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Antibacterial Activity Test Of Extract, N-Hexane Fraction, Ethyl Acetate, And Water Of Tangkalak Guava Leaves (Bellucia Pentamera Naudin) Against Staphylococcus Epidermidis ATCC 12228 Bacteria

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Abstract

Skin infections by Staphylococcus epidermidis ATCC 12228 are increasing due to antibiotic resistance, while guava leaves (Bellucia pentamera Naudin) have antibacterial potential based on traditional use and phytochemical content. To evaluate the antibacterial activity of ethanol extract and n-hexane, ethyl acetate, and water fractions of guava leaves against S. epidermidis ATCC 12228. In vitro experimental with disc diffusion, liquid dilution (MIC), and solid dilution (MBC) methods. McFarland 0.5 bacterial suspension (1.5x10^8 CFU/mL); 500 g of leaf simplicia powder from Galing Village, Sambas. Analytical balance, rotary evaporator, SPSS 27 (Shapiro-Wilk, Levene, Kruskal-Wallis, Tukey post-hoc). Ethanol extract inhibited 3.43-6.86 mm (weak-moderate); ethyl acetate fraction 3.67-5.42 mm (most active); MIC 20%; positive for alkaloids, flavonoids, tannins, saponins. Ethanol extract and ethyl acetate fraction have the potential as alternative antibacterial candidates for nosocomial infections.

Keywords: Antibacterial Activity, Bellucia Pentamera, Ethyl Acetate Fraction, Minimum Inhibitory Concentration, Staphylococcus Epidermidis.

INTRODUCTION

Skin infections remain a public health problem in developing countries like Indonesia, where data shows that approximately 26% of dermatology patients suffer from infectious skin diseases, with a high prevalence in late adolescence. The skin, as a protective barrier, plays a crucial role in regulating body temperature and preventing pathogen invasion, but damage to this barrier often triggers opportunistic infections. Traditional medicine remains prevalent among Indonesians to address these complaints, reflecting a reliance on readily accessible local natural resources.

*Staphylococcus epidermidis*ATCC 12228 is an opportunistic gram-positive bacterium that frequently causes nosocomial infections, particularly in immunocompromised patients, those using catheters, or the elderly. This bacterium produces*glycocalyx*which facilitates biofilm formation on foreign bodies, thereby increasing resistance to phagocytosis and conventional antibiotics. The prevalence of methicillin resistance in hospital isolates reaches 70-90%, exacerbating the challenges of treating medical device-associated infections.

Despite medical advances, bacterial skin infections in Indonesia continue to increase, influenced by urbanization and tropical environmental factors that favor the proliferation of pathogens such as S.*epidermidis*. Widespread antibiotic resistance in these bacteria demands exploration of therapeutic alternatives, as the biofilm mechanism reduces the effectiveness of conventional drugs such as ciprofloxacin.[Pertiwi*et al*., 2022] Local plant extract fractions have not been explored in depth against this specific strain, leaving a gap in the development of new antibacterial agents.[Wibawa*et al*., 2025]

Tangkalak guava leaves (*Bellucia pentamera*Naudin) has been traditionally used by the Meranjat ethnic group in South Sumatra to restore stamina and treat minor infections, supported by its vitamin C content which supports immunity. [Haryono*et al*., 2019] Previous research confirmed the presence of bioactive compounds such as alkaloids, flavonoids, terpenoids, tannins, and saponins in this plant, which have the potential to interfere with bacterial cell wall synthesis. [Nasra*et al*., 2023][Agusanty*et al*., 2023] However, the antibacterial activity of the leaves against S.*epidermidis*ATCC 12228 has not been tested, specifically on the n-hexane, ethyl acetate, and water fractions.[Agusanty*et al*., 2025]

The absence of data on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract and its fractions against this bacteria hampers the validation of its potential as an alternative drug candidate. [Syahmani*et al*[., 2022] Previous research has focused on other pathogens such as E. coli or S. aureus, leaving a gap for specific nosocomial strains. This urgently needs to be studied to support the development of locally based phytopharmaceuticals.

This study aims to evaluate the antibacterial activity of ethanol extracts and fractions of n-hexane, ethyl acetate, and water of guava leaves against S. epidermidis ATCC 12228, including the determination of MIC and MBC using disc diffusion and dilution methods. The urgency of the study lies in the urgent need for new antibacterial agents amid the global resistance crisis, where endemic plants such as B. pentamera can be a source of cheap and sustainable active compounds for rural communities in Indonesia. The novelty lies in the first test against this specific ATCC 12228 strain, complementing previous phytochemical data without overlapping with studies on other pathogens. [Nasra*et al*., 2023][Agusanty*et al*., 2023]

RESEARCH METHODS

This study used an experimental quantitative approach to test the causal relationship between the concentration of ethanol extract and fraction of guava leaves (Bellucia pentamera Naudin) as independent variables on the diameter of the inhibition zone and the MIC/MBC value in Staphylococcus epidermidis ATCC 12228 bacteria as dependent variables. [Sugiyono, 2021] The type of research is an in vitro laboratory with a post-test only control group design, involving three replications per concentration (10%, 20%, 30%) to ensure the reliability of the results. [Sudaryono, 2021] Antibacterial testing methods include disc diffusion for initial screening, liquid dilution for MIC, and solid dilution for MBC, according to pharmacopoeial standards that guarantee the accuracy of measuring the activity of bioactive compounds.

Data Analysis Instruments and Techniques

The main instruments include analytical balance, rotary evaporator, laminar air flow, incubator, autoclave, moisture balance, blender, 40 mesh sieve, microscope, and NA/NB media; materials include fresh B. pentamera leaves (9,300 g wet), 96% ethanol, n-hexane, ethyl acetate, water, DMSO, ciprofloxacin (positive control), and S. epidermidis ATCC 12228 culture. Data analysis techniques used SPSS version 27 for the Shapiro-Wilk normality test and Levene's homogeneity; if $p > 0.05$, continued with One-Way ANOVA and Post-Hoc Tukey, or Kruskal-Wallis and pairwise if not normal. [Emzir, 2022] This approach allows the detection of significant differences between fractions with a 95% confidence level, consistent with the quantitative positivism paradigm. [Creswell & Creswell, 2023]

Population and Sample

The study population was a colony of S. epidermidis ATCC 12228 bacteria from the Microbiology Laboratory, with samples in the form of a McFarland 0.5 standard suspension (1.5 × 10^8 CFU/mL) inoculated on NA/NB media. Extract and fraction samples were obtained from 500 g of B. pentamera leaf simplicia powder (yield 24.6%), fractionated into n-hexane, ethyl acetate, and water. [Sugiyono, 2021] Purposive sampling technique was applied to select fresh old leaves from Galing Village, Sambas, West Kalimantan, ensuring representativeness and minimal genetic variation. [Sudaryono, 2021]

Research Procedures

The procedure begins with plant determination at Tanjung Pura University, followed by preparation of the simplicia: wet sorting, washing, chopping, sun drying (6-10 days), dry sorting, blender grinding, 40 mesh sieve. Extraction of 96% ethanol maceration (1:10, 5 days), evaporation (50°C), multistage fractionation (n-hexane, ethyl acetate, water); standardization of simplicia/extract (organoleptic, water content <10%, drying loss <10%); phytochemical screening; sterilization of equipment (oven 170°C/2 hours, autoclave 121°C/15 psi); bacterial rejuvenation, McFarland suspension, Gram staining;

diffusion test (disc soaked for 30 minutes, incubation at 37°C/24 hours), liquid/solid dilution for MIC/MBC. [Emzir, 2022] All steps were carried out in triplicate in LAF to avoid contamination, followed by statistical analysis. [Creswell & Creswell, 2023]

RESULTS AND DISCUSSION

Plant Determination

This plant determination aims to determine the truth and suitability of the identity of a guava leaf sample (Bellucia pentamera Naudin) that will be used in the research.Plant determination in this study was carried out inHead of the Tanjung Pura University Laboratory, Pontianak. Showed that the results of this determination were that the plant used as a research sample was indeed a guava leaf plant (Bellucia pentamera Naudin).

Preparation of Simple Drugs

In the manufacture of simplicia of guava leaves (Bellucia Pentamera Naudin) taken from Galing District, Sambas Regency, West Kalimantan. The wet weight was obtained 9,300g after drying, the dry weight was obtained as much as 1,230g. The percentage of dry weight to wet weight was 13.22%. After going through the drying process, the guava leaves were then ground into powder, obtaining a powder weighing 1,010g with a yield percentage of 82.11%. The guava leaves were ground by blending and sieved with a 40 mesh sieve to reduce the size of the powder particles. The yield percentage can be seen in table 1.

Table 1. Percentage of Tangkalak Guava Leaf Soaking

information	Initial weight	Final Boobt	Percentage%
Material retrieval	9,300g		
Drying	9,300g	1,230g	13.22%
pollination	1,230g	1,010g	82.11%

Standardization of Simple Drugs

Organoleptic Test of Simple Drugs

One of the parameters of the standardization of simple drugs is organoleptic. The results of the organoleptic test of guava leaves (Bellucia Pentamera Naudin) can be seen in Table 2.

Table 2. Organoleptic Test Results of Tangkalak Guava Leaves

Parameter	Results
Smell	Tangkalak Guava Leaf Speciality
Color	Green
Flavor	A bit bitter
Form	Fine

Water Content Test of Simple Drugs

The moisture content of guava leaf powder was determined by weighing 2 grams of powder. The moisture content was measured using a moisture balance at 105°C, then the test process was allowed to run until the device beeped to indicate completion. The results of the guava leaf powder moisture content test can be seen in Table 4.3.

Table 1.Results of water content test of guava leaf powder

Replication	Sample weight (grams)	Drying shrinkage percentage %
I	2	5.83%
II	2	8.53%
III	2	6.09%
Average		**6.81±1.48**

Drying Shrinkage Test of Simplex

Drying loss was determined by weighing 2 grams of powder. The drying loss was measured in an oven at 105°C for 30 minutes. The results of the drying loss test for guava leaves are shown in Table 4.

Table 4. Results of Drying Shrinkage Test of Simplex

Test sample	Replication	Sample weight	Exchange rate weight	Exchange rate weight+sample	Percentage (%)
Powder	I	2 grams	47.170 g	48.983 g	9.35%
	II		41.523 g	43.328 g	9.75%
	III		45.362 g	47.174 g	9.4%
Average					**9.5±0.217**

Making Ethanol Extract from Tangkalak Guava Leaves

The preparation of guava leaf extract (Bellucia pentamera Naudin) was carried out using the maceration method. A total of 500 g of guava leaf powder was put into a maceration container, then 96% ethanol solvent was added with a ratio of material and solvent 1:10, which is 5 liters. The maceration was carried out for 5 days occasionally while stirring, after which it was filtered with flannel cloth to obtain the maceration results, then the maceration results were filtered again using filter paper to ensure there was no sediment in the final maceration results.(Sukadiasa et al., 2023)From 500 grams of powdered starting material used in the extraction process, 123 grams of extract was obtained. This value indicates an extraction yield of 24.6%, which is the ratio of the resulting extract weight to the starting material weight. A yield of 24.6% indicates that the extraction process is proceeding with fairly good efficiency.

Standardization of Tangkalak Guava Leaf Extract

Organoleptic Test of Extract

One specific parameter in extract standardization is the organoleptic test, which assesses extract quality based on sensory observations, including odor, color, taste, and appearance. The results of the organoleptic test of guava leaf extract can be seen in Table 5.

Table 5. Organoleptic Test Results of Tangkalak Guava Leaf Extract

Parameter	Results
Smell	Typical features of guava leaves
Color	Blackish green
Flavor	Bitter
Form	Thick

Determination of Water Content of Extract

The water content of the guava leaf extract was determined by weighing 2 grams of powder. The water content was measured using a moisture balance at 105°C, then the process was allowed to proceed until the device gave a beep signal indicating the test was complete. The results of the guava leaf extract water content test can be seen in Table 6.

Table 6. Percentage of Water Content of Tangkalak Guava Leaf Extract

Replication	Sample weight (grams)	Percentage (%)
I	2	8.23%
II	2	7.75%
III	2	8.10%
Average		**8.02 ±0.24**

Determination of Extract Drying Loss

Determination of drying loss of guava leaf extract by weighing 2 grams of guava leaf extract. Drying loss was measured using an oven with a temperature set at 105°C, waiting until the instrument beeps indicating the analysis is complete. This can be seen in Table 7.

Table 7. Percentage of Drying Loss of Tangkalak Guava Leaf Extract

Test sample	Replication	Sample weight	Exchange rate weight	Exchange rate weight+sample	Percentage (%)
Extract	I	2 grams	50.453 g	52.368 g	4.25%
	II		43.892 g	45.786 g	5.3%
	III		47.842 g	49.773 g	3.45%
Average					**4.33±0.927**

Ethanol-Free Test of Tangkalak Guava Leaf Extract

The ethanol-free test was conducted to ensure the absence of ethanol in the extract, thus obtaining a truly pure extract. Based on the observation results, the guava leaf extract reacted with 1% H_2SO_4, CH_3COOH, and then heated did not show any ester odor. This result was declared positive, indicating that there was no characteristic ethanol ester odor.

Phytochemical Screening

The resulting extract was then subjected to phytochemical screening to determine the metabolite content of the guava leaf extract. The results of the phytochemical screening of the guava leaf extract can be seen in Table 8 below.

Table 2. Phytotochemical Screening Results of Tangkalak Guava Leaf Extract

Compound	Reagent	Reference	Information	Results
Alkaloid	Dragendorff + hydrochloric acid	The precipitate is brownish orange or brick red	Brownish orange sediment	+
	Wagner	Brown sediment	Brown sediment	+
Flavonoid	HCl + Mg powder	Red sediment(Novia et al., 2023).	Orange	+
Saponin	Aquades + shake for 10 seconds	The foam is stable and remains for at least 10 minutes.(Kartikasari*et.al*., 2022).	Stable foam	+
Terpenoid	HCL + H2SO4	Chloroform + concentrated sulfuric acid(Kartikasari*et.al*., 2022).	The formation of two layers with red, orange, or bluish green colors	-
Tannin	FeCl3	Blackish green(Reiza et al., 2019).	Blackish green	+

Fractionation of Tangkalak Guava Leaf Extract

Fractionation is a separation process that aims to produce purer extract fractions through a liquid-liquid extraction method using solvents with different levels of polarity.(Suhaenah et al., 2023). The yield results of the guava leaf fraction can be seen in Table 9.

Table 9.Fraction Yield Results

Solvent	Fraction Weight	Yield (%)
n-Hexane	4.12	41.2%
Ethyl acetate	4.03	40.3%
Water	4.42	44.2%

Staphylococcus epidermidis ATCC 12228 Bacterial Activity Test

Gram staining

Gram staining aims to distinguish between two groups of bacteria, namely Gram-positive bacteria and Gram-negative bacteria. Gram-positive bacteria can retain the purple color of crystal violet so that when observed under a microscope they will show a purple color, while Gram-negative bacteria will not retain the purple color of crystal violet, but safranin can be absorbed into the cell wall so that when viewed under a microscope they will appear red.(Sandy, Wardani, Septiarini, et al., 2021).

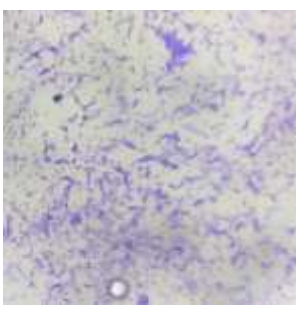

Figure 1.Gram Staining of Bacteria*Staphylococcus Epidermidis*ATCC 12228 (Personal Documentation)

Antibacterial Test Results Using the Diffusion Method

Testing the antibacterial activity of ethanol extract and fraction of guava leaves was carried out using the disc diffusion method. This method uses disc paper soaked in ethanol extract of guava leaves with concentrations of 10%, 20%, 30%, as well as fractions of n-Hexane, ethyl, and water with concentrations of 10%, 20%, 30%, ciprofloxacin dics 5 µg as a positive control (+) and DMSO 5% as a negative control (-). The disc paper was placed on the surface of the NA media that had been inoculated with the test bacterial suspension. The results obtained were the formation of a clear zone with the diameter shown in Table 10.

Table 10.Diffusion Antibacterial Activity Test Results

Test material	Concentration (%)	Zone Resistor (mm)			Mean (mm)±SD
		I	II	III	
Extract	10%	3.46	3.67	3.18	3.43±0.24
	20%	5.25	6.37	4.05	5.22±1.16
	30%	6.08	8.75	5.77	6.86±1.63
***n*-Hexane**	10%	0	0	0	0±0
	20%	0	0	0	0±0
	30%	0	0	0	0±0
Ethyl	10%	3.04	5.04	2.93	3.67±1.18
	20%	5.14	5.28	3.44	4.62±1.02
	30%	5.33	5.92	5.01	5.42±0.46
Water	10%	3.88	1.78	3.51	3.05±1.12
	20%	4.02	3.26	4.15	3.81±0.48
	30%	5.86	3.69	4.78	4.77±1.08
Ciprofloxacin	5 µg	22.41	21.24	24	22.55±1.38
DMSO	5%	0	0	0	0±0

Antibacterial Test Results Using the Liquid Dilution Method (MIC)

The MIC value was determined at several concentrations to determine the smallest amount of antibacterial active ingredient required to inhibit the growth of the tested bacteria. The MIC test results can be seen in Table 11.

Table 11. Antibacterial Test Results Using the Liquid Dilution Method

Concentration	Results	Information
20%	+	Clear
10%	-	Cloudy
5%	-	Cloudy
2.5%	-	Cloudy
Positive control	+	Clear
Negative control	-	Cloudy

Antibacterial Test Results Using the Solid Dilution Method (SDI)

The Minimal Bactericidal Concentration (MBC) is determined using the solid dilution method, namely by looking at the lowest concentration that appears clear, meaning that it does not show any bacterial colony growth after incubation for 24 hours at 37°C.

The results of the antibacterial activity test image of the extract by determining the solid dilution MBC value can be seen in Figure 2.

Figure 2. KBM Results

Data analysis

The data obtained were then subjected to statistical analysis using tests for normality and homogeneity using the Shapiro-Wilk and Levene's tests to determine significant differences both overall and between treatments. The results of the normality test can be seen in Appendix 10, Table 12.

Table 12. Normality Test Results

Treatment Group	Test Statistics	Sig
10% extract	.993	.843
20% extract	1,000	.962
30% extract	.827	.181
10% n-hexane	.	.
20% n-hexane	.	.
30% n-hexane	.	.
10% ethyl acetate	.789	.088
20% ethyl acetate	.807	.131
30% ethyl acetate	.971	.676
10% water	.877	.317
20% water	.857	.259
30% water	1,000	.995
k+	.799	.112
k-	.	.

Next, a homogeneity test was conducted to determine whether the data variance between two or more groups was the same (homogeneous) or different. This homogeneity test is one of the prerequisites for independent ANOVA analysis that must be met before further statistical analysis. The results of the homogeneity test showed a significance value (Sig) <0.001. The results of the homogeneity test can be seen in Appendix 12.

Based on the results of the statistical analysis, the inhibitory power variable showed a test statistic value of 4.179 with a significance value (Sig) < 0.001. A significance value smaller than 0.05 indicates that the test results are statistically very significant, so the null hypothesis (H_0) is rejected. Thus, it can be concluded that the treatment given has a significant effect on inhibitory power, and the observed differences did not occur by chance.

The one-way ANOVA test could not be used. Further data analysis was performed using the Kruskal Wallis test (non-parametric) as an alternative to the one-way ANOVA test.The results of the Kruskal Wallis test showed a significance value (Sig) < 0.001.

After that, the analysis was continued using Pairwise Kruskal Wallis with the test results listed in Appendix 13.

DISCUSSION

As an initial step to ensure the authenticity of the research material, plant identification was performed to ensure the identity of the sample used. This identification was performed by matching the morphological characteristics of the guava plant (Bellucia pentamera Naudin). This identification was intended to establish the validity of the sample used in the study clearly from the plant used, thereby reducing errors. The identification was conducted at the Faculty of Mathematics and Natural Sciences, Tanjung Pura University. The results of the identification showed that the sample used was indeed a guava plant with the Latin name (Bellucia pentamera Naudin).

After the determination is carried out, the preparation of the simplex is carried out.The initial stage is the collection and preparation of raw materials in the form of guava leaves (Bellucia pentamera Naudin). The guava leaves are collected from empty land precisely in the Galing Village area, Galing District, Sambas Regency, West Kalimantan Province. The guava leaves are selected to be fresh and old. After the guava leaves are collected, then wet sorting is carried out to separate damaged or unfit plant parts, the results of the wet sorting are weighed, it can be seen that the weight of the guava leaves obtained is 9,300 grams.

The collected 9,300 grams of guava leaves were then washed with running water to remove any dirt. The clean guava leaves were then wet sorted, chopped, dried, dry sorted, pollinated, and sifted. After that, the chopped leaves were dried in the sun under a black cloth. The purpose of drying is to ensure the medicinal plants last longer in storage and to prevent the growth of microorganisms and mold. After drying, dry sorting was carried out to separate foreign objects such as unwanted plant parts and other impurities remaining in the dried medicinal plants. The dry sorting resulted in 1,230 grams of medicinal plants. The dried medicinal plants were then processed into powder by grinding them using a blender specifically designed for medicinal plants. Converting the medicinal plants into powder form aims to increase the efficiency of extracting active compounds during the extraction process. One factor that influences the solubility of a substance is particle size, where the smaller the particle size, the larger the surface area, thus accelerating the dissolution process and increasing extraction efficiency.(Fatwami & Royani, 2023).

The medicinal plants are then sieved using a 40-mesh sieve to obtain a uniform powder. Particles that are too fine can cause clumping during the extraction process, while particles that are too coarse can inhibit the dissolution of the active compounds. Therefore, the fineness of the medicinal plant powder must be adjusted to ensure optimal extraction.(Maulidah et al., 2022)After that, the powdered herbal medicine can be stored in a tightly closed container. Then, the yield of the herbal medicine is calculated.

The yield of the Tangkalak guava leaf simplicia can be seen in Table 1 at 13.22%, these results have met the requirements for the determination of the yield, which is more than 10%, the higher the yield produced, the higher the content of substances that will be attracted to the raw materials. Based on the results of the yield of the simplicia that has been obtained from weighing 9,300 grams of Tangkalak guava leaves and 1,230 grams of dry.

Before proceeding to the extraction process, the powdered medicinal plants require quality standardization. This standardization is carried out to ensure the quality of the materials used for research. Standardization of the powdered medicinal plants includes organoleptic testing, water content, and drying loss.

Furthermore, organoleptic standardization of the simplicia was carried out, where the quality of the simplicia was observed based on the observation of the five senses and the results obtained included a distinctive odor, green color, and fine powder form. Organoleptic examination of the simplicia of guava leaves (Bellucia pentamera Naudin) was carried out at the Laboratory of Duta Bangsa University, Surakarta. The results of the organoleptic examination of guava leaves (Bellucia pentamera Naudin) can be seen in Table 2.

Testing the water content of the simplex can be seen in Table 3. The average result for guava (Bellucia pentamera Naudin) powder was 6.81%. The purpose of this test was to determine the maximum limit or range of the amount of compounds lost during the drying process. The water content obtained met the quality requirements (≤ 10%).(Samang et al., 2025)The main factor influencing the water content of the medicinal plant to meet the requirements is the drying method used, as the drying process is one of the post-harvest stages that plays a crucial role in determining the quality of the medicinal plant. Excessively high water content can facilitate the growth of microorganisms in the powder, potentially reducing the quality stability during the standardization process of guava (Bellucia pentamera Naudin) medicinal plant and causing physical changes such as decay.(Insan et al., 2025).

The drying loss obtained can be seen in Table 4, which is 9.5%. This result meets the requirements of the Indonesian Herbal Pharmacopoeia, which stipulates that the drying loss of an extract should not exceed 10%. The use of a temperature of 105°C is considered to provide an optimal balance between the efficiency of the evaporation process and the stability of the test results. The use of temperatures higher or lower than this temperature has the potential to affect and reduce the level of accuracy and consistency of the test results. If the drying loss value is too high, this can cause changes in the chemical composition of the extract, reduce the quality of the herbal medicine, and increase the risk of bacterial growth.(Samang et al., 2025).

It can be seen that the yield percentage of 96% ethanol extract of guava leaves (Bellucia pentamera Naudin) in Table 5 is 24.6%. The yield calculation was carried out to determine the amount of extract produced from the fresh herbal medicine used. The results showed that the yield obtained was influenced by the type of solvent used.(Syafriana et al., 2024)The solvent used for the extraction process of secondary metabolites in guava leaves is 96% ethanol, where this solvent has the ability to extract with wide polarity, so it can extract secondary metabolites that are polar, semipolar and even nonpolar.(Agung et al., 2024)The filtrate obtained was then filtered using a Buchner funnel to ensure there was no sediment. The filtrate was then evaporated using a rotary evaporator to obtain a solvent-free and thicker extract at a temperature of 40°C, with the aim of evaporating the solvent at a temperature that is not too high, so that the process is safer and avoids damage to the sample.(Anwar et al., 2022)Determining the yield of a sample aims to determine the amount of extract produced during the extraction process. The higher the yield, the greater the active compound content of the sample. The extract yield obtained in this study was greater than 10%. A good yield is considered good if the yield exceeds 10% (Rahadyana et al., 2024).

Next, the extract standardization is carried out which can be seen in table 6, namely the organoleptic test, namely the assessment of the quality of the extract based on observations of the five senses which include distinctive odor, blackish green color, bitterness, and thick liquid form. Organoleptic examination of guava leaf extract (Bellucia pentamera Naudin) was carried out at the Laboratory of Duta Bangsa University, Surakarta.

Determination of the water content of the extract is carried out to determine the amount of water contained in the extract, which is related to the level of purity and the possibility of contamination. Furthermore, this test aims to inhibit microbial growth that can affect the quality and shelf life of the extract. The test results in Table 7 show that the water content of guava (Bellucia Pentamera Naudin) leaf extract is 8.02%. This value meets the water content requirements for thick extracts, which is less than 10%, thus complying with applicable regulations.(Wandira et al., 2023).

The next test was the drying loss, which can be seen in Table 8, which was 4.33%. This value meets the requirements, which stipulate that the drying loss of an extract should not exceed 10%.(Samang et al., 2025).

In table 9, the ethanol-free test shows that the ethanol extract of guava leaves (Bellucia pentamera Naudin) is free from its solvent, namely 96% ethanol, which is shown to have no detectable ester odor, so it can be stated positively that it does not contain ethanol.(Kusniawati et al., 2025).

Based on the phytochemical screening results presented in Table 10, the ethanol extract of guava leaves (Bellucia pentamera Naudin) is known to contain various secondary metabolite compounds, namely alkaloids, flavonoids, tannins, and saponins. This finding is in line with research(Agusanty et al., 2025) The results showed that guava leaf extract contains compounds such as flavonoids, alkaloids, tannins, and saponins. One frequently used qualitative phytochemical screening technique is the color reaction test using specific reagents. This can also be influenced by the solvent used; in the plant extraction process, the solvent used is 96% ethanol. This is based on the universal and selective properties of ethanol, which allows it to dissolve compounds soluble in both polar and non-polar solvents. 96% ethanol solvent has broad extraction power and can optimize the binding of secondary metabolites.(Nufus et al., 2021).

Phytochemical tests for alkaloids showed that guava (Bellucia pentamera Naudin) leaf extract gave a positive result for alkaloids when reacted with Dragendorff and Wagner reagents. This test method works based on the principle of a precipitation reaction that occurs through a ligand exchange mechanism. Nitrogen atoms in the alkaloid structure, which have lone electron pairs, can interact with the reagent by replacing iodide ions, resulting in the formation of a precipitate as an indicator of the presence of alkaloid compounds (Kartikasari et al., 2022).

In the flavonoid identification test, the extract of guava leaves (Bellucia Pentamera Naudin) showed a color change in the solution to red. This color change indicates that the ethanol extract of pineapple peel positively contains flavonoid compounds. During the testing process, the extract

solution was heated for about 2–3 minutes because most flavonoids are easily soluble in hot water. After heating, HCl and magnesium (Mg) powder were added to the solution. The addition of these two reagents aims to reduce the benzopyrone group in the flavonoid structure, resulting in the formation of flavylium salts that produce a red to orange color as an indication of the presence of flavonoids.(Inderiyani & Herdaningsih, 2021).

The tannin test results on guava (Bellucia Pentamera Naudin) leaf extract showed a positive reaction, indicated by the formation of a blackish-green color. This color appears after the addition of $FeCl_3$, which interacts with the hydroxyl groups in the tannin structure. The reaction between iron (III) ions and the phenolic groups in tannins forms a dark-colored complex, indicating the presence of tannins in the extract (Pratiwi et al., 2023).

Test results showed that guava (Bellucia pentamera Naudin) leaf extract reacted positively with saponins. This was indicated by the formation of foam that remained stable and did not disappear quickly after the solution was shaken for several minutes. The appearance of this foam indicates the presence of glycoside compounds, which have the property of forming foam in water. These saponins can then be broken down through hydrolysis into sugars (glucose) and non-sugar components called aglycones (Jusna & Nasrudin, 2022).

The fractionation results of guava (Bellucia pentamera Naudin) leaf extract in Table 4.11 show that the n-hexane fraction yield was 41.2%, the ethyl acetate fraction 40.3%, and the water fraction 44.2%. The difference in yield values for each fraction is related to the diversity and amount of chemical compounds contained in the guava (Bellucia pentamera Naudin) leaf extract. Fractionation data shows that the water fraction produces the highest yield compared to the n-hexane and ethyl acetate fractions. This condition is thought to be due to the nature of n-hexane and ethyl acetate which are more volatile, so that during the evaporation process there is a shrinkage in the solvent volume. This evaporation affects the final number of fractions obtained, resulting in a lower yield (Maryam et al., 2024).

Gram staining is a method used to categorize bacteria based on their characteristics, namely gram-positive and gram-negative bacteria. Gram-positive bacteria have cell walls with a thick peptidoglycan layer and do not have a lipoprotein or lipopolysaccharide layer. On the other hand, gram-negative bacteria have cell walls with thinner peptidoglycan, coated by a lipoprotein or lipopolysaccharide layer. Based on Figure 1, the results of the gram staining show that the bacteria are purple because they are able to retain the main dye, namely crystal violet, which binds to the thick peptidoglycan layer. In addition, the bacteria show a morphology in the form of branched filaments or a combination of rod/filament shapes organized in irregular groups. These characteristics indicate that Staphylococcus epidermidis ATCC 2228 bacteria are included in the category of gram-positive bacteria.(Sandy, Wardani, Septiarini, et al., 2021).

Antibacterial activity testing using the diffusion method in this study was conducted by measuring the diameter of the inhibition zone on NA (Nutrient Agar) media. NA media, which is a yellowish-white powder, is included in the universal media category. Quoted from (Rinihapsari et al., 2023), this media is one of the most commonly used to support the growth of various types of bacteria. Because it contains agar as a solidifying agent, this NA media has a solid form. This solid media is used to observe the appearance and morphology of bacterial colonies. After being poured into a petri dish, the NA media is allowed to harden. Next, bacteria are inoculated into the media using a sterile cotton swab until evenly distributed over the entire surface of the media. The paper discs to be used are soaked in each sample concentration for approximately 15 minutes to allow the liquid to be properly absorbed. After that, the discs are placed on the media that has been inoculated with bacteria, then incubated at 37°C for 24 hours.

The results of the antibacterial activity test showed that the extractguava leaves (Bellucia pentamera Naudin)able to inhibit bacterial growth*staphylococcus epidermidis*ATCC 2228, which is characterized by the formation of an inhibition zone around the disc. Based on the data in Table 12, it can be seen that increasing sample concentration is directly proportional to the magnitude of the

inhibition power produced in each group. The results of the 10% concentration extract, amounting to 3.43 mm, are classified as weak, followed by 20% concentration, 5.22 mm, and 30% concentration, 6.86 mm, which are categorized as moderate. This is because the guava leaf extract(Bellucia pentamera Naudin)has the ability to inhibit bacterial growth*staphylococcus epidermidis*ATCC 2228This is because the extract still contains a complete mixture of secondary metabolites found in guava leaves, which function as antibacterials.

Of the three fractions tested, the ethyl acetate fraction was the most active fraction against bacteria.*staphylococcus epidermidis*ATCC 2228. With an average inhibition zone diameter at a concentration of 10% of 3.67 mm, it is classified as weak, while at a concentration of 20% of 4.62 mm is classified as weak and at 30% of 5.42 mm is categorized as moderate. Ethyl acetate is a semi-polar solvent that is able to dissolve both polar and nonpolar compounds. In addition, ethyl acetate is effective in attracting compounds of the flavonoid, tannin, and saponin groups.(Murdiyansah et al., 2020)This causes the ethyl acetate fraction to be the most active fraction, characterized by the formation of the greatest inhibitory power compared to other fractions.

The results of antibacterial activity tests on water fractions at concentrations of 10%, 20%, and 30% showed weak antibacterial activity at 3.05 mm, 3.81 mm, and 4.77 mm, respectively. This is because the water fractions contain antibacterial compounds only in very small amounts, making them insufficient to inhibit or kill bacteria.(Kristianto et al., 2020).

The results of the antibacterial activity test on the n-Hexane fraction showed no inhibition zone. The absence of an inhibition zone in the n-Hexane fraction at all concentrations indicates that n-Hexane is only capable of extracting nonpolar compounds, such as steroids, and this class of steroid compounds is known to have relatively weak antibacterial activity in inhibiting antibacterials.(Kusumastuti et al., 2021).

The results of the antibacterial test by liquid dilution with the determination of the Minimum Inhibitory Concentration (MIC) value on guava leaf extract with concentrations of 20%, 10%, 5%, 2.5% are presented in Table 4.13 andAppendix 10. In the extract of the test solution concentration of 20%, the solution looks clear and there are no visible lumps or membranes growing, while the extract at the test solution concentration of 10%, 5%, 2.5% the solution looks cloudy and there are lumps/membranes indicating the growth of staphylococcus epidermidis ATCC 2228 bacteria in the solution. It can be seen that the 20% concentration is a concentration that is able to inhibit the growth of staphylococcus epidermidis ATCC 2228 bacteria. This is indicated by the color of the solution which is not cloudy and the absence of lumps/membranes formed in the solution. Thus, the sample solution in the test solution with a concentration of 20% can be stated as the MIC value.

In the solid dilution test, it is expected that the 20% concentration which has been proven to be able to inhibit the growth of staphylococcus epidermidis ATCC 2228 bacteria in the media remains clear, which indicates that the extract test solution with a concentration of 20% is able to kill staphylococcus epidermidis ATCC 2228 bacteria. Minimum Killing Concentration (MBC), which indicates antibacterial activity, can be determined by inoculating samples from the test tube into NA media in a petri dish, then incubating for 24 hours at 37°C. MBC is determined as the lowest concentration in NA media that does not have growth of Staphylococcus epidermidis ATCC 2228. From the results obtained, it can be seen that the concentration of 20% has a minimum inhibitory concentration during the liquid dilution test treatment, during the solid dilution test the bacteria still grow, this can be influenced by the concentration being too low and on agar media, heating during media preparation can also potentially reduce the stability of the antibacterial compounds used, so that in the MBC test with a concentration of 20% there are still bacteria that grow indirectly, this concentration cannot kill bacteria on agar media.

The next data analysis is the normality test in the normality test in this study using the Shapiro–Wilk Method chosen because the number of samples in this study is less than 50. Based on the results of the analysis, it is known that the data is not normally distributed, indicated by a significance value of 1,000> 0.05 and 0.000 < 0.05. Because there is one data that does not meet the normality criteria,

the normality assumption with the Shapiro–Wilk method is declared not met. Because there are data that show a normal distribution and do not show a normal distribution.

This homogeneity test is one of the prerequisites for independent analysis of the ANOVA test that must be met before further statistical analysis is carried out. The results of the homogeneity testin attachment 12.shows a value <0.05, indicating that the data between groups do not have the same variance. This likely occurs because the data differences in the samples are quite large, as data with smaller variances tend to have a higher level of uniformity.

Based on the results of the Kruskal–Wallis test, the homogeneity test results listed in Appendix 12, obtained an Asymp. Sig value of 0.000 which is smaller than 0.05, indicating that there is a significant difference between the treatment groups.

The results of the Pairwise Kruskal-Wallis test showed that not all pairs of treatment groups had statistically significant mean differences. Some treatment pairs showed significance values greater than the set limit ($\alpha = 0.05$), so that the mean differences between the groups could not be declared significant. For example, comparisons between 10% extract and 30% extract, 10% extract with 30% ethyl acetate, 10% extract with 30% water, 30% extract and ciprofloxacin also showed significance values above 0.05. This indicates that the inhibitory power produced by the 30% extract is relatively equivalent to all groups with the comparison, so there is no statistically significant difference.

So it can be concluded that the four treatment pairs have the same average relative inhibitory power and are not significantly different.

CONCLUSION

This study concluded that the ethanol extract of guava (Bellucia pentamera Naudin) leaves and its ethyl acetate fraction showed moderate antibacterial activity against Staphylococcus epidermidis ATCC 12228, with an average inhibition zone of 3.43-6.86 mm for the extract and 3.67-5.42 mm for the ethyl acetate fraction at concentrations of 10-30%, while the n-hexane fraction was inactive and the aqueous fraction was weak. The extract positively contained alkaloids, flavonoids, tannins, and saponins, with an MIC of 20% but no MBC was detected at that concentration; Kruskal-Wallis analysis confirmed significant differences between treatments (Sig <0.001). These findings support the potential of local plant bioactive compounds as alternatives to conventional antibiotics amid nosocomial resistance.

However, limitations include the lack of MBC, possibly due to low concentrations or sensitivity of agar media to the active compounds, and the in vitro focus without toxicity testing or specific molecular mechanisms. Suggestions for further research include testing isolated fractions, vivo testing in skin infection models, and collaboration with the Indonesian Food and Drug Authority (BPOM) for phytopharmaceutical development. Practically, these results imply the use of guava leaves as an affordable traditional medicine for opportunistic skin infections in endemic areas such as West Kalimantan, supporting community treatment while reducing dependence on imported antibiotics.

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