

Antibacterial Effect of Lerak Fruit Decoction (*Sapindus rarak* DC) on the Growth of *Streptococcus mutans* as an Alternative Cavity Cleanser Material (In Vitro)

Nevi Yanti*^{}, Fitri Yunita Batubara^{}, Cut Nurliza^{}, Bryan Julio Hasim^{}

Department of Conservative Dentistry, Faculty of Dentistry, Universitas Sumatera Utara, Medan, 20155, Indonesia

*Corresponding Author: nevi.yanti@usu.ac.id

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ABSTRACT

Cavity cleanser is needed to remove smear layers and eliminate microbes. In this context, lerak fruit decoction (*Sapindus rarak* DC) is an important natural material used as an alternative to 2% CHX. The fruit effectively removes smear layers, possesses antimicrobial properties, and shows low surface tension. Therefore, this research aims to analyze the effectiveness of lerak fruit decoction as an alternative material for cavity cleanser material. The laboratory experiment commences with boiling 100 grams of lerak fruit in 100 ml of distilled water to obtain MIC and MBC values with the Dilution and Drop Plates Miles Misra method. The results of ANOVA and LSD tests show that lerak fruit decoction has antibacterial effect with MIC and MBC values of 12.5% and 25%, respectively. This research serves as the foundation for further investigations into lerak fruit decoction, considering the availability, biocompatibility, affordability, and ease of processing. Moreover, it can be concluded that 25% lerak fruit decoction has similar antibacterial ability to 2% CHX against *Streptococcus mutans*.

Keywords: Antimicrobial Effect, Cavity Cleanser, Lerak Fruit Decoction, *Streptococcus Mutans*

ABSTRAK

Dalam kedokteran gigi, setelah preparasi kavitas gigi yang mengalami karies dibutuhkan bahan cavity cleanser untuk membuang smear layer dan mengeliminasi mikroba di dalam kavitas. Salah satu bahan alami yang dapat dijadikan alternatif bahan cavity cleanser adalah rebusan buah lerak. Penelitian membuktikan bahwa buah lerak dapat membuang smear layer, memiliki efek anti mikroba, dan memiliki tegangan permukaan yang rendah. Penelitian ini bertujuan untuk menunjukkan efektivitas Rebusan Buah Lerak (*Sapindus Rarak* DC) sebagai bahan alternatif cavity cleanser. Eksperimen laboratorium ini dimulai dengan merebus 100 gram buah lerak dengan aquadest 100 ml hingga mendidih, kemudian mencari nilai KHM dan KBM bahan dengan Metode Dilusi dan Drop Miles Misra. Hasil uji ANOVA dan LSD menunjukkan rebusan buah lerak mempunyai efek antibakteri dengan nilai KHM sebesar 12,5% dan nilai KBM sebesar 25%. Diharapkan hasil penelitian ini dapat menjadi dasar penelitian lebih lanjut untuk mengembangkan rebusan buah lerak dikarenakan mudah diperoleh dan lebih biokompatibel serta pembuatannya yang lebih sederhana dan ekonomis. Dapat disimpulkan bahwa rebusan buah lerak 25% mempunyai kemampuan antibakteri yang sama dengan CHX 2% terhadap *Streptococcus mutans*.

Kata Kunci: Efek Antimikroba, Cavity Cleanser, Rebusan Buah Lerak, *Streptococcus Mutans*



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1. Introduction

Caries are produced from a complex interaction between microbial species in the tooth surface with dietary, saliva, and genetic influences. In this context, metabolic interactions found in dental biofilm produce acid and form extracellular polymer to promote the adherence of microbial. [1] *Streptococcus mutans* is primary cariogenic microorganism with the ability to produce a large number of acids, exceeding the saliva buffer capacity. This gives cariogenic bacteria an advantage to outcompete a non-cariogenic species in a low pH environment. In the second invasive phase, *S. mutans* co-aggregate and co-adhere with another microbial species. In the last phase, dental biofilm has reached a stable condition that changes the balance of an oral ecology, allowing bacteria to penetrate the tissue. Therefore, calcium hydroxyapatite dissolution occurs in enamel and dentin to form cavitation of the tooth. [1] Dentin caries should be treated with an invasive procedure by using burs or other instruments. In this condition, the smear layer consists of residual organic and inorganic components produced with the dental cavity wall contaminated by bacteria. [2]

Residual Bacteria, which survived in the cavity for more than a year, may proliferate through smear layer. Therefore, the toxin diffuses to the pulp, irritating and inflaming the tissue. [3] In media caries restoration, *S. mutans* has been reported as a cariogenic bacteria. [4]

The pretreatment with antibacterial agent on the cavity surface is useful for eliminating harmful effects of residual bacteria or the toxin. [2] A cleanser is an agent used to remove debris from cavity and decrease the post-restoration sensitivity of the treatment. [5] The ideal requirement should be bactericidal and bacteriostatic, biocompatible, easy to obtain, and handle. [6] The use of 2% Chlorhexidine digluconate as a cavity cleanser has become popular because of the wide spectrum antibacterial activity [2, 5], as shown in Figure 1a. However, 2% Chlorhexidine digluconate may induce allergy, dermatitis, desquamative gingivitis, or altered taste. [7] Different natural resources have been developed as antibacterial considering the disadvantages of using the current cavity cleanser. [8]

Natural plant-based drugs are used as an alternative to resistant microorganisms and can avoid the toxicity of achemical material. World Health Organization (WHO) recommend the use of plant-based drugs in the maintenance of public health, prevention, and cure for degenerative disease and cancer. In addition, the effort to improve the safety and efficacy of herbal drugs is supported. The use of plant-based drugs is considered to be more biocompatible because of the side effects which are lesser than synthetic. [9] According to the Ministry of Research, Technology and Higher Education, the Indonesian National Research Master Plan in 2017-2045 supported the development of nature-based drugs including herbal plants and marine biotics. [10]

Lerak (*Sapindus rarak* DC) is a natural material from the majority of plants in Java Island. In Palembang, Java, and West Java, this plant is called lamuran, lerak, and rerek, respectively. Additionally, the fruit has a round shape similar to marble with an old dark brown color and shining surface. [11] The pharmacologic efficacy includes antifungal, bactericidal and anti-inflammatory properties. [12] Approximately 28% of compounds in the fruits are saponin, alkaloid, polyphenol, flavonoid, and tannin. [11]

The decoction method offers significant benefits as a green extraction procedure for obtaining bioactive compounds from plant materials. This method uses water, which is eco-friendly, non-inflammable, non-hazardous, and prevents pollution. The characteristics of water polarity as a solvent can extract polar bioactive compounds [13] to form a hydrobond. [14]

Water has a greater polarity than methanol and ethanol, while methanol has greater polarity than ethanol. The value of the dielectric constant of a compound is directly proportional to polarity.

The values of the relative permittivity of water, ethanol, and methanol are 80.37, 24.3, and 33.62, respectively. Therefore, water can sufficiently extract alkaloid, flavonoid, saponin, and tannin compounds. [14, 15] Temperature positively affects the process, efficiency and speed of the extraction. [7]

The examination of lerak antibacterial effect has been conducted by several research. Fitria JR et al, 2022 showed that lerak fruit extract with concentrations of 6.25%, 12.5%, and 25% had similarities with 2.5% NaOCl solution + 17% EDTA ($p > 0.05$) in preventing adhesion and inhibiting the development of *F. nucleatum*. [16] According to Sabila N et al, 2022, lerak fruit extracts of 6.25%, 12.5%, and 25% had better inhibition against the hydrophobicity of *F. nucleatum* than 2.5% NaOCl + EDTA 17% solution. [17]

From the description, lerak can be developed as an alternative cavity cleanser material because of the pharmacological effect that fulfils the requirements of a cavity cleaner. Therefore, this research aims to evaluate the differences between lerak fruit decoction and 2% CHX by antibacterial effect. The null hypothesis is related to antibacterial effect against *S. mutans* as an alternative cavity cleanser but no analyses have been carried out on the topic. Antibacterial effect of lerak fruit decoction is analyzed as a cavity cleanser material against the growth of *S. mutans* by determining MBC (Minimum Bactericidal Concentration) and MIC (Minimum Inhibitory Concentration) values.



Figure 1. (a) 2% CHX. (b) lerak fruit

2. Materials and Methods

In this research, *S. mutans* colony was obtained through experimental design. The sample was cultured with Mueller Hinton Agar (MHA) Media and antibacterial test was carried out at the Laboratory of Dentistry Research Center of Airlangga University. Approximately 100 grams of lerak fruit, with the seeds removed, were cleansed with flowing water. Subsequently, the fruit was cut into small pieces and boiled with 100 ml of distilled water until the mixture reached a boiling point for 15 minutes. The solution was filtered using filter paper, resulting in a brownish color. The decoction was stored in a sterile glass container at the desired location before conducting antibacterial examination (Figure 2d).

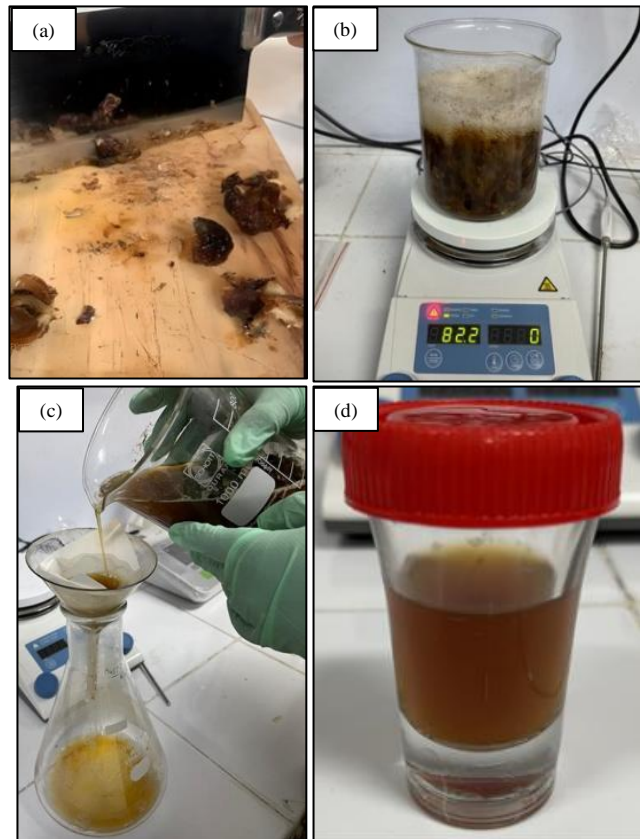


Figure 2. (a) Cutting lerak fruit into small pieces. (b) Boiling lerak fruit with distilled water (c) Filtering lerak fruit decoction. (d) Storing lerak fruit decoction

S. mutans bacteria were obtained from the Laboratory of Dentistry Research Center of Airlangga University, at Surabaya and cultured in MHA. Additionally, one or two doses of the bacteria were suspended in BHIB solution in a test tube. The mixture was homogenized and incubated again at a temperature of 37°C for 24 hours. The turbidity was adjusted to match the standard of 0.5 McFarland. After the solution reached the desired level, the sample was ready for testing.

In this research, antibacterial test was conducted using Drop Plates Miles-Misra method. The procedure followed the dilution method as specified by the Laboratory of Dentistry Research Center of Airlangga University and the concentrations tested ranged from 100% to 3.125% (Figure 3). After the dilution method was carried out in every test tube, 0.1 ml of the bacteria suspension was added using a micropipette into the test tube of negative control and 2% CHX.

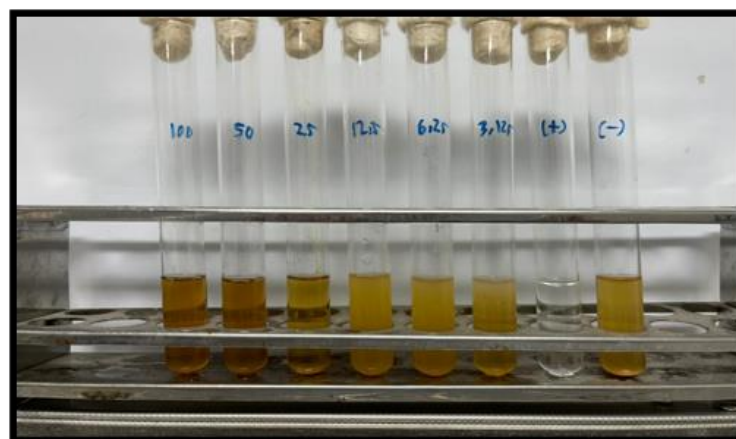


Figure 3. Result of lerak fruit decoction dilution after incubating 24 hours at a temperature of 37°C

The solution was incubated at a temperature of 37°C for 24 hours. Subsequently, one dose of 0.1 ml from the prepared solution using the dilution method was taken and placed onto MHA and the plates were incubated for 24 hours under anaerobic conditions. The bacterial colonies that grew on MHA were counted and compared with those from the negative and positive controls.

3. Results

The decoction produced from the fruit was brown color and viscous. Before testing antibacterial effects, lerak fruit decoction was stored in a sterile sealed glass cup and the test was conducted by determining MIC and MBC values. In this research, MIC value was determined in the dilution method. This was achieved by diluting 100% of lerak fruit decoction into 50%, 25%, 12.5%, 6.25%, and 3.125%. The treatment groups were filled with 0.1 ml from the bacterial suspension. MIC value was determined and the solution showed a clearer sight than the negative control. However, the weakness of natural products such as lerak fruit decoction was the brownish color formed. Another test was needed by placing the solution in a solidified growth media to count the sum of the bacteria colony using Drop Plates Miles Misra method. The solutions from the concentrations were placed in MHA using spreading method and the treatment group was made into 4 replications. Figure 4 represents antibacterial result of every treatment group replicated 4 times to obtain accurate results.

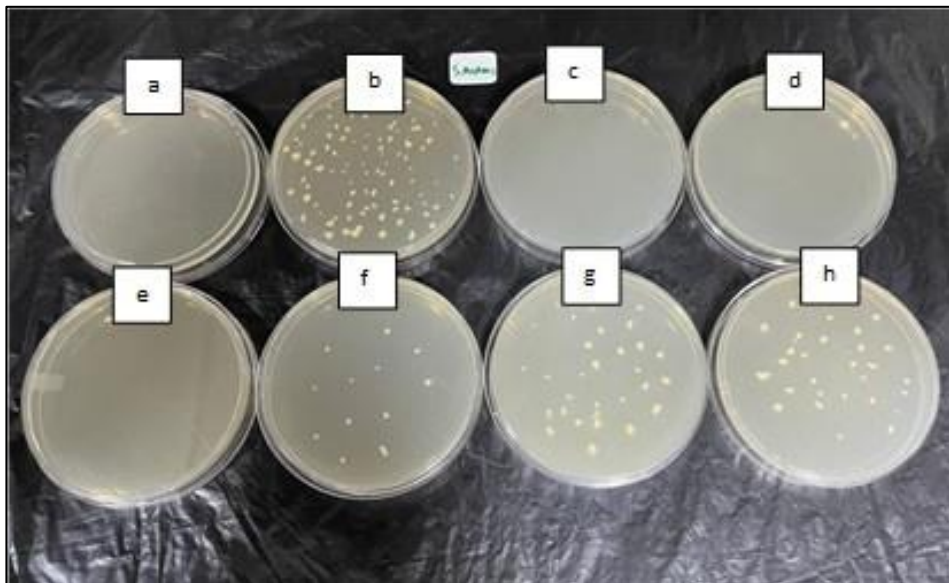


Figure 4. Antibacterial test result of the experimental material shows:

- a. 2% CHX
- b. negative control (BHIB)
- c. concentration of 100%
- d. concentration of 50%
- e. concentration of 25%
- f. concentration of 12.5%
- g. concentration of 6.25%
- h. concentration of 3.125%

One-way ANOVA test was used to observe antibacterial effect at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 2% CHX from the treatment group. The result showed a significant value of $p=0.000$ ($p<0.05$), hence, lerak fruit decoction had antibacterial effect against *S. mutans* (Table 1).

Table 1. One way ANOVA results of antibacterial effect of 2% CHX and lerak fruit decoction against *Streptococcus mutans*.

No.	Concentration	N	Mean	SD	Sig
1.	2% CHX	4	0	0	0.000
2.	100%	4	0	0	
3.	50%	4	0	0	
4.	25%	4	0	0	
5.	12.5%	4	12	1.414	
6.	6.25%	4	27.50	2.380	
7.	3.125%	4	48.75	2.500	
8.	Negative Control	4	159.75	5.058	

LSD test was carried out to observe the difference in the antimicrobial effect of lerak fruit decoction and 2% CHX against *S. mutans* at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 2% CHX. The result showed some treatment groups obtained at a significant value of $p=1.000$ ($p>0.05$). However, the significant differences in antibacterial effect with each other against the growth of *S. mutans* was not reported. The other treatment groups were obtained at a significant value of $p=0.000$ ($p<0.05$) due to the significant difference.

One-way ANOVA and LSD results showed that lerak fruit decoction had antibacterial effect with a significant value of $p=0.000$ ($p<0.05$) measured by using MIC and MBC values against the growth of *S. mutans*. MIC value was at 12.5% because bacteria were inhibited when a solution eliminated more than 90% of the growth. The decoction eliminated 92.5% of bacteria at a concentration of 12.5% by comparing the growth in the treatment groups from the negative control. Meanwhile, MBC value was at a concentration of 25% in eliminating the growth from 99.9-100% of MHA at a temperature of 37°C for 24 hours. Figure 5 shows antibacterial effectivity test of lerak fruit decoction and 2% CHX.

Table 2. LSD test results of antibacterial effect of 2% CHX and lerak fruit decoction against *Streptococcus mutans*.

No.	Treatment Group		P value
	Concentration	Concentration	
1.	100%	2% CHX	1.000
		Negative Control	0.000
		50%	1.000
		25%	1.000
		12.5%	0.000
		6.25%	0.000
		3.125%	0.000
2.	50%	2% CHX	1.000

		Negative Control	0.000
		25%	1.000
		12.5%	0.000
		6.25%	0.000
		3.125%	0.000
3.	25%	2% CHX	1.000
		Negative Control	0.000
		12.5%	0.000
		6.25%	0.000
		3.125%	0.000
4.	12.5%	2% CHX	0.000
		Negative Control	0.000
		6.25%	0.000
		3.125%	0.000
5.	6.25%	2% CHX	0.000
		Negative Control	0.000
6.	3.125%	2% CHX	0.000
		Negative Control	0.000

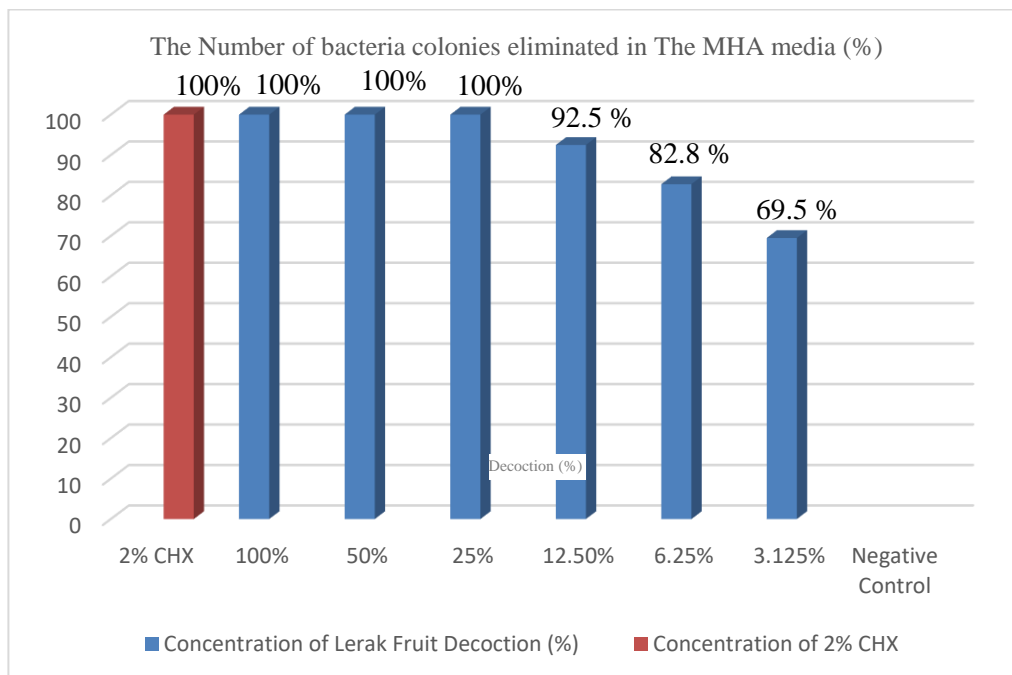


Figure 5. Antibacterial effectiveness test graphic results of lerak fruit decoction and 2% CHX against *Streptococcus mutans*.

4. Discussions

Many laboratory methods can be used to evaluate in vitro antimicrobial activity from plant-based material. [18] This research used the dilution and Drop Plates Miles Misra methods. In the first method, multiple dilutions on lerak fruit decoction were carried out, and every concentration of the experimental materials was obtained from half of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. The concentration was adjusted based on the standards for antibacterial testing and the experimental material was replicated 4 times to obtain more accurate results.

After the data was obtained, analytic tests such as one-way ANOVA and LSD were carried out. One-way ANOVA test showed that lerak fruit decoction had antibacterial effects on the growth of *S. mutans* with a significance value of $p = 0.000$ ($p < 0.05$). LSD test showed that there was a significant difference in antibacterial effects between the treatment and control group with each value $p = 0.000$ ($p < 0.05$).

MIC and MBC values in this research were 12.5% and 25% . This was because lerak fruit decoction with a 25% concentration killed 99.9% of *S. mutans*. LSD and ANOVA results showed no difference ($p > 0.05$), between the concentration of 25% lerak fruit decoction with 2% CHX. An average number of 0 CFU/ mL bacterial colonies was also reported on MHA. Therefore, lerak fruit decoction produced the same antibacterial effect as 2% CHX against the growth of *S. mutans* when used at a concentration of 25%.

Based on these results, lerak fruit is an alternative for cavity cleanser due to the bacteriocide and bacteriostatic characteristics. This is also supported by Nevi Yanti et al and Risya DM on antibacterial effect against other microorganisms such as *Fusobacterium nucleatum* and *Enterococcus faecalis*.

Several research showed that lerak extract had antibacterial effectiveness. Risya DM, in 2009, proved that the extract had antibacterial effect against *Enterococcus faecalis*, with an MBC value of 25%, as reported in this research. [13] According to Nevi Yanti et al, in 2022, lerak extract inhibited *F. nucleatum*, with concentrations of 6.25%, 12.5% and 25%. [16] In addition, the extract had better inhibition against the hydrophobicity of *F. nucleatum* than 2.5% NaOCl solution + 17% EDTA. [17] *S. mutans* was tested while Risya DM, in 2009 analyzed *Enterococcus faecalis*. These bacteria had the same morphology and structures, hence, the material used reported similar antibacterial activity effects in killing the cells.

The structure of the bacterial cell wall also determines the penetration, binding, and activity of antibacterial material. *S. mutans* is a Gram-positive bacteria with a cell wall composed of 40%–80% peptidoglycan/murein of 40 layers. The cell wall of *Enterococcus faecalis* bacteria also consists of 40% peptidoglycan, the rest is teichoic acid and polysaccharides. In Gram-positive bacteria, teichoic acids are connected to peptidoglycan by covalent bonds. This compound is hydrophilic and functions as a transport medium for positively charged ions to enter and leave the cell wall. The water-soluble nature causes the cell walls of Gram-positive bacteria to be more polar and easily penetrate the peptidoglycan layer. [19]

The difference in the results may be caused by the differences in the methods used to produce formulations of lerak fruit. Differences in formulation refer to the solvents used to extract the active substances from lerak fruit. In this research, the decoction method used water as a solvent, resulting in high polarity. Therefore, this method selectively attracts water-soluble active compounds. [20] The extract formulation can attract alkaloid compounds, flavonoids, glycosides, saponins, tannins, steroids, and terpenoids. The decoction attracts polar compounds such as alkaloids, flavonoids, glycosides, saponins, and tannins. [14, 15] Non-polar compounds such as steroids and terpenoids cannot be found in the decoction. [15] Previous results used the inhibition zone method, while Drop Plates Miles Misra was adopted in the current research. In previous research, concentrations were predetermined after the dilution method was conducted.

Antibacterial effect is anticipated due to the presence of numerous compounds in lerak fruit decoction. The compounds are dominated by 28% saponins as well as alkaloids, polyphenols, flavonoids, and tannins.[21] The phytochemical components are consistent with the research conducted by Putra et al. Each of the phytochemical compounds shows a mechanism of action as

antibacterial agent. [22]

Table 3. Result of secondary metabolites analysis of lerak fruit. [23]

No.	Secondary Metabolites	Raegent	Results
1.	Alkaloids	Dragendroff	+
		Bouchardat	+
		Meyer	+
2.	Flavonoids	Powdered Mg + Amyl Alcohol + HCl	+
3.	Glycosides	Molish + H ₂ SO ₄	+
4.	Saponins	Hot Water	+
5.	Tannins	FeCl ₃	+
6.	Steroids/Triterpenoids	Lieberman-Bourchat	+

Lerak fruit contains triterpenoid saponins, which have the ability to interact with cell membranes and reduce surface tension. [24] Alkaloids are a group of basic compounds containing natural nitrogen with low molecular weights. [25] These compounds work as antibacterial by inhibiting the formation of the peptidoglycan layer. [22]

Polyphenols have a unique molecular structure to accept electrons from radicals generated in biological systems to stop oxidative chain reactions in cells. [24, 26] Phenols work as antibacterial compound by denaturing cell proteins and inhibiting the synthesis of nucleic acid. [21] Flavonoids are included in a group of natural materials with low molecular weight phenolic compounds. [27] The compounds work by binding to proteins, interfering with the metabolic processes. [21] Tannins are a heterogeneous group of water-soluble polyphenolic compounds found in plants. [28] This compound works by inhibiting bacteria through protoplasmiccoagulation. [21]

GC-MS research led to the identification of several bioactive compounds from lerak fruit capable of showing antibacterial power. Table 4 shows the bioactive components derived from benzyl chloride, 1-dodecanamine, N,N-dimethyl, 1-Tetradecanamine, N,N-dimethyl,4-(3-dimethylaminopropoxy) benzaldehyde, 1-(dimethylamino)butan-2-ol, Acetamide,2-(diethylamino)-N-(2,6-dimethylphenyl), 9-octadecenoic acid, Hexadecanoic acid, Cis-13-Octadecenoic acid, Quinoline, 1-azanaphthalene, 6-Octaenoic acid, 2-(Benzylmethylaminomethyl)-2-norbornanone, and Cis-13-Eicosanoic. [23]

Table 4. GC MS analysis of lerak fruit. [23]

Compound	Content (%)
Benzyl chloride	6.51
1-dodecanamine, N,N-dimethyl-	19.91
1-Tetradecanamine, N,N-dimethyl	6.57
4-(3-dimethylaminopropoxy) benzaldehyde	3.28
1-(dimethylamino)butan-2-ol	3.18
Acetamide,2-(diethylamino)-N-(2,6 dimethylphenyl)	1.41
9-octadecenoic acid	1.84

Hexadecanoic acid	6.82
Cis-13-Octadecenoic acid	1.46
Tetrahydroquinoline4,4,-	4.55
6-Octadecanoic acid	27.46
2-(Benzylmethylaminomethyl)-2-Norbanone	3.20
Cis-13-Eicosenoic acid	5.54

Lerak possesses characteristics as a promising alternative for cavity cleanser. Nevi Yanti et al. showed that 25% ethanol extract removed the smear layer in the apical 1/3 of the root canal. [12] According to Nevi Yanti et al (2022), 6.25%, 12.5%, and 25% of lerak fruit prevent excessive porosity in the tooth root canal better than 2.5% NaOCl irrigation solution + 17% EDTA ($p < 0.05$). [16] In addition, the fracture resistance of teeth irrigated with 12.5% and 25% lerak was better than 2.5% NaOCl + EDTA 17% solution. [17]

The materials used in the formulation of a decoction are more affordable, easily processed, and yield non-toxic by-products compared to a standard ethanolic extract. Since the research was conducted in vitro, the effects may differ when applied directly to a tooth cavity and cariogenic bacteria are polymicrobial. Therefore, research on polymicrobial biofilms should be carried out before testing the oral cavity of living subjects (in vivo) such as animals. This allows observation of the decoction's ability to eliminate various bacteria present in the cavity. Moreover, different perspectives are provided regarding the clinical applicability of lerak fruit decoction as an alternative to conventional cavity cleanser.

Antibacterial effect of *S. mutans* was tested using decoction of lerak fruit without separating the compounds. The gingival tissue around the cavity was not irritated during the clinical process. Therefore, further research is needed on the toxicity test of lerak fruit decoction. Based on the discussion, antibacterial effect against *S. mutans* as an alternative cavity cleanser is acceptable.

5. Conclusion

In conclusion, lerak fruit decoction was reported to possess antibacterial effect against the growth of *S. mutans* with MIC and MBC values of 12.5% and 25%, respectively. According to LSD and ANOVA results, the same antibacterial effect of 2% CHX was produced on the growth of *S. mutans* at a concentration of 25%.

6. Conflict of Interests

The authors declare no conflict of interest concerning the publication.

7. Ethical Approval

Applicable and approved by the committee of ethics with the letter No: 389/KEP/USU/2021.

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