

ANTIBACTERIAL EFFECTIVENESS OF LERAK FRUIT ETHANOL EXTRACT (*Sapindus rarak* DC) AND 2% CHLORHEXIDINE IN *Enterococcus faecalis* (EFEKTIVITAS ANTIBAKTERI EKSTRAK ETANOL BUAH LERAK (*Sapindus rarak dc*) DAN CHLORHEXIDINE 2% PADA *Enterococcus faecalis*)

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ABSTRACT

The failure of a root canal treatment can be caused by the facultative anaerobic bacteria *Enterococcus faecalis*, which should be eliminated throughout root canal irrigation. Chlorhexidine 2% is a popular antibacterial liquid used in root canal irrigation. Natural substances can also be used to make antibacterial irrigation liquids. One natural substance with antibacterial properties is lerak fruit extract (*Sapindus rarak* DC), containing 28 per cent of saponins, alkaloid compounds, polyphenols, antioxidant chemicals, and flavonoid groups and tannins. This study was conducted to determine the effectiveness of 25% lerak fruit extract (*Sapindus rarak* DC) against *E. faecalis* compared to 2% Chlorhexidine. This study is a pure experimental study using the agar diffusion method against three experimental groups chlorhexidine 2% as a positive control, lerak fruit extract (*Sapindus*

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rarak DC) as a treatment group, and aquadest as a negative control. Each group was repeated ten times on Muller Hinton Agar (MHA) media which was applied using a paper disc. Then the diameter of the inhibition zone was calculated using a caliper. The results in this study, 25% lerak (*Sapindus rarak* DC) fruit extract had an average inhibition zone diameter of 11.775 mm, and an average inhibition zone diameter of 2% chlorhexidine was 19.745 mm. The difference in the diameter of the inhibition zone was significantly different in all groups. Conclusion ethanol extract of lerak fruit (*Sapindus rarak* DC) 25% can inhibit the growth of *E. faecalis* and can be used as an alternative to other antimicrobial agents as disinfection of root canal irrigation.

Keywords: antibacterial; chlorhexidine; lerak fruit

ABSTRAK

Kegagalan perawatan saluran akar dapat disebabkan adanya bakteri fakultatif anaerob Enterococcus faecalis, yang seharusnya mampu dieliminasi melalui irigasi saluran akar. Chlorhexidine 2% merupakan cairan antibakteri yang biasa digunakan pada irigasi saluran akar. Obat antibakteri dapat juga diperoleh dari bahan alami. Ekstrak buah lerak (Sapindus rarak DC) adalah salah satu bahan alami antibakteri yang mengandung saponin 28%, senyawa alkaloid, polifenol, senyawa antioksidan dan golongan flavonoid, juga tannin. Penelitian ini dilakukan untuk mengetahui efektivitas 25% ekstrak buah lerak (Sapindus rarak DC) terhadap E. faecalis dibandingkan dengan Chlorhexidine 2%. Penelitian ini merupakan penelitian eksperimen murni dengan metode difusi agar, terhadap 3 kelompok eksperimen klorheksidin 2% sebagai kontrol positif, ekstrak buah lerak (Sapindus rarak DC) sebagai kelompok perlakuan, dan aquades sebagai kontrol negatif. Masing-masing kelompok diulang 10 kali pada media Muller Hinton Agar (MHA) yang diaplikasikan menggunakan kertas cakram kemudian dihitung diameter zona hambatnya menggunakan jangka sorong. Hasil penelitian ini ekstrak buah lerak (Sapindus rarak DC) 25% memiliki diameter zona hambat rerata 11,775 mm dan diameter

zona hambat rerata klorheksidin 2% adalah 19,745 mm. Perbedaan diameter zona hambat berbeda nyata pada semua kelompok. Ekstrak etanol buah lerak (Sapindus rarak DC) 25% dapat menghambat pertumbuhan E. faecalis dan dapat digunakan sebagai alternatif agen antimikroba lain sebagai desinfektan irigasi saluran akar.

Kata kunci: antibakteri; buah lerak, klorheksidin

INTRODUCTION

Root canal therapy is a procedure used to remove bacteria from the root canal wall that might cause infection and tissue growth. The removal of germs from the root canal system is critical to the effectiveness of root canal therapy.^{1,2} Root canal therapy is divided into three stages: biomechanical preparation, disinfection, and root canal obturation. Endodontic treatment relies on biomechanical preparation to remove microorganisms found in root canals. Proper irrigation might have an impact on the root canal cleansing potential. The optimal irrigation solution must have the ability to destroy microorganisms, dissolve necrotic tissue, be lubricating, remove the smear layer, and not irritate good tissue.^{2,3} Facultative bacteria, particularly *Enterococcus faecalis*, are commonly responsible for root canal treatment failure. Infections caused by these bacteria have a frequency of 24-77%. *E. faecalis*

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resistance factor may survive in nutritional deficiencies and at alkaline pH. *E. faecalis* can also compete with other bacteria for dentinal tubule invasion.⁴ The smear layer can also infiltrate into the dentinal tubules in the same circumstances, causing microorganisms to be coated by the smear layer and making *E. faecalis* challenging to remove.⁵ The failure of the root canal treatment can be avoided by using the proper irrigation solutions. Chlorhexidine 2% is one of the suitable irrigation fluids shown to be more efficient against *E. faecalis*.⁶

Chlorhexidine 2% is effective against *E. faecalis* in root canals and dentinal tubules. Irrigation with 2% chlorhexidine solution for 2 minutes removed microorganisms in the dentinal tubules up to 100 μ m. Chlorhexidine 2% has low cytotoxicity, broad-spectrum antibacterial, and is also water-soluble. The irrigation can damage the integrity of the cell membrane so that it can precipitate

cytoplasmic fluid. Its antibacterial effect attracts cation molecules from 2% chlorhexidine with anion molecules from the bacterial cell membrane. The disadvantage Chlorhexidine 2% is less effective against gram-negative bacteria and cannot dissolve necrotic tissue or remove the smear layer. Therefore 2% Chlorhexidine cannot be used as a sole irrigation solution in root canal treatment.^{6,3}

Lerak fruit (*Sapindus rarak DC*) is one of the alternative natural ingredients that can be developed as root canal irrigation. The active compounds contained in lerak fruit (*Sapindus rarak DC*) are 28% saponins, alkaloid compounds, polyphenols, antioxidant compounds, and flavonoids, as well as tannins.⁷⁻⁹ Ethanol extract of lerak fruit (*Sapindus rarak DC*) can be used for alternative irrigation. The root canal has low surface tension. The benefit of low surface tension is that the liquid can wet the entire surface of the root canal wall. Research on the effect of various preparations of lerak extract (*Sapindus rarak DC*) found that lerak fruit (*Sapindus rarak DC*) can lift the smear layer. At the same time, 2% Chlorhexidine cannot remove the smear layer.^{8,3} Innovation of ethanol extract of lerak fruit (*Sapindus rarak DC*) as a root canal irrigant is known to have antibacterial and

antifungal effects. In the study of *E. faecalis*, ethanol extract of lerak fruit (*Sapindus rarak DC*) had an antibacterial effect with a minimal bactericidal concentration of 25%.⁹ No study has compared the natural antibacterial ingredients of the ethanolic extract of lerak fruit (*Sapindus rarak DC*) 25% compared with 2% chlorhexidine against *E. faecalis*. The purpose of this study was to explain the differences in the antibacterial effectiveness of the 25% ethanol extract of lerak fruit (*Sapindus rarak DC*) against *E. faecalis* compared to 2% chlorhexidine.

METHOD

The study was conducted in vitro with agar diffusion method on *E. faecalis* bacterial plates. The bacteria were identified as *E. faecalis* after being reidentified. A total of 9 samples of bacterial plates were divided into three groups, namely ethanol extract of lerak fruit (*Sapindus rarak DC*) 25% (group 1), 2% chlorhexidine (group 2), and distilled water as a control. The 25% ethanolic extract of lerak fruit (*Sapindus rarak DC*) was obtained from the Laboratory of the Research Institute for Spices and Medicinal Plants (Balittro) by weighing the extract using an analytical balance of as much as 1.25 grams and then dissolved in 5 ml of distilled water. Anova then tested the results

obtained if the data were normally distributed, and the Post Hoc test continued. The presentation of the effects of data processing is then displayed in tabular form.

RESULT

The effectiveness test of 25% lerak fruit ethanol extract (*Sapindus rarak DC*) against *E. faecalis* compared to 2% Chlorhexidine can be seen in Figure 1.

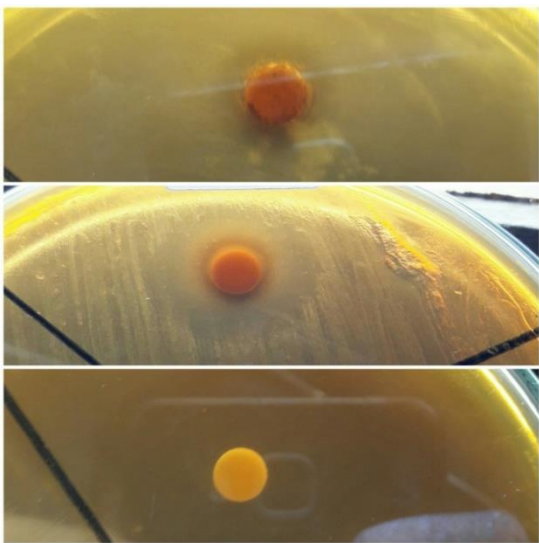


Figure 1. The effectiveness test of 25% ethanol extract of lerak fruit (*Sapindus rarak DC*) against *E. faecalis* compared to (B) Chlorhexidine 2% and (C) Aquades as a negative control.

Based on Table 1, it was identified that the 25% ethanol extract of lerak fruit (*Sapindus rarak DC*) affects inhibiting the growth of *E. faecalis* bacteria. Still, the 2% Chlorhexidine inhibition diameter is greater than that of the 25% ethanol extract of lerak

fruit (*Sapindus rarak DC*).

Table 1 Diameter of inhibition of 25% lerak fruit (*Sapindus rarak DC*) ethanol extract against *Enterococcus faecalis* compared to 2% chlorhexidine

Sample	I	II	III
	Extract of	Chlorhexidine	Control
	lerak fruit	2%	
	(mm)	(mm)	(mm)
1	12.75	21.10	0
2	13.50	23.05	0
3	11.50	16.50	0
4	10.25	14.60	0
5	12.30	18.55	0
6	13.05	21.35	0
7	11.10	23.50	0
8	11.10	20.25	0
9	11.10	18.05	0
10	11.10	20.50	0

The ethanolic extract of lerak fruit (*Sapindus rarak DC*) 25% affected inhibiting the growth of *E. faecalis* bacteria. Still, the diameter of the diffusion zone of chlorhexidine 2% was greater than 25% extract of lerak fruit (*Sapindus rarak DC*).

Table 2. Diffusion zone of *E. faecalis* inhibition

Group	N	Diffusion zone (mm)			P
		Min.	Max.	Mean ± SD	
25% extract of lerak fruit	10	10.25	13.50	11.775 ± 1.056	0.000
chlorhexidine 2%	10	14.60	23.50	19.745 ± 2.819	
control	10	0.00	0.00	0.00	

ANOVA test showed that there was a significant difference in the diffusion zone

between 3 groups. The largest diameter of inhibition formed by the extract of lerak fruit (*Sapindus rarak DC*) is 25%, 13.50 mm, and 2% Chlorhexidine, 23.50 mm (Table 2).

DISCUSSION

The study used the agar diffusion method to determine the diameter of the clear zone (inhibition zone) formed around the paper disc from the 25% ethanol extract of lerak fruit (*Sapindus rarak DC*) against *E. faecalis* compared to 2% Chlorhexidine. Table 2 shows a significant difference between 25% lerak fruit ethanol extract (*Sapindus rarak DC*) and 2% Chlorhexidine, with the average diameter of inhibition formed by 25% lerak fruit ethanol extract (*Sapindus rarak DC*) is 11.775 mm. At the same time, the average diameter of the inhibition created by 2% Chlorhexidine is 19.745 mm.

The mechanism of 2% chlorhexidine eliminates *E. faecalis* is by damaging the cell membrane integrity to precipitate cytoplasmic fluid. The antibacterial effect works by attracting cation molecules from 2% chlorhexidine with anion molecules from the bacterial cell membrane. Meanwhile, the mechanism of lerak extract (*Sapindus rarak DC*) eradicates *E. faecalis* by saponins, alkaloid compounds,

polyphenols, antioxidant compounds, and flavonoid groups, as well as tannins. Inhibition of bacterial growth occurs due to the reaction of a chemical compound in lerak fruit as an antibacterial. Flavonoid compounds are one of the chemical compounds that are bacteriostatic. Its mechanism of action is by denaturing bacterial cell proteins and damaging the cytoplasmic membrane. Other compounds contained in Lerak fruit, such as saponins can also damage the cytoplasmic membrane. Cytoplasmic membrane Damage can cause the permeability of the cell membrane to decrease so that the transport of substances into and out of the cell becomes uncontrolled.¹⁰

Alkaloids have the ability to an antibacterial. The alkaloids mechanism is by interfering with the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not fully formed. It is suspected that the alkaloids work first to damage the cell wall, and then the flavonoids work to damage the bacterial cell membrane. Tannins are thought to shrink cell walls. Damage to the cell wall will inhibit the growth of bacterial cells, and eventually, the bacteria will die. In general, the work of a chemical as an antibacterial agent can result in changes that damage the inhibition of the growth of these bacterial cells.^{11,12}

The results of previous studies showed that lerak extract (*Sapindus rarak DC*) had low surface tension. The benefit of low surface tension is that the liquid can wet the entire surface of the root canal wall. The results showed that lerak extract (*Sapindus rarak DC*) with a 5% - 25% concentration had a lower surface tension than 2.5% NaOCl. In addition, because *Enterococcus faecalis* is covered with a smear layer in the dentinal tubules, irrigation materials are needed to eliminate the smear layer. Research on the effect of various preparations of lerak extract (*Sapindus rarak DC*) on the formation of micro-fissures at the apical root canal did not find any significant difference between the micro-fissures produced by 0.01% lerak ethanol extract (*Sapindus rarak DC*) and 0.008% lerak fruit saponins with micro gaps created by the combination of 5% NaOCl and 18% EDTA. It shows that irrigation with 0.01% lerak extract (*Sapindus rarak DC*) and 0.008% irrigation with lerak fruit saponins (*Sapindus rarak DC*) can remove the smear layer, while 2% Chlorhexidine cannot remove the smear layer.^{8,3} In this study, *E. faecalis* was carried out directly while *E. faecalis* would be covered by a smear layer in the dentinal tubules. Therefore, an irrigation fluid is needed that can lift the smear layer to eliminate

Enterococcus faecalis. The development of lerak extract (*Sapindus rarak DC*) as a root canal irrigant is known to have antibacterial and antifungal effects. In the study of *E. faecalis*, lerak extract (*Sapindus rarak DC*) had an antibacterial effect with a minimum killing concentration (KBM) of 25%.⁹ In this study, the 25% ethanol extract of lerak fruit (*Sapindus rarak DC*) can inhibit *E. faecalis* but is not as good as 2% chlorhexidine. The research result and data analysis show the 25% ethanol extract of lerak fruit (*Sapindus rarak DC*) can inhibit the growth of *E. faecalis* and can be used as an alternative to other antimicrobial agents as root canal irrigation disinfection.

CONCLUSION

Ethanol extract of lerak fruit (*Sapindus rarak DC*) 25% can inhibit the growth of *E. faecalis* and can be used as an alternative to other antimicrobial agents as disinfection of root canal irrigation.

CONFLICT OF INTEREST

We declare that there is no conflict of interest in the scientific articles.

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