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ANTIBACTERIAL ACTIVITY TEST OF CALCIUM HYDROXIDE AND MAULI BANANA (*Musa acuminata*) STEM EXTRACT GEL MIXTURE AGAINST *Enterococcus faecalis*

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ABSTRACT

Background: The use of calcium hydroxide as an intracanal medicament has several weaknesses that can cause side effects, including chronic inflammation in the periapical tissue and resistance to several bacteria, one of which is *Enterococcus faecalis*. The bacteria are the most resistant microorganism to calcium hydroxide due to a high pH tolerance capability. This weakness is the reason for finding alternative materials that can combine with calcium hydroxide. Mauli banana stem has bioactive compounds such as tannins, saponins, alkaloids, and flavonoids which have antibacterial and anti-inflammatory effects. Therefore, combined with calcium hydroxide may exhibit good characteristics as an intracanal medicament. **Purpose:** To analyze the antibacterial activity of the calcium hydroxide and mauli banana (*Musa acuminata*) stem extract gel mixture against *Enterococcus faecalis*. **Methods:** The research is a pure experimental study with a post-test only with control group design that uses 4 treatment groups, including calcium hydroxide and mauli banana stem extract gel mixture at a concentration of 25%; 37,5%; 50%; and control with each of 9 replications. The parameter measured was the diameter of the inhibition zone that formed on Mueller Hinton Agar. **Result:** One Way Anova and Bonferroni Post Hoc tests showed significant differences between each treatment group. **Conclusion:** The mixture of calcium hydroxide and mauli banana stem extract gel had antibacterial activity against *Enterococcus faecalis*. The mixture of calcium hydroxide and mauli banana stem extract gel at 50% concentration obtained the highest inhibition zone.

Keywords: Antibacterial activity, Calcium hydroxide, *Enterococcus faecalis*, *Musa acuminata*.

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INTRODUCTION

Endodontic treatment or root canal treatment is a treatment that aims to treat an infected root canal system by removing infected tissue and preventing the root canal from recontaminating by bacteria after treatment. The principle of endodontic treatment is known as the "Endodontic Triad" which includes the preparation, sterilization, and filling or obturation of the root canal.^{1,2} The sterilization stage includes root canal irrigation and the addition of intracanal medicaments.¹

Calcium hydroxide is an intracanal medicament used in endodontics.³ The use of calcium hydroxide has several weaknesses that can cause side effects, including chronic inflammation in the periapical tissue and resistance to several

bacteria, one of which is *Enterococcus faecalis*.³⁻⁶ The bacteria are the most resistant microorganism to calcium hydroxide due to a high pH tolerance capability.^{3,7}

This weakness is the reason for finding alternative materials that can combine with calcium hydroxide. Research by Bretas et al (2017) proved that calcium hydroxide combined with propylene glycol and aloe vera gel showed antibacterial activity against *Pseudomonas aeruginosa* and *Enterococcus faecalis* and had high anti-inflammatory effectiveness.⁸ Effendy et al (2019) research reported that calcium hydroxide combined with propolis had higher antibacterial activity than the combination of calcium hydroxide with saline.⁹

Mauli banana (*Musa acuminata*) is a typical South Kalimantan banana. These plants are easy to find in South Kalimantan because they are abundant.¹⁰ Mauli banana is one of the natural plants that have the ability as antibacterial, antifungal, anti-inflammatory, and immunomodulatory.¹¹⁻¹⁴ Mauli banana stems contain bioactive components such as flavonoids, saponins, alkaloids, ascorbic acid, lycopene, and tannins. The most abundant bioactive compounds in the mauli banana stem extract are tannins. Tannins function as antibacterial and can reduce inflammation, increase epithelial formation, and cause vasoconstriction effects on blood vessels.^{11,12}

Mauli banana stem extract gel with a concentration of 25% had been non-toxic. It showed antibacterial, antifungal, and anti-inflammatory effect equivalent to aloe vera gel. In comparison, a higher concentration of 37,5% showed a higher anti-inflammatory effect than aloe vera gel with a lower presence of TNF- α and NF- κ B.^{10,11,13-15} Astuti et al (2018) proved that the 37,5% concentration of mauli banana stem extract gel could act as an immunomodulator better than calcium hydroxide.¹⁴ Apriasari et al (2020) research reported the 50% concentration of mauli banana stem extract gel had the highest anti-inflammatory effect compared to the concentration of 25% and 37,5%.¹¹

Mauli banana stem (*Musa acuminata*) contains antibacterial and anti-inflammatory properties when combined with calcium hydroxide, which can stimulate the formation of hard tissue through the release of (Ca⁺) ions and has an antibacterial effect through the release of (OH⁻) ions may exhibit good characteristics as an intracanal medicament.^{11,16} The release of hydroxyl ions by calcium hydroxide causes an increase in the pH of surrounding tissue, which can cause chronic inflammation.⁴ This chronic inflammation can be prevented by adding mauli banana stem extract gel. Mauli banana stem extract gel has bioactive compounds such as tannins which contain polyphenols. Polyphenols are able to show anti-inflammatory effects by inhibiting tumor necrosis factor α (TNF- α) and nuclear factor kappa β (NF- κ B) signals. The inhibition of NF- κ B and TNF- α are the key to the anti-inflammatory effect of the mauli banana stem extract gel.¹¹

Regarding the antibacterial and anti-inflammatory effects of the mauli banana stem extract gel and the absence of preclinical tests about the antibacterial activity of calcium hydroxide and mauli banana stem extract gel mixture, research is needed on the antibacterial activity test of that mixture against *Enterococcus faecalis*.

MATERIALS AND METHODS

The research has obtained a research permit and ethical clearance from the Ethical Committee of the Faculty of dentistry, University of Lambung Mangkurat Banjarmasin No.031/KEPKG-FKGULM/EC/IV/2022. This study is a pure experimental study with a post-test only with a control group design using 4 groups they are the mixture of calcium hydroxide and mauli banana stem extract gel with the concentration of 25%; 37,5%; 50%; and calcium hydroxide as control with 9 repetitions, so the total sample is 36 samples.

Mauli banana stem extract gel was carried out by boiling 100 ml of aquadest, adding *Sodium Carboxymethyl Cellulose* (Na-CMC), then homogenized. The ethanol-free of mauli banana stem extract was added and stirred quickly until homogeneous. Propylene glycol and nipagin were added to the gel mass. Aquadest is added up to 100 grams. Mauli banana stem extract gel is ready with a viscous consistency and dark brown color. The mixture of calcium hydroxide and mauli banana stem extract gel was carried out by combining calcium hydroxide and mauli banana stem extract gel in a ratio of 1:1. The form of that mixture is paste.

Antibacterial test with the hole diffusion method was carried out by making 4 holes in the media that had been inoculated with *Enterococcus faecalis* bacteria using a cork borer, then adding 0,5 grams of each mixture of calcium hydroxide paste and waiting about 2 hours for the diffusion of the test material in the medium occurs. Incubated at 37°C for 24 hours. The zone of inhibition is considered as the distance in millimetres between the edges of the clear zone. The data obtained from the results were statistically analyzed using the One Way Anova test and continued with the Bonferroni Post Hoc test to find out which groups had significant differences.

RESULTS

The results of the antibacterial activity test of calcium hydroxide and mauli banana (*Musa acuminata*) stem extract gel mixture against *Enterococcus faecalis* obtained the average values presented in table 1.

Table 1 The Average Value (Mean) and Standard Deviation of Inhibitory Zone of Calcium Hydroxide and Mauli Banana Stem Extract Gel Mixture Against *Enterococcus faecalis*.

Groups	N	Mean (mm) ± Standard Deviation
CH+MASE Gel 25%	9	22,06±0,59 ^a
CH+MASE Gel 37,5%	9	24,68±0,36 ^b
CH+MASE Gel 50%	9	27,69±0,66 ^c
CH (control)	9	17,39±0,56 ^d

*Value with different superscript letters shows significant difference ($p < 0,05$).

Based on the table above, we can see that the highest average inhibition zone is calcium hydroxide and mauli banana stem extract gel mixture with a concentration 50%, and the lowest is the control group.



Figure 1 The Inhibition Zone of Calcium Hydroxide and Mauli Banana (*Musa acuminata*) Stem Extract Gel Mixture Against *Enterococcus faecalis*.

All treatment groups had a significance value (p) $< 0,05$. It means that there was a significant difference in the average inhibition zone between the calcium hydroxide and 25% mauli banana stem extract gel mixture with calcium hydroxide and 37,5% mauli banana stem extract gel mixture; calcium hydroxide and 25% mauli banana stem extract gel mixture with calcium hydroxide and 50% mauli banana stem extract gel mixture; calcium hydroxide and 25% mauli banana stem extract gel mixture with control; calcium hydroxide and 37,5% mauli banana stem extract gel mixture with calcium hydroxide and 50% mauli banana stem extract gel mixture; calcium hydroxide and 37,5% mauli banana stem extract gel mixture with control; and also calcium hydroxide and 50% mauli banana stem extract gel mixture with control against *Enterococcus faecalis* bacteria.

DISCUSSION

The results of the antibacterial activity test of calcium hydroxide and mauli banana (*Musa acuminata*) stem extract gel mixture against *Enterococcus faecalis* showed that calcium hydroxide combined with mauli banana stem extract gel with a concentration of 25%; 37,5%; and 50% have higher antibacterial activity against

Enterococcus faecalis than control group. This is in line with Punathil et al (2020) and Effendy et al (2019), which proved that calcium hydroxide has increased antibacterial activity against *Enterococcus faecalis* when combined with other materials that also have antibacterial effects.^{9,17} Mauli banana (*Musa acuminata*) stem is one of the natural plants that have the ability as an antibacterial.¹¹ Latanza et al (2020) research reported that mauli banana stem extract could be against *Staphylococcus aureus*, which is also included in gram-positive bacteria and facultative anaerobes.¹⁸

The antibacterial activity test of calcium hydroxide and mauli banana stem extract gel mixture can be seen from the diameter of the inhibition zone formed. According to the classification of bacterial growth inhibition response by David and Stout, the antibacterial activity of calcium hydroxide and mauli banana stem extract gel mixture with concentrations of 25%; 37,5%; and 50% against *Enterococcus faecalis* obtained an average inhibition zone of 22,06 mm; 24,68 mm; 27,69 mm respectively classified as very strong, and the control with an average inhibition zone of 17,39 mm classified as strong.¹⁹ The result showed an increased average diameter of the inhibition zone along with an increase in the concentration of mauli banana stem extract gel combined. Octavia et al (2019) proved that the increased average inhibition zone of *Enterococcus faecalis* bacteria was directly proportional to an increase in the concentration of extract added. This is due to the increase in secondary metabolites or bioactive compounds contained in the extract along with the increase in concentration.²⁰ Bioactive compounds that have an antibacterial effect in the mauli banana stem extract gel are 67,59% tannin; 14,49% saponins; 0,34% alkaloids; and 0,25% flavonoids.^{11,13}

Calcium hydroxide mixed with mauli banana stem extract gel has a synergistic effect against *Enterococcus faecalis* by lowering the surface tension of the bacterial cell wall causing cell leakage and making intracellular compounds released, inhibiting the formation of polypeptides on the bacterial cell wall, causing lysis of the bacterial cell wall, disrupting the stability of the peptidoglycan constituent components of the bacterial cell wall and make the bacterial cell wall layer is incomplete formed, causing coagulation of bacterial cell proteins so denaturation of protein happen, have strong alkaline properties with a pH of 12-12,5 so the mixture of these medicaments

can hydrolyze lipids in the polysaccharide layer of bacterial cell walls, damaging the cytoplasmic membrane of bacteria which causes protein denaturation and inhibits the DNA replication process, so the growth of *Enterococcus faecalis* is inhibited.^{16,18,21}

This study proved that the average diameter of the calcium hydroxide inhibition zone as a control had a significant difference with the calcium hydroxide and mauli banana stem extract gel mixture groups. This is because the calcium hydroxide used in this study is impure, but commercial calcium hydroxide with the trademark UltraCal XS contains only 35% calcium hydroxide and additional compositions such as aquadest and barium sulfate as radiopaque materials.

Another factor that affects the results of this study is the existence of limitations in the study. This limitation is a limited research discussion only to determine the antibacterial activity of calcium hydroxide and mauli banana stem extract gel mixture with a ratio of 1:1 against *Enterococcus faecalis*. The result of this study indicates that the mauli banana stem extract gel has a chance to combine with calcium hydroxide as an alternative intracanal medicaments against *Enterococcus faecalis*. However, further research is needed to fulfill the requirements of alternative intracanal medicaments, such as ensuring that materials are non-toxic, biocompatible, and non-irritating.

According to the result of the study, it can be concluded that there is an antibacterial activity of calcium hydroxide and mauli banana (*Musa acuminata*) stem extract gel mixture against *Enterococcus faecalis* and all treatment groups have a significant difference in the average diameter of the inhibition zone on the growth of *Enterococcus faecalis* bacteria with the highest average obtained by the mixture of calcium hydroxide and mauli banana stem extract gel with a concentration of 50%.

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