

Differences in Minimum Inhibitory Concentrations of Probiotics and Prebiotics on *Staphylococcus aureus*

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Differences in Minimum Inhibitory Concentrations of Probiotics and Prebiotics on *Staphylococcus aureus*

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Abstract

Currently, the use of antibiotics has increased sharply and use in the long term will have a bad impact on health. So the emergence of efforts in the form of making natural antibiotics by utilizing materials that are easily available in the environment. One of them is milk, where milk is a food product produced by farm animals, for example cows. This milk can be used as a probiotic. This probiotic is made by adding a bacterium and the bacterium that is often used is *Lactobacillus casei*. In addition to milk, the material that can be used is telang flower, which is one of the typical plants of Ternate, Maluku with purple, white, pink, and blue petals. Telang flower is often used as a traditional medicine because it has many benefits and content in it. Therefore, in this study Telang flower was used as a prebiotic tested on *Staphylococcus aureus* bacteria. The study was carried out in vitro by the method of laboratory experiments. The design used is Post Test control group only. The test is repeated at each concentration 3 times to obtain accurate results. The inhibition zone is measured using a caliper. The result of the inhibition zone formed from 3 repetitions is taken as the average value. The test results of the minimum inhibitory concentration of probiotics against *Staphylococcus aureus* bacteria showed results if at a concentration of 1.25% can form an inhibitory zone of 9.47 mm. While the minimum inhibitory concentration test on prebiotics against *Staphylococcus aureus* bacteria showed results at a concentration of 2.5% can form an inhibitory zone of 9.02 mm. This suggests that probiotics and prebiotics can be used as antibacterials, since at the lowest concentrations used they can form an inhibitory zone. So the higher the concentration of probiotics and prebiotics used, the inhibition zone formed will be greater.

Keywords: probiotics, prebiotics, *Staphylococcus aureus*

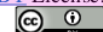
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INTRODUCTION

Bacterial infections are one of the major global health problems, especially in tropical countries. Bacteria such as *Escherichia coli* and *Staphylococcus aureus* often cause infections in the throat area and gastrointestinal tract and can also cause systemic inflammation (Hermawati, 2016). *Staphylococcus aureus* is a bacterium with Gram-positive properties that can live on the surface of a person's body without giving side effects. These bacteria are usually found around the mouth, nose, vital organs, and rectum. However, if the area of the skin there is a wound or puncture, then these bacteria will enter the wound and cause an infection (Misna & Diana, 2016). *Staphylococcus* bacteria can cause a wide variety of suppurative infections of varying severity in soft tissues, bone tissues, endovascular tissues, and respiratory organs (Erikawati et al., 2016). It is believed that infectious diseases rank as the second leading cause

of death worldwide. The increasing threat of drug-resistant microorganisms presents a serious global public health challenge (CDC, 2022).

Staphylococcus aureus, widely acknowledged as a major pathogen in both clinical and community settings, is notoriously difficult to treat with penicillin and other antimicrobial drugs (Aky et al, 2020). The resistance is attributed to the production of β -lactamase enzymes, and the first documented case of a penicillin-resistant strain of *Staphylococcus aureus* was reported in 1945 (Täläpan, 2023).

Currently, reports of *Staphylococcus aureus* resistance to various antibiotics continue to appear. In 2014, a study was conducted on the sensitivity pattern of *Staphylococcus aureus* bacteria in Dr. Soeradi Tirtonegoro Klaten. From this study, 64.8% of isolates were resistant to tetracycline antibiotics, 53.7% were resistant to erythromycin antibiotics, and 40.7% were resistant to cloxacillin (Nuryah et al, 2019). *Staphylococcus aureus* becomes the bacterium that causes nosocomial infections. Infection caused by these bacteria is a serious problem because there is an increase in bacterial resistance to various antibiotics. The World Health Organization (WHO) says that every 2 years + 2 million people develop bacterial infections resistant to 2 or more antibiotics (Ma'ruf et al., 2018).

Probiotics are dietary supplements that contain live microbes. Which can provide benefits for the host who consumes it. This probiotic has great potential as an anti-inflammatory (Aviany & Pujiyanto, 2020). Milk-based probiotics are added with a bacterium. Milk is one of the food ingredients derived from farm animals with high nutritional content, besides the protein and minerals in it are good for body health (Rachman et al., 2018). Probiotic products are one of the results of biotechnology applications with a series of lactic acid bacteria utilization processes (Quigley, 2019). One species of bacteria used in making probiotic products is *Lactobacillus*. The condition that bacteria can be used as probiotic microbes is that they grow well in vitro, have high stability and viability and are safe for humans (Sunaryo et al., 2014). Lactic acid bacteria that produce bacteriocins that are bactericidal in nature have proven to be very useful for developing the safety of fermented food products. To date, only one bacteriocin produced by lactic acid bacteria has been found and has been used in food products, namely nisin. *Lactobacillus acidophilus* can produce bacteriocin, namely Acidophilin A. The antimicrobial effect of *Lactobacillus acidophilus* can be caused by the activity of bacteriocins and organic acids. The antimicrobial effect of *Lactobacillus acidophilus* may be due to the activity of bacteriocins and other organic acids as well as microbial competition with other bacteria. Various activities shown by probiotics include cholesterol lowering, anti-colon cancer, and anti-dermatitis (Hermawati et al., 2016).

Prebiotics are one of the components of functional foods that are being developed at this time. Where this prebiotic has benefits especially in humans because it is able to stimulate the growth and activity of probiotic bacteria (Setiarto et al., 2016). The prebiotic used in this study is Telang flower extract. Telang flowers (*Clitoria ternatea* L) are classified as leguminosae plants that have pharmacological benefits. In Indonesia, this plant is commonly found in Java, Sumatra, Maluku, and Sulawesi (Hawari et al., 2022).

In this study, milk containing *Lactobacillus* is utilized as a probiotic, leveraging the potential of Lactic Acid Bacteria (LAB) strains. These strains show promise due to their capacity to produce bactericidal bioactive peptides known as bacteriocins and enzymes that effectively control both biofilm formation and the proliferation of harmful pathogens. Species within the *Lactobacillus* genus are known to contain bacteriocins; for instance, *L. acidophilus* is capable of producing lactacin B or lactacin F, while *L. casei* B80 generates casein 80. Some specific LAB strains have demonstrated significant effectiveness in combating biofilm-forming *Staphylococcus aureus* (Hermawati et al, 2020). Telang flower is used as a prebiotic because it contains chemical components, such as anthocyanins, flobatanins, saponins, tannins, proteins, carbohydrates, phenols, flavonoids, anthraquinone triterpenoids, volatile oils, steroids, and flavol glycosides. Flavonoids are one of the compounds that have the ability to inhibit the synthesis of nucleic acids in microbial cells, interfere with the activity of cell

membranes and interfere with microbial metabolic processes (Nasir et al., 2022). This study was conducted by utilizing probiotics and prebiotics. Therefore, this study aims to determine the relationship between probiotics and prebiotics. While probiotics and prebiotics used today have been developed to work as antibiotics.

The purpose of the study was to test the minimum inhibitory power of probiotics and prebiotics against *Staphylococcus aureus* bacteria in vitro so that the difference can be known and the method used was laboratory experimental with Post Test-only Control Group Design.

METHOD

This study used laboratory experimental method with Post-Test-Only Control Group design that tested the minimum inhibition zone of probiotics and prebiotics against *Staphylococcus aureus* bacteria. A series of research activities were carried out at the testing Unit of the Faculty of Pharmacy, Airlangga University in August - October 2023.

In this study using bacterial strains *Staphylococcus aureus* ATCC 6538 obtained from pure culture at the Faculty of Pharmacy, University of Airlangga. In addition, the tools and materials used include digital balance sheets, petri dishes, incubators, ovens, autoclaves, vortexes, and micropipettes.

Then the probiotics used in this study are made from fresh cow's milk that has gone through a series of processing. Where fresh cow's milk as much as 100 mL cooked at a temperature of 70°C and allowed to cool. After cooking, *Lactobacillus casei* bacteria are added. The mixture of milk and bacteria is tightly closed and then stored at room temperature for 24 hours. Furthermore, prebiotic production is done by maceration extraction method on telang flowers. Extraction used methanol solvent. Conducted on nutrient Agar media with base layer (30 mL) and seed layer (20 mL) using *Staphylococcus aureus* test bacteria ATCC 6538 made inoculum with 25% transmittance at wavelength 580 nm. A total of 10 inoculums of test bacteria were added to the seed layer in a petri dish, allowed to solidify. Made a hole print, filled with 100 mL probiotic with 100% concentration, probiotic solution with 50% concentration, kanamycin as positive control, sterile distilled water as negative control. Next incubated at a temperature of 32.5°C for 24 hours. The Diameter of the zone formed around the hole is measured using a caliper (mm).

The Data to be collected is the primary data from the results of laboratory experimental research. Primary Data in the form of clear research results or not Nutrient media in order to test the bacteria *Staphylococcus aureus* which received probiotic and prebiotic treatment. The existence of this inhibition is indicated by the diameter of the inhibition zone.

Processing and analysis of data is done using descriptive statistics, which shows the size of the central tendency (mode, median and mean). The researchers measured the inhibition zone formed with 3 repetitions. Repetition is done 3 times with the aim to reduce the potential for data errors that may occur (Retnaningsih et al., 2019).

Calculate mode, median and mean values using concentration 10%, 5%, 2.5%, 1.25%, and 0.65%. The mean value of inhibitory zone diameter between probiotics and prebiotics was compared to see if there was a difference in the minimum inhibitory concentration test using probiotics and prebiotics.

RESULTS AND DISCUSSION

Results

Probiotics used in the study was fresh cow's milk added *Lactobacillus casei* bacteria with a series of processes through which. While the prebiotics used in the study is an extract of telang flowers. These probiotics and prebiotics are made with a wide variety of concentrations.

The method of this study using agar diffusion in the presence of a clear colored inhibition zone formed in the bulkhead pitting area, the agar Nutrient media inoculated with *Staphylococcus aureus* ATCC 6538 with base layer (30 mL) and seed layer (20 mL). The concentrations of probiotics and prebiotics used are 10%, 5%, 2.5%, 1.25%, and 0.65%. The

Diameter of the drag zone formed around the well is measured using a caliper (mm). The following is the diameter of the probiotic inhibition zone formed can be seen in Figure 1.

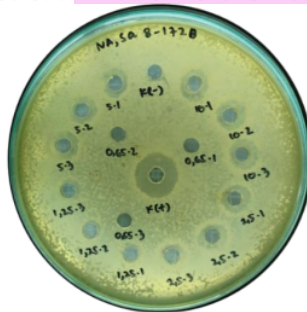


Figure 1. Inhibition of probiotics against *Staphylococcus aureus*, sterile aqueous solution (K-), and kanamycin (K+)

In Figure 1, the diameter of the inhibition zone formed against *Staphylococcus aureus* bacteria is shown on a nutrient medium so that with each pitting a probiotic is added. The minimum inhibition test of probiotics against *Staphylococcus aureus* ATCC 6538 resulted an inhibition zone of 9.47 mm at a concentration of 1.25%. When viewed from Figure 2, the greater the concentration of probiotics used, the greater the diameter of the inhibition zone formed.

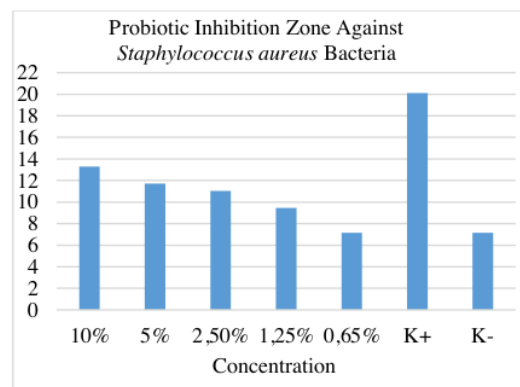


Figure 2. Probiotic inhibition zone, sterile Aquadest solution (K-), and Kanamycin (K+)

While the following is the diameter of the prebiotic inhibition zone formed can be seen in Figure 3.

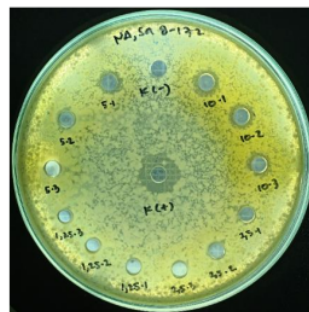


Figure 3. Prebiotic resistance to *Staphylococcus aureus*, sterile Aqua dest solution (K+), and kanamycin (K+)

9 Prebiotic minimal inhibition Test against *Staphylococcus aureus* ATCC 6538 resulted in an inhibition zone of 9.02 mm a concentration of 2.5% which can be seen in Figure 4. In addition, Figure 4 shows that the higher the concentration of prebiotics used, the larger the diameter of the resistance zone formed.

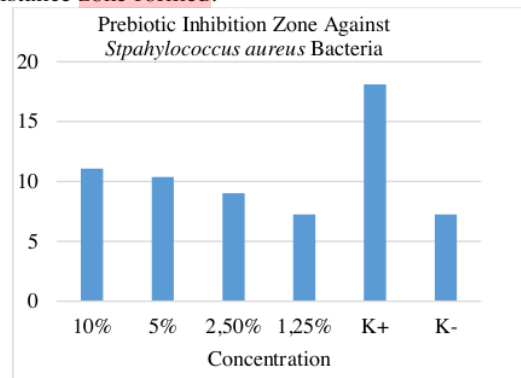


Figure 4. Prebiotic inhibition zone, sterile Aquadest solution (K-), and Kanamicin (K+)

Discussion

Staphylococcus aureus is one of the bacteria that causes food poisoning in humans. The bacterium can produce enterotoxins with its toxic ability to survive high temperatures, although it dies by heating the toxin produced intact (Retnaningsih et al., 2019). According to (Larasati et al., 2020) polysaccharide capsules are one of the components of the dindin of *Staphylococcus aureus* bacteria. Capsule production will further increase the virulence of the bacteria and the capsule is also resistant to antibiotics.

Antibiotics are drugs derived from all or certain parts of microorganisms and are used to treat bacterial infections. These antibiotics are 2 kinds, which have the property of killing bacteria and limiting the growth of bacteria. Irrational use of antibiotics can be a problem, in the form of resistance to antibiotics (Ihsan et al., 2016). Therefore, it is currently necessary to make changes to not always use antibiotics. Because the use of antibiotics for a long time will also have a bad impact on health. So there is a need for alternative materials that can be used to replace factory-made antibiotics. One of them is utilizing natural materials. This natural material can come from plants or materials produced by farm animals.

In this study the materials used are fresh cow's milk and Telang flowers. Fresh cow's milk will be processed into probiotics with the addition of *Lactobacillus casei* bacteria and Telang flower will be processed into prebiotics by making it into Telang flower extract. This study used Nutrient media so that previously the media had been inoculated with *Staphylococcus aureus* ATCC 6538 bacteria and made pitting. Then these wells are filled with various concentrations of probiotics and prebiotics. The concentration used is 10%, 5%, 2,5%, 1,25%, and 0.65%. Telang flower, or butterfly pea (*Clitoria ternatea* L.), is a compound flower that is identical to the purple color of its petals. Besides purple, telang flowers also have distinctive blue, pink, and white colors (Hermawati & Islamy, 2023). The telang flower used in the study came from Sendang District, Tulungagung Regency, East Java.

The positive control used was Kanamicin. Kanamicin is an antibiotic that belongs to the aminoglycoside group by inhibiting the process of protein synthesis of mycoroorganism and belongs to the broad-spectrum antibiotic class, so it can interact with Gram-positive and Gram-negative bacteria (Widyasari et al., 2014). Therefore, in this study, the antibiotic Kanamicin was used. The negative control used in this study was sterile aquadest.

Prebiotics from this Telang flower extract contain flavonoid compounds. Flavonoids in low concentrations are able to damage the cytoplasmic membrane causing damage to metabolites with the effect of inactivating the enzyme system of bacteria, while in high

concentrations are able to damage the cytoplasmic membrane and precipitate cell proteins (Afifi & Erlin, 2017).

Probiotic milk is capable of producing bacteriocins and organic acids that act as antibacterial agents. In fact, it can inhibit the growth of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL) strains (Hermawati, 2016). The diverse compounds present in various probiotic bacteria highlight the bacteriocin production by specific strains like *Lactobacillus acidophilus* (lactacin B and acidocin A) and *Lactobacillus casei* (casein). These bacteriocins contribute to stronger resistance against pathogenic bacteria. Previous studies have shown that probiotic milk containing lactic acid bacteria producing bacteriocins exhibits bactericidal characteristics crucial for food safety in fermentation products. Probiotics have various activities, such as cholesterol-lowering, supported by the Total Plate Count (TPC) data of *L. acidophilus* and *L. casei* probiotic milk. These counts, meeting the required standards for intestinal residence targeting, indicate the minimal probiotic requirement for health effects, such as the production of acids or other compounds like bacteriocins. This correlation between metabolite production, biomass, and the non-growth-associated pattern is significant for creating probiotic milk formulations, particularly those containing *L. acidophilus* and *L. casei*, either as single or multi-strain formulas (Hermawati & Aryati, 2020).

Based on previous research conducted by Rahmiati & Simanjuntak (2019) *Lactobacillus casei* bacteria belong to the group of lactic acid bacteria. Lactic acid bacteria have the ability to produce antibacterial compounds commonly called bacteriocins. This bacteriocin serves to kill and inhibit the growth of other microorganisms. So that the research conducted is in line with previous research.

The results obtained from research that has been done obtained the minimum inhibitory zone on probiotics with a concentration of 1.25% with a diameter of 9.47 mm inhibitory zone and on prebiotics with a concentration of 2.5% with a diameter of 9.02 mm inhibitory zone. This shows that the inhibition zone formed from probiotics is larger than that of prebiotics.

CONCLUSION

In this study probiotics and prebiotics can provide an inhibitory zone. Where probiotics with a concentration of 1.25% form an inhibitory zone of 9.47 mm, while prebiotics with a concentration of 2.5% form an inhibitory zone of 9.02 mm. The inhibitory zone formed from the lowest concentration has a size above the inhibitory zone formed from the negative control. This suggests that the inhibition zone formed from probiotics is greater than that of prebiotics. Therefore, probiotics and prebiotics have the ability and potential if used as antibacterial.

RECOMMENDATION

This research is expected to be further developed using other pathogenic bacteria. With the hope that probiotics and prebiotics still have the ability to inhibit bacteria and probiotics and prebiotics are increasingly symbolized their use.

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