

GUAVA (*Psidium guajava* L.) LEAF EXTRACTS CAN AS A GROWTH INHIBITOR *Staphylococcus aureus*

Putu Rusmiany

UNIVERSITAS MAHASARASWATI DENPASAR

Sumantri

UNIVERSITAS MAHASARASWATI DENPASAR

Thomas Jonathan Senduk

UNIVERSITAS MAHASARASWATI DENPASAR

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Abstract: *Staphylococcus aureus* is one of the bacteria found in the oral cavity. These bacteria are beneficial and play a role in normal physiological and defense development in humans, but in certain circumstances these bacteria can turn into pathogens due to predisposing factors, such as poor oral hygiene. One alternative used to maintain oral hygiene is guava leaf extract. Guava contains active compounds in the form of flavonoids, tannins, and alkaloids. Plants containing flavonoids have antibacterial activity, so guava leaves have the potential to inhibit the growth of *Staphylococcus aureus* bacteria in the oral cavity. The purpose of this study was to determine the potential inhibition zone contained in guava leaves against *Staphylococcus aureus* bacteria. This study used 4 concentrations, namely 25%, 50%, 75%, and 100% with each repetition 4 times. Guava leaf extraction was carried out by maceration method using 96% ethanol as solvent. Inhibition zone testing was carried out using the Kirby Baurer method. The zone of inhibition was determined by measuring the clear zone around the disc paper on agar media. The results of statistical tests using the Kurskal-Wallis test showed that there were significant differences at various concentrations on the growth of *Staphylococcus aureus* bacteria. The conclusion is that guava leaves can inhibit the growth of *Staphylococcus aureus* bacteria with an effective concentration of 100%. Inhibition zone testing was carried out using the Kirby Baurer method. The zone of inhibition was determined by measuring the clear zone around the disc paper on agar media. The results of statistical testing using the Kurskal-Wallis test showed that there were significant differences at various concentrations on the growth of *Staphylococcus aureus* bacteria. The conclusion is that guava leaves can inhibit the growth of *Staphylococcus aureus* bacteria with an effective concentration of 100%. Inhibition zone testing was carried out using the Kirby Baurer method. The zone of inhibition was determined by measuring the clear zone around the disc paper on agar media. The results of statistical testing using the Kurskal-Wallis test showed that there were significant differences at various concentrations on the growth of *Staphylococcus aureus* bacteria. The conclusion is that guava leaves can inhibit the growth of *Staphylococcus aureus* bacteria with an effective concentration of 100%.

Keywords : *Staphylococcus aureus*, guava leaf, antibacterial

Introduction

Dental and oral health is an integral part of overall health and life, so it needs to be cultivated throughout the community. Healthy teeth are teeth that are neat, clean, supported by strong, pink gums. Healthy teeth and mouth do not smell bad. Poor oral conditions such as the presence of calculus and stains, dental caries, and toothless conditions can cause problems in life. everyday.1

The oral cavity is the gateway for the entry of various kinds of microorganisms into the body, these microorganisms enter with food or drink. Bacteria commonly found in the mouth include *Streptococcus mutans*, *Streptococcus viridans*, *Staphylococcus aureus* epidermidis, *Staphylococcus pneumonia*, and *Staphylococcus aureus*.1 These microorganisms are beneficial and play a role in normal physiological development and defense in humans. However, not all of these microorganisms are pathogenic, in the oral cavity the microorganisms that enter will be neutralized by antibacterial substances produced by the salivary glands and normal flora bacteria.

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Normal flora is a group of microorganisms that live on the skin and mucous membranes of healthy or sick humans. The presence of normal flora in certain body parts has an important role in the body's defense because it produces a substance that inhibits the growth of other microorganisms. The normal flora in the oral cavity consists of *Streptococcus mutans*/*Streptococcus viridans*, *Staphylococcus aureus*, *Lactobacillus* sp and *Pseudomonas aeruginosa*. Even though they are normal flora, in certain circumstances these bacteria can turn into pathogens due to predisposing factors, such as poor oral hygiene.

Staphylococcus aureus are often found as normal flora bacteria on the skin and mucous membranes in humans, but these bacteria can also cause infections in both humans and animals. *Staphylococcus aureus* is known as a pathogenic microorganism associated with various clinical syndromes. Besides being present in the mouth, *Staphylococcus aureus* can also infect other tissues or organs causing disease with characteristic signs such as necrosis, inflammation, and abscess formation. Therefore, it is necessary to search for natural antibacterial compounds that do not cause negative impacts. against humans, namely by utilizing the active substances that kill bacteria contained in plants

In this day and age, many people are returning to using herbal plants as an alternative treatment. Factors that encourage people to utilize natural medicines include the high price of modern/synthetic medicines and the many side effects that are produced. One of the plants that is widely used and spread in various places is *Psidium guajava* with the regional name guava. *Psidium guajava* or the other what we often call guava is a plant originating from the Central United States, then the spread of this plant extends to Southeast Asia and the territory of Indonesia through Thailand. The guava plant (*Psidium guajava*) is known by the Indonesian people as a herbal medicine that can be used to treat various diseases.

The most commonly used part of the guava plant is the leaves. Traditionally people use guava leaves by boiling them in boiling water, then the results of the boiling are filtered and drunk regularly. Guava leaves (*Psidium guajava* Linn.) contain active ingredients, including tannins which are antibacterial (precipitates protein from bacteria), quercetin, polyphenolics, saponins, alkaloids and flavonoids that inhibit the growth of bacteria, essential oils, malic acid and oxalic acid.

According to Purwiyanto (2006), guava leaf extract (*Psidium guajava* L.) has been shown to be able to inhibit bacterial growth *Escherichia coli* and *Staphylococcus aureus*. Research conducted by Siti (2017) tested the use of guava leaves as antibacterial and fungi, the results of the study showed a concentration of 75% had an inhibitory power of 88 mm against *Candida albicans* and 124 mm against *Staphylococcus aureus*. Research conducted by Ayu (2016) on *Streptococcus mutans* bacteria, at a concentration of 100% the inhibition zone produced was 343.91 mm and at a concentration of 50% the inhibition zone was 243.12 mm.

Based on the description above, researchers are interested in conducting research on the effect of guava leaf extract (*Psidium guajava* L.) on the growth of *Staphylococcus aureus* bacteria.

RESEARCH METHODOLOGY

The research design used was Post test Only Control Group Design. The sample used in this study was *Staphylococcus aureus* bacteria obtained from the stock culture of bacteria stored in UPTD Balai Bali Provincial Health Laboratory. The guava leaves used in this study came from Banyuwangi. Guava Leaf Extract (*Psidium guajava* L.) is a result of the extraction of guava leaves (*Psidium guajava* L.), then the concentration is made 25%, 50%, 75%, and 100%. In this study, the sample was divided into 6 groups, namely group 1 treated with a negative control solution with sterile distilled water, Group 2 was treated with guava leaf extract with a concentration of 25%. Group 3 was treated with 50% guava leaf extract. Group 4 was treated with guava leaf extract with an extract concentration of 75%. Group 5 was treated with 100% guava leaf extract. Group 6 was treated with a positive control solution with *Chlorhexidine*. This study used the Inhibitory Zone test by means of *Staphylococcus aureus* bacterial suspensions that had been adjusted for turbidity levels were cultured into petri dishes containing Mueller Hinton Agar (MHA) using the spreading technique by colonizing the bacterial suspension. Blank disk that has been saturated with various solutions is then affixed to the surface of Mueller Hinton Agar (MHA) which has been suspended by bacteria. The distance between the blank disks with each other is at least 15 mm and the blank disks that have been affixed to the surface of the media so that they cannot be moved or shifted. The media that had been implanted with the discs were incubated at 37°C for 24 hours in an inverted position. Measure the zone of inhibition. The zone of inhibition is the clear area around the paper disc measured using a caliper.

RESEARCH RESULT

The test results were carried out on September 1, 2020. The test results of guava leaf extract were 25%, 50%, 75% and 100% in killing the colonies of *Staphylococcus aureus* bacteria on Mueller Hinton Agar media with four repetitions for each treatment.

Results more can be shown in Table 1 as follows

Table 1. : Bacterial inhibition zone test results

Staphylococcus aureus

Repetition	25%	50%	75%	100%	k+	k-
I	6.1	6.3	6.8	7.1	15.2	0
II	6.3	6.4	6.7	7.1	15.1	0
III	6.3	6.4	6.8	7.2	14.8	0
IV	6.2	6.4	6.9	7.1	14.6	0
Average	6.23	6.38	6.80	7.13	14.93	0

Table 2. Data Normality Test Results

	ro-Wilk df		
	Statistics		Sig.
25%	.863	4	.272
50%	.630	4	.001
75%	.945	4	.683
100%	.630	4	.001
K(+)	.939	4	.650

Information :

Positive Control : Chlorhexidine 0.2%

Negative Control : sterile Aquadest

Test Bacteria : *Staphylococcus aureus* Test Method : Agar Diffusion (*Kirby Baurer*)

Descriptively, the table above shows that the zone of inhibition of growth of *Staphylococcus aureus* bacteria was highest in the positive control group with an average value of 14.93. The 100% guava leaf extract treatment showed an inhibition zone for the growth of *Staphylococcus aureus* bacteria with an average value of 7.13 which was higher than the 25% treatment with an average growth inhibition zone of 6.23 for *Staphylococcus aureus*. Treatment of 50% guava leaf extract with an average growth inhibition zone of *Staphylococcus aureus* was 6.38. The guava leaf extract treatment was 75% with an average growth inhibition zone of 6.80 for *Staphylococcus aureus*, while the negative control showed no growth of *Staphylococcus aureus* bacteria.

The normality test in this study uses the Shapiro-Wilk test to prove that the data used are normally distributed or not. The Shapiro Wilk test was used on a small sample of less than 30 samples. The results of the inhibition zone for the growth of *Staphylococcus aureus* bacteria are shown in Fig.

- a. Lilliefors Significance Correction
- b. K(-) is constant. It has been committed.

Based on the results of the normality test, the data in Table 2. shows that the inhibition zone for the growth of *Staphylococcus aureus* bacteria colonies is data that is not normally distributed because it has a significance value of <0.05, namely in the 50% and 100% guava leaf extract treatment groups, as well as in the control treatment. negative with a constant value which is then carried out homogeneity test using Levene Statistics.

Test the homogeneity of the data using Levene Statistics at the 5% significance level. Data homogeneity test results shown in Table 5.4 as follows.

Table 3. The results of the homogeneity test of the growth inhibition zone colony bacteria *Staphylococcus aureus*

Levene Statistics	df1	df2	Sig.
10.354	5	18	.000

The results in Table 5.4 obtained a significance value of 0.001 which is smaller than 0.05, so it can be explained that the inhibition zone for the growth of *Staphylococcus aureus* bacteria colonies is not homogeneous because the significance value is <0.05.

Testing the data using the Kruskal Wallis test was due to the data being not normally distributed. Kruskal Wallis also called the H test is an alternative procedure of One Way Anova if the data obtained does not meet the 95% significance level or = 0.05. The test results are shown in Table 4. as follows:

Table 4. The results of the Kruskal Wallis test for the inhibition zone for the growth of *Staphylococcus aureus* bacteria colonies

Variable	N	Sig	Mean Difference (P)
25%	5		6.75
50%	5		10.25
75%	5	0.000	14.50
100%	5		18.50
K(+)	5		22.50
K(-)	5		2.50

Test results Table 4 of the data obtained by the Kruskal Wallis test power obtained p value = 0.000 where the value <0.05 indicates that there is a significant difference in the effectiveness of the guava leaf extract concentration of 25%, 50%, 75% and 100% in killing the colony growth of *Staphylococcus aureus* bacteria.

Difference effectiveness guava leaf extract concentration 25%, 50%, 75% and 100% in killing bacterial growth colonies *Staphylococcus aureus*

The results of the test using the Mann Whitney U Test, the difference in the antibacterial inhibition zone of guava leaf extract against the inhibition zone for the growth of *Staphylococcus aureus* bacteria colonies are shown in Table 5 as follows.

Table 5. is the result of the Mann Whitney U Test for antibacterial power.

Group	Z	Sig.
Extract 25% and 50%	-2.124	0.034
Extract 25% and 75%	-2,337	0.019
Extract 25% and 100%	-2.381	0.017
Extract 25% and (K+)	-2.323	0.020
Extract 25% and (K-)	-2.477	0.013
Extract 50% and 75%	-2.381	0.017

Extract 50% and 100%	-2.428	0.015
Extract 50% and (K+)	-2.366	0.018
Extract 50% and (K-)	-3.077	0.002
Extract 75% and 100%	-2.381	0.017
Extract 75% and (K+)	-2.324	0.020
Extract 75% and (K-)	-2.477	0.013
Extract 100% and (K+)	-2.366	0.018
Extract 100% and (K-)	-2,530	0.011
(K+) and (K-)	-2,460	0.014

Based on the results obtained in Table 5.6, it can be explained that the difference between one group and another is by looking at the significance value. Significant group differences obtained sig value <0.05. The results showed that the treatment between groups showed a significant difference. In the guava leaf extract treatment 25% showed a lower inhibition zone with an average value of 6.225, the guava leaf extract treatment 50% showed an inhibition zone with an average value of 6.375, the guava leaf extract treatment 75% showed an inhibition zone with an average value. the average was 6.80, the 100% guava leaf extract treatment showed an inhibition zone with an average value of 7.125. The test results indicate that guava leaf extract is 100% more effective in inhibiting bacterial growth *Staphylococcus aureus*.

DISCUSSION

This study aims to determine the effect of guava leaf extract on the activity of *Staphylococcus aureus* bacteria. Guava leaf extract was obtained using the maceration method, with simple considerations and procedures. The maceration procedure carried out in the manufacture of this extract does not use heating, so that natural ingredients do not decompose.¹³

Based on the research conducted, the results showed that the inhibition zones for the growth of *Staphylococcus aureus* bacterial colonies with concentrations of 25%, 50%, 75% and 100% showed a significant change due to guava leaf extract treatment, this was evidenced by the formation of an inhibition zone or clear zone on the media. so that. This clear zone indicates the inhibition of the growth of microorganisms by antimicrobial agents on the surface of the agar medium. This study is supported by previous research by Ajizah (2004) who found that the number of bacteria indicated by colony growth drew a linear decrease.¹⁴

The increase in the concentration of guava leaf extract was significantly effective in inhibiting bacterial growth colonies which could be seen from the 25% guava leaf extract treatment showed the growth of *Staphylococcus aureus* bacteria with an average value of 6.225 mm lower than the 50% treatment with the average growth of *Staphylococcus aureus*. an average of 6.375 mm. The guava leaf extract treatment was 75% with an average *Staphylococcus aureus* growth of 6.80 mm and the 100% guava leaf extract treatment with an average *Staphylococcus aureus* growth was 7.125 mm.

According to Bell (1984), if the diameter of the inhibition zone formed is greater than or equal to 6 mm, then the extract is categorized as having antibacterial activity and if the diameter of the inhibition zone formed is smaller than 6 mm or is not formed then the extract is categorized as having no antibacterial activity. ... The greater the concentration of guava leaf extract, the greater the active ingredient as an antibacterial.¹⁵ This is in accordance with research conducted by Yani (2020) who found that the average inhibition zones were 7.08 mm, 11.09 mm, 11.34mm, 15.49mm and 17.31mm. The test results showed that there was a significant difference in the activity of guava leaf extract to inhibit bacterial growth *Staphylococcus aureus* in vitro.¹⁶

In this study, the greater the concentration there was an increase in the diameter of the inhibition zone, but the diameter of the inhibition zone was relatively small. There are several factors that affect the diameter of the zone of inhibition of bacterial growth according to Sumarno (2000), namely the turbidity of the bacterial suspension. If the bacterial suspension is less cloudy

the diameter of the inhibition zone will be larger and vice versa if the bacterial suspension is too cloudy, the diameter of the inhibition zone will be smaller.

Incubation temperature can also be a factor that affects the diameter of the zone of inhibition of bacterial growth. To obtain optimal growth, incubation was carried out at 35°C. Temperatures that are less than 35°C can cause the diameter of the inhibition zone to be larger. This can happen to plates that are stacked more than 2 plates during incubation. Incubation at a temperature of more than 35°C, may cause poor diffusion of the extract. In this study, the temperature used during incubation was 37°C.

In addition, the thickness of the agar medium can also be one of the factors that affect the diameter of the bacterial inhibition zone. The thickness of the effective agar is about 4 mm. If it is less than 4 mm the diffusion of the extract will be faster, whereas if it is more than 4 mm the diffusion of the extract will be slower. In this study, measurements were not made on the agar media, so the thickness of the Mueller Hinton Agar (MHA) media used was not known with certainty.

The significant results in this study were due to the increase in the antibacterial activity obtained, based on the increase in concentration so that the content of the active substance as antibacterial also increased. The increase in concentration resulted in the high content of active ingredients that functioned as antibacterials so that they had the ability to inhibit bacterial growth as well. In addition to the concentration factor, other factors from the type of plant used as antibacterial and differences in the structure of the cell wall of a bacterium can also determine the ability to inhibit bacterial growth.

Guava leaves are plants that have antibacterial properties that can inhibit the growth of gram-positive and gram-negative bacteria. Antibacterial content contained in guava leaves are flavonoids, alkaloids, eugenol, tannins and saponins which have an antibacterial effect by damaging the membrane structure.

The content of flavonoids has an anti-bacterial effect through the ability to form complexes with extracellular and soluble proteins with the bacterial cell wall. Flavonoids in guava leaf extract at low concentrations can damage the cytoplasmic membrane causing leakage of important metabolites that inactivate bacterial enzyme systems. While at high concentrations it can damage the cytoplasmic membrane and precipitate cell proteins

Compound *flavonoids* is a polar compound that is easily soluble in polar solvents such as ethanol, methanol, butanol and acetone.²⁰ Flavonoid compounds and their derivatives have two specific physiological functions, namely as chemicals to overcome disease attacks (as antibacterial) and antiviral for plants. Flavonoids function as bacteriostatic and their mechanism of action is to denature bacterial cell proteins. Flavonoids work as inhibitors that will inhibit the replication and transcription of bacterial DNA.²¹ Flavonoid compounds can damage the cytoplasmic membrane which causes leakage of important metabolites and inactivates bacterial enzyme systems. This damage allows nucleotides and amino acids to seep out and prevent the entry of active ingredients into the cell, this situation causes the death of bacteria.

Tannins a substance that is widely distributed in plants and is used as energy in metabolic processes in the form of oxidation. According to Naim (2004), the mechanism of action of tannin as an antibacterial is related to the ability of tannin to inactivate bacterial cell adhesins (molecules attached to host cells) on the cell surface. In cell membrane damage, H⁺ ions from phenolic compounds and their derivatives (flavonoids) will attack the polar group (phosphate group) so that the phospholipid molecule will decompose into glycerol, carboxylic acid and phosphoric acid. This will cause phospholipids to be unable to maintain the shape of the cell membrane, as a result the membrane will leak and bacteria will experience growth inhibition and even bacteria will die, tannin compounds can also denature cell proteins, inactivate adhesins, enzymes, (transport substances from one cell to another) and inhibits nucleic acid synthesis, thereby disrupting the growth of *Staphylococcus aureus* bacteria.

The mechanism of action of alkaloids as antibacterial is by interfering with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not fully formed and causes cell death. They can work alone or together to inhibit bacterial growth

Staphylococcus aureus.²⁵ Another study states that the mechanism of action of alkaloids as antibacterial is that alkaloid compounds react with amino acid compounds that make up bacterial cell walls and bacterial DNA.²⁴ The reaction between amino acid compounds that make up bacterial cell walls and bacterial DNA results in changes in the structure and composition of bacterial amino acids, This is because most of the amino acids have reacted with the basic groups of the alkaloid compounds. Changes in the arrangement of the amino acid chains in DNA will result in changes in the genetic balance of amino acids in DNA, so that bacterial DNA will be damaged. Damage to the DNA of the bacterial cell nucleus will encourage lysis of the bacterial cell nucleus and thus the bacteria will become inactive and lysis.²⁶

Saponins is a group of glycoside compounds that are generally soluble in polar solvents, such as ethanol. Saponins too is compound active, foams when shaken in water.

Saponin compound extraction will give better results as an antibacterial when using 70% ethanol solvent (Kumalasari and Sulistyani, 2011). These results are in accordance with the screening test carried out on guava leaf extract using 96% solvent where the saponin

test carried out did not form a stable foam after shaking it vertically for 10 seconds so that in the saponin test with 96% ethanol solvent no saponin content was formed or results the saponin test with 96% solvent was negative.

The solvent used in this study was 96% ethanol solvent. Ethanol is used as a solvent because it is polar where ethanol is soluble in water and other organic solvents so that it can dissolve components that are easily soluble in water.²⁷ Physical properties of ethanol can dissolve both non and polar because the OH group in ethanol helps dissolve polar molecules and ions. the ion and its alkyl group CH₃CH₂- can bind non-polar materials, so that the ethanol solvent can dissolve bioactive compounds.²⁸ The solvent for 96% ethanol used is 96% ethanol because it easily dissolves active metabolite compounds that have antimicrobial effects such as phenols, tannins, flavonoids. and essential oils which are a class of bioactive compounds that can inhibit microbial growth.

Staphylococcus aureus. Looking at the facts of the research results, namely the decrease in the number of *Staphylococcus aureus* colonies, there is evidence of related research and the Kruskal Wallis and Mann Whitney U Test analysis that guava leaf extract (*Psidium guajava* L.) contains active ingredients that have an antibacterial effect against *Staphylococcus aureus*, it can be concluded that it was concluded that guava leaf extract (*Psidium guajava* L.) was proven to have an antibacterial effect that could inhibit the growth of *Staphylococcus aureus* bacteria, this proves that the hypothesis that has been compiled previously is proven.

Conclusion

Based on the research results of the inhibition zone test of guava leaf extract against *Staphylococcus aureus* bacteria using the maceration method, it can be concluded that guava leaf extract has the potential as an antibacterial in inhibiting the growth of *Staphylococcus aureus* bacteria. The higher the concentration, the greater the inhibition of the growth of *Staphylococcus aureus*.

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