



ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT AND METHANOL EXTRACT OF GUAVA LEAVES (*Psidium guajava* L.) Against *Staphylococcus aureus*

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ABSTRACT

Guava leaves contain secondary metabolites, consisting of tannins, polyphenolates, flavonoids, monoterpenoids, alcolloids, quinones, and saponins. The main component of guava leaves is tannins, the amount of which reaches 9-12%. Tannins are antibacterial by precipitating proteins. The antimicrobial effect of tannins is through reactions with cell membranes, inactivation of the function of genetic material. alkaloids, flavonoids can inhibit the growth of *Staphylococcus aureus*. This research aims to find out the activities antibacterial ethanol and methanol extract of guava leaves against *Staphylococcus aureus*. This study uses the disc diffusion method. Using 5 treatment groups, namely positive control using chloramphenicol antibiotics and for positive control using DMSO. For concentrations of 5%, 10%, 15% use ethanol extract and methanol guava leaves. This study is an experimental study, testing antibacterial activity using *Staphylococcus aureus* bacteria. The data obtained was analyzed using the One Way ANOVA test and continued with the DMRT/Ducan test to see the difference in each treatment. The average results of the diameter of the inhibition zone for ethanol extracts were 10.47 mm (5%), 11.95 mm (10%), 16.94 mm (15%). For methanol extracts it is 9.7 mm (5%), 12.1 mm (10%), 19.7 mm (15%). One Way ANOVA analysis followed by DMRT/Ducan showed that both shallot extracts have antibacterial potential and do not have significant differences. The conclusion of this study is that guava leaf ethanol and methanol extracts with concentrations of 5%, 10% and 15% have potential as antibacterial against *Staphylococcus aureus*.

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Introduction

Indonesia has many types of plants that can be used as a source of natural medicinal ingredients and have been widely used by the community for generations as a medicinal material to overcome health problems, one of which is easily obtained, namely guava, water guava, milk guava, one of the efforts to overcome the negative impact of the use of chemicals and antibiotics is the use of alternative medicinal ingredients that are safer, environmentally friendly, and can be applied and easily decomposed naturally (Hendi 2018). Guava (*Psidium guajava* L.) is one of the medicinal plants that has been utilized in traditional medicine. According to Adyana et al, 2004 guava leaves (*Psidium guajava* L.) are antibiotics and have been used for anti-diarrhea. Several studies that have been conducted prove that guava leaves contain several phytochemical compounds that can be used to prevent and treat a disease, because guava leaves contain many antioxidants, anti-diarrhea and anti-virus, and DHF (*Dengue Hemorrhagic Fever*) (Mittal et al., 2010). Phytochemical screening results of guava leaves are known to contain secondary metabolites consisting of tannins, polyphenolics, flavonoids, monoterpenoids, alkaloids, quinones, and saponins. The main component of guava leaves is tannin which reaches 9-12%. Tannins are antibacterial by precipitating proteins. The antimicrobial effect of tannins through reactions with cell membranes, inactivation of genetic material functions. alkaloids, flavonoids can inhibit the growth of *Staphylococcus aureus* (Rosida, 2012). *Staphylococcus aureus* is a gram-positive pathogenic bacterium that easily grows on most bacteriological media in aerobic or anaerobic conditions. bacteria are found around the human environment. this is due to the ability of bacteria to easily adapt to the environment through resistance to antimicrobials. these bacteria are found in the skin, skin glands, mucous membranes,

wounds and cause laryngitis, skin infections (boils) and central nervous system and lung infections (Jawetz et al., 2014). Antibiotic administration is one of the options in dealing with infectious diseases caused by bacteria. However, the increasing use of antibiotics is starting to cause new problems, especially because most of the antibacterial ingredients used are hazardous chemicals and are not safe for health (Muntasir et al., 2021).

Research that has been done before obtained ethanol extract from guava leaves has antibacterial activity against *Staphylococcus aureus*. The observation results showed that the decrease in the number of colonies along with the increase in the concentration of guava leaves extract at concentrations of 1%, 1.5%, 2%, and 2.5%, the minimum kill level (KBM) of guava leaves extract was 3%. Another study conducted by Hermawan et al (2012), guava leaves extract has antibacterial activity against *Streptococcus mutans* with concentrations of 1.5%, 2%, 2.5%, 3%, based on this study, the minimum inhibitory value (KBM) of guava leaves extract with methanol solvent is 2% and the minimum kill rate (KBM) is 3.5% (Ekoputro, 2011). Based on the description above, the authors are interested in re-examining the antibacterial activity test of ethanol and methanol extracts of guava leaves (*Psidium guajava* L.) against the growth of *Staphylococcus aureus* with different concentrations 5%, 10%, 15%, for the novelty of researchers using two different solvents, so that it can be seen the results of testing extracts from the solvents used, which are more effective in inhibiting the growth of *Staphylococcus aureus*.

Materials and Methods

Material

The materials used in this study were *Staphylococcus aureus* isolates, guava leaves, 70% ethanol, 70% methanol, 10% dimethyl sulfoxide (DMSO), blank disc

paper, chloramphenicol antibiotic disc paper, sterile swab samples, aluminum foil, distilled water, Mc Farland 0.5, Mueller Hinton Agar (MHA), Nutrient Agar (NA) media, physiological NaCl, 1% H₂SO₄, 1% BaCl₂, active compound identification reagents.

Procedures

Sample Collection

Guava leaves (*Psidium guajava* L.) as much as 3 kg, obtained from Mekar Ayu Plantation, Lampahan, Timang Gajah District, Bener Meriah Regency by picking from the top five leaves that are already old.

Plant Determination

Plant determination aims to verify the accuracy of the identity of plants that will be used in research, so as to prevent errors in the collection of materials.

Preparation of Simplisia

3 Kg of guava leaves, washed and drained, then dried in the sun for 3 days, then mashed into powder using a leaf grinder (motor grinder). After obtaining guava leaf powder, it is used for two extraction methods with two ethanol and methanol solvents by maceration (Pandey et al., 2011).

Extraction Process

1. Maceration method using ethanol solvent, 500 g guava leaves were washed, dried in the sun until dry, and pulverized into powder. The powder was soaked with 5 liters of ethanol solvent for 3x24 hours and the filtrate was taken for filtration. Maceration with stirring 1 time every 2 hours and 5 minutes of mixing is filtered using a funnel and filter paper to separate the filtrate from the pulp (Pandey et al., 2011). The filter results were evaporated solvent using a rotary evaporator, obtained 121.9 g of thick extract free of solvents.

2. Maceration using 70% methanol solvent, 500 g guava leaves were washed, dried in the sun until dry, and mashed into powder. The powder was soaked with 5 liters of 70% methanol solvent for 3x24 hours and the filtrate was taken for filtration. Maceration with stirring 1 time every 2 hours and 5 minutes of mixing is done on the pulp (Bawondes et al., 2012). The filter results were evaporated solvent using rotary evaporator, obtained 119.7 g of thick extract free of solvents.

Phytochemical Screening

The ethanol and methanol extracts of guava leaves were then subjected to phytochemical screening to determine the content of secondary metabolites contained in guava leaves. This screening is done qualitatively by looking at the color testing reaction using reagents. Secondary metabolite compounds to be tested are alkaloids, saponins, tannins, flavonoids, quinones, polyphenols, and steroids/triterpenoids (Pangemanan et al., 2020).

Antibacterial Activity Test

a) Sterilization

Glassware and media were sterilized using an autoclave at a temperature of 121°C for 15 minutes (Novel et al., 2010).

b) Preparation of 0.5 McFarland Standard Solution

A mixture of 9.95 mL of 1% H₂SO₄ with 0.05 mL of 1% BaCl₂ was vortexed until it formed a turbid solution (Dalynn Biological, 2022).

c) Culture Revival

One loop of *Staphylococcus aureus* and *Escherichia coli* cultures was inoculated into slanted NA media and then incubated at 37°C for 24 hours (Smith & Aferd, 2022).

d) Preparation of Test Bacterial Suspension

A loop of revived bacteria was transferred into a test tube containing 10 mL of sterile 0.9% NaCl solution. It was homogenized using a vortex and compared with the 0.5 McFarland standard solution. The 0.5 McFarland standard is equivalent to a cell suspension with a concentration of 1.5×10^8 CFU/mL (Kherid et al., 2020).

e) Preparation of Guava Leaves Concentrations

Ethanol and methanol extracts of guava leaves made in 3 concentrations in 5%, 10% and 15% by adding DMSO into thick guava leaves extract with a certain amount at each concentration.

d) Antibacterial Activity Test

The antibacterial activity was tested using the agar diffusion method (disc diffusion

Kirby and Bauer) (Smith & Alferd, 2022; Koeth & Linda, 2022). A sterile cotton swab was used to spread the bacterial suspension, equivalent in turbidity to 0.5 McFarland, evenly over the surface of the MHA medium. Paper discs containing extracts of ethanol and methanol extracts of guava leaves with concentrations of 5%, 10%, and 15%, negative control (DMSO), positive control (chloramphenicol) per disk, each placed on the agar surface. Then incubated at 37°C for 24 hours and the diameters of the inhibition zones—clear zones formed around the discs—were measured using calipers.

Results and Discussion

This study begins with the determination of plants in the Biosystematics Laboratory of the Biology Department of the Faculty of Mathematics and Natural Sciences, Syiah Kuala University to ensure that the plants used are true guava plant species (*Psidium guajava* L.), with number 143/UN11.1.8.4/TA.00.03/2024, the determination results show that the plants used in this study are true of *Psidium guajava* L. species (Rasnovi, 2024).

The processing of guava leaves includes washing, drying, and pulverizing. Washing aims to remove dirt that is attached, then the drying process is carried out to reduce the water content and prevent the growth of mold in guava leaves, so that it can be stored for a long time and the chemical composition in guava leaves does not change. Drying is done to reduce water content and stop the enzymatic process that occurs in the sample, besides that the sample will be more durable and make it easier for the solvent to attract active compound components in the sample during the extraction process. Furthermore,

the sample pulverization process aims to increase the surface area of the sample so that the contact between the solvent and the sample in the extraction process is greater. According to Anwar et al. (2017), the greater the contact between the sample and the solvent, the greater the possibility of damaged cell walls, thus facilitating the withdrawal of active compounds in guava leaves by the solvent.

Phytochemical Screening

Identification of active compounds contained in guava leaves (*Psidium guajava* L.) is done by phytochemical screening. This qualitative test is used as an initial guess to identify the group of compounds contained in nutmeg seeds that give activity as antibacterial. The active compound groups tested were alkaloids, saponins, tannins, flavonoids, quionones, polyphenols, steroids, triterpenoids. Based on the results of phytochemical tests, ethanol and methanol extracts of guava leaves are positive for alkaloids, saponins, quinones, polyphenols, steroids and triterpenoids (Table 1).

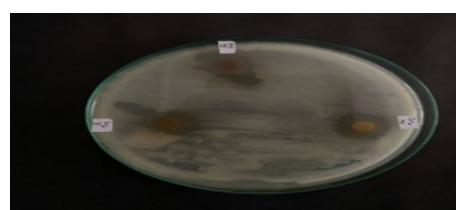
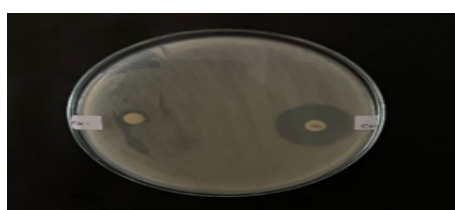
Table 1. Phytochemical Screening Results of Ethanol and Methanol Extracts of Guava Leaves

Jenis Metabolit Sekunder	Pereaksi	Hasil	Keterangan
Alkaloid	Dragendorff	+	Formation of an orange-brown precipitate
	Mayer	+	Formation of yellowish-white precipitate
	Wagner	+	Formation of brown precipitate
Saponin	Air	+	Foam formation
Tanin	FeCl ₃ 5%	-	No formation of a cloudy white solution
Flavonoid	Serbuk Mg	-	No formation of purple solution
Kuion	NaOH 1 N	+	Formation of yellow color
Polifenol	FeCl ₃ 5%	+	Formation of blue solution
Steroid	Liberman	+	Formation of green color
	Burchard		
Triterpenoid	Liberman	-	No formation of red color
	Burchard		

Antibacterial Activity Test

The antibacterial activity test of ethanol and methanol extracts of guava leaves against *Staphylococcus aureus* was carried out by Kirby and Bauer disc diffusion method. The principle of disc diffusion is to use discs as a carrier medium for test extracts with various concentrations. The test extract in the disk will diffuse into the agar media that has been inoculated with bacteria and will form an inhibition zone after incubation.

The results obtained showed that the treatment of 3 variations with concentrations of 5%, 10%, and 15%, negative control (DMSO) and positive control (chloramphenicol) had a significant effect on the inhibition zone formed, so it was necessary to continue with the Duncan Multiple Range Test (DMRT) further test. The results of measuring the diameter of the inhibition zone of ethanol and methanol extracts of guava leaves against *Staphylococcus aureus*.



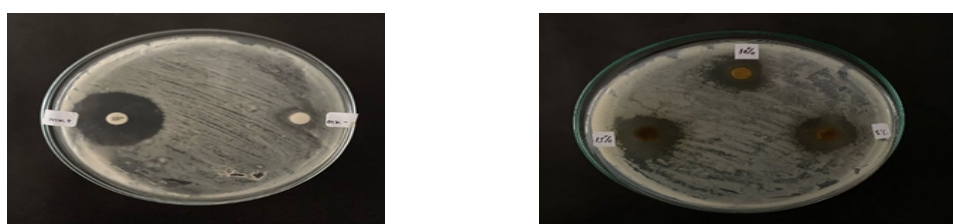
(a) negative and positive controls (b) bacterial inhibition of ethanol extract

Figure 1. Antibacterial activity of ethanol extract of guava leaf on MHA media after incubated for 24 hours at 37°C against *Staphylococcus aureus*.

Table 2. Results of Inhibition Zone Activity Test against *Staphylococcus aureus*

Treatment	Inhibition Zone Diameter (mm)
Negative control (DMSO 10%)	0,00 ± 0,00 ^a
Guava leaf ethanol extract 5%	10,47 ± 455,74 ^b
Guava leaf ethanol extract 10%	11,95 ± 1.940,08 ^b
Guava leaf ethanol extract 15%	16,94 ± 4.519,44 ^b
Positive control (chloramphenicol)	20,06 ± .811,01 ^c

Note : Numbers followed by the same letter are not significantly different, while those followed by different letters indicate significant differences according to DMRT $\alpha=0.05$; SD (N=3)



(a) negative and positive controls (b) bacterial inhibition of methanol extract

Figure 2. Antibacterial activity of ethanol extract of guava leaf on MHA media after incubated for 24 hours at 37°C against *Staphylococcus aureus*.

Table 3. Results of Inhibition Zone Activity Test against *Staphylococcus aureus*

Treatment	Inhibition Zone Diameter (mm)
Negative control (DMSO 10%)	0,00 ± 0,00 ^a
Guava leaf methanol extract 5%	9,7 ± 2,954,66 ^b
Guava leaf methanol extract 10%	12,1 ± 2,018,94 ^b
Guava leaf methanol extract 15%	13,2 ± 1,661,21 ^b
Positive control (chloramphenicol)	19,7 ± 3.798,82 ^c

Note : Numbers followed by the same letter are not significantly different, while those followed by different letters indicate significant differences according to DMRT $\alpha=0.05$; SD (N=3)

Tables 2 and 3, show that the treatment of methanol and ethanol extracts of guava leaves 5%, 10%, 15% has a significant effect on the inhibition zone formed, this can be seen from the sig value <0.05 , namely 0.00. The higher the concentration of ethanol and methanol extracts of guava leaves given, the greater the diameter of the inhibition zone formed on *Staphylococcus aureus*. The concentration of an antibacterial test material can affect the inhibition zone formed. The difference in the inhibition zone at each concentration is due to differences in the active substances contained at that concentration (Harvey et al., 2014). This is also in accordance with the statement of Madigan et al. (2021), that the concentration of antibacterial compounds is one of the factors that affect the efficiency and effectiveness of these antibacterials. Looking at the variation in the diameter of the inhibition zone formed,

according to Indryani et al. (2020) that if the diameter of the inhibition zone is 20 mm or more, the inhibitory activity is categorized as very strong, 11-20 mm is categorized as strong, 6-10 mm is categorized as moderate, and 5 mm or less is categorized as weak.

The results of measuring the diameter of the inhibition zone of guava leaves ethanol extract in inhibiting *Staphylococcus aureus*, showed that the highest inhibition zone was at a concentration of 15% of 16.94 mm and at the smallest concentration of 5% 10.47 mm, then at a concentration of 10% 11.95 mm. Based on the DMRT test on the treatment of ethanol extract of jambu biji leaves with a concentration of 10% is not significantly different from the concentration of 15%, but both concentrations are significantly different from the concentration of 5% and have a significant difference with the positive control treatment (chloramphenicol). The

results of measuring the diameter of the inhibition zone of guava leaf methanol extract in inhibiting *Staphylococcus aureus*, showed that the highest inhibition zone was at a concentration of 15% of 13.2 mm and at the smallest concentration of 5% 9.7 mm, then at a concentration of 10% 12.1 mm. Based on the DMRT (Duncan's Multiple Range Test) test on guava leaves methanol extract with concentrations of 5%, 10%, and 15% not significantly different but significantly different from the positive control (chloramphenicol) on the formation of inhibition zone diameter.

The formation of an inhibition zone around the disc paper is due to the activity of the chemical compounds contained in guava leaves, namely flavonoids, tannins and saponins that can inhibit *Staphylococcus aureus*. The content of chemical compounds has a different mechanism of action in inhibiting bacteria, such as tannins, which damage the bacterial cell membrane, astringent compounds from tannins can induce the formation of complex compound bonds to enzymes or micro-substrates (Rosidah et al., 2014).

Chloramphenicol antibiotics work by inhibiting protein synthesis in bacterial cells. Chloramphenicol will bind reversibly to the 50 S ribosomal unit, thus preventing the bond between amino acids and ribosomes. This antibiotic binds specifically to the acceptor (the initial binding site of the amino acyl T-RNA) or to the peptidyl moiety which is the critical binding site for peptide chain elongation (Zhang et al., 2022). In the diameter of the negative control inhibition zone (DMSO) 10%, neither the ethanol nor methanol extract treatment was formed, where it was seen that ethanol and methanol extracts could not inhibit the growth of *Staphylococcus aureus*, where the resulting inhibition zone was 00.00 mm. This shows that the 10% DMSO solution has no effect on bacteria. In addition, DMSO 10% is also used as a negative control to ensure that antimicrobial activity only comes from

ethanol and methanol extracts of guava leaves. The use of DMSO 10% as a solvent is because it is an aprotic solvent that can dissolve various kinds of polar and non-polar molecules that are difficult to dissolve (Galvao et al., 2014). In addition, DMSO 10% has no effect on the growth of both gram-positive and gram-negative bacteria (Bora et al., 2013).

Research conducted by Singh et al, (2016) ethanol and methanol extracts of guava leaves (*Psidium guajava* L.) have antibacterial activity against *Bacillus cereus* with the same extract concentration of 30% obtained an inhibition zone of 12.06 mm for ethanol extracts and 13.2 mm for methanol extracts, while for this study ethanol and methanol extracts from guava leaves (*Psidium guajava* L.) in inhibiting *Staphylococcus aureus* with 3 variations of concentrations, 5%, 10% and 15%, the largest inhibition zone was obtained at a concentration of 15%, ethanol extracts of 16.94 mm and methanol extracts of 15%. In inhibiting *Staphylococcus aureus* bacteria with 3 concentration variations 5%, 10% and 15%, the largest inhibition zone was obtained at a concentration of 15%, namely ethanol extract of 16.94 mm and methanol extract of 13.2 mm with a strong inhibition category.

Conclusions

Based on the results of the study it can be concluded that, ethanol extract of guava leaves (*Psidium guajava* L.) with 3 concentration variations 5%, 10%, 15%, against the growth of *Staphylococcus aureus* with a strong inhibition category. The ethanol extract of guava leaves (*Psidium guajava* L.) is able to form a larger inhibition zone 10.47 mm, 11.95 mm and 16.94 mm on *Staphylococcus aureus*, compared to the diameter of the inhibition in methanol extract, which is 9.7 mm, 12.1 mm and 13.2 mm.

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