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Cavendish Banana Peel Extract's Antibacterial Activities Potential as Disinfectant

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A B S T R A C T

Introduction: The COVID-19 pandemic makes personal hygiene more important than ever, and antibacterial substances such as disinfectants are crucial in maintaining said hygiene. This study aimed to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of cavendish banana peel extract (*Musa acuminata*) against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*.

Methods: The design of this study was a laboratory experiment that used the broth dilution method with test tubes using methanol as the extract's solvent. Sterile aquadest was used as the solvent, and Mueller-Hinton broth was used as the growth medium in tubes. All samples of the bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*) were provided by the Laboratory of Microbiology Faculty of Medicine, Universitas Airlangga.

Results: MBC was the only parameter found due to the color and particulates, which hindered the turbidity assessment of MIC. From the dilution test, the MBC of cavendish banana peel extract against the growth of bacteria *Escherichia coli* and *Staphylococcus aureus* was 25%, with no activity against *Bacillus subtilis*.

Conclusion: There were antibacterial activities of *Musa acuminata* peel extract against *Staphylococcus aureus* and *Escherichia coli*. Therefore, it has the potential to be used as a disinfectant.

Highlights:
1. The COVID-19 pandemic makes personal hygiene more important than ever, and antibacterial substances such as disinfectants are crucial in maintaining said hygiene.
2. The MBC of cavendish banana peel extract against the growth of bacteria *Escherichia coli* and *Staphylococcus aureus* was 25%, with no activity against *Bacillus subtilis*.
3. *Musa acuminata* peel extract has the potential to be used as a disinfectant.

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Introduction

Bananas are the most produced fruit in Indonesia. According to Statistics Indonesia/BPS (2018), banana production in Indonesia reached 7,264,383 tons in 2018. This figure shows that bananas are quite popular and widely available among the population. Bananas have a sweet taste and are easy to consume. The part that is commonly consumed is the flesh, while the rest is discarded.

Bananas (*Musa acuminata*) have many benefits and nutrients. Some of the nutrients found in bananas include carbohydrates, protein, fat, fiber, minerals, and vitamins. Bananas are also rich in flavonoids, phenolics, and fatty acids with anti-inflammatory, antibiotic, and anticancer effects. These substances are known to reduce the symptoms of diseases such as cancer, diabetes, and cardiovascular diseases. In addition, the nutrients in bananas have the ability to improve wound healing.[1–3]

The antibiotic properties of bananas are mainly found in the peel, which contains a significant amount of these substances. When ripe, the proportion of banana skin can reach up to 40% of the total fruit weight. Many antibacterial substances in banana skin are often wasted as people do not commonly consume them. With the increasing demand for disinfectants, it is necessary to discover disinfectants with easily obtainable antibacterial properties. Therefore, this study aimed to determine the antibacterial activity of Cavendish bananas (*Musa acuminata*).

Methods

The bananas selected were the ones fully yellow in colour with several brown spots on them. The peels were sun-dried for 5-7 days for around 10 hours of sunlight each day and ground into powder with a mechanical blender to the size of approximately 1x1 mm coarse powder. A total of 553 grams of dried banana peel was used with 4 ml of solvent for every gram of peel (1:4 ratio). The extraction method used was maceration using 80% methanol as the solvent. The solvent was replaced every 24 hours three times. The banana peel extract powder was placed in a container along with the solvent until all powder was submerged and allowed to stand for 24 hours with frequent stirring. After 24 hours, the powder was transferred to another container and re-macerated. The macerated product obtained was concentrated in a rotary evaporator at a minimum temperature of 40°C until a thick extract was formed. The extract was then transferred to a measuring cup and evaporated in a water bath at around 65°C, and the final product was weighed. The final product weighed 61 grams and was stored in an airtight container, which was then kept in a refrigerator at 4°C until further usage.

The broth dilution test used *Escherichia coli* ATCC 2592, *Staphylococcus aureus* ATCC 25823, and *Bacillus subtilis*. All of the microorganisms were provided by the Laboratory of Microbiology, Department of Clinical Microbiology, Faculty of Medicine, Universitas Airlangga University.

Banana peel extract was prepared in concentrations (v/v) of 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5625%, 0.78%, 0.39%, and 0.195% with distilled water. The prepared solutions were then poured using single-use pipettes. The dilution tests were conducted in accordance with the microdilution guideline of the Clinical and Laboratory Standards Institute/CLSI (2021).[4] Bacteria to be cultured were prepared in advance by inoculating the associated bacteria from an agar plate (using 3-5 colony isolates and taken with a sterile loop), adding to Mueller-Hinton Agar, and incubating at 35°C ± 2°C until it reached a turbidity of 0.5 McFarland (equivalent to 1-2×108 CFU/mL). The McFarland solution used as the reference for the turbidity of the bacterial growth medium was prepared by adding 0.5 mL of 1.175% $BaCl_2$ solution to 99.5 mL of H_2SO_4 solution and stirred continuously until turbidity was formed. The bacterial solution was added to the test tubes containing the banana peel extract until the final volume of the test tubes was 2 ml, and the number of colonies in the test tube was equivalent to 5×105 CFU/ml. The bacterial solutions were inoculated into the test tubes using pipettes. Two controls were prepared in the 10th and 11th tubes, sterility control (negative) and bacterial growth control (positive). After the incubation period (24 hours at 37°C), the tube's contents were assessed visually for their minimum inhibitory concentration (MIC). After a similar incubation period, the contents were streaked onto the nutrient agar plates to determine the minimum bactericidal concentration (MBC). All of the tests were conducted in triplicate.

Results

MIC was determined visually by assessing which tube had the lowest concentration of extract while still maintaining a clear suspension after an incubation period (no bacterial growth). As shown in Figure 1, the leftmost tube (50% concentration) was too murky to look for any bacterial growth, while the rest of the tubes were filled with banana peel fiber from the extract. Thus, making a visual assessment for MIC was not ideal. Banana peel extract still showed antibacterial activities, as shown in Table 1, for their MBC. MBC was determined by inoculating the suspension in each tube onto a MacConkey agar plate and assessing if there was any growth after being incubated for another 24 hours at 37°C.

Table 1. MBC values of cavendish banana peel extract

Bacteria	Concentration	Positive Control	Negative Control
Escherichia coli	25%	+	-
Staphylococcus aureus	25%	+	-
Bacillus subtilis	-*	+	-

*MBC was not found for this bacterium until the highest concentration reached (50%)
+ Bacterial growth occurred
- Bacterial growth did not occur

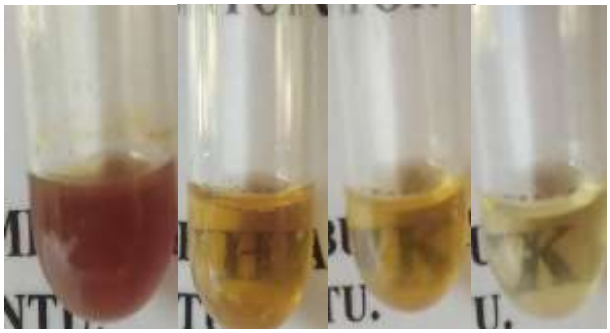

Figure 1. Several resulting test tubes of broth dilution tests from one of the replications (the leftmost tube had the highest extract concentration)

Figure 2. Streaking results of *Escherichia coli* test tubes from 50% (area 1) to 0.195% (area 9). K+ = positive control; K- = negative control

Figure 3. Streaking results of *Staphylococcus aureus* test tubes from 50% (area 1) to 0.195% (area 9). K+ = positive control; K- = negative control

Figure 4. Streaking results of *Bacillus subtilis* test tubes from 50% (area 1) to 0.195% (area 9). K+ = positive control; K- = negative control

Escherichia coli showed no growth on the plate at the two highest extract concentrations. A similar phenomenon was also observed in *Staphylococcus aureus*. While the other bacteria showed no growth in either of the two highest concentrations, *Bacillus subtilis* showed the capability to grow in all concentrations.

Discussion

Cavendish banana peel exhibited antibacterial activities to *Staphylococcus aureus* and *Escherichia coli* but not to *Bacillus subtilis*. The bacteria were chosen to represent their respective group, gram-positive bacteria for *Staphylococcus aureus*, gram-negative bacteria for *Escherichia coli*, and spore-forming gram-positive bacteria for *Bacillus subtilis*. Such phenomenon might originate from the secondary metabolites contained within the plant extract, which are known to already have antibacterial activities, e.g., phenolic compound, flavonoid, tannin, and terpenoids, and how they act on the cell wall.[5,6]

Banana peels are known to contain flavonoids, polyphenols, antioxidants, vitamins, fatty acids, vitamins, minerals, etc. Secondary metabolites such as terpenoids, alkaloids, and phenols are compounds that have antibacterial activity. In general, some of the antibacterial mechanisms proposed by the previous studies are changes in cell membrane permeability, changes in intracellular activity of target cells due to hydrogen bonding at the binding site of certain intracellular enzymes, and loss of structural integrity of the cell wall. These mechanisms make phenol compounds that are lipophilic more effective than those that are hydrophilic.[7–9]

Banana peels have been identified to contain quercetin, coumarin, catechin, caffeic acid, cinnamic acid, chrysene, ellagic acid, gallic acid, myricetin, routine, naringenin, tannin, and terpenoid compounds.[10–12] These compounds are categorized as phenolic compounds and other secondary metabolites. These compounds' antibacterial mechanisms can represent the mechanism of the antibacterial activity of cavendish banana extract. For example, quercetin is a flavonoid compound with MIC against *Escherichia coli* and *Staphylococcus aureus* and has more activity against gram-positive bacteria than gram-negative bacteria.[13] Coumarin, a phenol compound, has an MIC between 62.5 to 125 µg/mL towards *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus cereus*.[14] Aside from the mentioned compounds, compounds such as ß-sitosterol, malic acid, 12-hydroxystrearic acid, and succinic acid are also found in cavendish banana peels, and their MICs have been known too.[10]

The secondary metabolites have stronger activity against gram-positive bacteria than other types of bacteria (gram-negative or spore-forming bacteria) because the way these compounds work is centered on cell wall degradation and the cell wall characteristics of gram-positive bacteria. One of them is peptidoglycan, which plays an important role.[11,15]

In this study, *Bacillus subtilis* had more resistance to the extract than the other bacteria, even more than *Staphylococcus aureus,* which is also a gram-positive bacterium. This phenomenon is thought to result from its capability to form spores in stressful environments though this hypothesis needs further study. *Bacillus subtilis* has greater resistance towards stressors where said bacteria shows cell injury in the form of shortening of the cell length, the surface of the cell wall becoming rougher, and signs of loss of cell wall integrity.[16] As a comparison, in the same study, *Staphylococcus aureus* was exposed to the same substance but at a lower concentration, which indicated that *Staphylococcus aureus* was more susceptible than *Bacillus subtilis* and formed separated cocci (indicating replication failure). The spore formation was shown by Rao (2016), who used high-pressure carbon dioxide (6.5-20 MPa) and temperatures of up to 86˚C for up to 2 hours on *Bacillus subtilis*.[17] The damages that could be observed include cell fragment remains, deformities, and collapsed spores.

Disinfectants are substances meant to be applied on surfaces to eliminate microbial life (excluding endospores). Loyaga-Castillo (2020) showed that banana peel extract has activity against fungi (*Candida albicans*), which can be used as a topical antiseptic for related fungi.[18] In addition, banana peel extract also has antibacterial activities against resistant types of bacteria, indicating this extract can be used as an antibacterial agent.[19] Cavendish banana peel extract at a concentration of 25% is able to kill gram-positive and negative bacteria. This extract can be categorized as a low-level disinfectant (can be used on noncritical objects) if it can kill mycobacterium and fungi.[20] Based on this study, it can be said that the banana peel extract may be used as an alternative to the active ingredients of disinfectants.

Strength and Limitations

The strength of this study was the number of types of bacteria tested against the banana peel extract, which helped other researchers to have a general idea of how the extract behaves. The limitation of this study was that MIC was not discovered, and not all types of microorganisms were tested, thus, the extract can be stated as a particular level of disinfectant. Therefore, further studies are needed to determine the effect of banana peel extract against typical fungi and mycobacterium. Further research is also necessary to assess its applicability as a disinfectant on multiple types of surfaces. Lastly, similar research should also be conducted with a wider variety of microbes.

Conclusion

There were antibacterial activities of *Musa acuminata* peel extract against *Staphylococcus aureus* and *Escherichia coli*. Therefore, it has the potential to be used as a disinfectant.

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Conflict of Interest
The authors declared there is no conflict of interest.

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Ethical Clearance
This study did not involve humans or animals as the subject. No ethical clearance was required at the time this study was conducted.

Authors' Contributions
Conceived and planned the experiments: ELR, WR, LD, MRW. Conducted the experiments: ELR. Helped in the technical aspects of the experiment: WR. Contributed to the interpretation of the results: ELR, WR, LD, MRW. Took the lead in writing the manuscript: ELR. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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