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# ANTIMICROBIAL POTENTIAL OF KEPOK BANANA SHEATHS EXTRACT (*Musa paradisiaca formatypica*) ON THE GROWTH OF *Staphylococcus aureus* BACTERIA

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## ABSTRACT

Banana plant is one of the most famous and beneficial plants in the world. Almost all parts of this plant, namely fruit, leaves, flower buds, stems, pseudo stems (sheaths and flower stem) can be utilized. This study aims to identify antimicrobial active substances and the antimicrobial potential of Kepok Banana **(***Musa paradisiaca formatypica*) sheaths. The study was an experimental laboratory to design a post-test only control group. The maceration method is used to extract the active substance and identify the levels of the antimicrobial active substance using the TLC Spectrophotodensitometer and the diffusion method (disc) to test the antimicrobial potential. The research data were analyzed using the Kruskal Wallis test. The positive qualitative test contains flavonoids, phenol, saponins and alkaloids. The quantitative content of Kepok Banana sheaths extract contains phenol (1,9 mg/ml) dan flavonoid (93,3 mg/ml) dan tannin (2,7 mg/ml). The results showed differences in antimicrobial potential at various concentrations (55%, 70%, 85% and 100%) of Kepok Banana sheaths extract with positive and negative controls (sig = 0.000). There are no effective concentration in inhibiting the growth of*Staphylococcus aureus* bacteria with an average inhibition range of 4.47 mm (resistant category) based on the NCCLS standard.
**Keywords:** Kepok Banana sheaths extract; antimicrobial potential; *Staphylococcus aureus.*

## INTRODUCTION

Antibiotics are the main choice in the treatment of infectious diseases caused by *Staphylococcus aureus* bacteria. However, the problem that occurs is the existence of resistance and even multiresistance to *Staphylococcus aureus* bacteria. These microbes are resistant to penicillin oxacillin and other beta-lactam antibiotics. Therefore, it is necessary to develop traditional treatment methods using herbs that can inhibit the growth of *Staphylococcus aureus* bacteria. Banana plants are native to Asia, Indo-Malaysia and Australia which are included in the genus Musa (family: *Musaceae*). This plant is now widely spread in the tropics and sub-tropics,and is often used as food and traditional medicine, both parts of the fruit, leaves, stems, and roots. Previous research stated that among the different *Musa* species, *Musa paradisiaca* displayed efficient antibacterial activity followed by *Musa acuminata* against multi-drug resistant nosocomial infection causing pathogen.[1]

Every region in Indonesia has a regional name for banana plants. In Bali, Kepok banana are known as "BiuKepok, Biu Gedang Sabo", which are usually processed into fried bananas, cakes, chips, etc. Bananas are traditionally used to treat diarrhea, dysentery disorders, lesions/wounds, diabetes mellitus, uremia, hypertension and heart disease.[2] This plant often used to treat new wounds traditionally by applying banana sap to the injured part.[3] Banana plant sap contains phytochemical compounds that can accelerate the wound healing process in mice.[4] Banana plants affect several bacteria, one of which is *Staphylococcus aureus*. Phytochemical test of KepokBanana stems showed the content of tannins, alkaloids, and saponins, but not with flavonoids and steroids. The content inside is what makes the banana plant has a role as an anti-ulcer, anti-bacterial, and plays a role in woundhealing.[5]

Basically, almost all parts of the banana plant can be used starting from the roots, stems, midribs, leaves, to flowers and fruit. The extract of the banana plant (roots, stems, midribs, leaves, to flowers and fruit) is able to inhibit the growth of *Staphylococcus aureus* bacteria (very strong category).[6] Stem and root extracts from KepokBanana have activity in inhibiting the growth of *Staphylococcus aureus* bacteria. Methanol extract from Kepok Banana stems has greater effectiveness than root extract with concentrations of 6.25%, 12.5% and 25%.[7] The difference in the chemical content of some parts of the banana plant and the difference in the concentration of theextract attracted the attention of the author so that the purpose of this study was to determine the phytochemical

content and antimicrobial potential of Kepok Banana sheaths against the growth of *Staphylococcus aureus* bacteria *in vitro*.

## METHODS

This type of research is true-experimental, there is intervention on the treatment group and there is controlover external factors that have the potential to affect the experiment. The study was an experimental laboratory with post-test only-control group design. There are two groups chosen randomly. The first group was given treatment (experimental group) and the second group that was not treated (control group). Sheaths of kepok bananawere extracted and qualitative phytochemical tested in the Applied Chemistry laboratory at the Department of Medical Laboratory Technology, Polytechnic of Health, Denpasar. Antimicrobial potential test was conducted at Microbiology laboratory at the Faculty of Medicine, Udayana University. Quantitative phytochemical tests for antimicrobial active substances was conducted at Integrated Analytical Laboratory at Udayana University.

There were four treatments of kepok banana sheaths extract, namely concentrations of 55%, 70%, 85%, and 100%. The number of replications in the study was 8 times for each concentration, so the number of sampleswas 32 samples.

The research material used in this study is the sheath of kepok banana, aquadest, Mueller Hilton agar (MHA), Mc Farlland standard 0.5%, *Staphylococcus aureus* 3351, Ciprofloxacin antibiotic disk, 96% EtOH, 0,5% CMC solution (Carboxy Methyl Cellulose) and 0.85%.NaCl The tools used in this study was bottle of maceration (Pyrex), rotary evaporator, the petri dish (Pyrex), test tubes (Pyrex), round ose (Pyrex), tube flask (Pyrex), incubator (wagtech mir incubator), sterile container, measuring pipette (Pyrex volume 5 ml), petri dish, micropipette (termo 10µL, 50 µL, and 500 µL), beaker glass (Pyrex volume 250 ml), sterile cotton sticks, and digital scales.

Research procedure begins with taking kepok banana sheaths, washed under running water, then drain. Then cuts into small pieces and taken each as 250 grams, then dried in the sun indirectly for ± 5 days until the sample completely dry. After drying, followed by maceration method with 2 L of 96% ethanol solvent for 5 days(5 x 24 hours). Then re-macerated 2 times at room temperature 25°C - 30°C, filtered and the filtrate thus obtainedwere accommodated in the shelter container (bottle maceration). The entire filtrate that obtained concentrated byrotary evaporator at a temperature 50°C to obtain a viscous extract.

Identification of the antimicrobial active substance in sample (Total phenol determination). Total phenol was determined by Folin-Ciocalteu reagent with gallic acid as standard. A total of 0.25 mg of sample was added with 1.25 mL of Folin-Ciocalteu reagent which was diluted 10 times. Incubate for 4 minutes. Add 1 mL of 7.5%sodium carbonate. Incubation for 20 minutes at room temperature, then the absorbance was measured at a wavelength of 742 nm with routine as a standard. Results are expressed in gallic acid equivalent/mL extract (GAE/mL extract).

Identification of saponins, flavonoids and tannins by TLC-Spectrophotodensitometer. A total of 20 mg ofviscous extract was dissolved in pro-analytical methanol, 10 L was spotted on a TLC plate, eluted with ethyl acetate: formic acid: water (100:15:17) as mobile phase. Scan with a spectrophotodensitometer at a wavelength of 286 nm. The obtained chromatograms were identified with peaks and spectra.

Preparation of kepok banana sheath extract with concentrations of 55%, 70%, 85% and 100% made by mixing viscous extract of kepok banana sheaths with sterile CMC solution as a diluent. Each concentration is diluted into a volume of 1 ml. Mueller Hilton order made as much as 600 ml, placed in a tube flask, then sterilizedusing an autoclave. Colonies of bacteria (stock culture) were taken using round Ose and suspended into a tube containing 5 mL of 0.85% physiological NaCl solution.

Inhibition test was done by using paper disc diffusion method. The results of the test power Antibacterial based on measurements Diameter Inhibitory Power (DDH) growth of bacteria that formed around the paper disc.Empty paper discs are immersed in kepok banana sheath extract at various concentrations until all the liquid seepsinto the discs. Negative control used paper discs that were immersed in 1 ml of sterile 0.5% CMC solution. Positivecontrol used Ciprofloxacin antibiotic disc. A suspension of *Staphylococcus aureus* 3351 with a concentration of 0.5% Mc Farlland was prepared. Then the test bacteria suspension was inoculated evenly on a medium Mueller Hinton Agar (MHA), let stand until the bacterial suspension soaked into the media (5-15 min). Each disc that hadbeen saturated with kepok banana sheath extract then affixed to the surface of Mueller Hinton Agar media whichhad been scratched with bacterial suspension and slightly pressed with tweezers until it adhered perfectly. Positivecontrol and negative control were also attached to Mueller Hinton Agar media. Furthermore incubated at 37°C for 24 hours. After 24 hours, the diameter of the area that formed around the paper discs were observed and measured using a calliper.

The data obtained in the research analysis using the Kruskal Walls test to determine the potential of various concentrations of kepok banana sheaths extract. Then compared with the NCCLS table to see the most sensitive concentration of kepok banana sheaths extract.

## RESULTS

The results of the qualitative test with phytochemical screening on the antibacterial compound content of the Kepok banana sheaths extract obtained the following results:

Table 1. Results of antibacterial compounds qualitative test

| Compounds | Results |
|---|---|
| Quinone Alkaloid | - |
| Saponin Phenol | + |
| Flavonoid | + |
| Tannin | + |
| | + |
| | + |

Qualitative test results indicate a positive antibacterial compound are phenols and derivatives such as alkaloids, flavonoids, saponins, and tannins. Quantitative assay followed to determine the content of the antibacterial i.e. total phenols, flavonoids and tannins obtained the following results:

Table 2. Results of antibacterial compounds quantitative test

| Compounds | Results (mg/ml) |
|---|---|
| Phenol Flavonoid | 1.9 |
| Tannin | 93.3 |
| | 2.7 |

As previously mentioned, Kepok Banana sheaths extract made into four different concentration; 55%, 70%, 85%, and 100%. Positive control (antibiotic disk with Ciprofloxacin 30 gram) showed the presence ofinhibition zones in the eight replications with the diameter of inhibition zone obtained is 15.50 mm. The negativecontrol (96% ethanol) did not show any inhibition zone diameter (0.0 mm) in the eight replications. This is because96% ethanol does not contain antibacterial substances that can inhibit the growth of *Staphylococcus aureus* bacteria.

Table 3. Results of inhibition zone diameter measurement

| Replications | Inhibition zone diameter (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Negative Control | Positive Control | 55% | 70% | 85% | 100% |
| 1 | 0.0 | 15.0 | 0.0 | 5.0 | 6.0 | 7.0 |
| 2 | 0.0 | 15.0 | 0.0 | 5.0 | 6.0 | 7.0 |
| 3 | 0.0 | 16.0 | 0.0 | 5.0 | 6.0 | 7.0 |
| 4 | 0.0 | 16.0 | 0.0 | 5.0 | 6.0 | 7.0 |
| 5 | 0.0 | 15.0 | 0.0 | 5.0 | 6.0 | 7.0 |
| 6 | 0.0 | 15.0 | 0.0 | 5.0 | 6.0 | 7.0 |
| 7 | 0.0 | 16.0 | 0.0 | 5.0 | 6.0 | 7.0 |
| 8 | 0.0 | 16.0 | 0.0 | 5.0 | 6.0 | 6.0 |
| Average | 0.0 | 15.50 | 0.0 | 5.0 | 6.0 | 6.88 |


Figure 1. Colonies *Staphylococcus aureus* bacteria that have been treated

Kepok Banana sheaths extract at various concentrations (replications 1 to 8) showed the average inhibitionzone diameter was 4.47 mm. The measurement results when compared with the inhibition zone diameter of Ciprofloxacin antibiotic in the NCCLS table, included in the resistant category (≤ 15 mm).

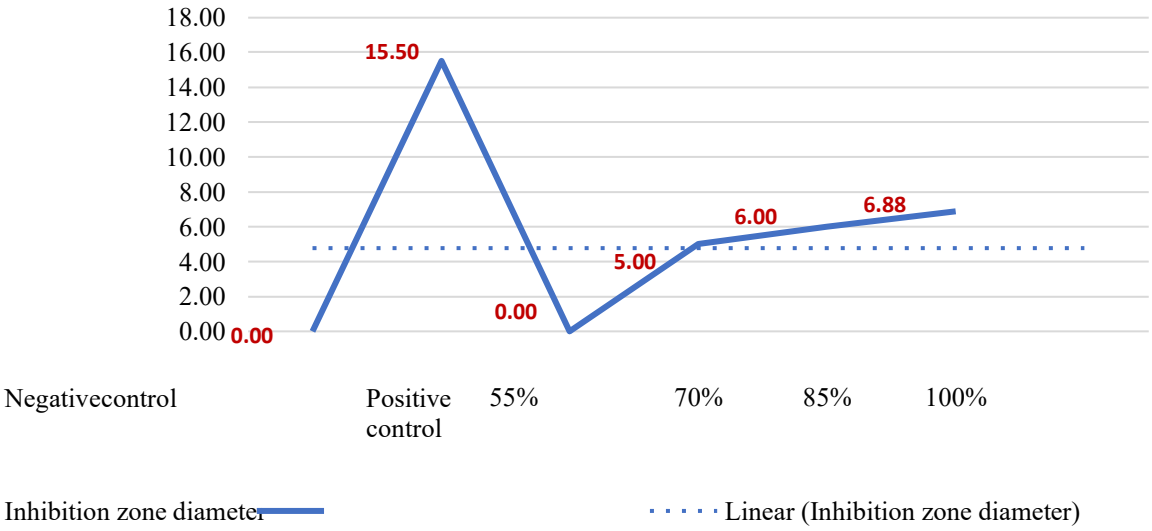


Figure 2. Inhibition zone diameter of Kepok Banana sheaths extract against the growth of *Staphylococcus aureus* bacteria *in vitro*.

The results of the Kolmogorov Smirnov statistical test, obtained a value of 0.000 <0.05, meaning that the data is not normally distributed, followed by the Kruskal Wallis test, the value of sig (0.002) is obtained. This value is less than the value of α (0.05). These results indicate there are differences in the antimicrobial potential of Kepok Banana sheaths extract in inhibiting the growth of *Staphylococcus aureus*. The results of the LSD (Least Significant Deference) test showed that there were differences in antimicrobial potency at various concentrations (55%, 70%, 85%, 100%) of Kepok Banana sheath extract with positive and negative controls is0.00 < 0.05.

## DISCUSSION

The results of qualitative tests on several parameters showed that alkaloids, saponins, flavonoids, phenol and tannins were detected (quinones were not detected), and further quantitative test results showed the presenceof phenolic content (1.9 mg/ml) and flavonoid (93.3 mg/ml) and tannins (2.7 mg/ml). These results are consistentwith previous research which states that flavanones, flavanols, hydroxycinnamic acid, dopamine and N-Acetyl serotonin are constituents of banana plant sap.[8] These compounds are secondary metabolites of banana plants. The content of secondary metabolites of a plant depends on the species and the level depends on the environmentin which the plant lives. Active substances contained in a banana tree, such as flavonoids, saponins and tannins that work hand in hand so as to have an antibacterial effect.[9] Flavonoids are known to function as phytoalexins, namely as antimicrobials that can inhibit the growth of bacteria and fungi, thereby helping to inhibit the spread ofpathogens in plants.[10] Similar studies have also stated that not only bacteria and fungi on banana plants can be inhibited, but bacteria and fungi that are pathogenic in humans can also be inhibited, such as *Staphylococcus sp* 16, 18, 24 and *Candida sp* 16.[11]

In addition to flavonoids, tannins are also secondary metabolites that are often found in plants. Tannins areastringent, polyphenolic, have a bitter taste. Tannins are commonly used for the treatment of skin diseases, antibacterial, treatment of diarrhea, haemostatic (stops bleeding) and haemorrhoids.[12]
Based on the results of the study, the concentration of Kepok Banana sheaths extract did not show the potential for the sensitive category to the growth of *Staphylococcus aureus* (compared to the NCCLS table). Thediameter of the inhibition zone formed at various concentrations of the Kepok Banana sheaths extract tested wasclassified into the resistant category with a small growth inhibition capacity of 4.47 mm at various concentrations.The results of this study are not in accordance with the results of previous studies that used sample stems and roots extract of kepok banana had activity in inhibiting the growth of *Staphylococcus aureus* bacteria significantly with extract concentration of 6.25%, 12.5%, 25%.[7] There are differences in inhibition zone diameter such as different concentrations of extracts; different extract dilution, the difference of sample extract, different of bacteriastrains will influence different despite coming from the same species and different varieties of plants used; influenced by the state of site, the nutrients obtained from around the area grows, etc.[7] The small diameter inhibition zone that shown in this study caused by the low content of secondary metabolites that act as antibacterialin the plant parts used as samples, which were plant parts close to the roots (there was no significant growth in these parts). The results of secondary metabolism that are processed in the leaves will be transported to parts of the body that need it such as parts that are growing or parts that require substances from secondary metabolism.[10]In previous studies, it was stated that banana plant extracts were able to inhibit the growth of fungi and several bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida sp*.[13] Histopathologically the administration of Kepok banana plant extract can provide a cosmetic effect by repairing damaged skin structureswithout leaving scar tissue or scar tissue and accelerating the process of re-epithelialization of epidermal tissue, the formation of new blood vessels in mice.[14]

## CONCLUSION

The phytochemical content of Kepok Banana sheaths extract qualitatively contains antibacterial compounds, suchas alkaloids, saponins, tannins, phenols, and flavonoids. Quantitative test of Kepok Banana sheaths extract showedthe content of phenol, flavonoids, and tannins. There were differences in antimicrobial potency at various concentrations (55%, 70%, 85%, 100%) of Kepok banana sheaths extract with positive and negative controls. There is no effective concentration of Kepok Banana sheaths extract (sensitive category) with potential as an antimicrobial based on NCCLS standards. Further research is recommended to use samples of banana plant partsthat are more diverse, different media and extract concentrations are more varied, and different growing locations.

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