

Antibacterial activity of mixed pineapple peel (*Ananas comosus*) extract and calcium hydroxide paste against *Enterococcus faecalis*

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ABSTRACT

Background: *Enterococcus faecalis* (*E. faecalis*) is the bacteria most commonly resulting from failed root canal treatment. Intracanal medicament is used to enhance the success of root canal treatment. A material widely used for this purpose is calcium hydroxide. However, its ineffectiveness in eliminating *E. faecalis* requires the addition of other antibacterial substances, such as iodoform which has the disadvantage of having toxic effects on tissues. Pineapple peel has antibacterial properties because it contains chemical compounds, such as flavonoid, saponin, tannin, as well as the enzyme bromelain. **Purpose:** The aim of the study was to determine the antibacterial activity of a mixture of pineapple peel extract at 6.25% and 12.5% concentrations and calcium hydroxide paste at a ratio of 1:1 compared to 100% calcium hydroxide and a mixture of calcium hydroxide and iodoform paste against *E. faecalis*. **Methods:** The research was laboratory-based experiment in nature. Sample groups were divided into two control groups (one featuring 100% calcium hydroxide paste and a second featuring a mixture of calcium hydroxide and iodoform paste) and two treatment groups (mixture of pineapple peel extract and calcium hydroxide paste in 6.25% and 12.5% concentrations with ratio 1:1). The method was using agar diffusion. The result data were analyzed by one way Anova test. **Results:** The highest average of the inhibitory zone occurred in group with a mixture of pineapple peel extract 12.5% and calcium hydroxide paste while the smallest average was that of group with a mixture of calcium hydroxide and iodoform paste. **Conclusion:** Mixture of pineapple peel extract in 6.25% and 12.5% concentrations and calcium hydroxide paste ratio 1:1 has higher antibacterial activity than paste of 100% calcium hydroxide and mixture of calcium hydroxide and iodoform paste against *E. faecalis*.

Keywords: calcium hydroxide; pineapple peel; zone inhibit; *Enterococcus faecalis*; intracanal medicament

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INTRODUCTION

Dental caries constitute the most prevalent infectious disease worldwide. If dental caries are ignored, bacteria may reach the pulp chamber through open dentinal tubules resulting in the colonization of microorganisms. This may, in turn, lead to infection of the root canal.¹ Root canal treatment is the treatment of choice for such infections. Failure of root canal treatment due to the persistence of bacterial infection within the root canal is referred to as a secondary infection.² Microorganism is a factor associated with root canal treatment failure.³

Enterococcus faecalis (*E. faecalis*) is the most commonly bacteria found in cases of failed root canal treatment.² Studies reported that 77% of failed root canal treatment cases suffered reinfection due to *E. faecalis* resistance. The high resistance of *E. faecalis* because of various virulence factors, including its ability to compete with other microorganisms in invading dentinal tubules and to survive in high temperatures and broad pH range.⁴

The principle of root canal treatment is removal of the organic and inorganic debris, microorganisms and toxic products from the root canal.⁵ Preparation procedures cannot eliminate bacteria effectively. Therefore, it is

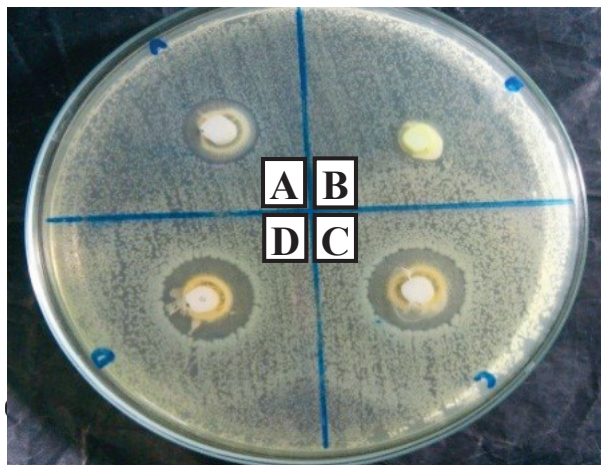


Figure 1. Diameter of inhibitory zone in each sample groups againsts *Enterococcus faecalis*.

necessary to administer intracanal medicaments prior to obturation of the root canal.⁶ One material widely used as an intracanal medicament is calcium hydroxide which is a bactericide, but it is not effective in eliminating microbes from dentin tubules because *E. faecalis* is resistant to it.⁷ The optimization of the antibacterial activity of calcium hydroxide should be supplemented with another antibacterial substance such as iodoform. Iodoform can increase antibacterial activity, but has some shortcomings such as it can be dissolved rapidly by root canal microorganisms and have toxic effects on tissues.⁸

It is necessary to develop a herbal material as an alternative to substitute these chemicals. One of the herbal materials possessing antibacterial properties is pineapple peel (*Ananas comosus*). Pineapple peel has antibacterial properties because it contains chemical compounds. A phytochemical test showed that ethanol extracted from pineapple skin contains alkaloids, phenols, flavonoids, phytosterols, steroid, saponins, tannins and terpenoids.⁹ In addition, there is another component of pineapple peel, namely; bromelain enzyme.¹⁰

Previous research indicated that the inhibitory zone of pineapple peel extract against *Staphylococcus aureus* measured 15.06 mm.¹¹ Other research indicated that the minimum inhibitory concentration (MIC) of pineapple peel extract against *Streptococcus viridans* was 1.56% and minimum bactericidal concentration (MBC) was 3.125%.¹²

According to previous studies, the minimum bactericidal concentration of pineapple peel extract against the growth of *E. faecalis* was 6.25%. Therefore, it was necessary to investigate the antibacterial activity of the pineapple peel extract (concentration of 6.25% and 12.5%) and calcium hydroxide paste mixture as an alternative intracanal medicament against *E. faecalis*. The aim of the study was to determine the antibacterial activity of a mixture of pineapple peel extract in 6.25% and 12.5% concentrations

and calcium hydroxide paste at a ratio of 1:1 compared to 100% calcium hydroxide and a mixture of calcium hydroxide and iodoform paste against *E. faecalis*.

MATERIALS AND METHODS

This research was approved by the Ethics Review Committee of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya, No. 147/KKEPK.FKG/VII/2016. Pineapple peel extract was obtained from Phytochemicals Laboratory, Materia Medica, Batu, Indonesia. *E. faecalis* ATCC 29912 bacteria were drawn from the stock of the Microbiology Laboratory, Faculty of Dental Medicine, Universitas Airlangga, Surabaya.

This research constituted a laboratory experiment with post test only control group design. Sample groups divided into four groups, including two positive control groups and two treatment groups. The positive control groups (group A and B) contained 100% calcium hydroxide paste (hydroxidocalcio PA), and a mixture of calcium hydroxide and iodoform paste (calplus). Meanwhile, the treatment groups (group A and B) received a mixture of 12.5% pineapple peel extract and calcium hydroxide paste, and mixture of 6.25% pineapple peel extract and calcium hydroxide paste.

Pineapple peel extract was extracted by macerating with 96% ethanol. The results of extraction produced 45 ml of 100% pineapple peel extract. The dilution processes of pineapple peel extract using distilled water to obtain 12.5% and 6.25% concentrations. Pineapple peel extract was combined with calcium hydroxide powder at a ratio of 1:1 and mixed until homogeneous. Preparation of *E. faecalis* bacteria begins with culturing *E. faecalis* in BHIB and incubating it in an anaerobic atmosphere at 37° C for 24 hours. Subsequently, a suspension of *E. faecalis* bacteria comparable to a McFarland standard 0.5 of 1.5×10^8 cfu/ μ was produced (1.5×10^8 CFU/ml).

The determination of the antibacterial activities of sample groups against *E. faecalis* employed an agar diffusion method. *E. faecalis* was cultured in nutrient agar media. A petridish containing nutrient agar media was divided into four zones, separated by a line marker on its underside. Each well zone contained 0.5 gram sample groups. The petridish was incubated anaerobically in an incubator at 37° C for 24 hours. The diameter of the inhibitory zone of the resulting culture was measured. The inhibitory zone could be seen as a clear area around the wells that showed no bacterial growth. Measurement of the diameter of the inhibitory zone to the nearest 0.1 mm was effected using calipers.

A Kolmogorov-Smirnov Normality statistic test was performed, while the difference between the groups was established by the conducting of a one-way Anova test, followed by a multifactorial comparison test using a Tukey HSD test and a preceding Levene homogeneity statistic test.

RESULTS

The subjects of the research into the antibacterial activity of a mixture of pineapple peel extract and calcium hydroxide paste against *E. faecalis* were divided into four groups, two treatment groups and two positive control groups with seven replications. The calculation results of the averages and standard deviations in each sample group can be seen in Table 1.

The results showed that the highest average of the inhibitory zone occurred in group C with a mixture of pineapple peel extract 12.5% and calcium hydroxide paste, while the smallest average was that of group B with a mixture of calcium hydroxide and iodoform paste.

The terms of parametric statistical tests were normality and homogeneity. A Kolmogorof Smirnov normality test showed that each sample group had a value of $p > 0.05$. Consequently, it can be seen that the data from all the research groups had normal distribution. The results of a subsequent homogeneity test using Levene test showed a value of $p > 0.125$, indicating that all the research groups had a homogen variance ($p > 0.05$).

With the prerequisite of normal and homogeneous distribution, a one-way Anova test was then conducted. Based on its results, the significance value obtained was 0.001, smaller than $\alpha = 0.05$, indicating that there was a significant difference in the inhibitory zone of all sample groups against *E. faecalis*.

Table 1. Averages (mm) and standard deviations of inhibitory zone in each sample groups against *E. faecalis*

Sample groups	n	Average (mm)	Standard deviation
A	7	17.5886	0.33682
B	7	0	0
C	7	21.5757	0.19739
D	7	19.8043	0.43355

Note:

Group A : 100% calcium hydroxide paste

Group B : Mixture of calcium hydroxide and *iodoform* paste

Group C : Mixture of pineapple peel extract 12.5% and calcium hydroxide paste (1:1)

Group D : Mixture of pineapple peel extract 6.25% and calcium hydroxide paste (1:1)

n : Sample size

Table 2. The result of Tukey HSD test

Sample groups	A	B	C	D
A	-	-	0.000*	0.000*
B	-	-	0.000*	0.000*
C	-	-	-	0.000*
D	-	-	-	-

Note:

* = there was a significant difference ($p < 0.05$)

A Tukey HSD test was performed to identify the significant differences between the sample groups, the results of which showed a significance value less than 0.05. This means that there were significant differences in the inhibitory zone between each group against *E. faecalis*. The probability value of the Tukey HSD test results can be seen in Table 2.

DISCUSSION

The results showed the inhibition zone of the mixture of pineapple peel extract and calcium hydroxide paste and 100% calcium hydroxide paste, except for the mixture of calcium hydroxide and iodoform paste. An Anova test showed there to be significant differences in the inhibitory zone in all sample groups against *E. faecalis*. The highest average inhibitory zone was found in the group containing a mixture of pineapple peel extract 12.5% and calcium hydroxide paste, while the smallest inhibitory zone occurred in the group featuring a mixture of calcium hydroxide and iodoform paste. Based on the research findings, the mixture of calcium hydroxide paste and pineapple peel extract with respective concentrations of 6.25% and 12.5% yielded 19.8 mm and 21.58 mm of inhibitory zone diameters. These inhibitory zone diameters are greater than that of 100% calcium hydroxide paste, which yielded 17.58 mm of inhibitory zone.

The inhibitory zone of the group containing 100% calcium hydroxide paste indicated that calcium hydroxide possesses antibacterial properties. These results were due to the mechanical action of calcium hydroxide against bacterial growth through the release of hydroxyl ions that can damage the cytoplasmic membrane of bacteria.¹³ Hydroxyl ions with an alkaline pH when coming into contact with the bacterial cytoplasmic membrane protein, will break the hydrogen bonds in the protein structure. The damage caused by hydrogen bonding will lead to changes in the polypeptide chain binding protein, and protein denaturation or loss of natural functions of the protein will occur. When hydroxyl ions come into contact with bacterial DNA, this will inhibit bacterial replication and mutations. This resulted in the breakdown of the structure and function of DNA, lead to bacterial cell death. Based on its antibacterial activities, calcium hydroxide was able to kill bacteria.¹⁴

Iodoform added to calcium hydroxide paste was expected to increase the antibacterial activities of calcium hydroxide. The antibacterial mechanism of iodoform through the release of iodine which increases protein precipitation and oxidation of essential enzymes. Iodoform plays an important role in controlling infection and has an intermediate antibacterial level.¹⁵ In this research the opposite occurred since the group B, which was a mixture of calcium hydroxide and iodoform paste, showed no inhibition zone against *E. faecalis*. It could potentially be influenced by the inadequacy of the iodoform and could be dissolved rapidly by root canal microorganisms,⁸ including *E. faecalis*.

The data relating to the results of a mixture of pineapple peel extract and calcium hydroxide paste showed that the inhibitory zone was higher than 100% calcium hydroxide paste. According to the results of phytochemical test referred to in previous research, the highest concentration of active compound in pineapple peel extract was that of flavonoids.¹⁶ Ether group in flavonoids tends to be unstable due to the easy release of electrons and have an opposite charge equal to that of the hydrogen atom of calcium hydroxide. This makes binding possible to form an alcohol group or hydroxyl. The release of hydroxyl ions can damage the bacterial cytoplasmic membrane. This reaction explains the ability of pineapple peel extract in synergism with calcium hydroxide to increase the inhibitory zone against *E. faecalis*.

Previous research showed that the inhibition zone increases linearly with the increasing concentration of the pineapple peel extract mixed with calcium hydroxide paste. One of the factors influencing the values of inhibitory zone is the concentration of antibacterial compounds.¹⁷ A mixture of pineapple peel extract 12.5% and calcium hydroxide paste produces a higher inhibitory zone than the group containing a mixture of pineapple peel extract 6.25% and calcium hydroxide paste. The higher inhibitory zone due to antibacterial compounds in pineapple peel extract is more elevated at higher concentrations. The higher antibacterial compound, the greater ability to inhibit bacteria within the extract.

Pineapple peel extract demonstrates antibacterial properties because it contains chemical compounds. In addition to flavonoids as the highest concentration of chemical compounds in pineapple peel extract, there are other active compounds, namely: saponins, tannins, and enzymes bromelain. Each of these chemical compounds has a specific role in inhibiting the growth of *E. faecalis*.

Flavonoid compounds have the ability to form complex bonds with extracellular protein through hydrogen bonding. Hydrogen bonds with extracellular proteins cause bacterial cell membrane structures containing proteins to become unstable. Consequently, the permeability of bacterial cell wall will be disrupted, resulting in bacterial cell death.¹⁸ Tannin compounds can damage bacterial cell membranes and result in reduced permeability, so the growth and activities at bacterial cells is inhibited.¹⁹ In other chemical compounds, saponin, as an antibacterial agent, can reduce the surface tension leading to the increasing permeability of cell membranes. Changes in cell membrane permeability cause release of intracellular compounds from the cell that leads to bacterial lysis.²⁰ The action mechanism of the bromelain enzyme as an antimicrobial lies in altering or damaging the wall structure of bacteria-containing protein. The bromelain enzyme will break down and denature the protein constituent of bacterial cell walls. As the cell weakens it can allow small molecules to leak out²¹

Antibacterial activity based on the value of the inhibitory zone was categorized into three groups, a weak

level inhibitory zone of 0.1 mm–11.5 mm, a middle level inhibitory zone of 11.5 mm–19.7 mm and a high level inhibitory zone of more than 19.7 mm.²² The results of inhibitory zone in group featuring a mixture of pineapple peel extract and calcium hydroxide were 19.8 mm and 21.58 mm respectively. This indicates that antibacterial activities of a mixture of pineapple peel extract and calcium hydroxide paste are included in the strong level. Based on these analysis, the mixture of pineapple peel extract and calcium hydroxide paste can be considered to be an alternative intracanal medicament in root canal treatment.

From the forgoing analysis, it can be concluded that a mixture of pineapple peel extract at 6.25% and 12.5% concentrations and calcium hydroxide paste with a ratio of 1:1 demonstrates a higher antibacterial activity than 100% calcium hydroxide paste and mixture of calcium hydroxide and iodoform paste against *E. faecalis*.

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