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Antibacterial Activity Test Of Extracts And Fractions*n*-Hexane, Ethyl Acetate, And Pineapple Peel Water (*Ananas Comosus*(L.) Merr.) Against Bacteria *Propionibacterium Acnes* ATCC 6919

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Abstract

Acne vulgaris is a common dermatological problem in adolescents in Indonesia's tropical climate related to the activity of Propionibacterium acnes bacteria. Long-term use of antibiotics risks causing resistance, so natural antibacterial alternatives are needed. This study aims to evaluate the antibacterial activity of ethanol extract and fractions of n-Hexane, ethyl acetate, and pineapple peel water against Propionibacterium acnes ATCC 6919, and to determine the most active fraction and MIC and MBC values. This study is a quantitative experimental study. The study population was the bacterial culture of P. acnes ATCC 6919, with samples in the form of pineapple peel extract and fraction. The research instruments included disc diffusion and liquid and solid dilution methods, while data analysis used the Shapiro-Wilk, Levene, and Kruskal-Wallis tests. The results showed that the ethanol extract had moderate to strong antibacterial activity, the ethyl acetate fraction showed the highest activity compared to other fractions, while the n-hexane fraction showed no activity. MIC and MBC values were obtained at a concentration of 20 percent. The conclusion of this study is that pineapple peel has the potential as a natural antibacterial source against P. acnes and the ethyl acetate fraction is the most active fraction.

Keywords: Acne Vulgaris, Antibacterial Activity, Ananas Comosus, Ethyl Acetate Fraction, Propionibacterium Acnes.

INTRODUCTION

Puberty is a crucial phase in adolescence, marked by the biological transition from childhood to adulthood due to hormonal changes, which significantly impact the skin, making adolescents more susceptible to dermatological problems such as acne (Oyopedo, 2020). In Indonesia, with its warm and humid tropical climate, skin conditions such as acne vulgaris are common, as they facilitate the growth of bacteria, parasites, and fungi, with a prevalence reaching 47-90% in adolescents aged 12-24 years (Wardani, 2020).

The skin as the body's outermost protector is composed of the epidermis, dermis, and subcutaneous, where the sebaceous glands and hair follicles are susceptible to blockage and infection, especially by *Propionibacterium acnes* (Dilla, 2023).

Acne vulgaris is caused by inflammation and blockage of the pilosebaceous duct due to the activity of P. acnes, a gram-positive anaerobic bacterium that produces lipase enzymes to break down lipids into free fatty acids, triggering inflammation and the release of pro-inflammatory cytokines (Sifatullah & Zulkarnain, 2021). The prevalence of acne is higher in males aged 16-19 years (95-100%) than in females (83-85%), and in Indonesia, tropical conditions exacerbate this infection (Wardani, 2020).

Conventional treatment uses antibiotics such as*ciprofloxacin* Broad-spectrum fluoroquinolones, but long-term use triggers increasing resistance, so safe alternatives from natural ingredients are needed (Dekotyanti, 2022).

Pineapple skin (*Ananas comosus* (L.) Merr.), organic waste rich in flavonoids, alkaloids, tannins, steroids, and saponins, has the potential as a natural antibacterial because flavonoids damage the cell walls of polar gram-positive bacteria such as P. acnes (Raudatul Patimah *et al*., 2021). Research shows that pineapple peel extract at a concentration of 4-8% produces an inhibition zone of 18.26-26.27 mm against P. acnes, more effective in the non-polar fraction (Nur Aeini Hartih, 2025).

However, there are not many studies on the specific activity of the fraction.*n*-Hexane, ethyl acetate, and water and the MIC/MBC values against strain ATCC 6919 (Zahrah *et al*., 2020).

This study aims to evaluate the antibacterial activity of extracts and fractions.*n*-Hexane, ethyl acetate, pineapple peel water against P. acnes ATCC 6919, identifying the most active fraction, and determining MIC and MBC; overcoming antibiotic resistance by utilizing abundant pineapple waste in Indonesia, and its novelty lies in testing specific fractions and standard strains for the development of natural topical drugs (Oyopedo, 2020).

RESEARCH METHODS

This research is quantitative experimental to test the antibacterial activity of ethanol extract and fraction of n-Hexane, ethyl acetate, and pineapple peel water (Ananas comosus (L.) Merr.) against Propionibacterium acnes ATCC 6919, which is measured through the inhibition zone, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) using the disc diffusion method and liquid and solid dilution (Sugiyono, 2023). The independent variables include the concentration of the fraction (30%, 40%, 50%), while the dependent variables are the diameter of the inhibition zone and the MIC/MBC value, with controlled variables including aseptic sterilization to prevent contamination (Sudaryono, 2022).

The main instruments include analytical scales, ovens, autoclaves, incubators, vacuum rotary evaporators, laminar air flow, micropipettes, and Nutrient Agar (NA) and Nutrient Broth (NB) media, with materials such as 96% ethanol, n-Hexane, ethyl acetate, 5% DMSO, and ciprofloxacin as positive controls. Testing techniques include standardization of simplex (organoleptic, water content <10%, drying loss), 96% ethanol maceration extraction, phytochemical screening (alkaloids, flavonoids, saponins, steroids, tannins), multilevel fractionation, and antibacterial tests with inhibition zone readings using the formula of the average horizontal and vertical diameters minus the disc diameter (Bryan *et al*., 2024).Data analysis used IBM SPSS version 25 with the Shapiro-Wilk normality test and Levene's Test of homogeneity; if normal ($p>0.05$), continued with One-Way ANOVA and post-hoc Tukey, while if not normal, used Kruskal-Wallis and pairwise comparison (Tjiptoningsih, 2023).

The study population was P. acnes ATCC 6919 bacterial culture from laboratory collection, with fresh pineapple peel simplicia samples taken from Ponggok District, Blitar Regency, East Java, after macro-microscopic determination at Batu Materia Medica Laboratory, the samples were made into dried simplicia with a 40 mesh sieve, extracted into 20 g of ethanol extract for fractionation (3 replications per fraction), and the bacterial suspension was adjusted to McFarland 0.5 (1.5 x 10^8 CFU/mL) for dilution test on 12 NA petri dishes per treatment.

The procedure begins with sampling pineapple peel, washing, drying, grinding, and standardization of the simplicia, followed by maceration of the simplicia 1:10 96% ethanol for 5 days, evaporation at 40-60°C until the extract is thick, as well as standardization tests of the extract (ethanol-free, water content, drying loss) and phytochemical screening. Fractionation was carried out in stages: 20 g of the extract was dissolved in ethanol-water, extracted with n-Hexane (non-polar), ethyl acetate (semi-polar), and water (polar) using a separating funnel (3x200 mL per solvent), evaporated in a 50°C water bath; the test solution was made 30-50% in 5% DMSO (Sugiarti *et al*., 2020; Kayogop, 2023). Antibacterial test: sterilization of equipment (oven 170°C/2 hours or autoclave 121°C/15 minutes), rejuvenation of NA slant bacteria 37°C/24 hours, Gram staining, McFarland suspension, NA streaking, placement of solution dip discs, incubation 37°C/24 hours for diffusion; serial liquid dilution 40%-5% NB + suspension for MIC (24 hours incubation, read cloudy/clear), and transfer to NA for MBC (Sandy *et al*., 2021).

RESULTS AND DISCUSSION

Plant Determination

Based on the results of the determination, it shows that the results of this determination are that the plants used as research samples are truly pineapple plants (Ananas comosus (L.) Merr.) The results of the determination of pineapple plants (Ananas comosus (L.) Merr).

Preparation of Simple Drugs

Table 1. Percentage of Pineapple Peel Simplex Yield

Information	Initial weight	Final weight	Percentage (%)
Taking materials	11,860 g		
Drying	11,860 g	2,120 g	17%
Pollination	2,120 g	1,900 g	89%

Standardization of Simple Drugs

Organoleptic test of simple drugs

Table 2. Organoleptic Test Results of Pineapple Skin (Ananas comosus (L.) Merr.)

Parameter	Results
Smell	Pineapple specialties
Color	Pale yellow
Flavor	A bit bitter
Shape	The powder is a bit coarse, like sand

Water content test of simple drugs

Table 3. Results of Water Content Test of Pineapple Peel Powder

Replication	Sample weight (grams)	Percentage (%)
I	2	7.8%
II	2	8.68%
III	2	9.28%
Average		**8.58±0.74**

Drying shrinkage test of simple drugs

Table 4. Results of Drying Shrinkage Test of Simplex

Test sample	Replication	Sample weight	Exchange rate weight before oven	Exchange rate weight + sample after oven	Percentage (%)
Powder	I		47.177 g	48.983 g	9.7%
	II	2 grams	41.527 g	43.328 g	9.95%
	III		45.369 g	47.174 g	9.75%
Average					**9.8±0.13**

Making Pineapple Peel Ethanol Extract

Table 5. Yield of Pineapple Peel Extract (Ananas comosus (L.) Merr.)

Powder weight	Extract weight	Percentage (%)
700 g	118 g	16.86%

Extract Standardization

Organole Testptik Extract

Table 6. Organoleptic Test Results of Pineapple Peel Extract

Parameter	Results
Smell	Pineapple specialties
Color	Brownish yellow
Flavor	Bitter
Shape	Keintal

Ethanol Free Extract Test

Table 7. Results of Ethanol-Free Test of Pineapple Peel Extract

Ethanol Free Test	Results	Reference
Pineapple peel extract (Ananas comosus (L.) Merr.) + H2SO4 + CH3COOH heated	+ No ester odor	Not tersmell the ester(Priamsari *et al.*, 2020)

Extract Drying Shrinkage Test

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

Test sample	Replication	Sample weight	Exchange rate weight before oven	Exchange rate weight + sample after oven	Percentage (%)
Extract	I	2 grams	50,440 g	52.368 g	3.6%
	II		43.898 g	45.786 g	5.6%
	III		47.836 g	49.773 g	3.15%
Average					**4.11±1.30**

Extract Water Content Test

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Table 9. Results of Water Content Test of Pineapple Peel Extract

Replication	Sample weight (grams)	Percentage (%)
I	2	5.03%
II	2	5.23%
III	2	7.95%
Average		**6.07±1.63**

Phytochemical Screening of Pineapple Peel Extract

Table 10. Phytochemical Screening Results of Pineapple Peel Extract

Compound	Reagent	Reference	Information	Results
Alkaloid	HCl + Mayer Water	The formation of a white precipitate indicates the presence of alkaloid compounds.(Kartikasari*eit.al.*, 2022).	White sediment	+
	Drageindroff + hydrochloric acid	The precipitate is brownish orange or brick red	Brownish orange sediment	+

Compound	Reagent	Reference	Information	Results
	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	+
Steroids	Liebeirman-Burchard	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	+

Pineapple Peel Extract Fractionation

Table 11. Results of Pineapple Peel Extract Fraction Yield

Solvent	Fraction Weight	Yield (%)
n-Heiksan	6.12	30.6%
E-ethyl acetate	6.03	30.15%
water	6.42	32.1%

Antibacterial Activity Test of Propionibacterium acnes

Gram staining

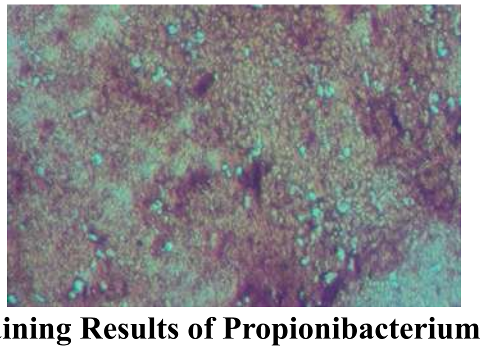

Figure 1. Gram Staining Results of Propionibacterium acnes ATCC 6919

Antibacterial Test Results Using the Diffusion Method

Table 12. Inhibition Zone of Propionibacterium acnes ATCC 6919

Test material	Concentration (%)	Zone Resistor (mm) I	II	III	Mean (mm)±SD
Extract	30%	7.7	8.1	7.9	7.9±0.2
	40%	9.9	10.6	10.9	10.3±0.5
	50%	11.0	11.8	11.6	11.4±0.4
***n*-Hexane**	30%	0	0	0	0±0
	40%	0	0	0	0±0
	50%	0	0	0	0±0
Ethyl	30%	2.7	3.0	2.8	2.8±0.1
	40%	5.2	5.4	5.5	5.3±0.1

Test material	Concentration (%)	Zone	Resistor	(mm)	Mean (mm)±SD
	50%	5.7	6.0	6.2	6.0±0.2
Water	30%	3.6	4.0	4.1	3.9±0.2
	40%	4.4	4.8	4.9	4.7±0.2
	50%	4.8	5.0	4.9	4.9±0.1
Ciprofloxacin	5 µg	27.5	28.8	29.8	28.7±1.1
DMSO	5%	0	0	0	0±0

Antibacterial Test Results Using the Liquid Dilution Method (MIC)

Table 13. Results of Antibacterial Activity Test by Liquid Dilution

Concentration	Results	information
30%	+	Jeirnih
20%	+	Jeirnih
10%	-	Turmoil
5%	-	Turmoil
Positive control	+	Jeirnih
Negative control	-	Turmoil

Figure 2. Antibacterial Activity Test of Liquid Dilution Extract (MIC)

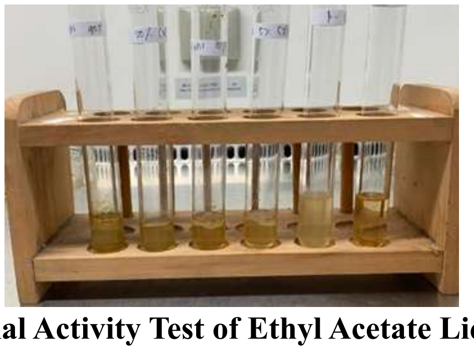

Figure 3. Antibacterial Activity Test of Ethyl Acetate Liquid Dilution (MIC)

Antibacterial Test Results Using the Solid Dilution Method (SDI)

Figure 4. Antibacterial Activity Test of Solid Dilution Extract (MBC)

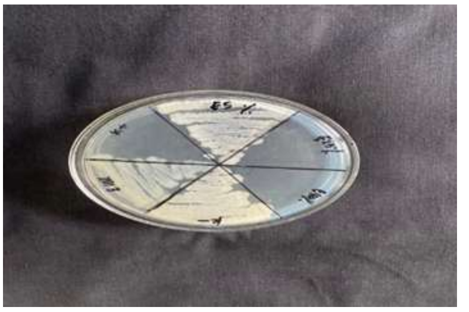

Figure 5. Antibacterial Activity Test of Solid Dilution Ethyl Acetate (MBC)

Data analysis

Table 14. Normality Test Results

Treatment group	Test statistics	sig
30% extract	1,000	1,000
40% extract	.949	.567
50% extract	.999	.927
30% ethyl	.775	.057
40% ethyl	.807	.132
50% ethyl	.987	.780
30% water	.983	.747
40% water	.993	.843
50% water	1,000	1,000
30% fn-hexane	.	.
40% fn-hexane	.	.
50% fn-hexane	.	.
k+	.994	.856
k-	.	.

Table 15. Results of Homogeneity Test

Variables	Test Statistics	Sig
Inhibitory force	10,484	0,000

Table 16. Kruskal Wallis Test Results

Variables	**Test Statistics**	**Sig**
Inhibitory force	**37,016**	**0,000**

DISCUSSION

This plant identification aims to determine the accuracy and suitability of the identity of a pineapple skin sample (Ananas comosus (L.) Merr.) that will be used in the research. The plant identification in this research was conducted at the Head of the Materia Medica Laboratory in Batu, East Java.

After determination, the crude drug was prepared. Pineapple (Ananas comosus (L.) Merr.) peel samples were taken from Ponggok District, Blitar Regency, East Java. After collecting the material, wet sorting was carried out to separate the pineapple peel from contaminants in the form of dirt attached to the pineapple peel (Ananas comosus (L.) Merr.). Next, the pineapple peel was washed thoroughly using running water and then drained. The cleaned pineapple peel was then sliced to the desired size to speed up the drying process. The drying process was carried out by drying in the sun and covered with a black cloth to protect the active compounds of the pineapple peel and accelerate drying because the black cloth is able to absorb heat. After the pineapple peel was completely dry, dry sorting was carried out to remove any dirt that might have been carried during the drying process. Pollination of crude drugs aims to increase the contact surface area between the crude drugs and the solvent, so that the solvent can penetrate the cell cavities of the crude drugs more optimally (Fatwami & Royani, 2023). The powdered simplicia was stored in a tightly closed glass jar and kept away from sunlight to maintain its quality. The results of the simplicia yield percentage in Table 1 showed a simplicia yield of >10%. This is in line with the research of Senduk *et al*. (2020) which stated that a good yield has a value above 10%. The high yield indicates the number of active components successfully extracted from the starting material.

Next, standardization of the simplicia was carried out, namely organoleptic testing, where the quality of the simplicia was observed based on observations of the five senses including smell, color, and shape. The organoleptic examination of the pineapple skin simplicia (Ananas comosus (L.) Merr.) was carried out at the Duta Bangsa University Laboratory in Surakarta. The pineapple skin simplicia was pale yellow, had a distinctive pineapple odor, a slightly bitter taste, and was in the form of a slightly coarse powder like sand.

The water content test of pineapple peel powder (Ananas comosus (L.) Merr.) obtained a result of 8.58 ± 0.74. The purpose of this test was to determine the maximum limit or range of the number of compounds lost during the drying process. The water content obtained was in accordance with quality requirements (≤ 10%) (Lestari, 2021). The main factor that influences the water content of the simplicia to meet the requirements is the drying method used, because the drying process is one of the post-harvest stages that has an important role in determining the quality of the simplicia (Lestari *et al*., 2023). Water content that is too high can facilitate the growth of microorganisms in the powder, thus potentially reducing the quality stability in the standardization process of pineapple peel simplicia (Ananas comosus (L.) Merr.) and causing physical changes such as decay (Putri, 2021). However, if the water content is too low, the pineapple peel simplicia can become brittle easily and this can have an impact on reducing the quality of the active compounds contained therein (Halimatushadyah *et al*., 2024).

The drying loss obtained was 9.8±0.13. These results meet 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, material stability, and the reliability of test results. The application of temperatures higher or lower than this temperature has the potential to affect and reduce the accuracy and consistency of the analysis results (Yambesei *et al*., 2025). 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 (Azis *et al*., 2024).

It can be seen that the yield percentage of 96% ethanol extract of pineapple peel (Ananas comosus (L.) Merr.) is 16.86%. The yield calculation was carried out to determine the amount of extract produced from the fresh simplicia used. The results showed that the amount of yield obtained was influenced by the type of solvent used (Ardiansa *et al*., 2024). The solvent used for the extraction process of secondary metabolites in pineapple peel was 96% ethanol, where this solvent has the ability to extract with wide polarity so that it can extract secondary metabolites that are polar, semipolar, and even nonpolar (Azis *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 40–60 °C, with the aim of evaporating the solvent at a temperature that is not too high so that the process takes place more safely and avoids damage to the sample (Yon Haryanto, 2023). 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 yield exceeding 10% is considered good (Rahadyana *et al*., 2024).

Next, extract standardization is carried out, namely organoleptic testing in the form of assessing the quality of the extract based on observations of the five senses which include smell, color, taste, and shape. Organoleptic examination of pineapple peel extract (Ananas comosus (L.) Merr.) was carried out at the Laboratory of Duta Bangsa University, Surakarta. The ethanol-free test showed that the ethanol extract of pineapple peel (Ananas comosus (L.) Merr.) was free from its solvent, namely 96% ethanol, which was shown to have no detectable ester odor so that it could be declared positive as not containing ethanol. The next test was the drying loss obtained at 4.11%. This value has met the requirements, which stipulate that the drying loss of an extract should not exceed 10%. Determination of the water content in the extract was carried out to determine the amount of water content in the extract, which is related to the level of purity and the possibility of contamination. In addition, this test aims to inhibit microbial growth that can affect the quality and shelf life of the extract. The test results showed that the water content of pineapple peel extract (Ananas comosus (L.) Merr.) was 6.07%. This value meets the water content requirements for thick extract, which is less than 10% (Rahadyana *et al*., 2024).

Based on the phytochemical screening results presented in Table 10, the ethanol extract of pineapple peel (Ananas comosus (L.) Merr.) is known to contain various secondary metabolite compounds, namely alkaloids, flavonoids, tannins, saponins, and steroids. This finding is consistent with research by Raudatul Patimah *et al*. (2021) which showed that pineapple peel extract contains compounds such as flavonoids, alkaloids, tannins, steroids, and saponins. One frequently used qualitative phytochemical screening technique is the color reaction test using specific reagents. In this method, a natural material sample is reacted with a specific reagent, resulting in a characteristic color change if it contains certain secondary metabolites. Certain reagents can be used to identify flavonoids, alkaloids, tannins, saponins, and other compounds. The resulting color change can indicate the presence of these compounds in the sample (Razoki *et al*., 2023).

Alkaloid testing results showed that pineapple (Ananas comosus (L.) Merr.) peel extract showed positive alkaloids using Mayer, Dragendorff, and Wagner reagents. The principle of this method is based on the occurrence of a precipitation reaction due to the ligand exchange process. Nitrogen atoms in alkaloid compounds, which have lone electron pairs, can replace iodo ions in the reagent (Kartikasari *et al*., 2022).

In the flavonoid test of pineapple peel extract (Ananas comosus (L.) Merr.), the identification results showed a change in the color of the solution to red, which indicates that the ethanol extract of pineapple peel positively contains flavonoid compounds. In the testing stage, the extract solution was heated for approximately 2–3 minutes because most flavonoids can dissolve in hot water. After the heating process, the solution was then added with HCl and Mg powder. The addition of Mg powder and HCl reduced the benzopyrone core in the flavonoid structure to form a red or orange flavyllium salt (Reiza *et al*., 2019).

Pineapple peel extract (Ananas comosus (L.) Merr.) showed positive results for saponins, which are considered positive if the foam formed when shaken persists for several minutes. The presence of foam indicates the presence of glycosides that can form bubbles in water, allowing them to be hydrolyzed into glucose and its aglycone compounds (Jusna & Nasrudin, 2022). The results of steroid testing on pineapple peel extract (Ananas comosus (L.) Merr.) showed negative results. This is due to the nonpolar nature of steroid compounds, which cannot be extracted properly using ethanol as a solvent (Kartikasari *et al*., 2022).

Tannin testing on pineapple (Ananas comosus (L.) Merr.) peel extract showed a positive result, indicated by the appearance of a blackish-green color. This color change occurs due to the addition of $FeCl_3$, which reacts with one of the hydroxyl groups in the tannin compound, resulting in a reaction that causes the color change (Pratiwi *et al*., 2023).

Pineapple peel extract fractionation results: the percentage of yield calculation from the n-hexane fraction was 30.6%, the ethyl acetate fraction was 30.15%, and the water fraction was 32.1%. The different yields of each fraction were related to the many types of compounds contained in the pineapple peel extract (Ananas comosus (L.) Merr.). The fractionation results showed that the water fraction was obtained in greater quantities than the n-hexane and ethyl acetate fractions. This was due to the volatile nature of the n-hexane and ethyl acetate solvents so that during the evaporation process there was a reduction in the solvent volume which resulted in low fraction yields obtained (Maryam *et al*., 2024).

Gram staining is a technique used to classify bacteria based on their characteristics into Gram-positive and Gram-negative bacteria. Gram-positive bacteria have cell walls with a thick peptidoglycan layer and no lipoprotein or lipopolysaccharide layer. In contrast, Gram-negative bacteria have cell walls with thinner peptidoglycan and are coated by a lipoprotein or lipopolysaccharide layer (Fenti Nurmailah *et al*., 2024). Based on Figure 1, the Gram staining results show that the bacteria appear purple because they are able to retain the primary dye, namely crystal violet, which binds to the thick peptidoglycan layer. In addition, the bacteria have a morphology in the form of branching filaments or a combination of rod-shaped/filament shapes arranged in irregular groups. These characteristics indicate that Propionibacterium acnes ATCC 6919 belongs to the Gram-positive bacteria group.

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

In the disc method, the antibacterial activity of a sample is indicated by the formation of a clear zone around a paper disc placed on an agar medium, indicating an area where bacterial growth is inhibited. The sample concentrations tested were 30%, 40%, and 50%. Ciprofloxacin was used as a positive control, while 5% DMSO served as a negative control. Based on the data in Table 12, the negative control, 5% DMSO, did not show any inhibition zone formation. The 5% DMSO used as a negative control is a solvent that has no ability to inhibit or support the growth of microorganisms. Furthermore, DMSO is also used as a solvent because of its ability to penetrate quickly into the extract without causing damage to the extract (Martha *et al*., 2024).

A positive control test was conducted to compare the diameter of the inhibition zone produced by the extract and fraction of n-hexane, ethyl acetate, and water of pineapple (Ananas comosus (L.) Merr.) peel with the antibiotic ciprofloxacin. The test results showed that ciprofloxacin produced an inhibition zone diameter against Propionibacterium acnes bacteria with an average value of more than 20 mm. This indicates that the inhibitory activity of ciprofloxacin is classified as very strong (Weinda *et al*., 2023).

The results of the antibacterial activity test showed that pineapple peel extract (Ananas comosus (L.) Merr.) was able to inhibit the growth of Propionibacterium acnes ATCC 6919 bacteria, which was indicated by the formation of an inhibition zone around the disc. Based on the data in 4.12, it can be seen that the increase in sample concentration is directly proportional to the magnitude of the inhibition power produced in each group. The results of the 30% concentration extract inhibition power were 7.988 mm (categorized as moderate), followed by 40% concentration of 10.521 mm and 50% 11.63 mm (categorized as strong). This is because pineapple peel extract (Ananas comosus (L.) Merr.) has the ability to inhibit the growth of Propionibacterium acnes ATCC 6919 bacteria. This is because the extract still contains a complete mixture of secondary metabolites found in pineapple peel which function as antibacterials.

Of the three fractions tested, the ethyl acetate fraction was the most active fraction compared to the n-hexane fraction and the water fraction against Propionibacterium acnes ATCC 6919 bacteria with an average inhibition zone diameter of 2.87 mm at a 30% concentration (weak), while at 40% and 50% concentrations, the inhibition zone diameters were 5.33 mm and 6.03 mm, respectively (moderate category). Ethyl acetate is a semipolar solvent capable of dissolving 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 inhibition power compared to other fractions.

Antibacterial activity tests on water fractions at concentrations of 30%, 40%, and 50% showed weak antibacterial activity, with concentrations of 3.9 mm, 4.7 mm, and 4.9 mm, respectively. This is because the water fractions contain antibacterial compounds in very small amounts, making them insufficient to inhibit or kill bacteria (Kristianto *et al*., 2020).

The antibacterial activity test results for 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 (Kusumastuti *et al*., 2021).

The results of bacterial testing by dilution of pineapple peel extract and ethyl acetate fraction (Ananas comosus (L.) Merr.) showed that the test solutions with concentrations of 40% and 20% appeared clear, indicating no bacterial growth. In contrast, the test solutions with concentrations of 10% and 5% appeared cloudy with the formation of a white membrane, indicating the growth of Propionibacterium acnes ATCC 6919. The Minimum Inhibitory Concentration (MIC) was determined from the lowest concentration that remained clear, namely at a concentration of 20%. The determination of the MIC value was carried out using several concentration variations. The antibacterial activity test using the dilution method used samples that had a minimum value (MIC) obtained from the diffusion test, namely the ethyl acetate extract and fraction. The presence of bacteria was indicated by the formation of turbidity in the liquid medium, while antibacterial activity was indicated by the medium remaining clear (Shina *et al*., 2024).

In the solid dilution test, it is expected that the concentrations of 40% and 20% which have been proven to be able to inhibit the growth of Propionibacterium acnes ATCC 6919 bacteria in the media remain clear, which indicates that the extract test solution with a concentration of 40% and 20% is able to kill Propionibacterium acnes ATCC 6919 bacteria. Minimal 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 incubated for 24 hours at 37°C. MBC is determined as the lowest concentration in NA media where there is no growth of Propionibacterium acnes ATCC 6919. The test results show that in a petri dish containing NA media that is scratched with the test solution at a concentration of 40% and 20%, Propionibacterium acnes bacteria do not grow at a concentration of 40% and 20%, which is indicated by the media remaining clear. Meanwhile, at concentrations of 10% and 5%, bacteria were still able to grow, as seen by the appearance of spots on the media. At a concentration of 20%, Propionibacterium acnes ATCC 6919 no longer showed growth, indicating that this concentration was effective in killing Propionibacterium acnes bacteria. The Minimum Killing Concentration (MBC) was set at 20%.

The next data analysis was the normality test. In this study, the normality test using the Shapiro–Wilk method was chosen because the number of samples was less than 50. Based on the analysis results, it was known that the data were not normally distributed, indicated by a significance value of 1.000 > 0.05 and 0.000 < 0.05. Because there was one data that did not meet the normality criteria, the normality assumption using the Shapiro–Wilk method was declared not met because there were data that showed a normal distribution and did not show a normal distribution.

The homogeneity test is a prerequisite for analysis (e.g., ANOVA) that must be met before further statistical analysis. The homogeneity test results in Table 15 show a value of <0.05, indicating that the data between groups do not have equal variance. This likely occurs due to significant differences in the data within the sample, as data with lower variance tends to have a higher degree of homogeneity.

Based on the results of the Kruskal–Wallis test listed in Table 16, the Asymp. Sig. value was 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 treatment group pairs had statistically significant mean differences. Some treatment pairs showed significance values greater than the established limit ($\alpha = 0.05$), so the mean difference between the groups could not be declared significant. For example, the comparison between 30% extract and 50% water resulted in a significance value of 0.354 (>0.05), indicating that the inhibitory power of the two treatments was not significantly different.

Furthermore, the 30% extract and 50% extract, the 30% extract and 40% ethyl acetate, and the 30% extract and ciprofloxacin pairs also showed significance values above 0.05. This indicates that the inhibitory power produced by the 30% extract was relatively equivalent to that of all the comparison groups, so there was no statistically significant difference.

Furthermore, comparisons between 50% ethyl acetate with 50% extract, 50% ethyl acetate with ciprofloxacin, and 50% extract yielded significance values of 0.329, 0.157, and 0.662, respectively. These values also exceed 0.05, so it can be concluded that the three treatment pairs have relatively similar average inhibitory power and are not significantly different.

CONCLUSION

This study concluded that the ethanol extract of pineapple peel (Ananas comosus (L.) Merr.) has moderate to strong antibacterial activity against Propionibacterium acnes ATCC 6919 with an average inhibition zone of 7.9-11.4 mm at a concentration of 30-50%, while the ethyl acetate fraction is the most active (2.8-6.0 mm, weak to moderate category), followed by the water fraction (3.9-4.9 mm, weak), and the n-Hexane fraction is inactive; the MIC and MBC values at a concentration of 20% for the extract and ethyl acetate fraction, supported by the content of flavonoids, alkaloids, saponins, and tannins that damage the cell walls of gram-positive bacteria. These findings are consistent with previous studies that show the potential of pineapple peel as an alternative antibiotic to overcome resistance in acne vulgaris in Indonesia's tropical climate.

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