 Flavonoid test

As much as 2 ml of extract was heated, then added to ethanol and homogenized. After that, add 0.2 gr of magnesium powder and 3 drops of HCL. The solution will change color to red, orange or yellow to indicate flavonoids.

### 2.3 Tannin test

Extract added to 1 ml of solution Fe (III) Chloride 10%. If the solution changes color, it will show dark blue, blue black, or black greenish, indicating the presence of tannins.

Result data for antibiofilm test Pisang Batu extract (*Musa balbisiana* Colla) against bacteria have been analyzed using ANOVA one way (one way analysis). Moreover, a Post Hoc Duncan study was carried out to see whether a group dominant treatment could be used to extract Pisang Batu.

## 3 Results and discussion

Based on the results obtained, at concentrations 25-100% and positive control, biofilm inhibition took place and outcome statistics showed significant differences. In the results diagram obtained (Fig. 1), it is obvious that when concentrations ranged from 25% to 75%, there was an increase in mark inhibition, but when concentrations reached 100%, there was a decline, leading to a sigmoid pattern (Fig. 1). This is compared backwards with results of research which stated that the higher the concentration, the higher the value of the inhibition so that shows a linear pattern [3, 5, 13-14]. Ciprofloxacin has the ability to slow or inhibit harmful bacteria and annoying biofilm maturation to lower production matrix extracellular polysaccharides (EPS) [15]. Additionally, ciprofloxacin inhibits DNA gyrase activity during growth and production of bacteria [16].

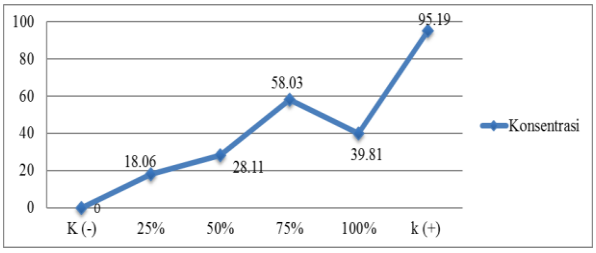


**Fig. 1**. Percentage activity of extract biofilm inhibition Pisang Batu (Musabalbisiana Colla) against Bacteria Staphylococcus aureus

Based on the study obtained that on concentration small, the compound active from the extract is capable to penetrate with Good into the bacteria but the inhibitory power is small whereas for concentration large , the compounds contained in extract have a strong inhibitory power, however no able to penetrate with good into the bacteria , so activity of the most biofilm inhibition good is located on no concentration too small and no too large [17]. Improvement in concentration did not have a meaningful influence on inhibiting biofilm [18]. This is due to the fact that there is possibility of saturation proteins forming a bond with compounds present in the extract. Besides that, when saturation receptor is present, the compound is no longer capable of hindering quorum sensing based on the expression of genes involved in biofilm formation. This means that the improvement in concentration does not have a significant impact on biofilm formation. Further research should be conducted to investigate other potential preventative measures of biofilm formation.

Biofilm formation consists of 3 stages, namely attachment, maturation and release. Stage sticking is influenced by proteins existing adeshi on surface bacteria while the maturation process is influenced by intercellular polysaccharides adhesion (PIA) produced by genes intracellular adhesion (IcaA) which is a component critical to biofilm formation. So for mechanism biofilm inhibition was carried out with a number of ways; preventing bacterial biofilm attachment with prevent the attachment process , inhibit the maturation process, and inhibit stage beginning formation matrix Extracelluler Polymeric Substance (EPS) which is something the resulting layer by bacteria as protector in the process of biofilm formation [19]. Furthermore, the second way is with penetrate into the matrix Extracelluler Polymeric Substance (EPS) and degrade pre-existing EPS formed by bacteria during the phase planktonic. Lastly, Quantum Sensing (QS) is a signal connector used as tool communication by bacteria form colonies and form biofilms [20]. Compounds contained phytochemicals in plants capable prove antibifilm activity with mechanism inhibiting bacterial adhesion on surface solids, capable hinder growth exopolysaccharides, and can bother signal communication (quorum sensing) between bacteria [21]. The study was also supported that revealing compound phytochemicals capable of stimulating biofilm formation [22].

Most Pisan Batu plants contain a compound that is part hump [23]. Based on results of phytochemical testing, extract Pisang Batu consists of a number of compounds that are flavonoids, saponins and tannins. Flavonoids contained in extract Pisang Batu is Wrong One compound class most phenols are present. Flavonoids also include polar compounds that can remain in polar solvents like methanol, ethanol, and butanol, water and others [24]. Compound flavonoids are capable of hindering biofilm formation by bacteria *Staphylococcus aureus* by inhibiting intracellular adhesion (IcaA), which IcaA will mediate the formation of PIA which is a component significant in biofilm formation. When IcaA is obstructed, PIA (intercellular polysaccharide) formation adhesions may also be obstructed, which may cause regulators to impair biofilm formation [25].

The saponins found in extract Pisang Batu are glycoside complexes, which are capable of lower voltage on the water surface, which results in foam if shaken. The decrease in water pressure occurs because there is a destructive soap compound bonding hydrogen to water. As saponins contain glycone, sugar, and aglycone groups, these compounds are called glycosides [26]. Saponins have biofilm inhibition with a mechanism that is with method damage biofilm matrix, and make blemishes on layer lipids so that possible penetration occurs [27].

Furthermore contained compounds in Pisang Batu extract namely tannins, resulting in a change in color (green black) [28]. Change of color occurs due to the presence of a hydroxyl group so that the tannins react with Fe 3+ ions and form a compound complex [29]. Tannins are compounds soluble in polyphenols in water. Tannin compounds will precipitate if reacted with alkaloids and when dissolved in water will form a colloid. Tannins can react with organic solvents like ethanol, methanol, acetone, and others [30]. Tannin compounds can inhibit biofilm formation. These compounds can hinder intercellular expression and adhesion (IcaA) and icaD play a role in biofilm formation [31].

## 4 Conclusion

Based on results study obtained that there is effect of anti-biofilm activity Pisang Batu extract (*Musa balbisiana* Colla) against bacteria *Staphylococcus aureus* with the highest inhibition antibiofilm extract Pisang Batu namely 58.03% obtained at concentration 75%. Furthermore, it is necessary to carry out tests on compound phytochemicals from the extract Pisang Batu (*Musa balbisiana* Colla) which is acting as anti-biofilm agent.

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