EFFICACY OF ANTIBACTERIAL PROPERTIES OF MANIHOT ESCULENTA CRANTZ PEEL EXTRACT AGAINST STAPHYLOCOCCUS AUREUS

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ABSTRACT

Background*: Staphylococcus aureus is a common commensal and pathogenic bacterium found on human skin that can cause various diseases. Soaps and alcohol-based hand sanitizers contain chemicals that can negatively affect skin health, causing irritation and dryness, especially in individuals with risk factors such as eczema. Studies have shown that cassava peel extract contains active metabolite compounds that exhibit antibacterial properties.* ***Objective****: This study aims to evaluate the antibacterial efficacy of cassava (Manihot esculenta Crantz) peel extract by assessing its inhibitory and bactericidal effects against Staphylococcus aureus.* ***Methods****: This research is an in vitro experimental study with a non-equivalent control group design. The extraction of cassava peel is conducted using a maceration method with 96% ethanol as the solvent. The antibacterial activity test is performed using the broth microdilution method on a 96-well microplate. The test solution employed is cassava peel extract (Manihot esculenta Crantz) at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values are measured using a microplate reader and recorded as optical density (OD) values.* ***Results****: The cassava (Manihot esculenta Crantz) peel extract exhibited antibacterial effects against Staphylococcus aureus. The MIC value of cassava (Manihot esculenta Crantz) peel extract was found at a concentration of 6.25%, while the MBC value was found at a concentration of 25%.* ***Conclusion****: Cassava peel has been shown to possess antibacterial effects against Staphylococcus aureus.*

Keywords*: Cassava peel; Manihot esculenta crantz; Staphylococcus aureus; Antibacterial effect; Minimum inhibitory concentration; Minimum bactericidal concentration*

INTRODUCTION

Staphylococcus aureus is an opportunistic bacterium, meaning it is mostly harmless to a healthy host but can cause infection when the host's resistance is compromised. This bacterium is commonly found in mucosal tissues (such as the nose) and on the skin. *Staphylococcus aureus* infections are among the most frequent, occurring both in community settings and hospitals. Individuals with immunocompromised conditions, chronic diseases (such as cancer, diabetes mellitus, vascular diseases, eczema, etc.), or those who use injected drugs are at higher risk of *Staphylococcus aureus* infection.[1,2]

Maintaining hygiene by regularly washing hands with soap or, in the absence of water, using alcohol-based hand sanitizers is one of the simplest ways to prevent bacterial infections.[3] However, soaps and hand sanitizers contain various chemical compounds that can have adverse effects on skin health, such as irritation, dryness, redness, and itchiness, especially in individuals with risk factors.[4,5] Therefore, alternative solutions are needed to address this issue.

Indonesia ranks 4th among the top global producers of cassava. While the starchy tuberous roots of cassava are widely utilized, the peels are often discarded as waste and, with no proper management, can lead to environmental concerns.[6,7] Notably, cassava peels are rich in secondary

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metabolites, including alkaloids, steroids, terpenoids, flavonoids, saponins, and glycosides, which are highly beneficial.[8]

Secondary metabolites present in cassava (*Manihot esculenta* Crantz) peels are known to exhibit antibacterial properties. Consequently, this study aims to evaluate the antibacterial activity of cassava peel extracts by assessing their inhibitory and bactericidal effects against *Staphylococcus aureus*.

METHODS

This study is an in vitro experimental study employing a non-equivalent control group design. It utilizes cassava (*Manihot esculenta* Crantz) peel extract and *Staphylococcus aureus* bacteria, divided into two groups: a control group and a test group. The research samples consist of subcultures of *Staphylococcus aureus* obtained from the pure isolate *Staphylococcus aureus* ATCC 25923, sourced from the Clinical Microbiology Laboratory of the Balai Besar Laboratorium Kesehatan (BBLK) Surabaya.

The study involved identifying the plant and preparing cassava (*Manihot esculenta* Crantz) peel extract through maceration using 96% ethanol as the solvent. The extract underwent phytochemical screening to identify secondary metabolites present in the extract product. Subsequently, the extract was diluted into various concentrations—100%, 50%, 25%, 12.5%, 6.25%, and 3.125%—using 10% DMSO as the diluent.

The antibacterial assay was conducted using the broth microdilution method with Mueller Hinton Broth as the culture medium. Prior to subculturing, the bacteria were identified to confirm their purity and identity. Bacterial growth was assessed by measuring turbidity with a microplate reader, yielding optical density (OD) values. These OD measurements were then used to calculate the extract's inhibitory and bactericidal effects.

RESULTS

Tabel 1. Phytochemical Screening Result

Compound class	Result
Flavonoid	Positive
Tannin	Positive
Alkaloid	Positive
Steroid	Negative
Terpenoid	Positive
Saponin	Positive

Phytochemical screening results indicate that the extract contains compounds recognized for their antibacterial properties.

Table 2. Bacteria identification result

Test	Results
Macroscopic	
Colony observation on blood agar	Colony is small, white to grayish in color, with a smooth, shiny surface, and surrounded by a clear beta-hemolytic zone.
Microscopic	
Gram Stain	Gram-positive bacteria, cocci-shaped, and grow in clusters.
Biochemical Properties	
Catalase	Positive
Coagulase	Positive
Mannitol fermentation (Mannitol Salt Agar)	Positive growth, can ferment mannitol

Based on a series of bacterial identification tests, the test bacterium was confirmed as *Staphylococcus aureus*.

Table 3. Percentage of inhibition

Group	Percentage (%)
Ku1	100
Ku2	100
Ku3	100
Ku4	75.2
Ku5	95.4
Ku6	91

After 24 hours of incubation, the microplate was read using a microplate reader. The percentage of inhibition was calculated, and any result above 100% was capped at 100%.

Table 4. Normality test result

Shapiro-Wilk	
Group	**Significance value**
Ku1	0.921
Ku2	0.644
Ku3	0.628
Ku4	0.959
Ku5	0.196
Ku6	0.410

The results of the normality test indicate that the data are normally distributed, allowing for the use of parametric analysis tests.

Table 5. Parametric analysis test result

One-way ANOVA		
Group	**Significance value**	**Description**
OD	< 0.001	Reject H_0

The results of the parametric test using one-way ANOVA were significant, leading to the rejection of the null hypothesis (H_0) and acceptance of the alternative hypothesis (H_1).

DISCUSSION

The minimum inhibitory concentration (MIC) is the lowest concentration of a substance that can visibly inhibit bacterial growth, typically defined as ≥90% inhibition or ≤10% microscopic growth. In contrast, the minimum bactericidal concentration (MBC) is the lowest concentration of a substance that can kill bacteria, defined as ≥99.9% inhibition or ≤0.1% colony growth. MIC measures bacteriostatic activity, while MBC measures bactericidal activity.[9]

The results of the antibacterial assay using the broth microdilution method show that wells containing higher concentrations of cassava peel extract exhibited darker brown coloration and precipitates at the bottom after 24 hours of incubation. This increased extract concentration resulted in higher optical density (OD) readings, which may be attributed to the dark color of the extract. Consequently, it became challenging to visually assess bacterial growth due to interference from the extract's color.

Higher concentrations of cassava peel extract (such as 100%, 50%, and 25%) resulted in precipitates at the bottom of the wells. These precipitates are likely due to interactions between the extract's compounds and the bacteria or because higher extract concentrations contain more metabolites, leading to increased insolubility and precipitate formation.

Higher concentrations of cassava peel extract (100%, 50%, and 25%) resulted in precipitates at the bottom of the wells. These precipitates likely formed due to interactions between bacterial cells and compounds in the extract or because elevated extract concentrations contain higher levels of metabolites, leading to increased insolubility and precipitate formation. The presence of precipitates can interfere with the accuracy of microplate reader measurements by causing light scattering, thus affecting optical density (OD) readings. Additionally, at these concentrations, the OD readings of the negative control group were higher than those of the test group. This anomaly may be attributed to bacteria metabolizing other components of the extract, such as carbohydrates, resulting in a clearer solution compared to the negative control without bacteria.

Based on the research findings, the minimum bactericidal concentration (MBC) of the 96% ethanol extract of cassava (*Manihot esculenta* Crantz) peels against *Staphylococcus aureus* was determined to be 25%, with an inhibition percentage of 128.1%, rounded down to 100%. The minimum inhibitory concentration (MIC) was found at 6.25%, exhibiting a 91% inhibition rate. These results indicate that cassava peel extract possesses antibacterial effects against *Staphylococcus aureus*. Phytochemical screening of the extract identified secondary metabolites such as flavonoids, tannins, alkaloids, terpenoids, and saponins, all known for their antibacterial properties. This is consistent with previous studies demonstrating the presence of these compounds in cassava extracts and their associated antimicrobial activities.

CONCLUSION

Based on the conducted research, it can be concluded that cassava (*Manihot esculenta* Crantz) peel extract exhibits antibacterial effects against *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) of the extract is 6.25%, while the minimum bactericidal concentration (MBC) is 25%.

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