ANTIBACTERIAL ACTIVITY OF 50% AND 96% ETHANOL EXTRACT OF HORN BANANA PEEL (Musa acuminata x Musa balbisiana) AGAINST Propionibacterium acnes

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ABSTRACT

Propionibacterium acnes is the main bacterium responsible for acne, with over 50% of its strains resistant to synthetic drugs. Treatment with natural substances, such as horn banana peel extract, can be an alternative. The polarity of the solvent, influenced by ethanol concentration, affects the compounds extracted and their antibacterial activity. This study aims to identify secondary metabolite compounds in 50% and 96% ethanol extracts of horn banana peel, evaluate the antibacterial activity of both extracts against *Propionibacterium acnes*, and determine the differences in antibacterial activity based on variations in solvent concentration. The banana peel extract was obtained using the *Microwave Assisted Extraction* (MAE) method. Phytochemical screening was conducted using the tube test method. Antibacterial activity was assessed using the well diffusion method. The 50% ethanol extract of horn banana peel had a higher yield (16,12%) compared to the 96% ethanol extract (10,02%). Both extracts contained active compounds such as alkaloids, flavonoids, tannins, polyphenols, and saponins. The 50% ethanol extract at concentrations of 5%, 10%, and 15% exhibited greater antibacterial activity against *Propionibacterium acnes*, with average inhibition zones of 18,68±1,32 mm, 14,11±0,35 mm, and 11.07±0.32 mm, respectively. In contrast, the 96% ethanol extract showed average inhibition zone diameters of 15,17±1,33 mm, 11,35±0,92 mm, and 8,06±0,86 mm. The differences in antibacterial activity are believed to be due to the varying polarities of the two solvents, with 50% ethanol being the more polar solvent. *Two-way* ANOVA analysis revealed a significant difference between the two factors (ethanol concentration and extract concentration) concerning the inhibition zones (*p-value* 0,004). The results indicate that both 50% and 96% ethanol extracts of horn banana peel contain secondary metabolite compounds such as alkaloids, flavonoids, tannins, polyphenols, and saponins. Both extracts exhibit antibacterial activity against *Propionibacterium acnes*, with a significant difference in activity between the 50% and 96% ethanol extracts.

Keywords: Horn banana peel, antibacterial, Microwave Assisted Extraction, Propionibacterium acnes.

INTRODUCTION

One of the infectious diseases caused by bacteria is acne. *Propionibacterium acnes* is the main bacterium responsible for acne vulgaris. This gram-positive bacterium produces lipase, which breaks down triglycerides and sebum into free fatty acids. The accumulation of this bacterium leads to inflammation and the formation of comedones, which are integral to the development of acne (Williams *et al*., 2019).

Acne significantly impacts adolescents and those concerned about their appearance. It not only causes aesthetic issues but can also lead to mental health problems, as individuals often receive negative comments from others. Approximately 30-50% of acne sufferers are reported to experience psychological issues such as anxiety and low self-esteem (Veronica *et al*., 2020).

The two most common treatments for acne are topical treatments, which are applied directly to the affected areas for localized effects, and oral treatments, which address acne systemically. Infections caused by *Propionibacterium acnes* can generally be treated with antibiotics. However, antibiotic resistance is on the rise, with many countries reporting that over 50% of *Propionibacterium acnes* strains are resistant to antibiotics (Madelina and Sulistiyaningsih, 2018). As an alternative to antibiotic treatments for acne caused by *Propionibacterium acnes*, the utilization of plants or plant parts with phytochemicals that have potential antibacterial properties might offer a viable solution (Shintawati, 2023).

One city known for its banana production is Lumajang, which is nicknamed the "Banana City." One of the distinctive types of banana from Lumajang, Malang, and Trenggalek is the horn banana. Chips made from horn bananas are a popular product in the region. However, the production process generates banana peel waste that has not been optimally utilized and has low market value (Zuhroh, 2016).

Several studies have indicated that banana peels can inhibit bacterial growth. Previous research showed that ethanol extract from kepok banana peels (*Musa balbisiana*) contains alkaloids, flavonoids, saponins, tannins, and quercetin, which exhibit antibacterial properties against *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Propionibacterium acnes* (Kasminah, 2016). Other studies have demonstrated that banana peels can inhibit other bacteria, such as *Escherichia coli* (Rizqiyah, 2021). Additionally, previous research revealed that ethanol extract from horn banana peels contains secondary metabolite compounds such as alkaloids, tannins, flavonoids, saponins, and polyphenols, which can inhibit antibacterial activity against *Propionibacterium acnes* (Shintawati, 2023).

In prior studies, antibacterial activity tests were conducted on 70% ethanol extracts from horn banana peels against *Propionibacterium acnes* (Shintawati, 2023). Previous research utilized ethanol concentrations of 50%, 70%, and 96% to demonstrate the influence of varying ethanol concentrations on the levels of polarity (Mubarak *et al*., 2018). The polarity of a solution is determined by the ethanol concentration, where higher ethanol concentrations result in lower solvent polarity (Noviyanti, 2016). The polarity of the solvent will affect the compounds that are extracted and, ultimately, will influence antibacterial activity.

Based on the above description, researchers are encouraged to conduct a study on the extraction of horn banana peels using 50% and 96% ethanol solvents against the primary bacteria causing acne, *Propionibacterium acnes*. This study aims to identify the secondary metabolite compounds in the 50% and 96% ethanol extracts of horn banana peels, evaluate the antibacterial activity of both extracts against

Propionibacterium acnes, and determine the differences in antibacterial activity based on the variations in solvent concentration used, with extraction employing the *Microwave Assisted Extraction* (MAE) method using 50% and 96% ethanol solvents.

MATERIAL AND METHODS

The tools utilized in this research include an autoclave, oven (Memmert), rotary evaporator (IKA), Biological Safety Cabinet (Biobase), incubator (Memmert), vortex mixer (Ohaus), microwave (Sharp), magnetic stirrer, micropipette (Dragon Lab), analytical balance (Sojikyo), blender, mesh sieve (40), UV-VIS spectrophotometer (Shimadzu), caliper, cork borer, 500 mL beaker (Pyrex), 100 mL graduated cylinder (Pyrex), Petri dishes, stirring rod, glass funnel (Iwaki), 500 mL Erlenmeyer flask (Iwaki), 10 mL volumetric flask (Iwaki), 5 mL volumetric flask (Iwaki), vials, test tubes (Iwaki), test tube rack, dropper pipette, 1 mL measuring pipette, ball pipette, L-shaped rod, ose wire, cuvette, Petri dishes, and Bunsen burner.

The materials used in this research include banana peel, Propionibacterium acnes (ATCC® 11828™), 50% ethanol (Merck), 96% ethanol (Merck), nutrient agar (Merck), Mueller-Hinton agar (Merck), 10% DMSO (Merck), clindamycin 1%, Mayer's reagent, Dragendorff's reagent, 70% ethanol (Merck), 2N HCl, Liebermann-Burchard reagent, distilled water, 1% BaCl, 1% H2SO4, sterile NaCl, FeCl3, spirit, filter paper, cotton, sterile gauze, aluminum foil, and brown paper.

Extraction of Horn Banana Peel

The horn banana peel was washed with running water and drained. The simplicia was cut and then dried for four days in an oven at 40ºC. After drying, it was blended into a fine powder and sieved using a mesh size of 40 (Fitriahani, 2017).

The powdered simplicia was then extracted using a solvent ratio of 1:10, with 50 grams of dried simplicia and 500 mL of ethanol. The extraction process employed *Microwave Assisted Extraction* (MAE) for five minutes at a temperature of 40ºC. Subsequently, the extract was filtered through filter paper and evaporated using a rotary evaporator at 50ºC. The extraction was replicated three times (Rejeki *et al*., 2020).

Phytochemical Screening

The content of secondary metabolite compounds was identified using test tube methods. In this study, a blank sample was used as a comparison, prepared by dissolving 50% and 96% ethanol extracts of banana peel in 2 mL of distilled water. The phytochemical screening aimed to test for alkaloids, flavonoids, saponins, tannins, polyphenols, terpenoids, and steroids.

Alkaloid Test

The alkaloid test was conducted using two reagents: *Dragendorff's* reagent and *Mayer's* reagent. An extract of 0,5 grams was placed in a test tube and dissolved in hydrochloric acid (HCl), followed by the addition of 2-3 drops of *Dragendorff's* reagent. A positive result for alkaloids with *Dragendorff's* reagent is indicated by a red color accompanied by a precipitate. For *Mayer's* reagent, 0,5 grams of the extract was similarly dissolved in HCl and treated with 2-3 drops of Mayer's reagent. A positive

result for alkaloids is marked by a yellow or orange color accompanied by a precipitate (Tiwari *et al*., 2017).

Flavonoid Test

An extract weighing 0,5 grams was placed in a test tube, to which 2 mL of 70% ethanol was added, followed by the addition of 5-6 drops of concentrated HCl. A red color indicates the presence of flavonoids, while an orange color indicates the presence of flavones (Tiwari *et al*., 2017).

Saponin Test

In this test, 0,5 grams of extract were mixed with 2 mL of distilled water until fully combined. The mixture was then shaken vigorously. A positive result is indicated by the formation of stable foam with a height of 1-3 cm that remains constant for 10 minutes (Saraswati, 2015).

Polyphenol Test

For the polyphenol test, 0,5 grams of extract were added to 3 mL of warm water. Subsequently, 1-2 drops of 1% $FeCl_3$ were added to the extract. A color change to dark blue or greenish-black indicates the presence of polyphenolic compounds (Saraswati, 2015).

Tannin Test

In the tannin test, 0,5 grams of extract were combined with 3 mL of warm water, followed by the addition of 1-2 drops of 1% $FeCl_3$. A color change to dark blue or greenish-black indicates the presence of tannins (Saraswati, 2015).

Terpenoid and Steroid Test

For the terpenoid and steroid test, 0,5 grams of extract were placed in a test tube, and 2-3 drops of Liberman-Burchard reagent were added. A color change to blue or green indicates the presence of steroids, while a purple-red color indicates the presence of terpenoids (Tiwari *et al*., 2017).

Media Preparation

Nutrient Agar (NA) for bacterial rejuvenation is prepared by dissolving 20 grams of NA media in 1 liter of distilled water on a magnetic stirrer. Once completely dissolved, the media is poured into several test tubes, and the mouths of the test tubes are covered with sterilized gauze wrapped around cotton. The media are placed vertically in a beaker for sterilization using an autoclave (Katrin *et al*., 2015).

Mueller Hinton Agar (MHA) for antibacterial growth is prepared by dissolving 34 grams of MHA media in 1 liter of distilled water in an Erlenmeyer flask on a magnetic stirrer. The solution is then sterilized in an autoclave (Shelin *et al*., 2023).

Sterilization of Equipment and Media

Equipment is first cleaned by washing and drying items such as Erlenmeyer flasks, measuring cylinders, test tubes, Petri dishes, and beakers. The bacterial growth media and equipment are then sterilized for 15 minutes in an autoclave at a temperature of 121°C. The sterilized equipment is placed in an oven along with additional tools such as L rods, inoculating loops, and tweezers, which are wrapped in

aluminum foil. The oven sterilization is carried out for 30 minutes at a temperature of 180°C to remove moisture from the equipment and to sterilize metal instruments (Wulandari *et al*., 2022).

Rejuvenation of *Propionibacterium acnes*

The rejuvenation of *Propionibacterium acnes* is performed by streaking bacteria from a pure culture using one inoculating loop onto the surface of slant agar. The test bacteria are grown on *Nutrient Agar* (NA) media and incubated for 24 hours at 37°C (Shelin *et al*., 2023).

Preparation of *McFarland* 0,5 Solution

The *McFarland* 0,5 solution is commonly used to compare the turbidity of bacterial cultures in liquid media, corresponding to a density between 1 x 10^7 cells/mL and 1 x 10^8 cells/mL. In distilled water, 0,05 mL of barium chloride ($BaCl_2$) is added to 9,95 mL of sulfuric acid (H_2SO_4) and then stored in a light-protected area (Aviany *et al*., 2020).

Preparation of Bacterial Suspension

One inoculating loop of rejuvenated test bacteria is suspended in 10 mL of physiological saline in a sterilized test tube and homogenized for 15 seconds using a vortex mixer. After that, turbidity is measured by visually comparing it to the McFarland 0,5 standard, and the density is quantified using a UV-Vis spectrophotometer with a wavelength of 625 nm (Rosmania and Yanti, 2020).

Antibacterial Activity Test

The concentrations of 50% ethanol and 96% ethanol extracts of banana peel used in this study were 5%, 10%, and 15%. In this study, the negative control was 10% DMSO, while the positive control was 1% clindamycin. The well diffusion method was employed for testing in this research. A total of 100 µL of *Propionibacterium acnes* bacterial suspension was introduced into a sterile *Mueller-Hinton Agar* (MHA) petri dish and spread evenly using a sterile spreader. Subsequently, wells measuring 6 mm in diameter were created using a punch. The ethanol extracts of banana peel at different concentrations were then added to the wells with 20 µL using a sterile micropipette. The petri dishes were incubated at 37°C for 24 hours to allow for the diffusion of the compounds. The inhibition zones were measured using a caliper (Shintawati, 2023). This study included five replicates for each concentration of the ethanol solvent.

Data Analysis

The diameter of the inhibition zones was analyzed using *two-way* ANOVA to compare the samples resulting from two factors: the concentration of solvent (50% ethanol and 96% ethanol) and the concentration of extract (5%, 10%, and 15%) on the inhibition results. This was followed by Duncan's test to compare all combinations of treatment involving the solvent concentrations (50% ethanol and 96% ethanol) and extract concentrations (5%, 10%, and 15%) to assess significant differences among them.

RESULT AND DISCUSSION

Extraction of Horn Banana Peel

The horn banana peel used in this study was young peel, characterized by its green color and relatively hard texture. The powdered simplicia of the horn banana peel was extracted using the *Microwave Assisted Extraction* (MAE) method with solvents of 50% and 96% ethanol. The extraction yielded a thick extract with a brownish-black color. This is attributed to the high content of phenolic compounds, such as tannins, present in young horn banana peels. These phenolic compounds typically exhibit dark colors ranging from brown to black and are soluble in polar solvents like ethanol.

Subsequently, the yield of the extract was calculated to determine the amount of active compounds present in the extracted material, as well as to establish the ratio of the amount of extract obtained to the initial weight of the powdered simplicia. Data on the thick extract and yield can be seen in Table 1.

The study found an average thick extract using 50% ethanol solvent of 8,06 grams, while the extract using 96% ethanol yielded an average of 5,01 grams. The average yield percentage of the 50% ethanol extract from horn banana peel was 16,12%, whereas the average yield percentage of the extract using 96% ethanol was 10,02%. The yield percentage of the 50% ethanol extract from horn banana peel was higher than that of the 96% ethanol extract.

Data analysis using the *independent sample t-Test* yielded a *p-value* (Sig.) of less than 0,05, specifically 0,000. This indicates a significant difference between the yield of the 50% ethanol extract from horn banana peel and the yield of the 96% ethanol extract.

The high yield of the ethanol extract from horn banana peel, exceeding 10%, can be attributed to several factors. First, the high content of active compounds in the horn banana peel significantly contributes to the weight or yield of the obtained extract. Another factor is the effectiveness of the solvent used; 50% ethanol has been shown to be more effective in extracting polar compounds from horn banana peel. With higher polarity and better penetration ability, 50% ethanol optimizes the extraction process, resulting in a higher yield compared to 96% ethanol. Additionally, careful processing and concentration of the extract can preserve the active compounds contained in the horn banana peel, contributing to an increased yield. The combination of these factors—high active compound content, effective extraction with a polar solvent, and optimized processing—enables the ethanol extract yield from horn banana peel to exceed 10%. This is supported by previous research, which indicated that a 70% ethanol extract from horn banana peel had an average yield value of 10,3% (Shintawati, 2023).

The findings indicate that the 50% ethanol extract from horn banana peel has a higher yield than the 96% ethanol extract. This difference is influenced by the polarity of the solvent, with 50% ethanol exhibiting higher polarity due to its higher water content. This higher polarity allows 50% ethanol to extract and dissolve more polar compounds from young horn banana peel, resulting in a higher extract yield (16,12%) compared to 96% ethanol (10,02%). Previous studies have also shown that a 50% ethanol extract from rose apple leaves yielded a higher percentage than extracts with 70% and 96% ethanol (Syamsul *et al*., 2020). Additionally, other research has

demonstrated that a 50% ethanol extract from cassava peel had a greater yield compared to 70% and 96% ethanol extracts (Uzma *et al*., 2023).

Plant type, extraction technique, temperature, time, and solvent type are several variables that can influence yield values (Tamrin, 2022). Apart from the type of solvent used in the extraction process, the concentration of the solvent can alter its polarity, resulting in different extract yields. The total amount of extract obtained can vary depending on the solvent's polarity and its ability to dissolve the compounds in the material being extracted (Riwanti *et al*., 2020).

The yield percentage of the 50% ethanol extract from horn banana peel is greater than that of the 96% ethanol extract. This indicates that the secondary metabolites present in horn banana peel are relatively easily soluble in ethanol at lower concentrations. A 50% ethanol solution, comprising equal parts ethanol and water, is a more polar solvent compared to 96% ethanol. Water effectively attracts all polar active compounds, while ethanol extracts all polar properties of the active compounds. The active compounds in the sample significantly contribute to the weight or yield of the obtained extract (Kristiani and Susanti, 2020).

Phytochemical Screening

The content of secondary metabolites was identified using a tube method, where testing was conducted on the extraction results to ensure that the compounds remained intact after filtration and evaporation processes were completed. Each phytochemical screening test included a blank and was replicated three times for both the 50% and 96% ethanol extracts of horn banana peel to identify the results of the phytochemical screening. The nominal data from the phytochemical screening results, replicated three times, are generally considered an initial approach that can provide some indications about the consistency of the results. The results of the phytochemical screening for the 50% and 96% ethanol extracts of horn banana peel can be seen in Table 2.

Based on the research findings, both the 50% and 96% ethanol extracts of horn banana peel tested positive for alkaloids, flavonoids, saponins, tannins, and polyphenols, while testing negative for terpenoids and steroids.

This study indicates that the 50% and 96% ethanol extracts of horn banana peel contain similar active compounds, with the presence of compounds known to possess potential antibacterial activity. The difference in ethanol concentration during extraction did not significantly affect the types of compounds present, but it may influence the quantity or relative concentration of each compound. Previous research supports that ethanol extracts from horn banana peel contain active compounds, including alkaloids, polyphenols, flavonoids, tannins, and saponins (Shintawati, 2023).

Variability in phytochemical screening results can be attributed to several factors, such as the type of solvent used in the sample extraction, the amount of extract used, and the visualization method chosen (Fajriaty *et al*., 2018).

The solvents used in this study are classified as polar solvents. The polarity of 50% ethanol is higher than that of 96% ethanol due to the fact that 96% ethanol contains only 4% water, while 50% ethanol contains 50% water in its mixture. Water has a high dielectric constant of 80,1 making it a very strong polar solvent. The dielectric constant of 50% ethanol is also higher at 48,1. In contrast, the dielectric constant of 96% ethanol is only 18,75; while it is still considered a polar solvent, it is not

as polar as 50% ethanol due to its lower water content. The polarity of a solvent is influenced by the molecular structure of the ethanol itself. The degree of polarity of a solvent is expressed by its dielectric constant (Uzma *et al*., 2023). The results of the phytochemical screening demonstrate that the extracts of horn banana peel contain polar secondary metabolites, such as alkaloids, flavonoids, saponins, polyphenols, and tannins (Suryandari and Kusumo, 2022).

Steroid compounds are classified as non-polar, which explains why they are not attracted to polar solvents (Kholidah, 2020). Previous studies have shown that phytochemical screening of ethanol extracts from various types of bananas, including kepok, mas, and nangka banana peels, did not reveal the presence of steroid compounds (Rahmi *et al*., 2021). Other research has indicated that the ethanol extract of kepok banana peel also lacked steroid content (Nababan *et al*., 2022). However, this finding contrasts with another study that reported positive results for steroid content in n-butanol extracts of local banana peels. This discrepancy may arise because n-butanol is a non-polar solvent capable of attracting non-polar compounds such as steroids (Ananta *et al*., 2018).

Previous research has indicated that banana peels contain tannins at a concentration of 4.97% (Hudiansyah *et al*., 2015). Other studies have found that banana peels contain tannins at a concentration of 3.8% (Anhwange *et al*., 2009). Overall, these research findings suggest that banana peels indeed contain a significant amount of tannins, with percentages ranging from approximately 3.5% to 4.9%. The substantial presence of tannins in banana peels highlights their potential as a source of natural materials that can be utilized for antibacterial purposes.

Antibacterial Activity Test

The bacterium used in this study was *Propionibacterium acnes* (ATCC® 11828™). The test bacterium was first rejuvenated to restore its metabolism and obtain an active bacterial isolate, ensuring optimal growth and preventing contamination. During the rejuvenation process, the bacteria were incubated for 24 hours before being suspended. The bacterial suspension was prepared using 0,9% physiological saline, which maintains the ionic balance of the microbial cells and supports bacterial viability. The turbidity of the bacterial suspension was measured and compared to the *McFarland* standard of 0,5 (Rosmania and Yanti, 2020).

The antibacterial activity test required a *McFarland* standard of 0,5 as a turbidity standard for the bacterial suspension, ensuring that the suspension remained within a specific range. The turbidity of *McFarland* 0,5 was compared with the bacterial suspension using a UV-Vis spectrophotometer at a wavelength of 625 nm. Table 3 shows the absorbance of *McFarland* 0.5 and the bacterial suspension at a value of 0,086. The readings at a wavelength of 625 nm yielded absorbance values of 0,08 – 0,1, which is equivalent to a bacterial count of 1,5 x 10^8 CFU/mL (Rosmania and Yanti, 2020).

The antibacterial activity test was conducted using the well diffusion method, which involved five treatments observed in this study: 50% ethanol extract and 96% ethanol extract of horn banana peel at concentrations of 5%, 10%, and 15%, clindamycin at 1% as a positive control, and DMSO at 10% as a negative control. The data on the inhibition zones can be seen in Table 4.

The results for the 50% ethanol extract of horn banana peel showed an average inhibition zone diameter of 11,07±0,45 mm at a concentration of 5%. At 10% concentration, the average inhibition zone diameter increased to 14,11±0,35 mm. Further, at 15% concentration, the average inhibition zone diameter was 18,68±1,32 mm. In the positive control group (K+), the average inhibition zone diameter was 22,10±1,34 mm, while no inhibition zone was observed in the negative control group (K-). For the 96% ethanol extract of horn banana peel, the average inhibition zone diameter at 5% concentration was 8,60±0,86 mm. At 10% concentration, the average inhibition zone diameter was 11,35±0,92 mm, and at 15% concentration, it was 15,17±1,33 mm. The average inhibition zone diameter for the positive control (K+) was 20,64±1,74 mm, while no inhibition zone was formed in the negative control (K-).

Table 4 illustrates the inhibition zone diameters resulting from the application of the 50% and 96% ethanol extracts of horn banana peel. Statistical analysis indicated a significant difference between the positive control and the various concentrations of the 50% and 96% ethanol extracts of horn banana peel (5%, 10%, and 15%). The positive control demonstrated a significant difference, producing the largest inhibition zone against *Propionibacterium acnes* compared to the negative control and the variations in extract concentrations (5%, 10%, 15%). Clindamycin, as a pure antibiotic, exhibited stronger antibacterial activity than the active compounds in the horn banana peel extracts. The extracts are known to contain various active compounds with different mechanisms of action, which means their effectiveness in inhibiting *Propionibacterium acnes* is not as strong as that of clindamycin, which has a specific mechanism of action. The anti-inflammatory mechanism of topical antibiotics involves suppressing leukocyte chemotaxis and reducing pro-inflammatory free fatty acids on the skin surface. The negative control in this study did not exhibit any antibacterial activity, as 10% DMSO is not bactericidal (Rizki *et al*., 2021).

Table 4 shows that the concentration of the extract affects the diameter of the inhibition zone obtained. As the concentration of the 50% and 96% ethanol extracts of horn banana peel increases, the inhibition zone also becomes larger. Higher concentrations allow more active compounds to interact with the bacteria, enhancing their effectiveness in inhibiting bacterial growth and activity. This is supported by previous research, which stated that extract concentration is a crucial factor influencing antibacterial activity; the inhibitory effect increases with higher concentrations (Trisia *et al*., 2018).

In this study, the 50% ethanol extract of horn banana peel demonstrated a larger average inhibition zone compared to the 96% ethanol extract. The significant difference in polarity between 50% and 96% ethanol plays a crucial role in the observed inhibition zone values. This is why the 50% ethanol extract exhibited a larger inhibition zone compared to the 96% ethanol extract in antibacterial activity tests. Polar compounds in the horn banana peel extract, such as alkaloids, flavonoids, saponins, polyphenols, and tannins, are better extracted using 50% ethanol, resulting in greater antibacterial activity.

It is likely that the inhibition zones formed are due to the secondary metabolites contained in the 50% and 96% ethanol extracts of horn banana peel, which function to inhibit *Propionibacterium acnes*. The phytochemical screening results for both the 50% and 96% ethanol extracts revealed the presence of flavonoids, alkaloids, tannins,

polyphenols, and saponins. These compounds possess different mechanisms of action as antibacterials.

Polyphenols act as antibacterials by altering the permeability of bacterial cell membranes. This disruption interferes with bacterial cell formation and growth. Previous studies have demonstrated that banana peel extracts contain polyphenolic compounds capable of inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* (Fitriahani, 2017).

Tannins are the most abundant components found in horn banana peel. They exert their antibacterial effects by binding to proteins on bacterial cells. This binding forms H+ ions, creating an acidic environment. Such acidic conditions can denature proteins and inactivate enzymes within bacteria, disrupting metabolism and potentially leading to bacterial cell death. Several studies have shown that banana peel extracts contain tannins with antibacterial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Propionibacterium acnes* (Saraswati, 2015).

Flavonoids disrupt membranes and form complex interactions with cell walls, thereby inhibiting the growth of *Propionibacterium acnes*, which is a Gram-positive bacterium (Narulita, 2017). Previous research indicates that flavonoids are highly effective in halting the growth of bacteria, viruses, and fungi (Fitriahani, 2017).

Alkaloids interfere with the components of peptidoglycan in bacterial cells, preventing the formation of intact cell walls and resulting in cell death. Phytochemical screening from previous research has shown that the horn banana peel extract contains alkaloids that act as antibacterials (Shintawati, 2023).

Saponins can damage bacterial cell membranes. The disruption of the bacterial cell membrane due to saponin exposure results in plasma membrane rupture, loss of cytoplasm, and disruption of substance transport and metabolism. This condition can inhibit growth and even cause bacterial cell death. Previous studies have also supported the notion that saponins exhibit bacteriostatic properties or act as antimicrobial agents (Saraswati, 2015).

In the *two-way* ANOVA analysis conducted, the results indicated that for the ethanol concentration variable, the *p-value* (Sig.) was 0,000, which is less than 0,05. This suggests a significant difference in the inhibition zone values influenced by varying ethanol concentrations. Additionally, for the extract concentration variable, the *p-value* (Sig.) also indicated a value of 0,000, indicating a significant difference in the inhibition zone values influenced by differences in extract concentrations. Furthermore, the *two-way* ANOVA results showed a *p-value* (Sig.) of 0,004 for the interaction between ethanol concentration and extract concentration. This indicates an interactive effect between ethanol concentration and extract concentration on the inhibition zone values. Thus, both ethanol concentration and extract concentration have significant individual effects on the inhibition zone values, and there is also an interaction effect between these two factors on determining the inhibition zone values.

Based on the analysis results, it can be concluded that both ethanol concentration and extract concentration significantly influence the inhibition zone values for the growth of *Propionibacterium acnes*. To observe specific differences among treatment groups, post-hoc testing using Duncan's test was performed.

From Table 5, it is evident that the 50% ethanol extract at concentrations of 5%, 10%, and 15% shows significant differences compared to the 96% ethanol extract at equivalent concentrations. At a 5% extract concentration, the 50% ethanol extract had

an average inhibition zone diameter of 11,07±0,45 mm, while the 96% ethanol extract only achieved 8,60±0,86 mm. This difference is statistically significant. Similarly, at an extract concentration of 10%, the 50% ethanol extract produced an inhibition zone with an average diameter of 14,11±0,35 mm, whereas the 96% ethanol extract only measured 11,35±0,92 mm. This difference is also significant according to Duncan's test. Even at the highest extract concentration of 15%, the 50% ethanol extract showed an average inhibition zone diameter of 18,74±1,32 mm, while the 96% ethanol extract only reached 15,17±1,33 mm. This considerable difference is also statistically significant.

Overall, the 50% ethanol extract demonstrated stronger inhibitory activity against the growth of *Propionibacterium acnes* compared to the 96% ethanol extract at the same extract concentrations. This difference is statistically significant at a 95% confidence level.

CONCLUSION

Based on the results of the conducted research, it can be concluded that both the 50% and 96% ethanol extracts from horn banana peel (*Musa acuminata x Musa balbisiana*) contain secondary metabolites, specifically alkaloids, flavonoids, tannins, polyphenols, and saponins. Furthermore, the 50% and 96% ethanol extracts of horn banana peel exhibit antibacterial activity against *Propionibacterium acnes*. Notably, there is a significant difference in the antibacterial activity between the 50% and 96% ethanol extracts of horn banana peel.

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TABLE

Table 1. Results of 50% and 96% Ethanol Extracts of Horn Banana Peel

Concentration Solvent	Powder (grams)	Replication	Thick Extract (grams)	Average ± SD (grams)	Yield (%)	Average ± SD (%)
50% Ethanol	50	1	8,38	8,06±0,37	16,76	16,12±0,74
		2	8,16		16,32	
		3	7,65		15,3	
96% Ethanol	50	1	4,63	5,01±0,38	9,26	10,02±0,77
		2	5,01		10,02	
		3	5,4		10,8	

Table 2. Results of Phytochemical Screening

Compound	Reagent	Test Results		Conclusion	
		50% Ethanol	96% Ethanol	50% Ethanol	96% Ethanol
Alkaloids	*Dragendroff* and *Mayer*	The color change to red accompanied by precipitation indicates a positive result for Dragendorff's reagent, while the color change to yellow accompanied by precipitation indicates a positive result for Mayer's reagent.	The color change to red accompanied by precipitation indicates a positive result for Dragendorff's reagent, while the color change to yellow accompanied by precipitation indicates a positive result for Mayer's reagent.	(+)	(+)
Flavonoids	70% Ethanol and HCl 2N.	The color change to red.	The color change to red.	(+)	(+)
Saponins	Aqueous and HCl 2N.	The formation of stable foam with a height of 1-3 cm that remains constant for more	The formation of stable foam with a height of 1-3 cm that remains constant for more	(+)	(+)

		than 10 minutes.	than 10 minutes..		
Polyphenols	$FeCl_3$	The color change to dark green.	The color change to dark green.	(+)	(+)
Tannins	$FeCl_3$	The color change to dark green.	The color change to dark green.	(+)	(+)
Steroids	*Liberman-Burchard*	No color change.	No color change.	(-)	(-)
Terpenoids	*Liberman-Burchard*	No color change.	No color change.	(-)	(-)

Notes
(+) Contains chemical compounds from horn banana peel
(-) Does not contain chemical compounds from horn banana peel

Table 3. Results of *McFarland* 0.5 Solution and Bacterial Suspension Measurements

Solution	Absorbance
Mc Farland 0,5	0,086
Suspension of *Propionibacterium acnes* bacteria	0,086

Table 4. Results of Inhibition Zone Diameter Data for 50% and 96% Ethanol Extracts of Horn Banana Peel Against *Propionibacterium acnes* Bacteria.

Diameter of Clear Zone for *Propionibacterium acnes* (mm)						
Sample	**Replication**	**Extract Concentration**			**Control Group**	
		5%	**10%**	**15%**	**K+**	**K-**
50% Ethanol Extract of Horn Banana Peel	1	11,57	14,19	18,89	20,61	0
	2	10,93	14,30	19,25	22,42	0
	3	10,53	13,60	16,58	20,79	0
	4	10,81	13,93	18,82	23,22	0
	5	11,53	14,53	20,17	23,49	0
	Average ± SD	**11,07±0,45**	**14,11±0,35**	**18,68±1,32**	**22,10±1,34**	**0**
	Replication	**Extract Concentration**			**Control Group**	
96% Ethanol Extract of Horn Banana Peel		**5%**	**10%**	**15%**	**K+**	**K-**
	1	9,81	12,71	17,48	23,14	0
	2	8,53	11,22	14,96	18,77	0
	3	7,63	11,17	14,25	19,92	0
	4	9,07	11,54	14,91	21,57	0
	5	7,99	10,12	14,28	19,62	0
	Average	**8,60±0,8**	**11,35±0,**	**15,17±1,**	**20,64±1,**	**0**

± SD	**6**	**92**	**33**	**74**	

Table 5. Average Inhibition Zone Diameter Results for 50% and 96% Ethanol Extracts of Horn Banana Peel Against *Propionibacterium acnes*

Concentration Solvent	**Average Inhibition Zone Diameter (mm)**					
	5%	**10%**	**15%**	**K+**	**K-**	**Average**
Etanol 50%	11,07±0,45 c	14,11±0,35 d	18,74±1,32 e	22,10±1,34 g	0[a]	13,20±7,80
Etanol 96%	8,60±0,86[b]	11,35±0,92 c	15,17±1,33 d	20,60±1,74 f	0[a]	11,14±7,09

Note: Notations with different letters indicate a significant difference.