

ANTIBACTERIAL ACTIVITY TEST OF BANANA PEEL ESSENTIAL OIL AGAINST ESCHERICHIA COLI (E. COLI) BACTERIA IN BIOCHEMISTRY LABORATORY

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ABSTRACT

A study on the antibacterial activity of essential oil from banana peel was conducted. The research was conducted in a span of two months starting from sample collection, sample distillation process, and testing the antibacterial activity of essential oil. This study was conducted to determine the antibacterial activity of banana peel essential oil as a natural antibacterial against Escherichia coli (E. coli) bacteria considering the resistance of synthetic antibacterials to pathogenic bacteria. Isolation of essential oil in banana peel using steam distillation method and antibacterial activity test using disc paper method. The distilled essential oil was purified using anhydrous sodium sulphate (Na_2SO_4) to separate the oil from water and other impurities without affecting the purity of the essential oil. The results of the antibacterial activity test of banana peel essential oil against Escherichia coli (E. coli) bacteria with a concentration of 50% obtained an inhibition zone of 10.5 mm and at a concentration of 25% of 9.1 mm including the medium category. More than 50% of banana peel essential oil is thought to be more effective because at that concentration level, antibacterial compounds that have potential as antibacterial agents have reached sufficient levels and banana extract can be used in making hand sanitizer.

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1. INTRODUCTION

The use of antibacterials revolutionised the medical world and became an important milestone in safeguarding human health from deadly bacterial infections. The existence of antibacterials facilitates the treatment of various diseases, from simple to complex infections. However, over time, the extensive and inappropriate use of antibacterials has led to the problem of antibacterial resistance. Bacteria that were previously easy to destroy have now developed the ability to withstand antibacterials. This rapid growth of resistance occurs due to evolutionary selection triggered by the excessive and inappropriate use of antibacterials. Bacteria that were intrinsically sensitive to antibacterials have now evolved into antibacterial-resistant strains.

Antibacterial resistance is a major threat that can cause fatal consequences for human health, so it is necessary to conduct scientific studies and explore effective antibacterial agents as innovative solutions. The development and search for antibacterial substances from traditional plants can be a solution to overcome synthetic antibacterials that are resistant to pathogenic bacteria. Antibacterial resistance to pathogenic bacteria can be caused by excessive use so that its function is no longer effective. According to (Nurjanah et al., 2020), it was proven that 43% of *Escherichia coli* (*E. Coli*) bacteria were resistant to antibacterials from 2,494 people spread throughout Indonesia. The increasing resistance of *E. coli* bacteria to synthetic antibacterials has urged the research world to explore the diversity of flora as an alternative source of antibacterial compounds.

Further research into natural antibacterials from plants is becoming very important amidst the increasing failure of conventional antibiotic therapy (Nurjanah et al., 2020). With proper exploration, plants have the potential to be a valuable alternative source in developing new therapies against resistant bacterial infections. In addition to providing new hope in medicine, the use of natural antibacterials also supports a more environmentally friendly and sustainable approach to medicine. Thus, the development of antibacterials from plants can be an effective strategy in combating the growing antibacterial resistance crisis. So recently, scientists' interest in the use of plants for natural chemicals has grown and is of interest to investigate. Antibacterial substances in natural materials can be found in several plant parts, one of which is banana peel. Limitations in the effectiveness of synthetic antibacterials have prompted further research into natural materials suggesting that we still have a limited understanding of the antibacterial potential contained in banana peel essential oil.

Banana (*Musa* sp.) is one of the tropical plants with high production levels in Indonesia (Wikantika, 2021). Bananas can grow and bear fruit at an optimal temperature of around 29 - 30°C. In general, banana peels are discarded without further utilization, even though they have potential in the development of medicines due to their bioactive components and abundant availability. This study aims to explore the antibacterial effectiveness of certain terpenoid compounds present in banana peel essential oil as innovative antibacterial agents. Banana peels contain phenolic compounds that are useful as antibacterial, antioxidant, anti-fungal, and anti-inflammatory agents (Ecevit et al., 2022).

Based on research conducted by (Ida Ayu Raka Astiti Asih, Wiwik Susanah Rita, I Gusti Bagus Teguh Ananta, 2018), *Bacillus cereus*, *Salmonella enteritidis*, *E. Coli*, *Bacillus subtilis*, *Staphylococcus aureus* can be inhibited by using ethyl acetate extract of Clavendis banana (*Musa*, AAA cv. Cavendish) with an inhibition diameter between 9 - 12 mm. The content of antibacterial compounds in banana peel, there are other compounds that still have to be further investigated for antibacterial activity, namely terpenoid compounds in banana peel essential oil (Elangovan & Mudgil, 2023). Banana peels contain one of the typical compounds that are by-products of plant secondary metabolism, namely essential oils. Essential oils can be obtained through the steam distillation method. Research on essential oil as an antibacterial by (Hanifah, 2018), showed that essential oils from temulawak, patchouli, and clove leaves have potential as antibacterials and are effective in inhibiting bacterial growth. Banana fruits that have been consumed have their peels discarded without further utilisation. This is a great potential for the development of medicines from waste considering that banana peel waste can be utilised as an antibacterial. The essential oil content in banana peels can be used as a natural antibacterial substitute for long-term use of

synthetic antibacterials. Long-term use of synthetic antibacterials can cause adverse effects on human health.

Although previous studies have investigated the antibacterial properties of banana peels, comprehensive research on terpenoid compounds is still lacking, leading to a considerable knowledge gap at present. Given the high content of antibacterial compounds in banana peel and the increasing antibacterial resistance, it is necessary to test the antibacterial activity of banana peel essential oil against *E. coli* bacteria, and then the utilization of banana peel essential oil can be realized through natural products, namely hand sanitizers. This research brings a new dimension by focusing on terpenoid compounds present in banana peel essential oil, which have the potential to change the paradigm in antibacterial treatments and address the growing challenge of bacterial resistance. In brief, this study explores the antibacterial possibilities of terpenoid compounds present in banana peel essential oil, with the aim of providing valuable insights into the development of novel antibacterial agents that can address the growing challenge of bacterial resistance.

2. METHOD

2.1. Material

The materials used in this study are 20 kg dried banana fruit peels as sample, water as solvent, sodium sulfate (Na_2SO_4) anhydrous to purify essential oils, nutrient agar (NA), nutrient broth (NB), *Escherichia coli* bacteria (FMIPA UNSRI Laboratory) as test bacteria, aluminum foil, cotton, 96% ethanol as an active ingredient in hand sanitiser products, glycerol and aloe vera as a moisturiser in hand sanitiser products, banana fruit peel essential oil and tetracycline (PT. Novapharin Pharmaceuntical) as positive control in antibacterial test. The equipment used in this study were steam distillation circuit, 20 kg balance, analytical balance (Ohaus), glassware, 500 mL separatory funnel (Pyrex) for efficient and accurate separation of the aqueous and volatile oil phases, petri dish (Pyrex), bunsen to ensure cleanliness and sterility during the antibacterial activity test process, micropipette, LAF (Laminar Air Flow) to create a clean, sterile, and controlled work environment, ose needle, L-blade, paper disc (Whatman), tweezers, autoclave, incubator, oven, and caliper.

2.2. Pretreatment of Banana Peel Samples

Banana peel samples were dried at room temperature without direct sunlight for several days. Then the banana peel samples were weighed to ensure that the banana peel samples were perfectly dry when a constant sample weight was obtained. The results of the weighing were then cut into pieces of 3-5 cm in size. The cut samples were then stored at room temperature under closed conditions (Aryani et al., 2020). The isolation of banana peel essential oil was carried out using the steam distillation method. The use of steam distillation methods in essential oil isolation can provide a number of significant advantages in terms of efficiency, quality, and process safety. The steam distillation method can produce essential oils with a high level of purity compared to other methods and is cheap because the solvent used is water. The isolation of 20 kg of banana peel is expected to produce 8.5 mL of essential oil. A total of 20 kg of dried banana fruit peels

were put into the material container in the distillation device which had been filled with water. Distillation was carried out at a pressure of 40 barr with a temperature of 120°C for 6-8 hours. Collect the distillation results and separate the essential oil obtained using a 500 mL separatory funnel. Purification of the obtained essential oil is done using anhydrous Sodium sulfate (Na_2SO_4) to remove impurities in the oil. The purified essential oil was then put into a dark glass bottle with a lid and stored at a temperature of 10°C.

2.3. Antibacterial Activity Test of Banana Peel

In testing the antibacterial activity of banana peel essential oil, equipment sterilization was carried out. Instrument sterilisation prevents the possibility of cross-contamination between test samples and other materials or microorganisms that could interfere with experimental results. It also protects the researcher from exposure to pathogenic microorganisms that can cause infection or disease. Sterilization of tools is done by using an autoclave with a temperature of 121°C at high pressure for 15 minutes (Alda et al., 2022). The bacterial culture media used are NA, NB, and solid media of ganyong tubers. Making NA and NB media was done by dissolving 39 g of NA and NB media into 1 L of distilled water and heating on a hot plate and stirring using a magnetic stirrer. The dissolved media was put into an erlenmeyer and cotton as a lid. Sterilize the media with an autoclave for 15 minutes at 121°C (Juariah, 2021). Furthermore, making solid media from ganyong tubers by boiling ganyong tubers that have been peeled, washed, and cut into pieces as thick as 1 cm x 1 cm as much as 300 g. The softened ganyong tubers are taken by filtering the boiled water and then adding water to the boiled water until it reaches 1 L. Add 10 g white sugar and 15 g agar-agar to the boiled water then reheat while stirring continuously. The boiling medium is poured into a sterile Petri dish. Cool the media, cover using sterile paper and store at 4°C.

Antibacterial activity was tested using two methods, namely the disc paper diffusion method (Kirby Bauer) and the solid dilution method. The paper disc method allows the placement of test compounds in varying concentrations on paper discs, allowing the identification of the optimal concentration for antibacterial activity whereas the solid dilution method allows for a more accurate determination of the MIC, which is the lowest concentration of a compound capable of inhibiting bacterial growth.. In the disc paper diffusion method, the inoculum in the form of *E. coli* bacteria was inoculated on liquid NB media by taking one bacterial colony and incubated using an incubator at 37°C. Inoculates were then incubated by adding sterile NB until the turbidity level reached Std. Mc Farland (10^8 CFU/ml). The more significant the level of turbidity in the bacterial culture, the density of bacteria used in the test is in accordance with the predetermined standards. Next, 200 µL bacterial culture was inserted into a Petri disk containing sterile agar media and then flattened using an L-edge. 15 µL of banana peel essential oil was dripped on a disc paper with a diameter of 6 mm and then placed on a petri disk containing media. incubation was carried out for 24 hours at 37°C. The zone of inhibition will be visible as an area around the paper disc where bacterial growth is inhibited or there is no growth at all. Based on the size of the zone of inhibition observed, evaluate the antibacterial activity of the test solution. The larger the zone of inhibition, the stronger the antibacterial activity of the tested compound. Measurement of the diameter of the inhibition zone formed in millimeters using a caliper (Triastuti et al., 2020).

Antibacterial activity can be classified based on the zone of inhibition formed with inhibition limit parameters as follows in the Table 1.

Table 1. Antibacterial Activity Strength Categories

Diameter (mm)	Antibacterial Activity Category
>20 mm	Very strong
10-20 mm	Strong
5-10 mm	Medium
<5 mm	Weak

In the Table 1 solid dilution method, a solid medium is used from the boiled water of ganyong tubers which has been dripped with banana peel essential oil as much as 4-5 drops and then flattened using an L-edge. Let the media stand until the essential oil dries. Take 1 ose of *E. coli* bacterial inoculum and then grow it at an angle on solid media. Incubate for 24 hours at 37°C. The antibacterial effectiveness can be seen from the presence or absence of white spots of bacterial colonies on the Petri disk (Fitriana et al., 2020).

2.4. Preparation of Hand Sanitizer from Banana Peel Essential Oil

From 80 mL of 96% ethanol was mixed with 5 mL glycerol and 12 mL aloe vera juice in a 100 mL beaker until homogeneous. Add 3 mL banana peel essential oil, stir until homogeneous using a magnetic stirrer on a hot plate. The use of ingredients in hand sanitisers has its own uses to inhibit growth and kill bacteria quickly. The use of ethanol as an active ingredient is useful for killing bacteria. The use of glycerol and aloe vera juice serves to provide moisture to avoid irritation to the skin. And essential oil serves as a typical banana aroma giver in hand sanitisers and adds to their organoleptic value.

3. RESULTS AND DISCUSSION

3.1. Banana Peel Pretreatment

The banana peels used as samples were ensured to be of good quality with sample criteria including thick banana peels, not ripe, and not exposed to water. Samples were separated based on maturity level and sampling time for the drying process. The separation of the samples aims to classify the samples based on the time span required for the samples to dry completely. The banana peels were dried at room temperature without direct sunlight to maintain the quality of the essential oil. Drying aims to reduce the water content in the sample so that the oil in the sample easily comes out (Aryani et al., 2020). The drying process of banana peel samples takes place in different time frames depending on the level of ripeness. Overripe banana peels take about 10 days to dry completely while medium-ripe banana peels take about 7 days to dry can be shown in the Figure 1. Banana peel samples are categorized as perfectly dry when the sample weight is constant.



Figure 1. Banana Peel Sample Preparation

3.2. Isolation of Banana Peel Essential Oil by Steam Distillation Method

From 20 kg of banana peel samples that have been isolated by steam distillation method for 6-8 hours, 4.7 mL of essential oil was obtained. The obtained banana peel essential oil is then added with anhydrous sodium sulfate (Na_2SO_4) which serves to remove the water content of the essential oil (Anggraini et al., 2018).

3.3. Antibacterial Activity Test

3.3.1. Disc Paper Diffusion Method (Kirby Bauer)

Antibacterial activity testing with the disc paper method (Kirby Bauer) was carried out using several concentration series to determine the effect of banana peel essential oil inhibition on *Escherichia coli* (E. coli) bacteria. The concentration series of essential oil used in this study were 25% and 50%. The positive control used is tetracycline, referring to previous research that tetracycline can be used as a comparison of antibacterial response to bacterial growth from essential oils under the same conditions (Nurviana, 2018).

The results of the antibacterial activity test of banana peel essential oil against E. coli bacteria with a concentration of 25% and 50% can be seen in the following Table 2.

Table 2. Antibacterial Activity Test Results.

Repetition	Control (+)	Control (-)	25% (mm)	50% (mm)
I	30,6	-	9	10,2
II	28,2	-	8,5	9,8
III	30,5	-	9,8	11,6
Average			9,1	10,5

Table 3. Average Antibacterial Activity Test Results

Test sample	Average <i>Escherichia coli</i>
Control negatif	-
Control positif	29,7 mm
Concentration 25%	9,1 mm
Concentration 50%	10,5 mm

Where (+) is Tetracycline and (-) is DMSO 1%. The Table 2 and Table 3, parameter measured in the antibacterial activity test is the formation of an inhibition zone around the disc paper that has been soaked in banana peel essential oil. The test results can be seen in the following Fdigure 2, the 25% concentration had an inhibition zone diameter of 9.1 mm and the 50% concentration was 10.5 mm. Based on the category of inhibition zone diameter by (Herda Ariyani, Muhammad Nazemi, Hamidah, 2018) in table 1, the antibacterial activity of banana peel essential oil against *E. coli* bacteria is classified as moderate. Based on the zone of inhibition formed at 25% and 50% concentrations, it is known that the 50% concentration produces a better zone of inhibition. The test showed that the zone of inhibition was influenced by the concentration, characterised by a significant difference in the diameter of the zone of inhibition between the two concentrations. The level of compound used affects the antibacterial properties of the compound. The higher the percentage of essential oil used, the greater the possibility of spreading the antimicrobial substance in the medium, which has an impact on increasing the diameter of the inhibition zone formed. It can be concluded that concentrations of more than 50% banana peel essential oil are thought to be more effective because at these concentration levels, the antibacterial compounds that have the potential to act as antibacterial agents have reached sufficient levels.

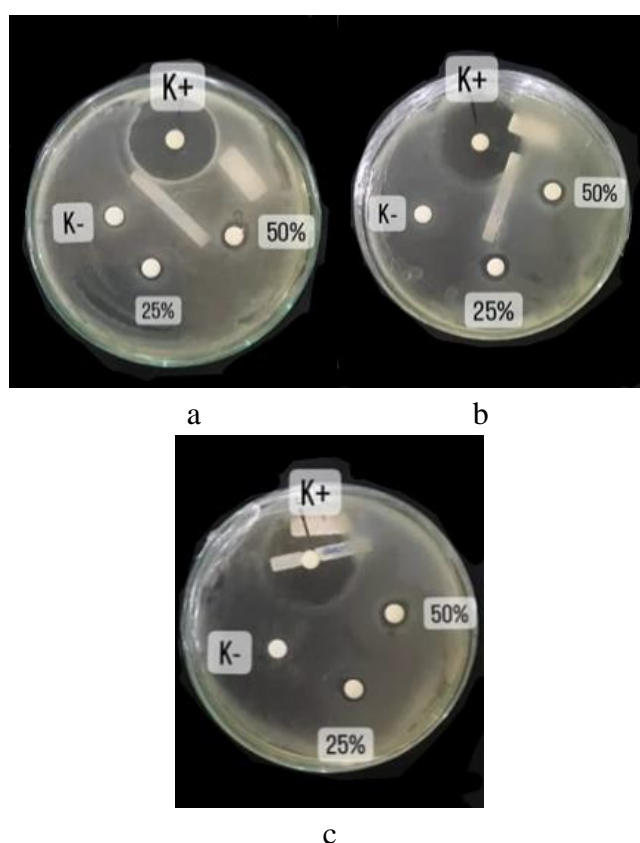


Figure 2. Zone of Inhibition of Banana Peel Against *E. Coli* Bacteria (a) Antibacterial Activity Test (b) Repetition I (c) Repetition II

3.3.2. Solid Dilution Method

In the antibacterial activity test with the solid dilution method on the solid media of ganyong tuber decoction water, 50% concentration of banana peel essential oil was used. The solid dilution method in antibacterial testing aims to determine the minimum concentration of compounds that can inhibit bacterial growth. This process involves mixing banana peel essential oil with solid medium that has been inoculated with *E. coli* bacteria. The use of ganyong tubers as a medium is effective in testing antibacterial activity. Based on research conducted by (Aptari, 2021), the nutrient content in the ganyong tuber media is effective as a medium for growing bacteria. The effectiveness of banana peel essential oil against *E. coli* bacteria in the solid dilution method can be seen in following Table 4.

Table 4. Antibacterial Activity Test Results

Test sample	Observation result
Control negatif	bacterial colonies formed
Control positif	no bacterial colonies formed
Concentration 25%	bacterial colonies formed
Concentration 50%	no bacterial colonies formed

The results showing the formation of bacterial colonies can be seen in the following figure.

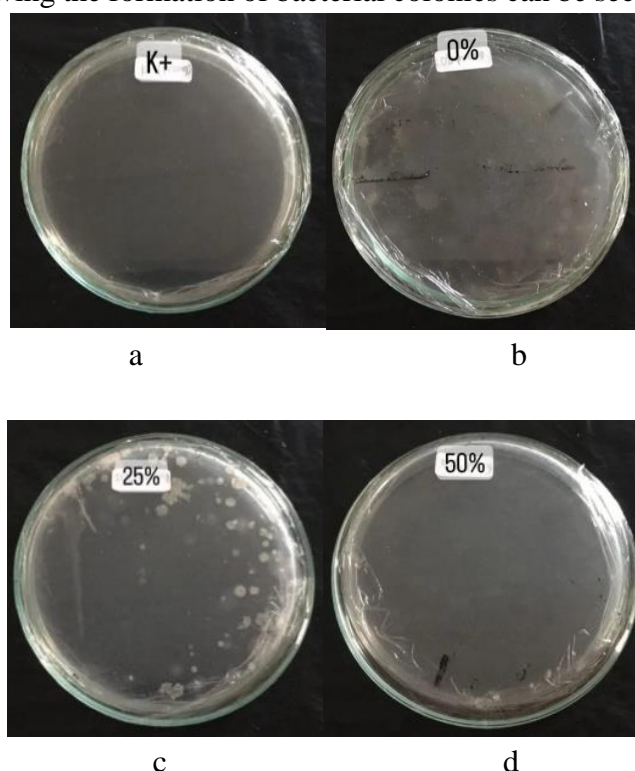


Figure 3. Antibacterial Activity Test on *E. Coli* Bacteria (a) Positive Control (b) Concentration Series 0% (c) Concentration Series 25% (d) Concentration Series 50%.

From the results of the study, both Figure 2 and Figure 3 show that the higher the concentration of banana extract, the more it can inhibit bacterial growth. This is because Banana peels contain phenolic compounds that are useful as antibacterial, antioxidant, anti-fungal, and

anti-inflammatory agents. This is supported by research conducted by Ecevit, Barros, Silva, & Reis (2022) which states that Banana peels contain phenolic compounds that are useful as antibacterial, antioxidant, anti-fungal, and anti-inflammatory agents too. Based on research conducted by (Ida Ayu Raka Astiti Asih, Wiwik Susanah Rita, I Gusti Bagus Teguh Ananta, 2018), *Bacillus cereus*, *Salmonella enteritidis*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* can be inhibited by using ethyl acetate extract of Clavendis banana (*Musa*, AAA cv. Cavendish) with an inhibition diameter between 9-12 mm. The content of antibacterial compounds in banana peel, there are other compounds that still have to be further investigated for antibacterial activity, namely terpenoid compounds in banana peel essential oil (Elangovan & Mudgil, 2023). Next, banana extract can be used in making hand sanitizer.

3.4. Hand Sanitizer Making

After testing the antibacterial activity, essential oil with a concentration that has the best inhibitory activity against *E. coli* bacteria is obtained and then formulated into a hand sanitizer. The use of essential oil in the formula is 3 mL with a concentration of 50% in the total volume of 100 mL liquid preparation. . Based on research conducted by (Bahri et al., 2021), on patchouli essential oil, the use of 3 mL of essential oil in a hand sanitizer obtained an inhibition of 13.9 mm. In addition, banana peel essential oil is useful to add a distinctive banana aroma to the hand sanitizer. The addition of glycerol and aloe vera juice is also done to avoid irritation to the hands due to the use of 96% alcohol and serves as a moisturizer. Hand sanitizer formulations can be seen in the following Table 5.

Table 5. Formulation of Liquid Hand Sanitizer

No.	Materials	Total
1.	Etanol 96%	80 mL
2.	Glycerol	5 mL
3.	Aloe vera juice	12 mL
4.	Essential oil	3 mL
Total		100 mL

4. CONCLUSION

Based on the research conducted, it was concluded that from 20 kg of dried banana peel samples, 4.7 mL of essential oil was obtained through the steam distillation method. The diameter of the inhibition zone of banana fruit peel essential oil is classified as moderate, namely obtained 10,5 mm at a concentration of 50% and 9,1 mm at a concentration of 25%. Suggestions for further research, identification of compound components contained in banana peel essential oil using GC-MS so that the main compounds contained therein are known.

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