Sriwijaya Journal of Dentistry

Sriwijaya Journal of Dentistry (SJD)
Volume 4 Issue 2 2023 : 25-35
https://sjd-fk.ejournal.unsri.ac.id/index.php/sjd/index

Antibacterial Potency of Various Concentrations of Pineapple Peel Extract (*Ananas comosus*) against *Enterococcus faecalis*

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Abstract

Introduction: *Enterococcus faecalis* is a Gram-positive bacterium commonly found in cases of failed root canal treatment. Irrigation material with antibacterial effects are important in supporting the success of root canal treatment. Pineapple peel as a natural ingredient is reported to contain compounds such as flavonoids, saponins, tannins, and bromelain enzymes that have been proven antibacterial effects. **Purpose:** To determine antibacterial potency of pineapple peel extract (*Ananas comosus*) with concentrations of 1.56%, 3.125%, 6.25% and 12.5% against *E. faecalis*. **Methods:** In vitro quasi-laboratory experimental study with a post-test only control group design consisting of pineapple peel extract with concentrations of 1.56%, 3.125%, 6.25%, 12.5%, and aquadest as negative control with repetition 6 times. The inhibition test was determined using the disc diffusion method on Mueller-Hinton Agar media. The MIC value can be determined using the broth dilution method and the MBC value can be determined using the solid dilution method. The inhibition zone diameter data were analyzed using One Way ANOVA test and Post Hoc Tukey test. **Results:** Pineapple peel extract with concentrations 12,5% had the largest mean inhibition zone, which is was 3.13 mm. The MIC and MBC values of pineapple peel extract were determined at a concentration of 6.25%. **Conclusion:** Pineapple peel extract (*Ananas comosus*) has antibacterial potency against *Enterococcus faecalis*.

Keywords: Antibacterial; *Enterococcus faecalis*; Pineapple peel

Introduction

Root canal treatment is a treatment procedure that aims to clean necrotic pulp tissue and eliminate all microorganisms present in the root canal to provide a supportive environment for the healing process.[1,2] Root canal irrigation is an important step in supporting the success of root canal treatment.[3] The root canal irrigation material that is often used in endodontic treatment is sodium hypochlorite (NaOCl). NaOCl is known for its strong antimicrobial activity and can kill microorganisms very quickly even at low concentrations. NaOCl is used in concentrations varying from 0.5% to 8%. In vitro studies show that 2.5% NaOCl can kill all *E. faecalis* bacteria within 10 minutes, but NaOCl has drawbacks such as bad taste, toxic, not effective in dissolving inorganic tissue and smear layer of root canal walls.[4] This deficiency of

NaOCl is the basis for the need to develop other materials from nature that have antibacterial effects and are safer to use.

The use of natural materials as antibacterial alternatives has been widely studied to overcome the shortage of synthetic materials. Pineapple with the Latin name *Ananas comosus* is a fruit plant that has an antibacterial effect and is widely developed in Indonesian plantations.[5,6] Research by Fitriyanti et al. (2019) reported that pineapple peel extract has an antibacterial effect.[7] Phytochemical tests showed that the ethanol extract of pineapple peel contains alkaloids, phenols, flavonoids, phytosterols, steroids, saponins, tannins, terpenoids, and bromelain enzymes.[8] Previous research conducted by Putri et al. (2016) also reported on pineapple peel extract which has an antibacterial effect where the minimum inhibitory concentration (MIC) was obtained at a concentration of 3.125% and the minimum bactericidal concentration (MBC) at a concentration of 6.25%.[9]

The results of this study indicate that pineapple peel extract has potential as an irrigating agent that has antibacterial effect. Based on this description, the researcher is interested in conducting a study which aims to determine antibacterial potency of various concentrations of pineapple peel extract (*Ananas comosus*) against *E. faecalis*.

Methods

This research is an in vitro quasi-laboratory experimental study with a post-test only control group design. The research was conducted on 27 February-11 March 2023 at Chemical Engineering Laboratory, Sriwijaya State Polytechnic to create pineapple peel extract and continued with testing the inhibition zone, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of the pineapple peel extract with concentrations 1.56%, 3.125%, 6.25%, 12.5%, and aquadest as negative control against *E. faecalis* ATCC 29212 which was conducted on 15-18 May 2023 at Inter-University Research Laboratory, Gajah Mada University.

Pineapple peel is taken from pineapple in Prabumulih City with *Queen* type that has matured at around 15-18 months of age with the characteristics of fruit aroma starting to appear, fruit skin color is yellow, has deep eyes, crown is more open, leaf edges are spiny, fruit stalks become wrinkled and the color of the flesh is dark yellow with a small fruit core.[6,10,11] The

samples were divided into five group: pineapple peel extract concentrations 1.56%, 3.125%, 6.25%, 12.5%, and aquadest as negative control with repetition 6 times which was calculated using Federer's formula.[9]

A total of 1 kg of pineapple peel is washed with running water then air-dried and then the pineapple peel is cut into small pieces (± 1 x 1 cm) and dried in an oven at 40°C for 2 x 24 hours then pulverized using a blender into powder.[12,13] 500 grams of pineapple peel powder was weighed and then put into a 1000 ml Erlenmeyer tube and soaked in 500 ml of 96% ethanol for 3x24 hours at room temperature. Every 1x24 hours, macerated pineapple peel was filtered using filter paper to separate the residue.[13,14] The solvent filtrate was evaporated using a Rotary Vacuum Evaporator with a temperature of 50°C to obtain a thick extract with a concentration of 100%. Pineapple peel extract was diluted with concentrations of 1.56%, 3.125%, 6.25% and 12.5%.[15]

Determination of the diameter of inhibition zone can be done using the disc diffusion method using a paper disk. A sterile cotton swab is dipped in the bacterial suspension until it gets wet. A sterile cotton swab is squeezed and pressed against the inner wall of the test tube and then smeared evenly on the Mueller-Hinton Agar medium. Paper disks were dipped in a solution of pineapple peel extract with concentrations of 1.56%, 3.125%, 6.25%, 12.5% and aquadest as a negative control. Petri dishes were incubated at 37°C for 1 x 24 hours. The inhibition zone around the paper disk was measured as shown in Figure 1 using a sliding caliper in millimeters.[14,15]

Inhibition zone : (Measurement 1 + Measurement 2 + Measurement 3) /3

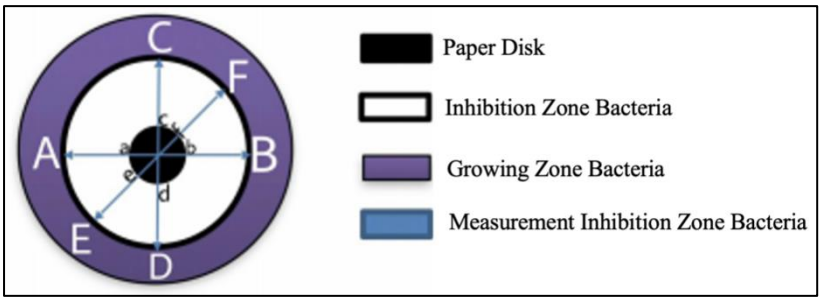

Figure 1. Measuring the diameter of the inhibition zone[16]

Table1. Inhibition zone diameter categories[17]

Inhibition zone diameter	Inhibition zone
Very Strong	> 20 mm
Strong	10-20 mm
Normal	5-10 mm
Weak	< 5 mm

The data obtained from the observations will be processed using SPSS. The normality test was carried out using the Shapiro-Wilk test and the homogeneity test was carried out using the Levene's test, if the data obtained was normally distributed and homogeneous ($p > 0.05$), then a test could be carried out parametric with One Way ANOVA and continued with Post Hoc Tukey test ($p < 0.05$).

Determination of the MIC value can be done using the liquid dilution method. Prepare as many as five reaction microtubes that have been labeled. Pineapple peel extract solution concentrations of 1.56%, 3.125%, 6.25%, 12.5% and aquadest as a negative control was added as much as 5 ml into the reaction microtube according to the mark previously made. 0.1 ml of *E. faecalis* suspension was also added to each microtube. The inoculated microtubes will be incubated for 24 hours at 37±1°C then when the incubation period is complete, the microtubes are removed and observed whether there is turbidity in the solution. A clearer solution indicates the presence of *E. faecalis* whose growth is stunted. Observations showing the extract with the lowest concentration capable of inhibiting the growth of *E. faecalis* without any turbidity were recorded as MIC.[18]

MBC values can be determined using the solid dilution method. The solution from the liquid dilution test was streaked streakwise on MHA media and incubated at 37^{o}C for 24 hours and then observed with the naked eye whether there was bacterial growth in the streaks on the media compared to the control. The results obtained will determine the lowest concentration of the pineapple peel extract solution. The lowest extract concentration that did not show bacterial growth was recorded as the MBC value.[18,19,20]

Results

The results of the average diameter of the inhibition zone in table 2 show that the pineapple peel extract group with a concentration of 12.5% had the largest average diameter of

the inhibition zone which was 3.13 mm, followed by the pineapple peel extract group concentrations of 6.25% (2.32 mm), 3.125% (1.69 mm), and 1.56% (1.16 mm), whereas in the negative control group did not show any inhibition zones.

Table 2. Results of measurement of inhibition zone diameter of pineapple peel extract group and control group on *E. faecalis*

Sample	Inhibition zone diameter (mm)						Average inhibition zone diameter (mm)
	1	2	3	4	5	6	
Pineapple peel extract 1,56%	1,17	1,2	1,07	1,13	1,2	1,2	1,16
Pineapple peel extract 3,125%	1,73	1,67	1,77	1,67	1,77	1,57	1,69
Pineapple peel extract 6,25%	2,4	2,3	2,27	2,3	2,3	2,37	2,32
Pineapple peel extract 12,5%	3,1	3,1	3,2	3,13	3,16	3,1	3,13
Aquadest	0	0	0	0	0	0	0,00

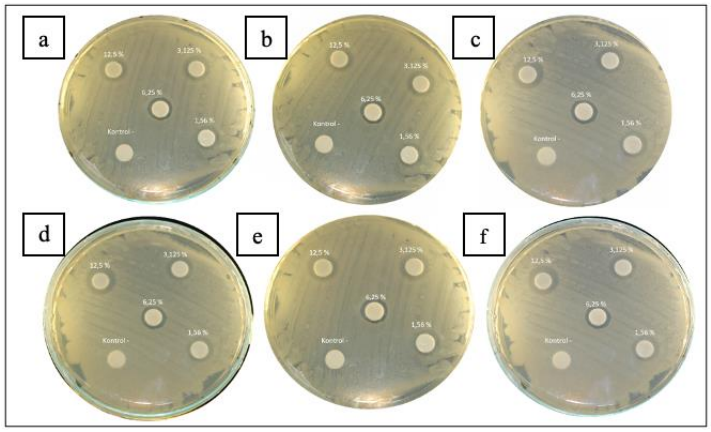

Figure 2. The results of the inhibition zone test with the disc diffusion method using pineapple peel extract concentrations of 1.56%, 3.125%, 6.25%, 12.5%, and control negative : (a) test 1, (b) test 2, (c) test 3, (d) test 4, (e) test 5, (f) test 6

The results of the MIC test were observed visually after incubated for 1x24 hours as shown in Figure 3. The solution with the same turbidity level as the bacterial suspension according to the 0.5 Mc Farland turbidity standard indicates the presence of bacterial growth. The results obtained showed that pineapple peel extract concentrations of 1.56%, 3.125%, and aquadest as negative control on microtubes appeared to have the same level of turbidity as the standard 0.5 Mc Farland bacterial suspension and there were still white lumps in the solution indicating the growth of *E. faecalis* is not inhibited. Microtubes with pineapple peel extract at a concentration of 6.25% and 12.5% showed clarity, so the MIC of pineapple peel extract was

set at a concentration of 6.25%. The results of the MIC test using the liquid dilution method on microtubes showed the effect of pineapple peel extract in inhibiting the growth of *E. faecalis*.

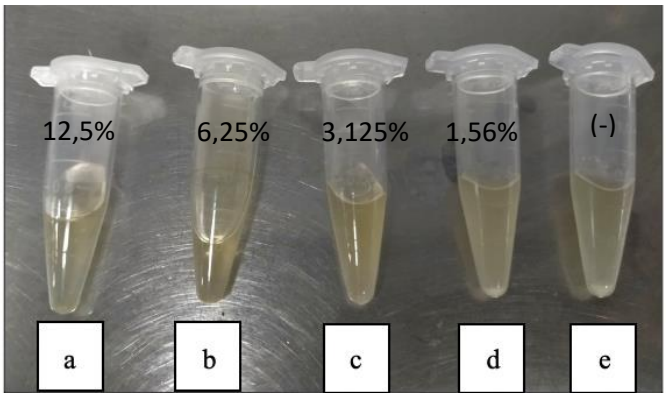

Figure 3. MIC test results using the liquid dilution method

Table 3. Minimum inhibitory concentration test results

Sample	Observation Result											
	1		2		3		4		5		6	
	+	–	+	–	+	–	+	–	+	–	+	–
Pineapple peel extract 1,56%		✓		✓		✓		✓		✓		✓
Pineapple peel extract 3,125%		✓		✓		✓		✓		✓		✓
Pineapple peel extract 6,25%	✓		✓		✓		✓		✓		✓	
Pineapple peel extract 12,5%	✓		✓		✓		✓		✓		✓	
Aquadest		✓		✓		✓		✓		✓		✓

Note : (+): clear, (-): turbid

The lowest concentration of pineapple peel extract that can kill *E. faecalis* was determined by the solid dilution method at concentrations of 1.56%, 3.125%, 6.25%, 12.5%, and aquadest as negative control using microtubes from the previous MIC test. Subculture was carried out on MHA media and then incubated for 1x24 hours.

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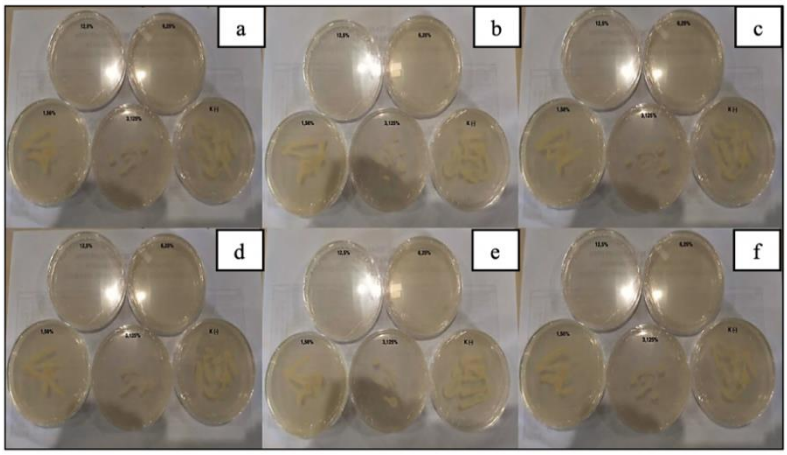

Figure 4. MBC test results with solid dilution subculture method using extract pineapple peel with concentrations of 1.56%, 3.125%, 6.25%, 12.5%, and negative control after incubated 1x24 hours: (a) test 1, (b) test 2, (c) test 3, (d) test 4, (e) test 5, (f) test 6.

Table 4. Minimum bactericidal concentration test results

Sample	Observation Result											
	1		2		3		4		5		6	
	+	–	+	–	+	–	+	–	+	–	+	–
Pineapple peel extract 1,56%	✓		✓		✓		✓		✓		✓	
Pineapple peel extract 3,125%	✓		✓		✓		✓		✓		✓	
Pineapple peel extract 6,25%		✓		✓		✓		✓		✓		✓
Pineapple peel extract 12,5%		✓		✓		✓		✓		✓		✓
Aquadest	✓		✓		✓		✓		✓		✓	

Note : (+): grow (-): not grow

The results of the MBC test in table 4 show that pineapple peel extract with a concentration of 1.56%, 3.125%, and aquadest as a negative control still has *E. faecalis* growth on MHA media while pineapple peel extract with a concentration of 6.25% and 12.5% could kill *E. faecalis* which was characterized by the absence of bacterial colonies growing on MHA media. Based on the results of the MBC test, it can be determined that MBC of pineapple peel extract was set at concentration of 6.25% and it can be concluded that pineapple peel extract has effectiveness in killing *E. faecalis*.

Discussion

The results of this study showed that pineapple peel extract had an antibacterial potency against *E. faecalis*. Based on the category of antibacterial power, pineapple peel extract

concentration 12.5% has the largest average diameter of the inhibition zone (3.13 mm), which is included in the weak category. The results indicate that pineapple peel extract can inhibit the growth of *E. faecalis*, but antibacterial power is still weak because the secondary metabolites contained at that concentration are less so that the ability to inhibit bacteria is reduced and to produce stronger antibacterial power, higher concentrations are needed. This is in accordance with the research by Sari et al. (2018) who reported an increase in the diameter of the inhibition zone when the extract concentration was increased.[7]

The results of the minimum inhibitory concentration (MIC) test showed that 6.25% pineapple peel extract was determined as the lowest concentration which was bacteriostatic against *E. faecalis*. MIC testing in this study was carried out by visual observation, but this method has a weakness, namely at the level of concentration of the solution if the concentration of the extract is increased, the color of the solution will become more concentrated so that the observations are less accurate and subjective. Further testing is needed in addition to assessing MIC by measuring the absorbance value using a UV-Vis spectrophotometer in order to obtain more accurate and thorough results.[21] Research by Wiharningtias et al. (2016) explained that the UV-Vis Spectrophotometer can be used to determine absorbance values as a more accurate determinant of solution turbidity.[22]

The results of the minimum bactericidal concentration (MBC) test showed that a concentration of 6.25% pineapple peel extract was determined as the lowest concentration that was bactericidal against *E. faecalis*. The minimum bactericidal concentration (MBC) of antibacterial compounds can be equal to or greater than the MIC value.[7] The results of this study are in line with Putri et al.'s research. (2016) who reported that the MBC value of pineapple peel extract was set at a concentration of 6.25%.[9]

The results of this study indicate that pineapple peel extract has antibacterial activity due to the mechanism of antibacterial power present in pineapple peel extract. Antibacterial activity can be influenced by several factors, namely the concentration of the extract, the type of bacteria being inhibited, and the content of antibacterial compounds.[23] Pineapple peel extract has antibacterial power because it contains active compounds in the form of flavonoids, saponins, tannins, and bromelain enzymes.[24] Flavonoids and tannins are compounds that are polar so that they more easily penetrate the Gram-positive peptidoglycan layer which is also

polar which can cause bacterial inhibitory activity in Gram-positive bacteria to be greater than in Gram-negative bacteria.[25] Saponins can also suppress bacterial growth because they can bind to lipopolysaccharides so that the permeability of the cell wall will be damaged and reduce the surface tension of the cell wall.[26] The bromelain enzyme is able to inhibit bacterial growth by breaking down proteins in the bacterial cell membrane which can reduce the surface tension of the cell wall.[27]

Conclusion

Pineapple peel extract (*Ananas comosus*) has an antibacterial effect on *E. faecalis* with the largest average diameter of the inhibition zone, which is 3.13 mm, with the minimum inhibitory concentration (MIC) value at 6.25%, and the minimum bactericidal concentration (MBC) at a concentration of 6.25%.

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