Korespondensi Penulis dengan
Malaysian Journal of Medicine & Health Sciences

Article History

Submission	: 11 Februari 2020
Hasil Review-1	: 12 Februari 2020
Pengiriman Revisi-1	: 12 Februari 2020
Hasil Review-2	: 11 April 2020
Pengiriman Revisi-2	: 12 April 2020
Accepted	: 17 April 2020
Galley Proof	: 12 Juni 2020
Revisi Galley Proof	: 15 Juni 2020
Published	: 05 Juli 2020

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Malaysian Journal of Medicine & Health Sciences - Manuscript ID MJMHS-2020-0109

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

Journal:	*Malaysian Journal of Medicine & Health Sciences*
Manuscript ID	Draft
Manuscript Type:	Supp: OBSM
Keywords:	Ambonese banana stem extract, Enterococcus faecalis, MIC, MBC, Fluorescent microscope

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. **Materials and 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%. **Conclusions:** Ambonese banana stem extract effectively inhibited the growth of *E. faecalis* bacteria.

Key words: Ambonese banana stem extract, Enterococcus faecalis, MIC, MBC, Fluorescent microscope.

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 stemswere 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˚ 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˚ 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, USA). 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 of saponin, 0.28 mg/mL of favonoid, 1.50 mg/mL of tannin and 0.30 mg/mL of antraquinone.

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 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 1).

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 fluoresce. In intact membranes, PI is unable to diffuse into cells due to the large molecular weight size (figure 2).

DISCUSSIONS

Ambonese banana extract has been proven to contain compounds that are antimicrobial. The most dominant contentsare flavonoids at 8.18%, saponins 6.73%, and tannins 4.38%. 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*.

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 Budi's research in 2013, 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 whicharea quite important differencefrom 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)

We used methods and rationale for the use of fluorescent dyes that indicate bacterial viability to assess the survival of bacteria attached to and internalized by host cells. There were living bacterial on positive group, but no bacterial on MBC concentration. To identify extracellular bacteria, infected cells are first exposed to a fluorescent reagent, such as a lectin or bacteria-specific antibody. The infected cells are then permeabilized and exposed to DNA-specific dyes that are differentially accessible to bacteria with intact vs. degraded membranes, as a surrogate for bacterial viability. The membrane permeable dye SYTO9 identifies the total bacterial population, while propidium iodide is only accessible to those bacteria that have compromised membranes and are thus considered nonviable. Propidium iodide and SYTO9 have been used to evaluate bacterial viability in biofilms, discriminate pathogenic from nonpathogenic bacteria, and enumerate viable water-borne bacteria (22,23).

CONCLUSIONS

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.

ACKNOLEDGMENTS

We thank you for the support of the Research Center for the completion of this research

REFERENCES

1. Sabir A. Root canal overfilling as an influencing factor for the success of endodontic treatment. Dent J. 2005; 38(4): 194-7.
2. Trilaksana A, Saraswati A. Efficacy of green tea leaf extract (camellia sinensis) with NaOCl 2.5% againts enterococcus faecalis as an alternative solution for root canal irrigation. J Dentomaxillofacial Sci. 2016;1(1):58-62.
3. Liu H, Wei X, Ling J. Biofilm formation capability of Enterococcus faecalis cells in starvation phase and its susceptibility to sodium hypochlorite. J Endo. 2010;36(4):630-5.
4. Wang QQ, Zhang CF, Chu CH, Zhu XF. Prevalence of Enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis. Int J Oral Sci. 2012;4(1):19–23.
5. Al-Nazhan S, Al-Sulaiman A, Al-Rasheed F, Alnajjar F, Al-Abdulwahab B, Al-Badah A. Microorganism penetration in dentinal tubules of instrumented and retreated root canal walls. In vitro SEM study. Restor Dent Endod. 2014;39(4):258–64.
6. Vatkar NA, Hegde V, Sathe S. Vitality of Enterococcus faecalis inside dentinal tubules after five root canal disinfection methods. J Conserv Dent. 2016;19(5):445–9.
7. Karadi R V, Arpan S, Pranav P, Parvez A. Antimicrobial Activities of Musa paradisiaca and Cocos nucifera. Int J Res Pharm Biomed Sci. 2011;2:264-7.
8. Karuppiah P, Mustaffa M. Antibacterial and antioxidant activities of Musa sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pac J Trop Biomed. 2013;3(9):737–42.
9. Kumar, K P S, Bhowmik D, Duraivel S, Umadevi M. Traditional and medicinal uses of banana. J Pharmacog Phytochem. 2012;1(3):51-63.
10. Kapadia SP, Pudakalkatti PS, Shivanaikar S. Detection of antimicrobial activity of banana peel (Musa paradisiaca L.) on Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans: An in vitro study. Contemp Clin Dent. 2015;6(4):496–499.
11. Górniak I, Bartoszewski R, Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev. 2019:18(1):241–72.
12. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control. 2019;8:118.
13. Mailoa MN, Mahendradatta M, Laga M, Djide N. Antimicrobial activities of tannins extract from guava leaves (Psidium Guajava L) on pathogens microbial. International Journal of Scientific & Technology Research. 2014; 3(1): 239-240.
14. Johnson, MB, Criss, AK. Fluorescence microscopy methods for determining the viability of bacteria in association with mammalian cells. J Vis Exp. 2013;79. doi: 10.3791/50729.
15. Azwanida NN. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med Aromat Plants. 2015;4:196. doi:10.4172/2167-0412.1000196.
16. Snehlata K, Sheel R, Kumar B. Evaluation of phytochemicals in polar and non polar solvent extracts of leaves of Aegle marmelos (L.). J Biotech Biochem. 2018;4(5):31-8.
17. Budi HS. PDGF-BB, BMP-4 and BMP-7 expression induced Ambonese banana stem (Musa paradisiaca var. sapientum) extract on wound healing process following tooth extraction. Dissertation. Surabaya: Universitas Airlangga. 2013.
18. Coelho L C B B, Silva P M, Oliveira P M. Lectin as antimicrobial agents. J Appl Microbiol. 2018; 125(5):1238-52.

19. Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, Jurzysta M. Antimicrobial activity of Saponins from Medicago sp.: Structure-activity relationship. Phytother Res. 2006;20:454-7.
20. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Sci World J. 2013:162750.
21. Sieniawska E,Baj T. Tannins : Pharmacognosy Fundamentals, Applications and Strategies. In: Simone Badal, editor. Academic Press; 2017:199-232.
22. Yang L, Wu L, Zhu S, Long Y, Hang W, Yan X. Rapid, absolute, and simultaneous quantification of specific pathogenic strain and total bacterial cells using an ultrasensitive dual-color flow cytometer. Anal Chem. 2010 Feb 1;82(3):1109-16.
23. Hoefel, D., Grooby, W.L., Monis, P.T., Andrews, S., & Saint, C.P. Enumeration of water-borne bacteria using viability assays and flow cytometry: a comparison to culture-based techniques. J Microbiol Methods. 2003;55:585-97.

Figure legends

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%.

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.

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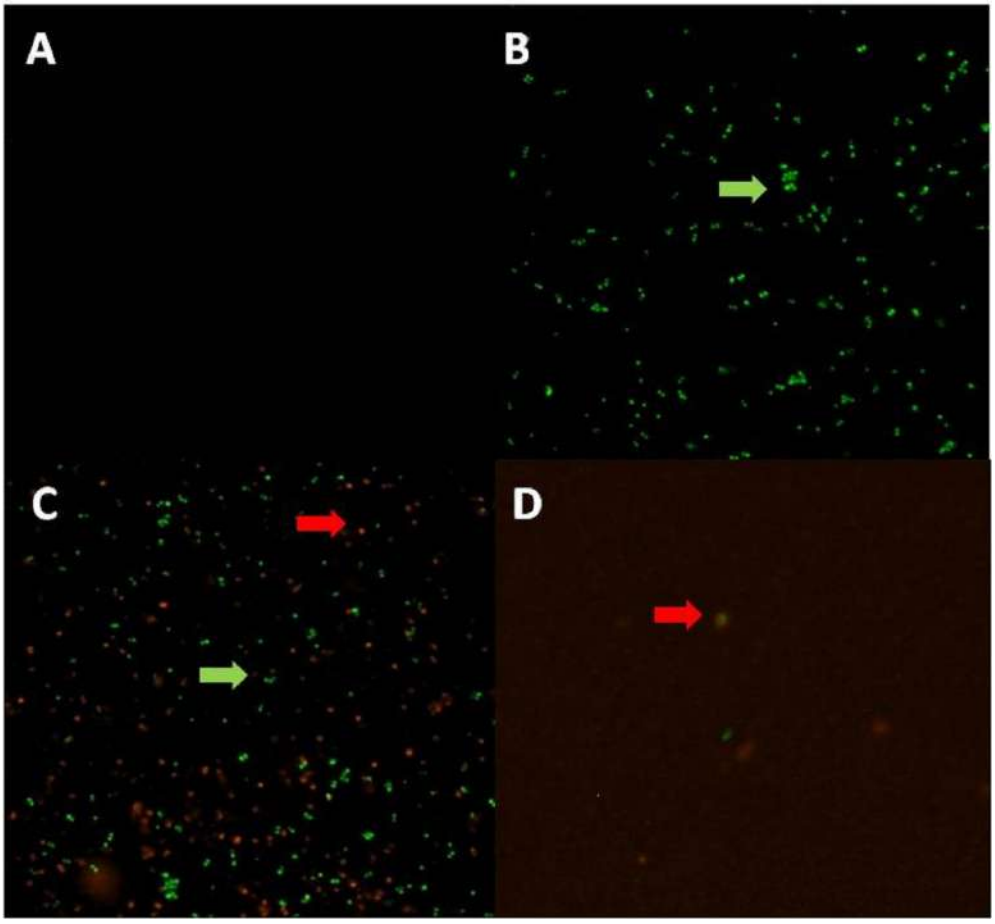

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Table legends

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

`	Number of repetitions	Colony (CFU / ml) $\bar{X}$
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

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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 of saponin, 0.28 mg/mL of favonoid, 1.50 mg/mL of tannin and 0.30 mg/mL of antraquinone.

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 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 1).

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 fluoresce. In intact membranes, PI is unable to diffuse into cells due to the large molecular weight size (figure 2).

DISCUSSIONS

Ambonese banana extract has been proven to contain compounds that are antimicrobial. The most dominant contentsare flavonoids at 8.18%, saponins 6.73%, and tannins 4.38%. 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*.

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 Budi's research in 2013, 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 whicharea quite important differencefrom 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)

We used methods and rationale for the use of fluorescent dyes that indicate bacterial viability to assess the survival of bacteria attached to and internalized by host cells. There were living bacterial on positive group, but no bacterial on MBC concentration. To identify extracellular bacteria, infected cells are first exposed to a fluorescent reagent, such as a lectin or bacteria-specific antibody. The infected cells are then permeabilized and exposed to DNA-specific dyes that are differentially accessible to bacteria with intact vs. degraded membranes, as a surrogate for bacterial viability. The membrane permeable dye SYTO9 identifies the total bacterial population, while propidium iodide is only accessible to those bacteria that have compromised membranes and are thus considered nonviable. Propidium iodide and SYTO9 have been used to evaluate bacterial viability in biofilms, discriminate pathogenic from nonpathogenic bacteria, and enumerate viable water-borne bacteria (22,23).

CONCLUSIONS

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.

ACKNOLEDGMENTS

We thank you for the support of the Research Center for the completion of this research

REFERENCES

1. Sabir A. Root canal overfilling as an influencing factor for the success of endodontic treatment. Dent J. 2005; 38(4): 194-7.

2. Trilaksana A, Saraswati A. Efficacy of green tea leaf extract (camellia sinensis) with NaOCl 2.5% againts enterococcus faecalis as an alternative solution for root canal irrigation. J Dentomaxillofacial Sci. 2016;1(1):58-62.
3. Liu H, Wei X, Ling J. Biofilm formation capability of Enterococcus faecalis cells in starvation phase and its susceptibility to sodium hypochlorite. J Endo. 2010;36(4):630-5.
4. Wang QQ, Zhang CF, Chu CH, Zhu XF. Prevalence of Enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis. Int J Oral Sci. 2012;4(1):19–23.
5. Al-Nazhan S, Al-Sulaiman A, Al-Rasheed F, Alnajjar F, Al-Abdulwahab B, Al-Badah A. Microorganism penetration in dentinal tubules of instrumented and retreated root canal walls. In vitro SEM study. Restor Dent Endod. 2014;39(4):258–64.
6. Vatkar NA, Hegde V, Sathe S. Vitality of Enterococcus faecalis inside dentinal tubules after five root canal disinfection methods. J Conserv Dent. 2016;19(5):445–9.
7. Karadi R V, Arpan S, Pranav P, Parvez A. Antimicrobial Activities of Musa paradisiaca and Cocos nucifera. Int J Res Pharm Biomed Sci. 2011;2:264-7.
8. Karuppiah P, Mustaffa M. Antibacterial and antioxidant activities of Musa sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pac J Trop Biomed. 2013;3(9):737–42.
9. Kumar, K P S, Bhowmik D, Duraivel S, Umadevi M. Traditional and medicinal uses of banana. J Pharmacog Phytochem. 2012;1(3):51-63.
10. Kapadia SP, Pudakalkatti PS, Shivanaikar S. Detection of antimicrobial activity of banana peel (Musa paradisiaca L.) on Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans: An in vitro study. Contemp Clin Dent. 2015;6(4):496–499.
11. Górniak I, Bartoszewski R, Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev. 2019:18(1):241–72.
12. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control. 2019;8:118.
13. Mailoa MN, Mahendradatta M, Laga M, Djide N. Antimicrobial activities of tannins extract from guava leaves (Psidium Guajava L) on pathogens microbial. International Journal of Scientific & Technology Research. 2014; 3(1): 239-240.
14. Johnson, MB, Criss, AK. Fluorescence microscopy methods for determining the viability of bacteria in association with mammalian cells. J Vis Exp. 2013;79. doi: 10.3791/50729.
15. Azwanida NN. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med Aromat Plants. 2015;4:196. doi:10.4172/2167-0412.1000196.
16. Snehlata K, Sheel R, Kumar B. Evaluation of phytochemicals in polar and non polar solvent extracts of leaves of Aegle marmelos (L.). J Biotech Biochem. 2018;4(5):31-8.
17. Budi HS. PDGF-BB, BMP-4 and BMP-7 expression induced Ambonese banana stem (Musa paradisiaca var. sapientum) extract on wound healing process following tooth extraction. Dissertation. Surabaya: Universitas Airlangga. 2013.
18. Coelho L C B B, Silva P M, Oliveira P M. Lectin as antimicrobial agents. J Appl Microbiol. 2018; 125(5):1238-52.
19. Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, Jurzysta M. Antimicrobial activity of Saponins from Medicago sp.: Structure-activity relationship. Phytother Res. 2006;20:454-7.
20. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Sci World J. 2013:162750.

21. Sieniawska E,Baj T. Tannins : Pharmacognosy Fundamentals, Applications and Strategies. In: Simone Badal, editor. Academic Press; 2017:199-232.
22. Yang L, Wu L, Zhu S, Long Y, Hang W, Yan X. Rapid, absolute, and simultaneous quantification of specific pathogenic strain and total bacterial cells using an ultrasensitive dual-color flow cytometer. Anal Chem. 2010 Feb 1;82(3):1109-16.
23. Hoefel, D., Grooby, W.L., Monis, P.T., Andrews, S., & Saint, C.P. Enumeration of water-borne bacteria using viability assays and flow cytometry: a comparison to culture-based techniques. J Microbiol Methods. 2003;55:585-97.

Figure legends

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%.

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.

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Improve the method

Reviewer: 2

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Comments to the Author
The manuscript with the title "Antimicrobial Activity of Musa paradisiaca var. sapientum on Enterococcus faecalis Viability" (MJMHS-2020-0109) is quite interesting. However, to be published in Malaysian Journal of Medicine and Health Sciences, then in this manuscript needs some improvement. Here are some improvements that need to be considered:

1. The author needs to give reasons for choosing water as a solvent in the maceration process. This is considering that if the reason for choosing water as a solvent is as mentioned in the introduction (polar, inexpensive and easy to obtain), the author needs to give another more scientific reasons. Because many compounds are more polar when compared to water, such as methanol and ethanol. In addition, the price of ethanol is also quite cheap, easily obtained, easily purified and can be reused for the next maceration process.
In addition, the author also needs to provide a more detailed explanation of the evaporation from the obtained extracts using rotary-vacuum evaporator. This is due to the fact that in this research, water will be evaporated (generally has a higher boiling point than organic solvents).
2. In this study what standard compound is used to determine the levels of saponins, flavonoids, tannins. This is considering that saponins, flavonoids, tannins are class of compounds. And the standard compound used in this study may be only one compound from each class of these compounds. Therefore, the author needs to provide an interpretation about how standard compound (one compound) can be used to determine the levels of saponins, flavonoids, tannins.
3. In the results section the authors said that "The concentration of each component was 1.30 mg/mL of saponin, 0.28 mg/mL of favonoid, 1.50 mg/mL of tannin and 0.30 mg/mL of antraquinone". However, in the discussion section the authors said that “The most dominant contents are flavonoids at 8.18%, saponins 6.73%, and tannins 4.38%". Therefore, the author needs to provide an explanation about how to convert concentration units in the form of mg/mL to %.
4. The writing of decimal on paper needs to be checked again. As in Table 1, the writing of decimal still uses commas.
5. The writing of reference needs to be checked again. This is because the writing of references is still not consistent and not in accordance with the existing guidelines.

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Thank you for your fine contribution. On behalf of the Editors of the Malaysian Journal of Medicine & Health Sciences, we look forward to your continued contributions to the Journal.

Sincerely,
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Reviewer: 1

Comments to the Author
The authors have made revisions in accordance with the comments that have been given by reviewers

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ORIGINAL ARTICLE

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

Hendrik Setia Budi[1,2], Wisnu Setyari Juliastuti[1], Brenda Regina Christy[3]

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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%. **Conclusions:** 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 fluoresce. 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*.

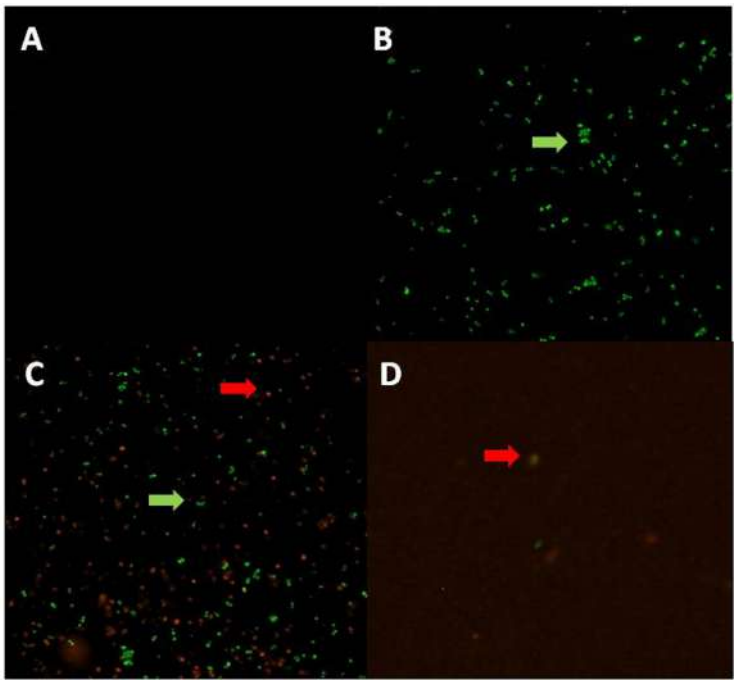

Figure 2: The viability of *E. faecalis* at 10x magnification fluorescent microscope. The using of a SYTO9 marker DNA probe and Propidium Iodide at A. The negative control, B. The Positive control, C. MIC and D. MBC. Description: red arrow dead bacterial cell, green arrow living bacterial cell.

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.

ACKNOWLEDGEMENTS

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REFERENCES

1. Sabir A. Root canal overfilling as an influencing factor for the success of endodontic treatment. Dent J. 2005;38(4):194-7.
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4. Wang QQ, Zhang CF, Chu CH, Zhu XF. Prevalence of Enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis. Int J Oral Sci. 2012;4(1):19–23.
5. Al-Nazhan S, Al-Sulaiman A, Al-Rasheed F, Alnajjar F, Al-Abdulwahab B, Al-Badah A. Microorganism penetration in dentinal tubules of instrumented and retreated root canal walls. In vitro SEM study. Restor Dent Endod. 2014;39(4):258–64.
6. Vatkar NA, Hegde V, Sathe S. Vitality of Enterococcus faecalis inside dentinal tubules after five root canal disinfection methods. J Conserv Dent. 2016;19(5):445–9.
7. Karadi RV, Arpan S, Pranav P, Parvez A. Antimicrobial activities of Musa paradisiaca and

Cocos nucifera. Int J Res Pharm Biomed Sci. 2011;2:264-7.
8. Karuppiah P, Mustaffa M. Antibacterial and antioxidant activities of Musa sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pac J Trop Biomed. 2013;3(9):737–42.
9. Kumar, K P S, Bhowmik D, Duraivel S, Umadevi M. Traditional and medicinal uses of banana. J Pharmacog Phytochem. 2012;1(3):51-63.
10. Kapadia SP, Pudakalkatti PS, Shivanaikar S. Detection of antimicrobial activity of banana peel (Musa paradisiaca L.) on Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans: An in vitro study. Contemp Clin Dent. 2015;6(4):496–9.
11. Gyrniak I, Bartoszewski R, Kryliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev. 2019:18(1):241–72.
12. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control. 2019;8:118.
13. Mailoa MN, Mahendradatta M, Laga M, Djide N. Antimicrobial activities of tannins extract from guava leaves (Psidium guajava L) on pathogens microbial. Int J Sci Tech Res. 2014; 3(1):239-40.
14. Johnson MB, Criss AK. Fluorescence microscopy methods for determining the viability of bacteria in association with mammalian cells. J Vis Exp. 2013;79. doi: 10.3791/50729.
15. Azwanida NN. A Review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015;4:196.
16. Snehlata K, Sheel R, Kumar B. Evaluation of phytochemicals in polar and non polar solvent extracts of leaves of Aegle marmelos (L.). J Biotech Biochem. 2018;4(5):31-8.
17. Budi HS, Astuti ER. The MMP-2, MMP-9 expression and collagen density of the Ambonese banana stem sap administration on wound healing. J Int Dent Med Res. 2019;12(2):492-7.
18. Coelho L C B B, Silva P M, Oliveira P M. Lectin as antimicrobial agents. J Appl Microbiol. 2018;125(5):1238-52.
19. Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, Jurzysta M. Antimicrobial activity of saponins from Medicago sp.: structure-activity relationship. Phytother Res. 2006;20:454-7.
20. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Sci World J. 2013:162750.
21. Sieniawska E,Baj T. Tannins : Pharmacognosy fundamentals, applications and strategies. In: Simone B, editor. Academic Press; 2017.
22. Yang L, Wu L, Zhu S, Long Y, Hang W, Yan X. Rapid, absolute, and simultaneous quantification of specific pathogenic strain and total bacterial cells using an ultrasensitive dual-color flow cytometer. Anal Chem. 2010;82(3):1109-16.
23. Hoefel D, Grooby WL, Monis PT, Andrews S, Saint CP. Enumeration of water-borne bacteria using viability assays and flow cytometry: a comparison to culture-based techniques. J Microbiol Methods. 2003;55:585-97.

Galley proof article for OBSM issue (MJMHS 16(SUPP4) July 2020

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ORIGINAL ARTICLE

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

Hendrik Setia Budi[1,2], Wisnu Setyari Juliastuti[1], Brenda Regina Christy[3]

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[3] Undergraduate Program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya 60132, Indonesia

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.

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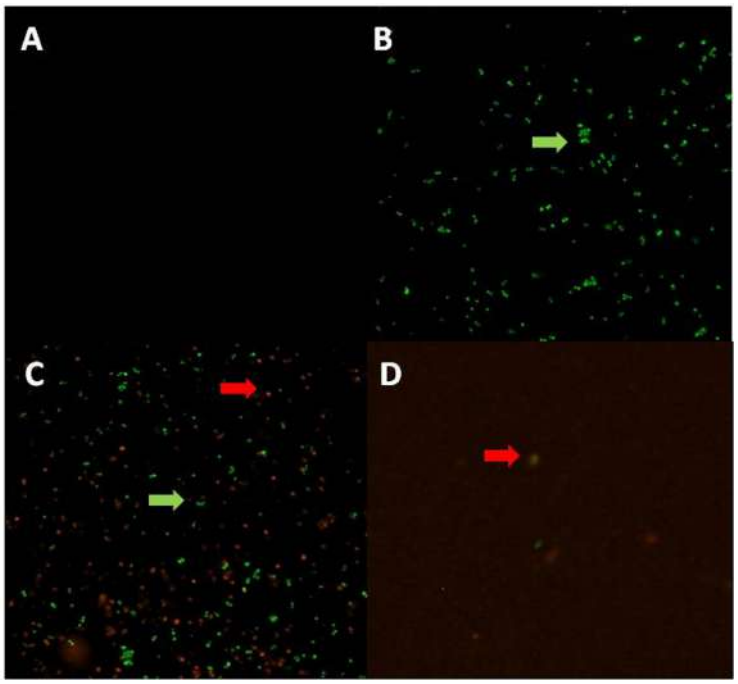

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3. Liu H, Wei X, Ling J. Biofilm formation capability of Enterococcus faecalis cells in starvation phase and its susceptibility to sodium hypochlorite. J Endo. 2010;36(4):630-5.
4. Wang QQ, Zhang CF, Chu CH, Zhu XF. Prevalence of Enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis. Int J Oral Sci. 2012;4(1):19–23.
5. Al-Nazhan S, Al-Sulaiman A, Al-Rasheed F, Alnajjar F, Al-Abdulwahab B, Al-Badah A. Microorganism penetration in dentinal tubules of instrumented and retreated root canal walls. In vitro SEM study. Restor Dent Endod. 2014;39(4):258–64.
6. Vatkar NA, Hegde V, Sathe S. Vitality of Enterococcus faecalis inside dentinal tubules after five root canal disinfection methods. J Conserv Dent. 2016;19(5):445–9.
7. Karadi RV, Arpan S, Pranav P, Parvez A. Antimicrobial activities of Musa paradisiaca and

Cocos nucifera. Int J Res Pharm Biomed Sci. 2011;2:264-7.
8. Karuppiah P, Mustaffa M. Antibacterial and antioxidant activities of Musa sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pac J Trop Biomed. 2013;3(9):737–42.
9. Kumar, K P S, Bhowmik D, Duraivel S, Umadevi M. Traditional and medicinal uses of banana. J Pharmacog Phytochem. 2012;1(3):51-63.
10. Kapadia SP, Pudakalkatti PS, Shivanaikar S. Detection of antimicrobial activity of banana peel (Musa paradisiaca L.) on Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans: An in vitro study. Contemp Clin Dent. 2015;6(4):496–9.
11. Gyrniak I, Bartoszewski R, Kryliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev. 2019:18(1):241–72.
12. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control. 2019;8:118.
13. Mailoa MN, Mahendradatta M, Laga M, Djide N. Antimicrobial activities of tannins extract from guava leaves (Psidium guajava L) on pathogens microbial. Int J Sci Tech Res. 2014; 3(1):239-40.
14. Johnson MB, Criss AK. Fluorescence microscopy methods for determining the viability of bacteria in association with mammalian cells. J Vis Exp. 2013;79. doi: 10.3791/50729.
15. Azwanida NN. A Review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015;4:196.
16. Snehlata K, Sheel R, Kumar B. Evaluation of phytochemicals in polar and non polar solvent extracts of leaves of Aegle marmelos (L.). J Biotech Biochem. 2018;4(5):31-8.
17. Budi HS, Astuti ER. The MMP-2, MMP-9 expression and collagen density of the Ambonese banana stem sap administration on wound healing. J Int Dent Med Res. 2019;12(2):492-7.
18. Coelho L C B B, Silva P M, Oliveira P M. Lectin as antimicrobial agents. J Appl Microbiol. 2018;125(5):1238-52.
19. Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, Jurzysta M. Antimicrobial activity of saponins from Medicago sp.: structure-activity relationship. Phytother Res. 2006;20:454-7.
20. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Sci World J. 2013:162750.
21. Sieniawska E,Baj T. Tannins : Pharmacognosy fundamentals, applications and strategies. In: Simone B, editor. Academic Press; 2017.
22. Yang L, Wu L, Zhu S, Long Y, Hang W, Yan X. Rapid, absolute, and simultaneous quantification of specific pathogenic strain and total bacterial cells using an ultrasensitive dual-color flow cytometer. Anal Chem. 2010;82(3):1109-16.
23. Hoefel D, Grooby WL, Monis PT, Andrews S, Saint CP. Enumeration of water-borne bacteria using viability assays and flow cytometry: a comparison to culture-based techniques. J Microbiol Methods. 2003;55:585-97.