

ORIGINAL ARTICLE

Antimicrobial Activity of *Musa paradisiaca* var. *sapientum* on *Enterococcus faecalis* Viability

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ABSTRACT

Introduction: Root canal treatment often fails because of bacteria that grow back after obturation, such as *Enterococcus faecalis* (*E. faecalis*). The previous study has found the percentage of root canal treatment failure as much as 32-70%. It was because the facultative anaerobic bacteria are able to penetrate into the dentinal tubules and found to be resistant. Ambonese banana stem is one of the herbal plants in Indonesia that has been widely used, including the sap on the stem. Active substances in the sap of Ambonese banana stems such as flavonoids, saponins, and tannins have antimicrobial efficacy. The purpose of this study is to prove the antibacterial activity of Ambonese banana stems extract on *E. faecalis* and analyzing bacterial viability through a fluorescent microscope. **Methods:** The sample of this study was *E. faecalis* (ATCC 29212) which bred on BHIB media, then serially carried out dilution with Ambonese banana stem extract with a composition of 100%, 50%, 25%, 12.5%, 6.25%, 3.125 %, 1,563%. and 0.781%. Cultured in the Agar Mueller-Hinton medium and then calculate the number of bacterial colonies. This examination was continued using a fluorescent microscope for determining the viability of bacteria. **Results:** We obtained MIC at a concentration of 1.563% and MBC at a concentration of 3.125%. The ability of Ambonese banana stem extracts to kill *E. faecalis* in MIC was 92.22%, while in MBC it was 100%. **Conclusion:** Ambonese banana stem extract effectively inhibited the growth of *E. faecalis* bacteria.

Keywords: Ambonese banana stem extract, *Enterococcus faecalis*, MIC, MBC, Fluorescent microscope

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INTRODUCTION

Root canal treatment is an endodontic treatment that is mostly done with the aim of eliminating bacteria and preventing re-infection to preserve the teeth as long as possible in the oral cavity. It is hoped that the function of chewing teeth returns to normal. The stages of root canal treatment are the preparation, sterilization, and refilling of the root canals which must be performed in asepsis. Root canal irrigation aims to eliminate necrotic tissue and wet the root canals of the teeth thereby reducing the number of microorganisms. Irrigation fluids must have a good antimicrobial ability with a minimum toxicity level (1,2). Root canal treatment can fail due to several factors such as host condition, preparation, microbes and others. The main cause is due to the bacteria *Enterococcus faecalis* (*E. faecalis*) which grows back after obturation of the root canal. The prevalence of *E. faecalis* ranges from 32% to 70% when root canal treatment fails (3,4). *E. faecalis* is very resistant

to medication during treatment because this bacterium has the ability to enter the dentinal tubules so that when prepared, it is not accessible from the liquid irrigation and instrumentation of other preparation tools (5,6).

One way to overcome this problem is to use antibiotics or medicament ingredients by developing alternative uses of herbal medicines that contain antimicrobial content (7,8). One natural ingredient that can be used is the Ambonese banana stem sap. Banana tree are tropical plants that are often used as herbal medicines. The banana tree can be used as an alternative in the treatment of wounds, fevers, insect bites, digestive disorders, and epilepsy (9).

Ambonese banana stem sap contains active substances that are antimicrobial, (10) in the form of flavonoids as much as 8.18%, saponins 6.73%, and tannins 4.38%. Flavonoids can cause cell membrane rupture and result in disruption of the exchange of substances needed by bacteria to maintain life so that it causes bacterial death (11). The mechanism of saponins as antimicrobial compounds is by reducing the surface tension of the bacterial cell wall and destroying membrane permeability and then cytoplasm leaks out of the cell

resulting in cell death (12). Tannins can form hydrogen bonds so that proteins are denatured and interfere with their physiological activities. Because cell membranes are damaged, food and nutrients needed by bacteria to produce energy cannot be absorbed, this has an impact on bacterial growth that is stunted or even dead (13). One method for observing cell membrane damage is to use a fluorescent microscope using DNA probes as a marker (14).

The extraction method is used to obtain the ambon banana stem sap, with maceration method because it is simple, easy, and can be applied by the community. This technique is done by immersing the sample in a solvent (15). This process causes the attraction of active substances in the solvent. The solvent used in this study is water. Water can be used as a solvent because it is polar, inexpensive and easy to obtain. The compounds to be extracted from Ambonese banana sap such as flavonoids, saponins, tannins are polar, so the extraction process uses polar solvents (16,17). Based on this, it is necessary to prove the antibacterial efficacy of Ambonese banana stem extracts that can inhibit the growth of *E. faecalis*.

MATERIALS AND METHODS

Samples

The sample was the stock of *E. faecalis* ATCC (American Type Culture Collection) 29212 obtained from the Research Center Institute of the Faculty of Dentistry, Airlangga University and bred using the media Brain Heart Infusion Broth (BHIB) in a test tube then standardized with 0.5 McFarland (1, 5 x 10⁸ CFU / ml). This study was approved by the Health Research Ethics Commission at the Faculty of Dental Medicine, Universitas Airlangga no. 738/HRECC.FODM/XI/2019.

Ambonese Banana Stem Extract Preparation and Dilution Method Test

This study used eight groups to test the antibacterial potency of Ambonese banana stem extract to *E. faecalis* with a concentration of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.563%, 0.781%, and two comparison groups, namely positive control and negative control, so that the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were obtained. The making of Ambonese stem banana extract was done by maceration method. The banana stems were cut at an angle, blended until soft, soaked in water solvent 2x24 hours and filtered with Whatman 42 paper, then researchers carried out evaporation of the filtrate using a rotary evaporator. Phytochemical screening was also carried out to test the compounds present in the extract qualitatively.

Each extract tube as well as positive and negative controls were added 0.05 ml of a suspension of *E. faecalis* bacteria and incubated anaerobically at 37°C

in 24 hours. Then 0.1 ml was taken from each extract and control tube and subcultured in the Mueller-Hinton agar medium with a spreader. It was then anaerobically incubated again at 37°C in 24 hours, after which the number of colonies can be calculated manually and expressed with a colony-forming unit (CFU) and compared with positive control and negative control to determine the MIC and MBC.

Observation of *E. faecalis* Viability through a Fluorescent Microscope

The dilution concentration that produced MIC and MBC values from Ambonese banana stem extracts was taken as much as 20 mL and then put in an Eppendorf tube. It was then suspended with a 3 µL SYTO9 marker DNA probe with a concentration of 50 nM (Cat. S34854, USA) and 3 µL 10 nM Propidium Iodide (Cat. P3566, US). Vortex was carried out then incubated for 15 minutes at room temperature and dark conditions. Observations were made on glass objects with a magnification of 10x using a fluorescent microscope (Olympus CH30, Japan). Cells with intact membranes produce green fluorescence with a maximum excitation of 535 nm, while cells with damaged membranes produce red fluorescence with a maximum excitation of 617 nm.

RESULTS

Phytochemical components

The Saponin, flavonoid, tannin, anthraquinone and lectin levels were analyzed using UV-Visible Spectrophotometer by comparing the absorbance values of standard compounds with the extract of ambon banana stem sap. Detection of saponin compounds with a wavelength of 215 nm, flavonoid with wavelengths of 226 nm, tannin with wavelengths of 275 nm, anthraquinones with wavelengths of 285 nm. The concentration of each component was 1.30 mg/mL (13%) of saponin, 0.28 mg/mL (2.8%) of flavonoid, 1.50 mg/mL (15%) of tannin and 0.30 mg/mL (30%) of anthraquinone.

Antibacterial activity of Ambonese banana stem sap extract

The results showed that the negative control group did not show bacterial growth, whereas the positive control group showed the growth of *E. faecalis* bacterial colonies. Giving Ambonese stem banana extract could inhibit the growth of bacterial colonies from 100-3.125% concentration. At concentrations below 3.125%, it began to show the growth of bacterial colonies (Figure 1).

In this study, three repetitions of antibacterial efficacy for each concentration of Ambonese banana stem extract were carried out, namely 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.563%, 0.781 as well as positive and negative controls. The results of counting the number of bacterial colonies on Mueller-Hinton Agar media



Figure 1: Growth inhibition of *E. faecalis* in Mueller-Hinton media induced Ambonese banana stem extract. Antimicrobial activity of each group at (-) negative control, (+) positive control, 1. concentration of 100%, 2. concentrations of 50%, 3. concentrations of 25%, 4. concentrations of 12.5%, 5. concentrations of 6.25%, 6. concentrations of 3.125%, 7. concentrations of 1.563%, 8. concentrations of 0.781%.

showed that at a concentration of 3.125% there was no growth of bacterial colonies. This shows that the administration of Ambonese banana stem extract with a concentration of 100-3.125% can inhibit bacterial growth, while the concentrations below that are 1.563% and 0.781% there is bacterial colonies growth of 13 colonies or 7.78% and 30 colonies or 17.9% (Table I).

Table 1: The number *E. faecalis* colonies induced Ambonese banana stem extract at 0.5 McFarland

	Number of repetitions	Colony (CFU / ml)
100%	3	0
50%	3	0
25%	3	0
12.5%	3	0
6.25%	3	0
3.125%	3	0
1.563%	3	13
0.781%	3	30
Control (+)	3	167
Control (-)	3	0

Viability of *E. faecalis*

The fluorescent microscope can be used to distinguish living bacteria and dead bacteria. The intact cell will have green fluorescence by coloring SYTO9 and PI. SYTO9 is a DNA staining that is able to diffuse into cells so that they are able to color cells either with intact or damaged membranes. Meanwhile for PI, only cells with damaged membranes produce a bond between PI and DNA so that the cells will have a red fluorescence. In intact membranes, PI is unable to diffuse into cells due to the large molecular weight size (Figure 2).

DISCUSSION

Ambonese banana extract has been proven to contain compounds that are antimicrobial. The most dominant contents were 13% of saponin, 2.8% of flavonoid, 15% of tannin and 30% of anthraquinone. In this study, Ambonese banana stem extracts were used with various concentrations, namely 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.563%. 0.781 with the aim to determine the ability of inhibitory and killing of Ambonese banana extract on the growth of *E. faecalis*.

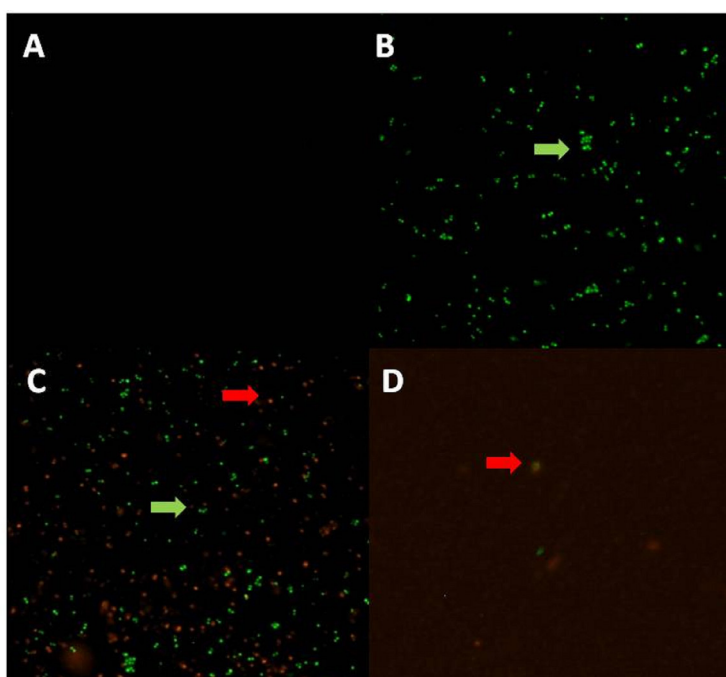


Figure 2: The viability of *E. faecalis* at 10x magnification fluorescent microscope. The using of a SYTO9 marker DNA probe and Propidium Iodide at A. The negative control, B. The Positive control, C. MIC and D. MBC. Description: red arrow dead bacterial cell, green arrow living bacterial cell.

The results of this study indicate Ambonese banana sap extract can inhibit the growth of *E. faecalis*. In accordance with the understanding of MIC is the minimum concentration that can inhibit the growth of 90% of bacteria and MBC is the minimum concentration that can inhibit 99.9% bacterial growth, then the MIC value is at a concentration of 1.56% because it can kill 92.22% of bacteria and MBC at a concentration of 3.125 % because bacterial growth is no longer found. These results indicate that the Ambonese banana stem extract had antibacterial activity against *E. faecalis*.

The results of *E. faecalis* colony count in this study are directly proportional to previous studies which stated that the Ambonese banana stem extract has antibacterial ability against human pathogenic bacteria such as *Staphylococcus aureus* (7). Research on the antibacterial activity of the Ambonese banana stem extract against *E. faecalis* has also been done before with the extraction method using ethanol 96%, there is an important difference in concentration for the MIC and MBC values obtained, namely the MIC at a concentration of 70% and the MBC at a concentration of 72.5%. Ambonese banana trees are influenced by topography, climate, soil type, rainfall, fertilization, and also the solvent used in extraction.

According to the previous studies, water extracts can attract other compounds in Ambonese banana plants, namely lectins. Lectin is a protein that is attached to certain carbohydrate groups that have antimicrobial properties by decreasing cell permeability until cell leakage occurs (17,18). It is suspected that in Ambonese banana sap extracts studied there are also lectin compounds so that it adds antimicrobial properties of the extract and produces MBC and MIC values which area quite important difference from previous research. Active substances containing antimicrobial compounds in Ambonese banana sap extract have been proven to inhibit the growth of *E. faecalis*. Saponin compounds work by lowering surface tension, resulting in increased cell permeability or leakage and causing intracellular compounds to come out (19). Flavonoids play a role in damaging proteins so that proteins cannot function anymore and interfere with the metabolism and physiological functions of bacteria. Disrupted metabolism results in permanent cell damage due to insufficient energy requirements to maintain its life (20). Tannin compounds react with bacterial cell membrane proteins that cause hydrogen bonds to form. The hydrogen bonds that are formed cause the denaturation of proteins and eventually the bacterial cell membrane is damaged due to disruption of cell wall permeability. This will cause the intake of nutrients and food needed for bacterial growth to decrease and cause the bacteria to die. In addition, tannins can also activate the adhesion ability of microbial cells so that they interfere with the attachment of bacteria (21).

There were living bacterial on the positive group, but no bacteria on negative group. The bacterial viability and death could be shown by microscope fluorescent. The increasing concentration of Ambonese banana stem extract causes *E. faecalis* death. It means the extracts capable to penetrate into bacteria thus damaging cell membranes. This was demonstrated using a fluorescent microscope through a SYTO9 DNA probe with a green label, and PI labeled in red. The living bacteria indicated green color on the fluorescent microscope, it means the PI unable to penetrate intact cell membranes. Whereas the dead bacteria is showed green fluorescent will turn red, that means PI able to penetrate in damaged membrane. The fluorescent dyes could be used to indicate bacterial viability (22,23).

CONCLUSION

Banana sap extract (*Musa paradisiaca var. sapientum*) has antibacterial efficacy with a Minimum Inhibitory Concentration (MIC) at a concentration of 1.563% and a Minimum Bactericidal Concentration (MBC) at a concentration of 3.125% against *E. faecalis* bacteria.

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