



Antibacterial Potency of Prabumulih's Pineapple Leaves Extract (*Ananas comosus*) Towards *Enterococcus faecalis*

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Abstract

Introduction: Unsuccessful elimination procedures of Gram-positive bacteria (*Enterococcus faecalis*) often lead to failure of endodontic treatment. It is necessary to use irrigation solutions to eliminate it from the root canal. The leaves of Pineapple (*Ananas comosus*) that are widely cultivated in Prabumulih have antibacterial properties due to active compounds such as bromelain enzymes, flavonoids, phenols, saponins, tannins, and alkaloids and have potential as an alternative material for root canal irrigation. **Purpose:** This study aimed to determine the antibacterial potency of Prabumulih pineapple leaf extract (*Ananas comosus*) against *E. faecalis*. **Methods:** This is an in vitro quasi-experimental laboratory study with a post-test-only control group study design. The test group consisted of Prabumulih pineapple leaf extract at concentrations of 1.56%, 3.125%, 6.25%, and 12.5% obtained using the maceration method. The positive control was NaOCl 2.5%, and distilled water was the negative control. The antibacterial activity of the extract using the disc diffusion method for the inhibition zone test, the dilution method for the minimum inhibitory concentration (MIC) test, and solid dilution for the minimum bactericidal concentration (MBC) test. **Results:** It showed that Prabumulih pineapple leaves extract has antibacterial potency against *E. faecalis* with the largest average inhibition zone at a concentration of 12.5% (2.324 mm), MIC at a concentration of 12.5%, and MBC also at a concentration of 12.5%. **Conclusion:** Prabumulih pineapple leaf extract has antibacterial potency against *Enterococcus faecalis*.

Keywords: antibacterial; *Enterococcus faecalis*; pineapple leaves

Introduction

Endodontic treatment is a biologically acceptable chemical and mechanical treatment procedure in the root canal of a tooth to eliminate pulpal and periradicular disease, promote healing, and repair the periradicular tissue.¹ It aims to eliminate infection by killing microorganisms in the root canal and preventing re-infection.^{2,3} However, if Gram-positive bacteria are not completely removed from the root canal, its persistence will lead to endodontic treatment failure.⁴

Enterococcus faecalis is the most common bacteria found in root canals, with a percentage of about 77%. Based on research conducted by Gomes et al., of the 53 species of bacteria in root canals, the most common bacteria found in failed endodontic treatment was *E. faecalis* (27%).⁵ The high resistance of *E. faecalis* is due to various virulence factors, including



its ability to compete with other microorganisms to invade the dentinal tubules and survive at high temperatures and pH.⁴

South Sumatra occupies the 7th position in 2021 as the largest pineapple producer in Indonesia based on data from the Central Statistics Agency (BPS). The city of Prabumulih is the largest producer of the Ratu variety pineapple (*Ananas comosus*) in South Sumatra and has been named the sweetest in Indonesia.^{5,6} Every harvest period, it is replaced with a new plant so that the leaves are discarded as waste. These large quantities of waste are underutilized and may cause severe environmental problems if not properly handled.⁸

Phytochemical testing by Thomas et al. (2019), showed the presence of active compounds in *A. comosus* leaf extract such as coumarins, terpenoids, phlobatannins, alkaloids, phenols, saponins, quinones, carbohydrates, proteins, cardiac glycosides, steroids, and flavonoids, as well as bromelain enzymes which have antibacterial effects.^{9,10} In line with the research conducted by Rega et al. (2016), *A. comosus* peel extract has an antibacterial effect on *E. faecalis* bacteria with a minimum inhibitory concentration (MIC) at a concentration of 3.125% with growth of less than 10% and a minimum killing concentration (KBM) at a concentration of 6.25%.¹¹ In order to mitigate the loss of valuable nutrients and minimize waste, there is significant potential to recover these compounds or transform them into valuable products, including one suitable for use as an irrigation solution in root canal treatment. The objective of this study was to examine the antibacterial activity of *A. comosus* leaf extract against *E. faecalis*.

Methods

The quasi-in vitro laboratory experimental research with a post-test-only control group design was carried out from February 27 to May 18, 2023, at the Chemical Engineering Laboratory of the Sriwijaya State Polytechnic, Palembang, South Sumatra and the Inter-University Research Laboratory at Gadjah Mada University, Sleman, Yogyakarta Special Region. The treatment groups are *A. comosus* leaf extract with concentrations of 1.56%, 3.125%, 6.25%, 12.5%, NaOCl 2.5% as the positive control group, and distilled water as the negative control group.



Before treatment, all tools were sterilized using an oven with a temperature of 160-170°C for 1-2 hours for heat-resistant glassware such as petri dishes, Erlenmeyer, ose, and others. For tools that cannot stand heat, sterilization was done using an autoclave at 121°C with a pressure of 15 ATM for 15 minutes.

The *A. comosus* leaf was sourced from the pineapple plantation in Prabumulih City. The extraction process involved the following steps: 1 kilogram of pineapple leaves were washed, drained, and cut into small pieces. Subsequently, they were dried under shade for three days and then in an oven for one day. Once dried, the leaves were blended into a powder. The pineapple leaf powder, weighing 500 grams, was then transferred into a 1000 mL Erlenmeyer flask. Next, 500 mL of 96% ethanol was added to the flask, and the mixture was shaken for one hour until homogeneous. The solution was left to macerate for 3 consecutive periods of 24 hours each at room temperature. After each 24-hour period, the solution was filtered using filter paper. The resulting filtrate was concentrated using a Rotary Vacuum Evaporator at 50°C until a 100% extract concentration was achieved. Finally, the extract was diluted to obtain concentrations of *A. comosus* leaf extract at 1.56%, 3.125%, 6.25%, and 12.5%.

Preparation of Mueller Hinton Agar Bacterial Culture Media

Mueller Hinton Agar (MHA) powder weighing 6.8 grams was dissolved in 200 mL of distilled water in an Erlenmeyer flask, and the mixture was homogenized by heating until completely dissolved. Subsequently, the media were sterilized by autoclaving for 15 minutes at 121°C and 1.5 ATM pressure. Finally, pour 25 mL of the Mueller Hinton Agar (MHA) medium into each Petri dish.

Preparation of *E. faecalis* Bacterial Suspension

The inoculated *E. faecalis* ATCC 29212 bacterial culture was obtained by using a sterile loop needle to transfer from a tube containing Brain Heart Infusion Broth (BHI-B). Subsequently, the cultures were incubated in an incubator for 24 hours at 37°C. Once incubated, the bacterial suspension grown on BHI-B media was transferred into a tube containing 0.9% NaCl solution until the turbidity of the suspension matched the McFarland standard (1.5 x 10⁸ CFU/ml).

Inhibitory test of Pineapple Leaf Extract



Measurement of inhibition test using a modified Kirby-Bauer method with paper disc diffusion. First, dip a sterile cotton swab into the bacterial suspension until it gets wet. Squeeze the sterile cotton swab and press it against the inner wall of the test tube, then scratch it evenly on the Mueller Hinton Agar medium. Dip the paper disk into the *A. comosus* leaf extract solution with concentrations of 1.56%, 3.125%, 6.25%, and 12.5%, 2.5% NaOCl, and distilled water. Incubate the petri dish for 24 hours at 37°C in an incubator. After the incubation period, the paper disks were examined for zones of inhibition of bacterial growth against *E. faecalis*, the vertical, horizontal and diagonal diameters were measured, recorded in millimeters (mm) with vernier calipers. (Figure 1.) The inhibition results were categorized by the diameter of the inhibition zone which was divided into weak (<5mm), medium (5-10mm), strong (10-20mm), and very strong (>20mm) based on the strength of the inhibition.

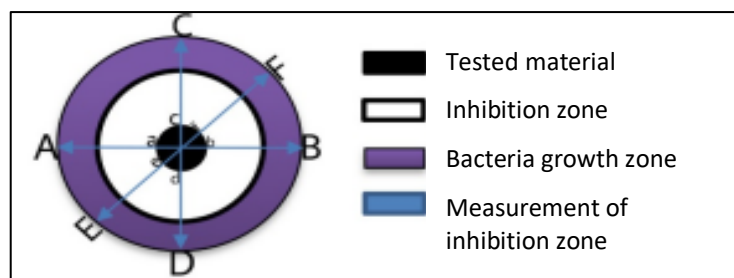


Figure 1. The measurement of inhibition zone

Minimum Inhibitory Concentration test (MIC)

The liquid dilution method was employed to ascertain the minimum inhibitory concentration (MIC). Six microtubes were prepared for each concentration of *A. comosus* leaf extract (1.56%, 3.125%, 6.25%, and 12.5%), with the inclusion of both positive and negative controls in each microtube. To each microtube, 0.1 mL of *E. faecalis* suspension was added. The inoculated microtubes were then incubated at 37°C for 24 hours before being removed. The MIC was determined by assessing the turbidity of the solution, with the solution exhibiting the clearest appearance and lacking any *E. faecalis* bacterial precipitate identified as having the lowest inhibitory concentration.

Minimum Bactericidal Concentration (MBC)



The minimum bactericidal concentration (MBC) was determined using the solid dilution method. A small portion of the solution obtained from the liquid dilution test was taken with a sterile cotton swab and streaked onto MHA media devoid of test bacteria or extracts, with each sample concentration labeled accordingly. The plates were then incubated for 24 hours at 37°C. Observations were made with the naked eye to assess the growth in the number of bacterial colonies on the MHA media. The MBC was determined by identifying the lowest concentration of the extract that exhibited no growth of *E. faecalis*. The data were analyzed statistically using SPSS software.

Results

Measurement of Inhibition Zone Diameter

The inhibition zone of *A. comosus* leaf extract against *E. faecalis* by disc diffusion method at concentrations of 1.56%, 3.125%, 6.25%, and 12.5%, positive control NaOCl 2.5%, and negative control can be seen in Figure 2 and Table 1.

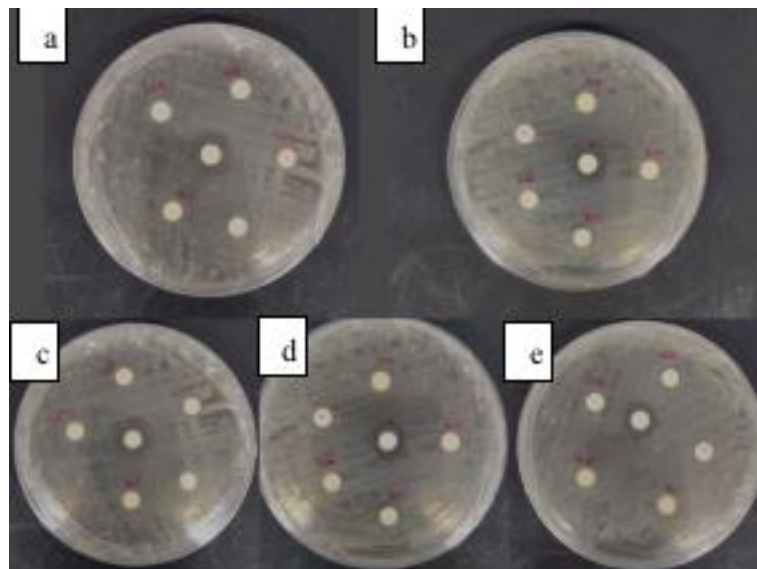


Figure 2. The results of the inhibition zone test using the disc diffusion method after 1x24 hours incubation: (a) Test 1, (b) Test 2, (c) Test 3, (d) Test 4, (e) Test 5

Table 1. Measurement of Inhibition Zone Diameter of the *A. comosus* Leaf Extract Test Group and the Control Group against *E. faecalis*



Groups	Test					Mean Diameter [nhibitory zone (mm) ± SD
	1	2	3	4	5	
<i>A. comosus</i> leaf extract 1,56%	1,13	1,16	1,13	1,1	1,2	1,144±0,03782
<i>A. comosus</i> leaf extract t 3,125%	1,76	1,73	1,76	1,76	1,73	1,748±0,01643
<i>A. comosus</i> leaf extract 6,25%	2,03	2,03	2,1	2	2,1	2,052±0,0455
<i>A. comosus</i> leaf extract 12,5%	2,33	2,33	2,33	2,33	2,3	2,324±0,01342
NaOCl 2,5%	6,76	6,73	6,8	6,76	6,73	6,768±0,05357
Distilled water	0	0	0	0	0	0±0,000

It was observed that all concentrations of *A. comosus* leaf extract could inhibit *E. faecalis* by measuring the diameter of the inhibition zone. As the concentration of the extract increased, there was a corresponding increase in the average diameter of the inhibition zone. According to Table 3, the concentration of 12.5% of Prabumulih *A. comosus* leaf extract produced the largest inhibition zone against *E. faecalis* with an average of 2.324 mm. The positive control of 2.5% NaOCl had the largest diameter inhibition zone of 6.76 mm, while the negative control showed no inhibition zone .

The results of the Kruskal-Wallis test showed that there is a significant difference between the mean diameter of the inhibition zone in each treatment group ($p < 0.05$). The results of the Mann-Whitney test in Appendix 13 show that there is a significant difference between the Prabumulih City pineapple leaf extract group and the control group marked with a significance value of $p < 0.05$.

Minimum Inhibitory Concentration Test Results (MIC)

The test was carried out using the liquid dilution method and was repeated five times using pineapple leaf extract with concentrations of 1.56%, 3.125%, 6.25% and 12.5%, positive control of 2.5% NaOCl and negative control of aquadest. Visual observation was carried out after incubation for 24 hours to see the results of the MIC test shown in Figure 3 and Table 2.

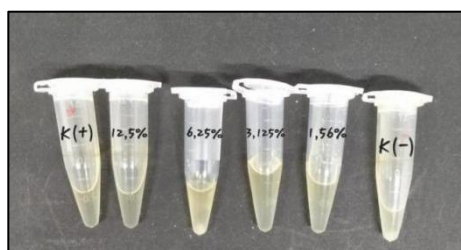


Figure 3. The results of the MIC test using the liquid dilution method using microtubes: (a) positive control, (b) pineapple leaf extract concentration 12.5%, (c) pineapple leaf extract concentration 6.25%, (d) pineapple leaf extract concentration 3.125%, (e) pineapple leaf extract concentration of 1.56%, (f) negative control.

Table 2. Minimum Inhibitory Concentration (MIC) Test Results

Groups	Repetition									
	1		2		3		4		5	
	+	-	+	-	+	-	+	-	+	-
<i>A. comosus</i> leaf extract 1,56%		✓		✓		✓		✓		✓
<i>A. comosus</i> leaf extract 3,125%		✓		✓		✓		✓		✓
<i>A. comosus</i> leaf extract 6,25%		✓		✓		✓		✓		✓
<i>A. comosus</i> leaf extract 12,5%	✓		✓		✓		✓		✓	
NaOCl 2,5%	✓		✓		✓		✓		✓	
Distilled water		✓		✓		✓		✓		✓

Exp: (+): Clear, (-): Unclear

The turbidity of the solution was observed, and the results showed that the negative control group and the pineapple leaf extract group with concentrations of 1.56%, 3.125%, and 6.25% still showed the growth of *E. faecalis* bacteria seen from the turbidity similar to the bacterial suspension. The 12.5% pineapple leaf extract solution and the positive control 2.5% NaOCl showed clarity, so the MIC of the pineapple leaf extract set a concentration of 12.5%.

Visual observation of clarity or the absence of white lumps on the microtubes is not enough to determine the results of the MIC test because the color of the extract will become darker as the concentration increases, especially at concentrations of 6.25% and 12.5%. Therefore, a follow-up test was carried out for the determination of MBC by subculture on Mueller Hinton Agar (MHA) media.

Minimum Bactericidal Concentration Test Results (MBC)

The solid dilution method was utilized to determine the minimum concentration of *A. comosus* leaf extract for *E. faecalis* eradication. Microtubes from previous MIC test results



were used to subculture on Mueller Hinton Agar (MHA) media, followed by a 24-hour incubation period. Test result are showed in Figure 4 and Table 3.

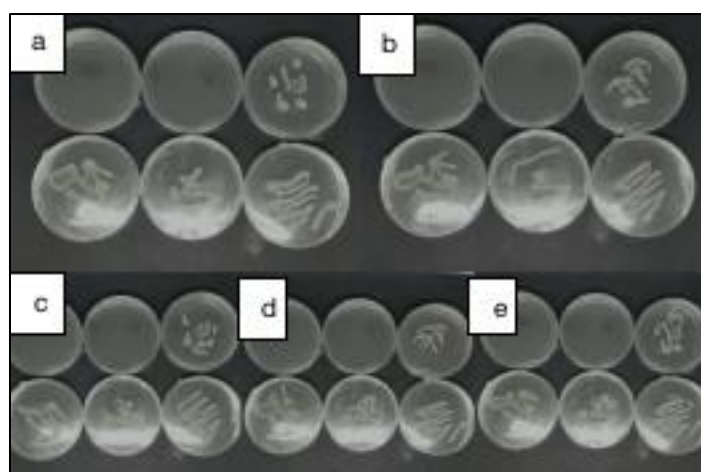


Figure 4. Results of the KBM test using the solid dilution subculture method using pineapple leaf extract with concentrations of 1.56%, 3.125%, 6.25%, 12.5%, positive control and negative control after 1x24 hour incubation: (a) Test 1, (b) Test 2, (c) Test 3, (d) Test 4, (e) Test 5

Table 3. Minimum Bactericidal Concentration (MBC) Test Results

Groups	Repetition									
	1		2		3		4		5	
	+	-	+	-	+	-	+	-	+	-
<i>A. comosus</i> leaf extract 1,56%	✓		✓		✓		✓		✓	
<i>A. comosus</i> leaf extract 3,125%	✓		✓		✓		✓		✓	
<i>A. comosus</i> leaf extract 6,25%	✓		✓		✓		✓		✓	
<i>A. comosus</i> leaf extract 12,5%		✓		✓		✓		✓		✓
NaOCl 2,5%		✓		✓		✓		✓		✓
Distilled water	✓		✓		✓		✓		✓	

Exp: (+): Grow, (-): Shrink

Table 3 shows that only extract with a concentration of 12.5% and a positive control of 2.5% NaOCl could kill *E. faecalis* bacteria which was characterized by the absence of bacterial growth, while at a concentration of 1.56%, 3.125 %, and 6.25% there were still colonies of *E. faecalis* bacteria growing on the media. Based on this, it can be determined that the MBC of *A. comosus* leaf extract was set at a concentration of 12.5%.



Discussion

The current study showed that pineapple (*Ananas comosus*) leaf extract from Prabumulih City had antibacterial power against *E. faecalis*. This finding aligns with Dewi et al. (2018), which reported antibacterial activity of pineapple hump extract against *E. faecalis*, with the most significant inhibition zone diameter observed in the 100% concentration group.

According to the results of the minimum inhibitory concentration (MIC) test, a concentration of 12.5% pineapple leaf extract was identified as the lowest concentration capable of inhibiting the growth of *E. faecalis*. The MIC value was determined by comparing the absorbance values before and after a 24-hour incubation at 37°C. If the absorbance value before incubation exceeded that after incubation, it indicated inhibition of bacterial growth; conversely, if the value after incubation was higher than before incubation, bacterial growth persisted.³

The findings from the minimum bactericidal concentration (MBC) test indicated that a concentration of 12.5% pineapple leaf extract was the lowest concentration capable of effectively eliminating *E. faecalis*. At concentrations of 6.25%, 3.125%, and 1.56%, bacterial colonies were still observed. It's noteworthy that the MBC of the antibacterial compound itself can be equal to or greater than the MIC value.^{14,15}

All of the test groups that were analyzed showed a lower average inhibition zone value when compared to the positive control of 2.5% NaOCl. This difference was statistically significant. This can be attributed to the strong antibacterial properties of NaOCl, particularly against *E. faecalis*. The antimicrobial action of NaOCl takes place in two ways. Firstly, when the chlorine ion comes into contact with organic debris or pulp tissue, hypochlorous acid is formed. This acid can then penetrate bacterial cells, oxidize the sulfhydryl groups of bacterial enzymes, and interfere with metabolism, ultimately leading to bacterial death. Secondly, NaOCl has a high pH of 11.0-11.5, which is effective for destroying anaerobic bacteria.¹⁶

The ability of the antibacterial power of pineapple leaf extract to inhibit the growth of *E. faecalis* bacteria is obtained from the content of active enzyme compounds such as bromelain, flavonoids, phenols, tannins, saponins, and alkaloids. The bromelain enzyme is a proteolytic enzyme that is able to inhibit and kill bacteria by hydrolyzing peptide bonds in the



bacterial cell wall and lowering the surface tension of the bacterial cell wall.¹⁷ Flavonoids and polyphenols are phenolic compounds that have polar properties so that these compounds mostly act on the peptidoglycan layer of Gram-positive bacteria which also has polar properties compared to the non-polar lipid layer where *E. faecalis* itself is a Gram-positive bacterium.^{18,19} Saponins cause bacterial death by reducing the surface tension of the cell wall, damaging membrane permeability, and interfering with cell metabolism while denaturing proteins in the bacterial cell membrane.^{19,20} Tannins are able to kill bacteria by interfering with peptidoglycan synthesis which will cause a loss of cell membrane permeability.^{5,11} Alkaloids work by interfering with cell wall synthesis causing the release of LTA (lipoteichoic acid) so that this enzyme hydrolyzes the cell wall and causes bacterial cell death.⁵

Irrigation solution for root canal treatment must meet ideal requirements to support the success of the treatment.. Further studies are needed to develop an irrigation solution derived from *Ananas comosus* leaves that can be used as an irrigation solution with efficacy equivalent to chemical-based irrigation solutions.

Conclusion

Pineapple leaf extract (*Ananas comosus*) from Prabumulih City exhibits antibacterial properties, effectively inhibiting and eradicating *Enterococcus faecalis* bacteria. The average inhibition zone begins at a concentration of 1.56%, measuring 1.144 mm. Moreover, higher concentrations lead to larger inhibition zones. The Minimum Inhibitory Concentration (MIC) is 12.5%, while the Minimum Bactericidal Concentration (MBC) is also 12.5%.

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