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Antibacterial Activity Test Of Extract And Fraction Of N-Hexane, Ethyl Acetate And Water Of Pineapple Peel (*Ananas Comosus* (L.) Merr.) Against *Staphylococcus Epidermidis* ATCC 12228 Bacteria

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Abstract

Staphylococcus epidermidis is a bacteria that causes mild skin infections accompanied by abscesses, with a high prevalence in Indonesia, requiring natural alternatives due to antibiotic resistance. This study aimed to evaluated antibacterial activity of 96% ethanol extract and n-hexane, ethyl acetate, water fractions of pineapple peel (Ananas comosus L. Merr.) against S. epidermidis ATCC 12228 and to determined MIC/MBC of the most active fraction. This quantitative and experimental study used the disk diffusion and liquid/solid dilution. methods. Population: pineapple peels from Blitar, East Java; sample: 700 g Simplicia powder. The nstruments included rotary evaporator, incubator, autoklaf, laminar air flow; data were analyzed using the Kruskal-Wallis (SPSS 25). Results showed that extract produced inhibition zones ranging from 5.63–11.43 mm, ethyl acetate fraction 3.80–7.55 mm, water 2.35–6.88 mm, n-hexane was inactive. The MIC and MBC values of the extract were 25%; ethyl acetate fraction MIC and MBC were 50%. Extract, ethyl acetate, and water fractions exhibited antibacterial activity agginst S. epidermidis, with extract being the most effective.

Keywords: Antibacterial Activity, Ananas Comosus, Ethyl Acetate Fraction, Minimum Inhibitory Concentration, Staphylococcus Epidermidis.

INTRODUCTION

According to World Health Organization data in 2020, 3,734 deaths in Indonesia were caused by skin diseases, equivalent to 0.22% of the total deaths, placing Indonesia in 75th place globally. More than 300 million cases of infectious skin diseases are estimated to occur annually worldwide, with the number of cases in Indonesia reaching 4.6% to 12.95% cases.

Skin diseases in Indonesia are often triggered by bacterial infections, with *Staphylococcus epidermidis* being a major cause of mild skin infections accompanied by abscesses and acne. This bacterium is a normal commensal flora of the skin, a Gram-positive coccus measuring 0.5-1.5 µm, but it can become an opportunistic pathogen when the immune system is weakened.

Staphylococcus epidermidis also contributes to unpleasant odor in the underarm and feet areas due to interaction with sweat and triggers the release of oleic compound through lipase enzyme, which exacerbates acne. The high prevalence of this infection requires further understanding of the dynamics of bacteria in the skin microbiome.

Conventional treatment of *Staphylococcus epidermidis* infections relies on chemical antibiotics, but overuse leads to antibiotic resistance, toxicity, and the need for increased doses. This resistance is increasingly concerning because the bacteria can form biofilms that enchance their tolerance to antibiotics and the host immune response, under certain conditions, these biofilms may disrupt skin homeostatis and contribute to infection, thereby necessitating safe and effective natural alternative therapies.

Pineapple (*Ananas comosus* (L.) Merr.) peel, often discarded as waste, contains phytochemical compounds such as flavonoids, tannins, saponins, alkaloids, terpenoids, and the enzyme bromelain, which have antibacterial, anti-inflammatory, and antioxidant potential. These compounds work by damaging the cell walls of Gram-positive bacteria through protein hydrolysis and reducing surface tension.

Previous research has shown that pineapple peel ethanol extract inhibits bacteria such as *Staphylococcus aureus* and *Streptococcus mutans* with a low MIC. However, specific data on *S.*

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epidermidis ATCC 12228 and comparisons of n-hexane, ethyl acetate, and water fractions are still limited. This necessitates evaluating the effectiveness of these fractions to optimize the use of pineapple waste as a natural antibacterial agent.

This study aims to determine the antibacterial activity of 96% ethanol extract and fractions of n-hexane, ethyl acetate, and pineapple peel water against *S. epidermidis* ATCC 12228, compare their effectiveness, and measure the MIC and MBC values of the most active fractions using disc diffusion and dilution methods. The urgency lies in overcoming antibiotic resistance through abundant and environmentally friendly natural sources, supporting traditional medicine in Indonesia. The novelty is the comprehensive evaluation of specific fractions against strain ATCC 12228 along with Kruskal-Wallis statistical analysis, complementing previous studies.

RESEARCH METHODS

This research is quantitative with an experimental approach to test the antibacterial activity of 96% ethanol extract and fractions of n-hexane, ethyl acetate, and pineapple peel water (*Ananas comosus* (L.) Merr.) against *Staphylococcus epidermidis* ATCC 12228 by measuring the inhibition zone, MIC, and MBC [Sandy *et al.*, 2021]. Conducted at the Pharmacy Laboratory of Duta Bangsa University Surakarta and the UPT Laboratory of Sebelas Maret University, the method includes 96% ethanol maceration extraction, stepwise fractionation, phytochemical screening, and disk diffusion and liquid/solid dilution tests with three replications for statistical accuracy [Shina *et al.*, 2024]. The experimental using independent variable (concentration 25%, 50%, 75%) to observe the effect on the dependent variable (inhibition zone diameter), with positive control of ciprofloxacin 5 µg/disc and negative control of DMSO 1% [Rahayu *et al.*, 2024][Fitriani & Nashihah, 2021].

The main instruments include analytical balance, autoclave, incubator, rotary evaporator, laminar air flow, microscope, and NA/NB media for bacterial culture; materials include pineapple peel, 96% ethanol, n-hexane, ethyl acetate, water, phytochemical reagents, and McFarland 0.5 standard bacterial suspension [Shina *et al.*, 2023]. Data analysis techniques used SPSS version 25 with Shapiro-Wilk, Levene, homogeneity normality tests, followed by one-way ANOVA or nonparametric Kruskal-Wallis (if data is not normal, $p < 0.05$ is significant), and Tukey post-hoc for comparison between groups [Sunari *et al.*, 2025]. Measurement of inhibition zone using the average formula (DV + DH - DC)/2 mm, classification: <5 mm weak, 5-10 mm moderate, 10-20 mm strong, >20 mm very strong [Harris *et al.*, 2017].

The study population was all ripe pineapple (*Ananas comosus* (L.) Merr.) peels from the plantations of residents of Ponggok District, Blitar Regency, East Java, which were fresh, greenish yellow in color and free from damage. Samples were taken purposively with the criteria for homogeneity, identified through determination at the Batu Materia Medica Laboratory, ensuring the authenticity of the plant [Indrawan *et al.*, 2024].

The procedure begins with sampling, washing, sun drying covered with plastic, grinding in a blender, and sieving through a 40 mesh sieve for the simplicia, followed by organoleptic standardization, water content (moisture balance <10%), and drying loss [Ramadhan *et al.*, 2024][Veninda *et al.*, 2023]. Extraction of 96% ethanol maceration (1:10, 5x24 hours), rotary evaporation, yield is calculated; standardization of extracts plus ethanol-free test; phytochemical screening (Dragendorff, Wagner, Mg/HCl, foam, Liebermann-Burchard, $FeCl_3$) [Shina *et al.*, 2024][Kartikasari *et al.*, 2022]. Multilevel fractionation (n-hexane, ethyl acetate, water) on 20 g of extract; test solution 25-75% extract and fractions; sterilization of tools/media; rejuvenation of NA bacteria slant 37°C/24 hours; Gram staining; 0.9% NaCl suspension; diffusion test (disc immersed for 15 minutes, incubation at 37°C/24 hours); dilution (serial 50-6.25%, NB incubation, NA subculture for MIC/MBC) [Sugiarti *et al.*, 2020][Fitriani & Nashihah, 2021][Jungjunan *et al.*, 2023].

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RESULTS AND DISCUSSION

Plant Determination

The pineapple plants used in this study were obtained from a pineapple plantation in Ponggok District, Blitar Regency, East Java. The plants were then identified at the Batu Herbal Materia Medica Laboratory in Batu City, East Java. The results showed that the samples were pineapple plants of the species *Ananas comosus* (L.) Merr. from the Bromeliaceae family.

Powder Simplicia Production

The results of storage and drying of the herbal powder are as follows:

Table 1. Results of Determination of Drying Loss of Pineapple Peel Powder Simplicia

No	Process	Initial weight	Final weight	Percentage (%)
1.	Material collection	11.86 kg	-	-
2.	Processing	11.86 kg	2.12 kg	17%
3.	Powdering	2.12 kg	1.9 kg	89%

Standardization of Simplicia

Organoleptic Test

Table 2. Organoleptic Characteristics of Pineapple Peel Simplicia

Parameter	Description
Odor	Typical pineapple scent
Color	Pale yellow
Taste	Bitter
Form	Fine powder resembling sand

Water Content Test of Simplicia

Table 3. Results of Simplicia Water Content Test

Replication	Sample Weight	Percentage of water content
1	2.00 grams	7.8%
2	2.00 grams	8.68%
3	2.00 grams	9.28%
Average		8.59%

Drying Shrinkage Test

Table 4. Results of Drying Shrinkage Test of Pineapple Peel Powder

Replication	Sample weight	Crucible weight	Crucible weight + sample	Percentage
1	2 grams	47.177 g	48,983g	9.7%
2	2 grams	41,527g	43,328g	9.95%
3	2 grams	45,369g	47.174g	9.75%
Average				9.8%

Production of 96% Ethanol Extract from Pineapple Peel

Table 5. Yield of Pineapple Peel Extract

Powder weight	Extract weight	Percentage
700 grams	118 grams	16.86%

Based on the research results, a total extract of 118 grams was obtained with a percentage of 16.86%.

Extract Standardization

Organoleptic Test

Table 6. Organoleptic Test Results of Pineapple Peel Extract

Parameter	Description
Odor	Characteristic
Color	Dark brown
Taste	Bitter
Form	Thick consistency

Water Content Test

Table 7. Results of Water Content Test of Pineapple Peel Extract

Replication	Sample Weight	Percentage of water content
1	2.00 grams	5.03%
2	2.00 grams	5.23%
3	2.00 grams	7.95%
Average		6.07%

Extract Drying Shrinkage Test

Table 8. Results of Drying Shrinkage Test of Pineapple Peel Extract

Replication	Sample weight	Crucible weight	Crucible weight + sample	Percentage
1	2 grams	50,440 g	42.368 g	3.6%
2	2 grams	43.898 g	45.786 g	5.6%
3	2 grams	47.836 g	49.773 g	3.15%
Average				4.12%

Ethanol Free Test

Table 9. Ethanol-Free Test Results of Pineapple Peel Extract

Reagent	Results
Extract + acetic acid + sulfuric acid (heating)	+ No ester – like odor

Phytochemical Screening of Pineapple Peel Extract

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Table 10. Phytochemical Screening Results of Pineapple Peel Extract

No.	Active Compounds	Reagent	Description	Results	Conclusion
1	Flavonoid	Mg powder and concentrated HCl	The presence of red or orange color change (Reiza *et al.*, 2019)	Color change to orange	Positive
2.	Alkaloid	HCl 2N + Mayer,	White precipitate forms (Kartikasari *et al.*, 2022)	The presence of white sediment	Positive
		Dragendorff	Brownish orange or brick red sediment (Kartikasari *et al.*, 2022)	Brownish orange sediment	Positive
		Wagner	Brown sediment (Kartikasari *et al.*, 2022)	Brown sediment	Positive
3.	Saponin	Ethanol 96% + Aquadest	Stable foam for 10 minutes (Kartikasari *et al.*, 2022)	Stable Foam	Positive
4.	Tannin	FeCl3 5%	Dark blue or blackish green color change (Reiza *et al.*, 2019)	Blackish green color	Positive
5.	Steroid	Lieberman-Burchard	A layer of red, orange or bluish green is formed (Kartikasari *et al.*, 2022)	Black solution	Negative

Pineapple Peel Extract Fractionation

Table 11. Percentage Yield Results of Pineapple Peel Fraction

No.	Solvent	Extract Weight	Fraction Weight	Yield
1.	*n*-Hexane	20 g	6.12 g	30.6%
2.	Ethyl acetate	20 g	6.03 g	30.15%
3.	Water	20 g	6.42 g	32.1%

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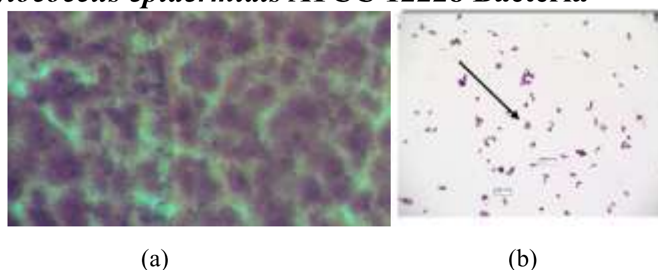
Identification of *Staphylococcus epidermidis* ATCC 12228 Bacteria



(a) (b)

Figure 1 (a.) Gram Staining Results of *Staphylococcus epidermidis* Bacteria
Source: personal documentation
(b.) *Staphylococcus epidermidis*
Source:Karimela *et al.* (2018)

Antibacterial Activity Test of Pineapple Peel Extract and Fraction (*Ananas comosus* (L.) Merr.) Against *Staphylococcus epidermidis* ATCC 12228

Diffusion Method

Table 12. Inhibition Zone Against *Staphylococcus epidermidis* ATCC 12228 Bacteria

Sample	Concentration	Resistance (mm)			Mean	Category
		I	II	III		
Extract	25%	5.19	6.39	5.32	5.63	Moderate
	50%	8.86	9,275	8.74	8.96	Moderate
	75%	10.48	11.27	12.53	11.43	Strong
Ethyl Acetate	25%	3.29	3.96	4.15	3.8	Weak
	50%	5.48	5.39	6.43	5.77	Moderate
	75%	6.86	7.94	7.85	7.55	Moderate
Water	25%	2.05	2.23	2.77	2.35	Weak
	50%	3.68	4.07	4.58	4.11	Weak
	75%	5.8	7.72	6.89	6.8	Moderate
***n*-Hexane**	25%	0	0	0	0	Weak
	50%	0	0	0	0	Weak
	75%	0	0	0	0	Weak
K-	DMSO 1%	0	0	0	0	Weak
	DMSO 1%	0	0	0	0	Weak
	DMSO 1%	0	0	0	0	Weak
Ciprofloxacin	5µg	20.52	19.05	16.29	18.62	Strong
	5µg	19	22.9	21.93	21.16	Very strong
	5µg	18	20.82	21.62	20.03	Very strong

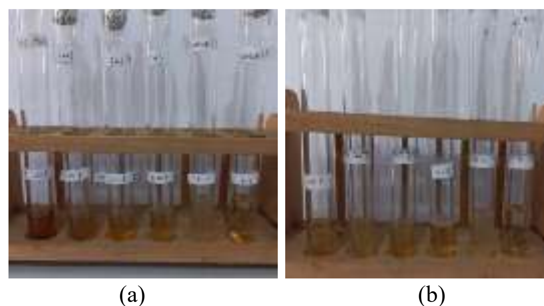
Dilution Method

Table 13. Antibacterial Activity Test Results of Pineapple Peel Extract Using the Dilution Method

Concentration	Results Liquid Dilution	Results Solid Dilution	Description
DMSO 1%	+	+	There is a bacterial growth
6.25%	+	+	There is a bacterial growth
12.5%	+	+	There is a bacterial growth
25%	-	-	There is no bacterial growth
50%	-	-	There is no bacterial growth
Ciprofloxacin 5 µg	-	-	There is no bacterial growth

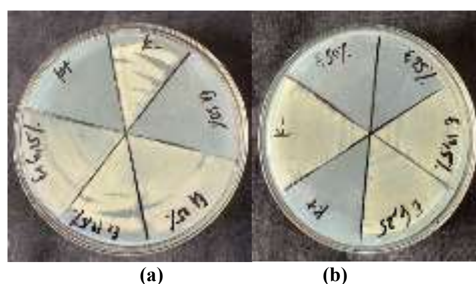
Table 14. Antibacterial Activity Test Results of Pineapple Peel Ethyl Acetate Fraction Using Dilution Method

Concentration	Results Liquid Dilution	Results Solid Dilution	Description
DMSO 1%	+	+	There is a bacterial growth
6.25%	+	+	There is a bacterial growth
12.5%	+	+	There is a bacterial growth
25%	+	+	There is a bacterial growth
50%	-	-	There is no bacterial growth
Ciprofloxacin 5 µg	-	-	There is no bacterial growth



(a) (b)

Figure 2. Antibacterial Activity Test of Pineapple Peel Ethyl Acetate Fraction and Extract using Liquid Dilution Method, (a) Extract, (b) Ethyl Acetate Fraction



(a) (b)

Figure 3. Antibacterial Activity Test of Pineapple Peel Extract and Ethyl Acetate Fraction Using Solid Dilution Method, (a) Ethyl Acetate Fraction, (b) Extract

Data analysis

Table 15. Results of Normality Test (Shapiro-Wilk and Levene's Test)

Sample	Test statistics	Sig
Extract 25%	.830	.189
Extract 50%	.751	.002
Extract 75%	.983	.749
Ethyl Acetate Fraction 25%	.906	.405
Ethyl Acetate Fraction 50%	.814	.149
Ethyl Acetate Fraction 75%	.812	.144
Water Fraction 25%	.923	.463
Water Fraction 50%	.994	.853
Water Fraction 75%	.836	.203
n-Hexane Fraction 25%	.	.
n-Hexane Fraction 50%	.	.
n-Hexane Fraction 75%	.	.
K+	.996	.879
K-	.	.

Table 16. Homogeneity Test Results

Levene Statistics	df1	df2	Sig
15,471	13	28	0.00

Table 17. Kruskal-Wallis Test Results

Kruskal-Wallis	**39,050**
df	13
Asymp. Sig	0.000

DISCUSSION

Plant Determination

Plant identification aims to determine the accuracy and suitability of the name or type of plant used in the study. Plant identification is an important process because plants are used for research on medicinal raw materials, so using the right type of plant will produce more objective data [Roring *et al.*, 2017]. Pineapple plant identification was carried out at the UPT Herbal Materia Medica Batu Laboratory, Batu City, East Java. The results of this identification were that the plant used as a research sample was a pineapple plant with the species *Ananas comosus* (L.) Merr. which belongs to the Bromeliaceae family. The pineapple plants used in this study were obtained from a pineapple plantation in Blitar, East Java.

Production of Simplicia Powder

The pineapple fruit used in this study was the skin of the pineapple. The selected pineapple skin was greenish-yellow in color [Harmonyzha *et al.*, 2023]. The initial stage was wet sorting by cleaning the skin of any dirt, then washing it with running water until clean. Wet sorting and washing aim to remove impurities and other unnecessary plant parts while separating the plant from parts damaged or rotten by insects [Handoyo & Pranoto, 2020]. After washing, the pineapple skin was drained to remove any remaining water from the washing process [Wicaksono *et al.*, 2022].

The ingredients were then sliced to increase the surface area, thereby accelerating the drying process [Handoyo & Pranoto, 2020]. The thickness of the slices affects the quality of the medicinal plants. Thin slices can accelerate drying by increasing the surface area, but they risk damaging or reducing the amount of active compounds that are sensitive to temperature, light, humidity, and microbes, which can reduce efficacy, change the quality of the medicinal plants such as color, odor, and taste, and increase the risk of damage during storage [Widodo & Subositi, 2021].

Drying of the medicinal plants is carried out by drying them in direct sunlight by covering the chopped plants with a black cloth. This covering aims to reduce damage to active compounds due to direct exposure to sunlight. After drying, dry sorting is carried out, which is the separation of pineapple skin from unwanted materials [Wijaya & Noviana, 2022]. Next, the dried pineapple skin is ground into powder using a blender, then sieved with a no. 40 mesh sieve [Ramadhan *et al.*, 2024]. Powdering is carried out to increase the particle surface area so that contact between the medicinal plants and the solvent increases and the extraction process can take place effectively. The medicinal plants powder is sieved using a 40 mesh sieve to obtain a uniform particle size and separate the coarse powder parts [Handayani & Susiloningrum, 2025].

Based on the data in Table 1, pineapple skin with an initial weight of 11.86 kg after drying produced a final weight of 2.12 kg and a drying effectiveness of 17%.

Simplicia Standardization

Standardization is a process for ensuring the quality of the final product to consistently meet certain parameters that have been set consistently. Simplicia standardization is carried out by determining specific and non-specific parameters so that the resulting medicinal plant has reliable quality and active compound content [Wibowo & Amalia, 2024]. The specific parameters in this study include organoleptic testing. The non-specific parameters include determining water content and drying shrinkage test.

Organoleptic testing was conducted by observing the simplicia using the five senses, including color, smell, taste, and shape. Based on the results of the organoleptic test of the pineapple peel simplicia shown in Table 2, the results obtained were a distinctive pineapple-like odor, a pale yellow color, a bitter taste, and a fine, sand-like powder. This is in accordance with the research of Rosyantari *et al*. (2022), which found that pineapple peel powder has a distinctive pineapple aroma with a moderate odor intensity, a distinctive pineapple taste, is fine, pale yellow in color, and has a dry and fibrous texture with a slightly rough sand-like texture.

The water content of pineapple peel powder was determined by weighing 2 grams of pineapple peel powder. The water content test used a moisture balance at 105°C. The testing process ended when the device beeped. Based on the results of the three replicate water content tests of pineapple peel Simplicia, as shown in Table 3, the average water content was 8.59%. The water content test aims to determine the remaining water content after the drying process, and the results obtained meet the requirements of the Indonesian Herbal Pharmacopoeia, which is no more than 10%. Excessive water content can cause microbial growth, thereby reducing the stability and quality of the simplisia [Andini & Putri, 2021].

The drying shrinkage test was conducted by weighing 2 grams of pineapple peel powder and then heating it in an oven at 105°C for 30 minutes. Based on the results of the drying shrinkage test of pineapple peel powder in Table 4, the average was 9.8%, so the pineapple peel powder has met the requirements of no more than 10%. The drying shrinkage test aims to determine the amount of compounds that evaporate or are lost during drying and maintain the quality of the simplicia because it is related to the potential for microorganism growth [Handayani *et al.*, 2024].

Preparation of 96% Ethanol Extract from Pineapple Peel

Pineapple peel powder that has been standardized is then extracted using the maceration method, whereby 700 grams of pineapple peel powder soaked in 7 liters of 96% ethanol with a ratio of 1:10. Ethanol is a universal solvent because it can attract polar and nonpolar compounds and is relatively non-toxic so it is safe to use. 96% ethanol was chosen in this study because 96% ethanol is more selective and has good absorption power, evaporates easily and can produce a thick extract in a shorter time than 70% ethanol [Adriana *et al.*, 2024]. Maceration is carried out for 3 days with one stir, then filtered and the residue can be reused for re-maceration by soaking for 2 days. Next, filtering is carried out using flannel cloth. The results of maceration and re-maceration are filtered again using filter paper to obtain pineapple peel extract filtrate. The filtrate obtained is concentrated using a rotary evaporator at a temperature of 40-60°C to remove the solvent without damaging the active compounds. The evaporation process was stopped after the solvent had completely evaporated, which was indicated by the absence of solvent droplets in the solvent flask [Indrawan *et al.*, 2024][Ramadhan *et al.*, 2024].

Extract Standardization

Based on the results of the yield percentage of 96% ethanol extract of pineapple peel in Table 5, the percentage obtained was 16.86% and is included in good yield because it reaches more than 10%. A higher yield value indicates a greater value of the extract produced. The requirement for a thick extract yield according to the Indonesian Herbal Pharmacopoeia is not less than 10% [Badriyah & Farihah, 2022]. The calculation of the extract yield is carried out to determine the amount of extract obtained. The yield value is declared good if it reaches more than 10% [Saerang *et al.*, 2023]. In addition, the calculation of the yield value aims to determine the amount of secondary metabolites extracted by the solvent [Sari *et al.*, 2021].

Extract standardization is a process to ensure the quality of a herbal medicinal product to meet the specific and non-specific parameters required to ensure the herbal plant extract has consistent quality and active compound content that can be accounted for as a medicinal ingredient [Fitriana, 2023]. Organoleptic testing is one of the extract standardization and includes specific parameters. Organoleptic testing is carried out as an initial stage of extract identification through observation of shape, color, odor, and taste [Syarif *et al.*, 2022]. Based on the results of the organoleptic test of

pineapple peel extract shown in Table 6, the results obtained were a distinctive odor, dark brown color, bitter taste, and thick texture.

The water content test for the extract was carried out by weighing 2 grams of pineapple peel extract, then analyzing it using a moisture balance at 105°C, waiting for the device to beep to indicate the test was complete. Based on the results of the water content test for the pineapple peel extract in Table 7, an average of 6.07% was obtained, thus meeting the requirements for extract water content, which is less than 10%. Thick extracts have a water content of around 5-30%. The water content test is related to the purity and quality of the extract, because the higher the water content, the extract becomes more susceptible to deterioration and spoilage due to microbial growth. High water content can trigger the decomposition of active compounds through enzymatic reactions, so water content is a factor that determines the quality, stability, and formation of an extract preparation [Sambode *et al.*, 2022].

The loss on drying test is a nonspecific parameter used to determine the maximum amount of compounds that may evaporate or be lost during heating. [Mewar & As'ad, 2023]. The drying loss test was conducted with 2 grams of pineapple peel extract. The drying loss was carried out using an oven at 105°C for 30 minutes. Based on the results of the drying loss test for the fruit peel extract in Table 8, an average of 4.12% was obtained, thus meeting the requirements for the extract drying loss test, which is below 10%. High drying loss has the potential to cause degradation of active compounds and impact the quality of the extract [Fikayuniar *et al.*, 2023]. Drying loss is used as one of the extract quality parameters to maintain its quality and prevent fungal growth [Sambode *et al.*, 2022].

The ethanol-free test was conducted to ensure the extract did not contain residual ethanol solvent because ethanol has disinfectant properties so it does not cause false positives in the antibacterial activity test. The ethanol-free test was conducted using an esterification reaction with acetic acid and sulfuric acid on pineapple peel extract accompanied by heating [Adriana *et al.*, 2024]. Based on the results of the ethanol-free test in Table 9, it shows a positive result indicating that the pineapple peel extract is free from ethanol solvent because there is no ester (fruity) aroma [Achmad *et al.*, 2024].

Phytochemical Screening of Pineapple Peel Extract

Phytochemical screening aims to provide an initial overview of the chemical compounds contained in the extract. In this study, the compounds tested included alkaloids, flavonoids, saponins, steroids, and tannins [A'yuni *et al.*, 2024]. Phytochemical screening in this study used the test tube method, a simple and rapid method for phytochemical screening. Pineapple peel extract is placed in a test tube and certain reagents are added to detect the presence of chemical compounds, indicated by a color change or reaction [Amelia *et al.*, 2023].

Based on the phytochemical screening data in Table 10, it shows that pineapple peel extract contains several secondary metabolite compounds such as flavonoids, alkaloids, tannins, and saponins. Pineapple peel extract showed positive results in the flavonoid identification test, which was indicated by a change in the color of the extract solution to red, indicating that the pineapple peel extract contains flavonoid compounds. The addition of Mg and HCl aims to reduce the benzopyrone core in the flavonoid structure to form a flavylium salt that produces a red to orange color [Reiza *et al.*, 2019].

Alkaloid testing is performed using three reagents: Mayer, Wagner, and Dragendorff. A positive result is indicated by the formation of a white precipitate in Mayer's reagent, an orange to brown precipitate in Wagner's reagent, and an orange precipitate in Dragendorff's reagent. Before adding the reagents, the sample solution is first treated with HCl, which increases the solubility of the alkaloid by forming a water-soluble alkaloid salt. The addition of acid is also necessary because alkaloids are basic, making them more stable and easier to analyze in acidic conditions [Reiza *et al.*, 2019].

The principle of alkaloid testing is based on a precipitation reaction due to ligand exchange, where the nitrogen atom in the alkaloid that has a lone electron pair binds to a metal ion from the reagent. Dragendorff's reagent contains Potassium tetraiodobismuthate (III) or K[BiI4], Mayer's

reagent contains potassium tetraiodomercurate (II) or ($K_2[HgI_4]$), while Wagner's reagent consists of potassium iodide and iodine which form a brown triiodide ion. The precipitate formed is a potassium-alkaloid complex resulting from a coordination bond between the metal ion and the alkaloid nitrogen [Reiza *et al.*, 2019].

The results of the tannin test on the ethanol extract of pineapple peel are characterized by a color change in the solution to blackish green. This color change occurs because the Fe^{3+} ion from the $FeCl_3$ reagent binds to the tannin compound. This bond is formed through the O in the tannin, which has a free electron pair, thus forming a complex compound between Fe and tannin [Widiawati & Qodri, 2023].

The saponin test results on pineapple peel ethanol extract are characterized by the formation of foam when the solution is shaken. The addition of HCl aims to maintain the foam for 5-10 minutes. The formation of foam in saponins is caused by the presence of polar and nonpolar groups in their structure. When shaken with distilled water, saponins will form micelles, with the nonpolar groups on the inside and the polar groups facing the outside [Widiawati & Qodri, 2023].

The phytochemical screening results of pineapple peel extract for steroid compounds showed negative results, indicated by the absence of color changes or reactions after the addition of Liebermann-Burchard reagent. The results of the identification of steroid compounds are in line with the study of Reiza *et al.* (2019) on pineapple peel extract which also showed negative results for steroids, but in the study of Indrawan *et al.* (2024) which showed positive results. The difference in results may be caused by the limitations of phytochemical tests in detecting steroids in certain samples. In addition, environmental factors such as soil conditions, climate and plant growth location can affect the type and amount of secondary metabolites produced by plants [Rahmasiah *et al.*, 2023].

Pineapple Peel Extract Fractionation

Fractionation is the process of separating a mixture into simpler components. One commonly used technique is liquid-liquid partitioning, which separates chemical compounds that may interfere with quantification or detection while simultaneously focusing the active components in the sample. This process aims to obtain extract fractions that are purer and have higher activity [Suhaenah *et al.*, 2023]. Fractionation is carried out based on the polarity of the solvent. Nonpolar compounds tend to dissolve in nonpolar solvents, while polar compounds are more soluble in polar solvents [Faidah *et al.*, 2024].

Pineapple peel extract fractionation was carried out using solvents with different levels polarities, namely, n-hexane, ethyl acetate, and water, each with three replications. Based on the results of the percentage yield of pineapple peel fractionation in Table 11, the yield percentage was 30.6%, in the ethyl acetate fraction the yield percentage was 30.15% and the water fraction yield percentage was 32.1%. A total of 20 g of pineapple peel extract was fractionated with a solvent ratio of 1:10 n-hexane, ethyl acetate, and water using the liquid-liquid method with a separating funnel. The principle of fractionation is based on the difference in polarity and specific gravity between the two fractions. Fractionation produced three fractions, namely n-hexane, ethyl acetate, and water. The highest yield was obtained in the water fraction (32.1%), followed by the n-hexane fraction (30.6%) and the ethyl acetate fraction (30.15%). This difference is influenced by the ability of each solvent to extract compounds from the extract. The water fraction contains highly polar compounds such as sugars, glycosides, carbohydrates, and saponins that are well soluble in water, indicating that pineapple peel extract contains more polar compounds than semi-polar or nonpolar compounds [Suryanto *et al.*, 2017].

Identification of *Staphylococcus epidermidis* ATCC 12228 Bacteria

Before testing the antibacterial activity of pineapple peel extract against *Staphylococcus epidermidis* ATCC 12228 bacteria, the bacteria were first rejuvenated to reactivate the bacterial isolate so that their growth would be optimal. Bacterial rejuvenation was performed by streaking 1-2 loops of a pure bacterial culture in a zigzag pattern on slant agar medium, then incubated at 37°C for 24 hours. Bacterial rejuvenation was carried out using Nutrient Agar media, with 5 mL poured into each

test tube [Fitriani & Nashihah, 2021]. Nutrient Agar is the most frequently used culture medium, NA media is also used as a medium for storing bacteria [Chezar *et al.*, 2025]. In addition, NA media is also widely used for the growth and isolation of various microorganisms, especially microorganisms that do not require special nutritional conditions (non-fastidious) [Herdiansyah *et al.*, 2023].

Identification of *Staphylococcus epidermidis* ATCC 12228 bacteria was performed using Gram staining and microscopy. Gram staining is a method for classifying bacteria into Gram-positive and Gram-negative bacteria based on differences in the structure and composition of their cell walls. These differences can be observed through the bacterial response to crystal violet and safranin dyes. The Gram staining method uses several dyes that are tested in stages, namely crystal violet, iodine, alcohol as a decolorizer, and safranin as a counterstain. The final staining results show that Gram-positive bacteria are purple and Gram-negative bacteria are red [Damayanti *et al.*, 2024].

Gram staining of bacteria aims to ensure that the bacteria used in this study are safe, in good condition and not damaged or contaminated by other bacteria or fungi. This is proven by obtaining a pure isolate of *Staphylococcus epidermidis* ATCC 12228. Based on the results of microscopic observations in Figure 1, it shows that *Staphylococcus epidermidis* ATCC 12228 bacteria are purple, which indicates that *Staphylococcus epidermidis* ATCC 12228 bacteria are Gram-positive, which have a thick peptidoglycan layer as the main component of the cell wall so that the Crystal violet dye does not fade during the decolorization process with alcohol [Deswita *et al.*, 2021].

Antibacterial Activity Test of Pineapple (*Ananas comosus* (L.) Merr.) Peel Extract and Fraction Against *Staphylococcus epidermidis* ATCC 12228

The results in Table 12 show that the negative control of 1% DMSO did not form a clear zone, indicating that 1% DMSO did not affect the inhibition zone formed from the concentration of pineapple peel ethanol extract. The use of a negative control in this study aims to provide a comparison to the test substance and to ensure that 1% DMSO as an extract solvent does not provide antibacterial activity that can affect the formation of the inhibition zone [Fitriani & Nashihah, 2021].

Positive controls were used as a comparison to show the inhibition zone produced by antibacterials whose effectiveness has been known [Fitriani & Nashihah, 2021]. Ciprofloxacin disks were chosen as positive controls because they can inhibit the growth of Gram-positive bacteria such as *Staphylococcus epidermidis* ATCC 12228 [Senja *et al.*, 2024]. The results showed that the average diameter of the ciprofloxacin disk inhibition zone formed was ± 20 mm, proving that ciprofloxacin has strong inhibitory power. According to the Clinical and Laboratory Standards Institute (CLSI) (2020), 5µg of Ciprofloxacin was declared sensitive to *Staphylococcus epidermidis* ATCC 12228 because it produced an inhibition zone diameter of ≥ 21 mm [Hamka, 2023].

Based on Table 4.12, the results of the antibacterial activity test show that pineapple peel extract and ethyl acetate and water fractions can inhibit the growth of *Staphylococcus epidermidis* ATCC 12228 bacteria. Pineapple peel extract at concentrations of 25%, 50%, and 75% produced an inhibition zone diameter with an average of 5.63 mm, 8.96 mm, and 11.43 mm, respectively. This is different from the research of Setiawan (2018) where 96% ethanol extract of pineapple peel with concentrations of 25%, 50%, and 75% can inhibit *Staphylococcus epidermidis* with an inhibitory power of 13.9 mm, 16.7 mm, and 20.84 mm, respectively. This can be caused by environmental factors that can affect the growth and content of plant compounds. Although using the same plant, differences in the area of origin allow for variations in nutrition, water, light, temperature, oxygen, and humidity. These differences in conditions can affect the metabolic process and the content of secondary metabolites [Jungjunan *et al.*, 2023].

The diameter of the inhibition zone is not the only benchmark for determining the strength of the antibacterial activity of a test material. The size of the inhibition zone is not only influenced by the level of toxicity of the test substance, but can also be influenced by the ability and speed of the substance to spread in the medium, its interaction with other components, and the environmental conditions of the in vitro test [Hafizah *et al.*, 2024]. In addition, the extract contains a mixture of polar and nonpolar compounds, while the n-hexane fraction is dominated by nonpolar compounds such as

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triterpenoids and alkaloids. The ethyl acetate fraction contains more polar compounds such as flavonoids and tannins [Parapat *et al.*, 2025]. The more diverse the secondary metabolite content in a sample, the greater the resulting inhibitory power [Yuliana *et al.*, 2021].

The ability to inhibit bacteria varies from the three fractions, this is related to the differences in the chemical content of each fraction. Based on the results of the antibacterial activity test among the three most active fractions, ethyl acetate showed the highest antibacterial activity with the largest average inhibition zone value. At concentrations of 25%, 50%, and 75%, the ethyl acetate fraction produced inhibition zone diameters of 3.80 mm, 5.77 mm, and 7.55 mm, respectively. These results indicate that the higher the sample concentration, the greater the inhibitory effect on bacteria [Zuhra *et al.*, 2025]. The ethyl acetate fraction showed greater inhibition power than the n-hexane and water fractions, although the difference with the water fraction was not very significant. This is thought to be related to the more diverse content of active compounds in the ethyl acetate fraction [Nasution *et al.*, 2023]. Ethyl acetate can attract compounds with a wider range of polarity, ranging from polar to nonpolar compounds. Based on the principle of like dissolve like, the ethyl acetate fraction can attract secondary metabolite compounds, namely polar alkaloids, polar flavonoids, saponins that have polar and nonpolar groups, as well as other compounds such as tannins and triterpenoids that also have dual polarity characteristics [Sitepu *et al.*, 2022].

The low yield of the ethyl acetate fraction results in a small amount of dissolved active compounds, resulting in weak antibacterial activity due to the lack of attracted semipolar compounds. The low levels of semipolar compounds can be influenced by the drying method. Oven drying produces higher levels of total flavonoids and total phenols compared to air- and sun-drying. The oven method also provides a lower water content, thus better maintaining the content of active compounds. Air-drying can reduce the quality of active compounds due to exposure to oxygen, light, fluctuating temperatures, humidity, and UV rays. These conditions can cause oxidation and photodegradation of compounds such as flavonoids, vitamin C, and terpenoids, thereby reducing their biological activity. In addition, excessive humidity slows drying and encourages microbial growth, while low humidity can make the drug too dry and brittle [Farren *et al.*, 2025].

Meanwhile, the water fraction at concentrations of 25%, 50%, and 75% produced inhibition zone diameters of 2.35 mm, 4.11 mm, and 6.88 mm, respectively, with a weak to moderate category. The water fraction contains a polar water solvent known to attract secondary metabolites such as phenolics, saponins, flavonoids, and tannins. Differences in the size of the resulting inhibition zone can be influenced by the solubility properties of the active compound. An antibacterial compound's effectiveness in inhibiting bacterial growth can be influenced by the characteristics of the test bacteria, the concentration of the active substance, and the length of contact time. In addition, the following factors influence the size of the inhibition zone, such as the sensitivity of the microorganism, the type of culture medium, incubation conditions, and the rate of compound diffusion in the medium. The rate of compound diffusion in the medium is influenced by the concentration of the microorganism, the composition of the medium, the incubation temperature, and the incubation time [Huda *et al.*, 2022].

Based on the results of the antibacterial activity test of pineapple peel, the n-hexane fraction was unable to produce inhibitory zone. Based on the results of the phytochemical test, pineapple peel extract showed positive results against alkaloids and tannins, but negative results against steroid compounds. N-hexane is a non-polar solvent that has more stable and selective properties in extracting compounds that cannot be dissolved in polar solvents, such as oil. n-hexane is a non-polar solvent that is effective in attracting non-polar compounds, including steroids, terpenoids, carotenoids, and triterpenoids. Therefore, the n-hexane fraction cannot inhibit bacteria, allegedly because the antibacterial compounds that play a more dominant role are semi-polar or polar, and the amount of antibacterial compounds is insufficient to inhibit bacterial growth so that they are not optimally attracted by n-hexane [Sitepu *et al.*, 2022].

Flavonoids, alkaloids, tannins, and saponins are known to possess antibacterial activity with varying mechanisms of action. Flavonoids work by damaging bacterial cell walls or increasing their

permeability, disrupting bacterial metabolism. Alkaloids inhibit bacterial cell wall formation by disrupting the peptidoglycan structure, preventing proper cell formation. Saponins work by reducing the surface tension of bacterial cell walls, causing cell damage and rupture [Mewengkang *et al.*, 2022]. Tannins exhibit antibacterial activity by inhibiting peptidoglycan formation. As a result, the bacterial cell wall structure is not fully formed. This weakens bacterial cells and leads to lysis due to osmotic and physical pressure, ultimately killing the bacteria [Akbar *et al.*, 2022].

The results of the antibacterial activity test on pineapple peel extract using the dilution method show in Table 13 that at 25% and 50% concentrations the solution did not appear to form white lumps or membranes indicating no growth of *Staphylococcus epidermidis* ATCC 12228 bacteria. Meanwhile, at concentrations of 12.5% and 6.25% the solution appeared cloudy and contained lumps or membranes indicating the growth of *Staphylococcus epidermidis* ATCC 12228 bacteria. The antibacterial activity test of the ethyl acetate fraction of pineapple peel using the same method in Table 14 shows that the solution at a concentration of 50% appeared clear without the formation of lumps or white membranes. However, at concentrations of 25%, 12.5% and 6.25% the solution appeared cloudy and was accompanied by lumps or membranes, indicating growth.

Figure 2 shows the MIC and MBC results of pineapple peel extract and ethyl acetate fraction. However, the determination of the Minimum Inhibitory Concentration (MIC) using the dilution method has limitations because turbidity observations cannot distinguish between live and dead bacterial cells. In addition, the color of the concentrated solution can affect the observation results so that the accuracy is low, so further testing is needed by measuring the absorbance value using a UV-Vis spectrophotometer to obtain more accurate results [Lolongan *et al.*, 2016]. The results of the solid dilution test in Figure 3 show that no bacterial growth was found on the scratched Nutrient Agar (NA) media, indicated by the media remaining clear. This indicates that the concentration of 25% pineapple peel extract and 50% pineapple peel ethyl acetate fraction is able to kill *Staphylococcus epidermidis* ATCC 12228 bacteria.

Currently, there is no research on the MIC and MBC tests of pineapple peel extract and ethyl acetate fraction against *Staphylococcus epidermidis*. However, in a study by Indrawan *et al.* (2024), the MIC and MBC of pineapple peel extract against other Gram-positive bacteria, namely *Streptococcus mutans*, were able to inhibit bacterial growth at a concentration of 0.78%. This can be caused by two factors that can affect the quality of the extract, namely biological factors and chemical factors. Biological factors include the type and source of the plant, soil conditions, plant parts used, harvest time, and the storage process of the material. Chemical factors include the type of active compounds contained in the plant, the extraction method, the type of solvent used, and the dosage form of the extraction results [Dianawati & Manisha, 2023]. Differences in activity are thought to be caused by variations in the proportion and amount of active compounds contained in the extract that are not yet known [Manurung *et al.*, 2025].

Data analysis

The results of the normality test in Table 15 show that in the n-hexane fraction treatment group at concentrations of 25%, 50% and 75% have a Sig value <0.05, so it can be concluded that the data is not normally distributed. Based on the results of the homogeneity test in Table 16, a significance value (Sig) <0.05 was obtained, which indicates that the data has unequal or inhomogeneous variations. This can be caused by quite large differences in data variations between treatment groups, so that the data does not meet the requirements for analysis using the One Way ANOVA test. The analysis was continued with the Kruskal-Wallis non-parametric test to determine any significant differences between the test material groups (extract, n-hexane fraction, ethyl acetate fraction and pineapple peel water fraction as well as negative and positive controls) with the inhibitory power of *Staphylococcus epidermidis* ATCC 12228 bacteria [Tjiptoningsih *et al.*, 2023].

Based on the results of the Kruskal-Wallis test in Table 17, a Sig of 0.000 < 0.05 was obtained. These results are in line with research (Dewi & Arlita, 2021) which indicates a significant difference

in the average diameter of the inhibition zone between treatment groups, so that further tests can be carried out to determine which treatment group shows a significant difference.
Based on the results of the Pairwise test, not all treatment group pairs showed significant differences in average. Some treatment pairs had sig values <0.05, indicating a statistically significant difference, while other pairs showed significance values >0.05, so the average differences that occurred could not be stated as significant. For example, the 50% n-hexane fraction with 50% water had a sig value of 0.173 >0.05, indicating no significant difference. 50% n-hexane with 50% and 75% extracts each had a sig value of 0.002, indicating a significant difference. 25% water fraction with 25% ethyl acetate, 50% showed sig results >0.05, indicating no significant difference, but the 25% water fraction with 75% ethyl had a sig value of 0.00 <0.05, indicating a significant difference. Meanwhile, the 25% water fraction with 50% extract has a sig value of 0.026 < 0.05, indicating a significant difference. 25% n-hexane with 75% ethyl has a sig value of 0.012, indicating a significant difference.

CONCLUSION

This study showed that 96% ethanol extract of pineapple (*Ananas comosus* L. Merr.) peel and its fractions have antibacterial activity against *Staphylococcus epidermidis* ATCC 12228, with the extract showing the highest inhibition (average inhibition zone 5.63–11.43 mm at concentrations of 25–75%), followed by ethyl acetate (3.80–7.55 mm) and water (2.35–6.88 mm) fractions, while n-hexane fraction was inactive. The extract contains flavonoids, alkaloids, tannins, and saponins that contribute to inhibition through damage to bacterial cell walls. The MIC value for the extract was 25% and MBC 25–50%, while the MIC and MBC of the ethyl acetate fraction were 50%, proven by disc diffusion and dilution tests, with Kruskal-Wallis analysis confirming significant differences ($p<0.05$). Limitations include variations in environmental factors affecting the consistency of active compounds as well as the less accurate visual dilution method without UV-Vis spectrophotometry.

Practical implications include the potential development of natural skin antiseptic products from pineapple waste to address opportunistic infections, reduce antibiotic resistance, and support a circular economy. Further research recommendations include the isolation of pure compounds from the ethyl acetate fraction, in vivo toxicity testing, and evaluation of resistant clinical strains for broader clinical validation.

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