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Test the effectiveness of Breadfruit (*Artocarpus altilis*) leaf extract on the growth of *Staphylococcus aureus*

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Abstracts

Infectious diseases caused by *Staphylococcus aureus* are a health problem in Indonesia. Antibiotics can be used as the treatment, but currently *Staphylococcus aureus* is resistant to antibiotics. Therefore, it is necessary to find alternative medicine. One of the plants that has characteristics to be used as herbal medicine is Breadfruit leaves. The purpose of this study was to determine the effect of concentration on breadfruit leaf juice which can inhibit the growth of *Staphylococcus aureus* bacteria. This type of research is experimental. The study population was pure *Staphylococcus aureus* bacteria on NAS (Nutrient Agar Slant) media. The sample in this study was pure *Staphylococcus aureus* transferred from pure culture media (NAS), while the number of repetitions of the sample was 5 times the treatment, in this study were P1 (100%), P2 (75%), P3 (50%), P4 (25%), P5(0%). Data on the growth of *Staphylococcus aureus* bacteria was obtained by indirect observation, namely through laboratory tests and data collection techniques using the ANOVA test with an error rate (0.05) and followed by the Tukkey HSD test through the SPSS16.0 program. Based on the results of the antibacterial test of breadfruit leaf juice (*Artocarpus altilis*) in ANOVA test, the probability number was 0.000 and there was an effect on the growth of *Staphylococcus aureus* bacteria. Followed by the Tukkey HSD test, it was obtained at a concentration of 25% which is the minimum inhibitory power, the effective concentration to inhibit the growth of *Staphylococcus aureus* at a concentration of 75%. From the data above, it can be concluded that there is an effect of breadfruit leaf juice (*Artocarpus altilis*) on the growth of *Staphylococcus aureus*.

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Keywords: *Artocarpus altilis*, Breadfruit Leaf, *Staphylococcus aureus*

1. Introduction

Infectious diseases are one of the biggest health problems not only in Indonesia, but also throughout the world. In addition to viruses as the cause, bacteria are no less important in causing infectious diseases. Infectious diseases are also one of the main causes of death in the world [1]. The most common bacteria that causes infection is *Staphylococcus aureus*. This bacterium is estimated to kill about 19,000 people per year in all parts of the world. In Indonesia in 2014, the incidence of nosocomial infectious diseases caused by *Staphylococcus aureus* bacteria reached 148,703 cases [2].

Staphylococcus aureus infection can also occur as a result of direct contamination of the wound, characterized by the characteristic form of an abscess. *Staphylococcus aureus* with low invasiveness can cause various skin infections such as acne, pyodema and impetigo. If *Staphylococcus aureus* is widespread and bacteremia occurs, endocarditis, acute hematogenous osteomyelitis, meningitis or pulmonary infection, and toxic shock syndrome may occur [3].

Staphylococcus aureus is a gram-positive bacterium that is spherical in shape, does not have spores, and does not move. These bacteria can be found in the nose, mouth, skin, eyes, and liver. Skin disorders caused by infection with *Staphylococcus aureus* bacteria include impetigo, which is a skin infection that causes the formation of small pus-filled blisters [4].

Treatment of *Staphylococcus aureus* infection can be done by giving antibiotics. Inappropriate use of antibiotics creates a new problem, namely resistance to antibiotics. Research in several countries has shown that *Staphylococcus aureus* is resistant to penicillin and its derivatives such as methicillin. Because *Staphylococcus aureus* produces beta-lactamase enzymes (penicillinase) which can break down the beta-lactam ring of penicillin so that the antimicrobial becomes inactive. Some of the dangers that can be caused by the use of antibiotics include allergies and supra-infection [5].

Therefore, it is necessary to find other alternative treatments to utilize natural ingredients for health, especially medicines derived from plants. The use of herbal medicine has become part of the natural treatment system. Besides being easy to obtain and inexpensive, found in nature, the use of herbal medicines is known to be safe and free of side effects [6]. Herbal medicines that are usually used for the treatment of infections caused by *Staphylococcus aureus* are derived from the iodine plant (*Jatropha multifida* Linn) and bone fractures (*Euphorbia tirucallin* Linn) [7].

One of the plants that have characteristics that can be used as herbal medicine is the breadfruit plant. Breadfruit (*Artocarpus altilis*) is one of the plants that is easily available and empirically has been used in certain communities in Indonesia as a traditional medicine [8]. Based on the observations of community researchers in Wedoro village, Sugihwaras sub-district, Bojonegoro, using breadfruit leaves to be used as a spice wrapper that is sold in shops or stalls. This plant contains chemical compounds that have antimicrobial, anti-inflammatory, anti-cancer, heart-protecting, and hepatoprotective properties [8]. The breadfruit plant has therapeutic benefits in various parts, such as the flowers can cure toothache, the bark is used to dilute the blood for women after giving birth, while the leaves can be used to treat skin, heart, kidney diseases and are used as inflammatory drugs. The ability of breadfruit leaves in treating several diseases is due to the compounds contained in it [9]. Breadfruit leaves contain several nutritious compounds such as tannins, saponins, and flavonoids [10]. Flavonoids function as antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes, denature bacterial cell proteins and damage cell membranes beyond repair. Tannins function to inhibit coagulation enzymes, inhibit bacterial cell wall synthesis and protein synthesis. Saponins interact with the bacterial wall so that the wall will lyse [11].

Based on research conducted by Fiana et al [12], tested the antimicrobial activity of the

ethanolic extract of breadfruit leaves against the bacteria *Escherichia coli*, *Bacillus subtilis*, and the fungus *Candida albicans*, *Micobsporum gypsiu*m. As a result, breadfruit leaf extract was able to inhibit the proliferation of bacteria and fungi. Inhibition of breadfruit leaf extract against *Escherichia coli*, 791 was higher than tetracycline antibiotics. Meanwhile, the inhibitory power of breadfruit leaf extract against *Bacillus subtilis*, 889 was greater than that of tetracycline. And breadfruit leaves were able to inhibit the growth of *Candida albicans* with a minimum inhibitory concentration of 13%.

Based on the observations of researchers, the community in Wedoro village, Sugihwaras sub-district, Bojonegoro usually uses the sap from the breadfruit plant (*Artocarpus altilis*) as a new wound medicine. However, until now there has been no research on the potential of breadfruit (*Artocarpus altilis*) leaves to inhibit *Staphylococcus aureus*. Therefore, this research was carried out with the title "The effect of breadfruit leaf juice (*Artocarpus altilis*) on the growth of *Staphylococcus aureus*."

2. Experiments Procedure

In this study, the research design used was an experimental design, namely by placing the target or object in the 0% group and the treatment group. Data on the growth of *Staphylococcus aureus* bacteria was obtained by indirect observation, namely through laboratory tests. Examination of the number of colonies on *Staphylococcus aureus* bacteria uses the Total Plate Number (ALT) calculation method. The tools used in this research are: Petri dish, Pasteur pipette, Autoclave, Heating Device, Sterile Gauze (filter), Water bath (water bath), Incubator, Test Tube, Measuring cup, Stirrer, Tube rack, Spirit fire, Foot three, Filler, Erlenmeyer, Ose, Measuring pipette, Centrifuge tube.

The materials used in this study were: Breadfruit Leaves, sterile Aquadest, MSA Media, *Staphylococcus aureus* bacteria suspension. And the reagents used in this study were: sterile Pz, H₂SO₄1%, 1% BaCl₂, sterile Aquadest, NaOH, and HCl.

Sample inspection procedure

First day of examination

1. Prepare the tools and materials needed
2. Ignite a sparkling fire with a lighter
3. Label each tube according to the concentration, namely the concentration of 100%, 75%, 50%, 25% and 0% or C (Control).
4. Heating a round loop over a sparkling flame, taking a suspension of *Staphylococcus aureus* on a standard Mc Farland I as much as 1 eye and mixing the suspension into each concentration randomly.
5. Re-cap the tube with a fatty cotton
6. Incubate at 37°C for 24 hours.

The second day

1. Observing each tube, whether there is turbidity or not.
2. Take the smallest concentration that starts to look cloudy, and retest on MSA solid media with the aim of ascertaining whether the germ is *Staphylococcus aureus*.
3. Heating the round loop over the flame of the spiritus, take one eye of the germ that was at the smallest concentration earlier.
4. Embedding on solid media by scratching on the surface of the media.
5. Incubate again at 37°C for 24 hours.

The third day

1. Observing the results on solid media whether colonies were formed which identified the bacteria as *Staphylococcus aureus*.
2. Record the smallest concentration and count the number of colonies
3. Record the observed results as data.

3. Result and Discussion

Research result

Based on the results of research on the effect of breadfruit leaf juice (*Artocarpus altilis*)

1 on the growth of *Staphylococcus aureus* in the Microbiology Laboratory of Health Analyst Study Program, Faculty of Health Sciences, University of Muhammadiyah Surabaya, the average number of colonies from each concentration was different. The results of the study of breadfruit leaf juice (*Artocarpus altilis*) as the inhibition of *Staphylococcus aureus* bacteria obtained the average of each concentration was different. The following is a graph of the average growth of *Staphylococcus aureus* bacteria.

Table 1. Results of the average *Staphylococcus aureus* bacterial colonies from the concentration of Breadfruit (*Artocarpus altilis*) leaf juice on Mannitol Salt Agar media.

No	Code	Results The average number of colonies of <i>Staphylococcus aureus</i> bacteria based on the concentration of breadfruit leaf juice (<i>Artocarpus altilis</i>) in %				
		0	25	50	75	100
1	A1	130	20	8	0	0
2	A2	150	28	10	0	0
3	A3	162	41	15	0	0
4	A4	188	75	40	0	0
5	A5	170	64	35	0	0
	Sum	800	229	108	0	0
	Average	160	46	27	0	0

The average yield of each concentration is different. At a concentration of 100%, 75% obtained an average of 0 colonies. 2 At a concentration of 50%, an average of 27 colonies was obtained, at a concentration of 25%, an average of 46 colonies was obtained, at a concentration of 0%, an average of 160 colonies was obtained.

The results of the data on the growth of *Staphylococcus aureus* colonies on Mannitol Salt Agar (MSA) media were tested for normality of the data to determine the statistical test that would use the one - sample Kolmogorov - Smirnov test. Based on the 4 results of the normality test, it shows that the data is normally distributed. Because > 0.05 . Therefore, 4 determine the effect of breadfruit leaf juice on the growth of *Staphylococcus aureus* using the ANOVA test. The calculated F value is 89,857

with a significant level (ρ) of 0.000 which is smaller than 0.05. So, the hypothesis (H_a) is accepted.

Then the data is continued with the Tukkey HSD test. Based on the results of the Tukkey HSD test, there were differences in the growth of *Staphylococcus aureus* bacteria at concentrations of 100% and 75% with concentrations of 50% and 25%.

Discussion

The results of the study that it was known that the concentration of 100% and 75% breadfruit leaf juice could kill *Staphylococcus aureus* bacteria. Research that shows the killing power of *Staphylococcus aureus* bacteria is due to the concentration of antimicrobial substances contained in breadfruit leaf juice which is very effective in killing bacteria. And the minimum inhibition at a concentration of 25%. At a concentration of 75%, the antibacterial content of breadfruit leaves was effective to inhibit *Staphylococcus aureus*. Economically, 75% concentration is easier to achieve, and does not require a long time in its application.

The ability of breadfruit leaf juice to kill and inhibit *Staphylococcus aureus* bacteria is due to the presence of flavonoids, tannins, and saponins [13]. The content of flavonoids in breadfruit leaves functions as an antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of the bacterial cell membrane. Its mechanism of action is by denaturing bacterial cell proteins and irreparably damaging cell membranes [11].

The content of saponins will interfere with the surface tension of the cell wall, so when the surface tension of the bacterial cell wall is disturbed, antibacterial substances will be able to easily enter the cell and will interfere with metabolism and will cause the bacterial cell to lack nutrients, so that bacterial growth is inhibited or dead. The antibacterial effects of tannins include reactions with cell membranes, inhibition of coagulase enzymes, inactivation of enzymes, and destruction or inactivation of the function of genetic material.

Based on the results of this study, breadfruit leaf juice can be considered as a

natural anti-bacterial to inhibit the growth and kill *Staphylococcus aureus* bacteria.

4. Conclusion

There is an effect of Breadfruit Leaf Extract (*Artocarpus altilis*) on the growth of *Staphylococcus aureus* bacteria. And the concentration of Breadfruit Leaf Juice (*Artocarpus altilis*) which is effective for killing *Staphylococcus aureus* bacteria is a concentration of 75%.

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