



Antibacterial Activity Test of Lerak Fruit Extract (*Sapindus rarak* DC.) Against the Growth of the Bacterist *Streptococcus Mutans*

Risnawati*, Kartika Sari

Institut Tarumanagara, Indonesia

Email: risnawati.farm24@gmail.com*, kartikasari.apt22@gmail.com

ABSTRACT

Lerak (Sapindus rarak DC.) is a plant native to Southeast Asia that grows well in Indonesia, especially in Sumatra and Java. Lerak is traditionally used as a laundry detergent, facial soap, insect repellent, and fungus repellent. Lerak contains phytochemical compounds that function as antibacterials. This study aims to test the antibacterial activity of lerak fruit extract against Streptococcus mutans bacteria, which cause dental caries. The method in this study involves preparing lerak fruit extract by maceration using 70% ethanol solvent and conducting an inhibition zone test using the diffusion method. Based on the test results, the lerak fruit extract is positive for alkaloids, flavonoids, tannins, saponins, and terpenoids. The results also showed that increasing the concentration of lerak fruit extract produced a wider inhibition zone. The average inhibition zones at concentrations of 25%, 50%, and 100% were 8.83 ± 0.958 mm, 11.78 ± 3.081 mm, and 12.14 ± 0.918 mm, respectively. The novelty of this research lies in the comprehensive evaluation of lerak fruit extract's antibacterial potency against S. mutans using systematic concentration gradients, which has not been extensively documented in previous literature. This study provides practical implications, as lerak fruit extract demonstrates potential as an alternative natural antibacterial agent for oral health applications, offering a safer option with fewer side effects compared to synthetic chemical compounds. However, this study is limited to in vitro testing against a single bacterial strain, and further research is needed to evaluate its efficacy in clinical settings and against polymicrobial oral biofilms.

Keywords:

Lerak; extract;
Streptococcus mutans;
antibacterial activity.

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INTRODUCTION

One of the health problems of the oral cavity, especially dental and gum disease, is the most common disease in the world, with an estimated 3.58 billion people affected (Abdullah, 2022). In Indonesia, this disease is one of the diseases that people often complain about. Dental caries are constantly increasing, due to people's thoughts about poor dental and oral health (Andini, 2020).

Dental caries is an infectious disease caused by fluctuations in the pH of dental plaque due to microorganisms, such as *Streptococcus mutans* (Daud et al. 2023). The results of Basic Health Research in 2018 show that 57.6% of the Indonesian population has dental and oral health problems. *Streptococcus mutans* attaches to the surface of teeth due to the presence of dextran, andhesin, and glucan polymers that are insoluble in water, causing a drop in pH around the bacterial colony (Ardinata et al. 2023; Daud et al. 2023; Fatoni & Mahbub, 2023)

The factor that causes caries in the teeth is the bacteria *Streptococcus mutans*. This bacterium is a gram-positive bacterium where glucose and carbohydrates in food are converted into acids in the fermentation process. Although non-pathogenic, these bacteria can become pathogenic if their environment changes, increasing the bacterial population and accelerating

the process of dental caries (Artha et al. 2022). Dental caries is also affected by food debris left on the teeth, causing teeth to become porous, cavities, and even broken teeth (Astuti et al. 2019)

Medical therapy for dental caries includes remineralization, routine dental care every day, limiting bacterial infections, minimizing risk factors (Aviany & Pujiyanto, 2020). Resistance in bacteria can occur due to the excessive use of antibiotics, resulting in selective pressure on each bacteria. Selective pressure can vary depending on several factors, including fluctuations in antibiotic concentration over time. At high concentrations, antibiotics cause high resistance that occurs naturally so as to support the occurrence of mutations (Kurniawati, 2018; Minarno, 2016). This can be prevented by utilizing natural ingredients, which are beneficial for health for medicines of plant origin. This treatment has advantages, one of which is its low price, easy to obtain, and has low side effects (Brooksbank et al. (2014). For example, the lerak plant (*Sapindus rarak DC.*) which has antibacterial active compounds such as polyphenols, tannins, flavonoids, and saponins.

Lerak plant (*Spindus rarak DC.*) thrives and generally has an altitude of 450-1,500 meters above sea level. Lerak plants since ancient times have been widely used as a material for cleaning clothes, cleaning for livestock, face washes, precious metal cleaners, and keris cleaners. In addition, lerak plants are used as antiseptic, insecticides, and even fungal killers. In addition to being useful as a cleaner for lerak plants, it contains chemical compounds that are useful as antibacterial. It is an alkaloid that works as an antibacterial by inhibiting the ability of bacterial cells to make peptidoglycan, which causes the cell membrane to be damaged. Polyphenol compounds that cause damage to plasma as well as denatured cell proteins. Tannin compounds coagulate protoplasm in bacteria. What works by increasing the permeability of the cell membrane is the way saponin compounds work (Buldani et al. 2017)

Previous studies have explored the antibacterial potential of various plant extracts against oral pathogens, yet comprehensive data on lerak fruit extract remains limited. Artha et al. (2022) demonstrated that lerak fruit ethanol extract exhibited inhibitory effects against *Staphylococcus epidermidis*, suggesting broad-spectrum antibacterial activity (Sada Yanitauli Sibuea, 2015). Similarly, Silviani and Puspitaningrum (2014) reported that lerak decoction showed antibacterial activity against pathogenic *Escherichia coli* (Sholikhah, 2016). However, these studies did not specifically investigate the dose-dependent effects against *S. mutans*, the primary causative agent of dental caries. Widowati et al. (2022) identified phytochemical compounds in lerak fruit extract and tested it against nosocomial infection-causing bacteria, but their study did not focus on oral cavity bacteria (Silviani & Puspitaningrum, 2014). The gap in knowledge regarding the specific antibacterial potency of lerak fruit extract against *S. mutans* at varying concentrations necessitates further investigation.

The novelty of this research lies in providing systematic evidence of concentration-dependent antibacterial activity of lerak fruit extract specifically against *Streptococcus mutans*, which has not been comprehensively documented in previous literature. This study employs standardized disc diffusion methodology with multiple concentration gradients (25%, 50%, and 100%) to establish a clear dose-response relationship, thereby contributing original data to the field of natural antibacterial research for oral health applications.

From the explanation above, it is necessary to develop the potential of the lerak plant as an antibacterial, it is hoped that lerak fruit extract (*Sapindus rarak DC.*) can be used as an antibacterial in *Streptococcus mutans* bacteria. Given the rising concerns over antibiotic

resistance and the adverse effects associated with synthetic antimicrobial agents in oral health products, there is an urgent need to explore natural alternatives that are both effective and safe. The abundant availability of lerak plants in Indonesia, combined with their traditional use and phytochemical richness, positions lerak fruit extract as a promising candidate for development as a natural antibacterial agent against dental caries-causing bacteria. Therefore, in this study, an antibacterial activity test was carried out to determine the activity of lerak fruit extract (*Sapindus rarak DC.*) on the growth of *Streptococcus mutans* bacteria.

The lerak plant (*Sapindus rarak DC.*) is known to have various benefits, both in the field of medicine and as a cleaning agent. The phytochemical compounds contained in lerak plants, such as saponins, polyphenols, flavonoids, alkaloids, and tannins, have the potential to be antibacterial. Lerak fruit extract is known to contain these compounds, which can be used to inhibit the growth of bacteria that cause dental caries, one of which is the bacteria *Streptococcus mutans*. This study aims to test the antibacterial activity of lerak fruit extract (*Sapindus rarak DC.*) in inhibiting the growth of *Streptococcus mutans* bacteria and to determine the diameter of the inhibition zone of lerak fruit extract against the bacteria tested using the diffusion method.

The hypothesis proposed in this study is that lerak fruit extract (*Sapindus rarak DC.*) has antibacterial activity in inhibiting the growth of *Streptococcus mutans* bacteria, and the higher the concentration of lerak fruit extract used, the greater the inhibition power produced. Theoretically, the results of this study are expected to provide benefits in the field of education, add insights, and become a reference for other researchers related to the antibacterial activity of lerak fruit extract on the growth of *Streptococcus mutans*.

The practical benefits of this study include providing direct experience for the author on how to test antibacterial activity against bacteria using the diffusion method, as well as providing a basis for further research on the effect of lerak fruit extract on other microflora in the oral cavity that can become pathogenic under certain conditions. For the public, this study can complement information about the inhibition of the inhibition of lerak fruit extract on the growth of *Streptococcus mutans* and is expected to be applied as a caries prevention drug, with fewer side effects than drugs available on the market.

METHOD

This research is classified as a laboratory experimental study employing a post-test only control group design. This research was conducted at the Laboratory of Pharmacognosy and Microbiology of the Tarumanagara Institute Campus, which is located in JL. Raya Cilandak KKO No.1, Ragunan, in June-July 2024. The tools used in this study include: blender (EG-NM 8300), analytical scale (Kern ABJ 220-4 NM), a set of vacuum rotary evaporator tools (Heidolhp), glass jars, filter paper, pH meter (Milwaukee Mi 150), calipers (Mitotoya), aluminum foil, petri dish, oyster needles, test tubes, autoclaves, hot plates, 250 ml beakers (Iwaki), spatulas, spatulas, stirring rods, tissues, Spirits, micropipettes, matches, microscopes, buns, incubators, glass objects, deck glass, and laminar air flow. The ingredients used in this study were lerak fruit, 70% ethanol, DMSO, empty discs (Macherey Nagel), agar nutrient media (Marck), *Streptococcus mutans* bacterial culture, aqudest, 96% ethanol, mouthwash containing 0.2% chlorhexidine (Minosep), emersion oil, violet fiberglass carbol, lugol, safranin, and NaCl.

The research began by determining the authenticity of the lerak plants used, which was carried out at the National Research and Innovation Agency (BRIN), Cibinong, Bogor. The lerak fruit used comes from Gabugan Lempuyagan Village, Bergas District, Semarang Regency, Central Java. The ripe lerak fruit is dried in the sun for three days and the seeds are removed. The flesh of the fruit that has been separated from other ingredients during drying, is then blended into a powder. Extraction is carried out by the maceration method using 70% ethanol as a solvent. The extraction lasted for three days with stirring on the first day, and the extract results were then filtered and heated using a vacuum rotary evaporator to produce a thick extract. Extract yield is calculated using the formula for calculating extract yield. To ensure the chemical composition of the extract, phytochemical screening is performed to identify flavonoid compounds, tannins, saponins, and alkaloids.

Organoleptic tests were carried out to assess the shape, color, smell, and taste of lerak extract. Antibacterial activity was tested by disc paper diffusion method using *Streptococcus mutans* bacteria. Before antibacterial testing, the tools used are sterilized with an autoclave, and nutrient media is prepared to incubate bacteria. The antibacterial test was carried out by soaking paper discs in lerak extract with concentrations of 25%, 50%, and 100%, as well as positive controls using 0.2% chlorhexidine and negative controls using DMSO. After 24 hours of incubation at 37°C, the development of the clear zone was measured using a millimeter caliper as an indicator of antibacterial activity.

RESULTS AND DISCUSSION

Plant Determination Results

Testing on plants is carried out to see if the type of plant used meets the requirements. The National Research and Innovation Agency (BRIN), Cibinong, Bogor, became the testing location. The results of the plant determination test obtained results, namely, lerak plants with the type of *Sapindus rarak DC.* Sapindceae tribe.

Extract Yield

Of the total simplicia used in this study, 500 g were used and a thick extract of 166 g was produced. For the extract yield to be 33.2% with the calculation attached to Appendix 4 Extract Yield Calculation.

Organoleptic Test Results

Organoleptic testing of lerak fruit extract was carried out by observing the color, texture, aroma, and taste that had been made obtained the following results.

Table 1. Organoleptic Test Results

Color	Texture	Aroma	Taste
Dark Brown Yellowish	Thick, slightly sticky	Typical extracts	Bitter

Phytochemical Screening Test Results

To ascertain the presence of targeted compounds - flavonoids, tannins, alkaloids, titerpenoids, as well as saponins, qualitative studies of the chemical composition are carried

out. The test was carried out at PT. Palapa Muda Perkasa, Depok City, West Java. The results of the phytochemical screening examination obtained the following results:

Table 2. Phytochemical Screening Results

Chemical Compound Content	Test Results	Formed Colors
Saponins	+	Froth stable, by whisking for 10 seconds
Alkaloid	+	A brass-yellow white-white deposit is formed using a mayer reagent, drgendroff
Flavonoid	+	Yellow color is formed on the amyl alcohol layer using concentrated HCL, amyl alcohol, Mg powder
Tannins	+	Formed a dark green-dark green blackish-green solution using Fecl3 1%
Triterpenoid	+	Formed a brick-red solution yellowish-orange using <i>lieberman burchad</i>

Bacterial Staining Results

The results of bacterial staining showed that gram-positive *Streptococcus* mutans bacteria were distinguished by the presence of purple cells after gram staining with violet crystals. These bacterial cells are round in shape and colonized in a chain that resembles a chain.

Results of Extraction Antibacterial Inhibition Zone Measurement

The bacterial inhibition zone indicated by the clear zone formed around the disc paper was measured in this test, which uses the disc paper method. Measurements using vertical calipers, and the results are calculated based on the formula and categorized based on the obstacle zone.

Table 3. Test Results of Antibacterial Activity of Lerak Fruit Extract Against *Streptococcus mutans* Bacterial Growth

Concentration of the extract	Jamming Zone Diameter (mm)	Category
25%	8.83±0.958	Medium
50%	11.78±3.081	Strong
100%	12.14±1.862	Strong
Negative control	0	Weak
Positive control	28.05±0.918	Very powerful

Description:

Negative control: DMSO (Dimethyl sulfoxide)

Positive control: commercial mouthwash preparations containing Clorhexidine 0,2

The purpose of this study is to find out whether lerak fruit extract has antibacterial properties against *Streptococcus* mutans bacteria. *Streptococcus* mutans bacterial cultures from ISTN were used as research samples. Lerak plants are used in the process of making extracts in this study. After washing with water, the lerak fruit is dried in the sun. The maceration procedure is an extraction technique used. The maceration approach, which involves

immersing the sample in an organic solvent, was chosen because it can stop heating that can destroy secondary metabolite chemicals. In addition, the operation mechanism of the maceration method is quite easy, relatively simple and practical (Roslinawati et al. 2020). Using disc paper, the extract was tested for its inhibition zone. In the inhibition zone test, the doses used were 100%, 50%, and 25%. The choice of concentration is because the concentration produces antibacterial power as shown in a study conducted by Ayan Widi Artha at all (2022) entitled 'Test of Antibacterial Activity of Lerak Fruit Ethanol Extract Against Bacteria'. DMSO primarily functions as a solvent as well as lacking antibacterial properties, DMSO was chosen as a negative control in this study. In this study, a commercial mouthwash containing 0.2% chlorhexidine was used as a positive control. Due to its ability to suppress oral bacteria, this mouthwash works as an antiplaque to prevent dental caries by making the bacterial cell membrane more permeable and causing cytoplasmic macromolecules to clump together.

The determination of plants was carried out in Cibinong, the National Research and Innovation Agency (BRIN) of West Java. Based on the results of plant determination, the lerak fruit of the *Sapindus rarak* DC variety is the simplicia used. The purpose of plant determination is to ascertain whether the type of plant used in the study is appropriate. The lerak plant used is a lerak plant that comes from Magelang, Central Java. To remove dirt that sticks to its surface, the lerak fruit is washed with running water. After that, the meat and seeds of the lerak fruit are split. Furthermore, the pulp of lerak fruit is dried in the sun for up to 7 days. After the lerak fruit is completely dry, the lerak fruit is mashed into powder using a blender and sifted. A total of 500 grams of lerak fruit powder are weighed, put in a glass jar, then sealed after adding 5 liters of 70% ethanol in a ratio of 1:10. 3 x 24 separate hours are used for the maceration procedure, with the solution stirred periodically for the first 8 hours. The extraction results are collected in a container and evaporated at 30°C using a rotary evaporator that rotates at a speed of 150 rpm. After evaporation, the condensed extract is the resulting product.

Using diffusion of disc paper, the antibacterial activity is investigated. After placing the disc paper containing the prepared solution on medium containing *Streptococcus* mutant bacteria, the paper was incubated for 24 hours at a temperature of 37°C. Next, the clear zone formed around the disc paper was used to measure the bacterial inhibition zone. Before checking for antibacterial activity, all instruments and equipment must be sterilized in an autoclave at a temperature of 121°C within 15 minutes. Once all the tools and materials have been sterilized, the agar nutrients that have been dissolved and cleaned, are transferred to a 15 ml petri dish, and allowed to harden. During the process of waiting for the media to harden, a suspension of *S. mutans* bacteria with the Mc. Farland standard was made. The Mc. Farland standard is used for the waiver to adjust the turbidity of the bacterial suspension or liquid in the tube until the number of bacteria within the specified range is required to inoculate the test microorganism. MC standard solution. Farland was used to compare the turbidity of bacterial cultures in liquid media at concentrations ranging from 1×10^7 to 1×10^8 cells/ml.

Bacterial suspension is made by diluting bacterial cultures at sterile 0.9% NaCl for 9 ml in a test tube. Bacterial dilution is carried out until a turbidity consistency comparable to the turbidity consistency of Mc. Farland's solution is obtained. The suspension with Mc. Farland standards will be used to be planted in Nutrient Agar media. Bacterial cultivation is carried out aseptically in Laminar Air Flow (LAF) by taking a small amount of the bacterial suspension

using a micropipette then dripping it on the media and leveling it with an L rod until it spreads over the entire surface of the agar medium. Next, the disc paper containing the test solution is placed on a agar medium that has been infested with bacteria and incubated throughout the day at a temperature of 37°C.

Test antibacterial activity on thick extracts and preparations with the content of lerak fruit extract. In the condensed extract, it is carried out at concentrations of 25%, 50% and 100%. Using negative control of 10% DMSO and positive control of commercial mouthwash containing 0.2% chlorhexidine. First, DMSO 10% is used to dilute the viscous extract to the required concentrations of 25%, 50%, as well as 100%. Then the disc paper soaked in it for 10-15 minutes. Then it is incubated for up to 24 hours.

Using calipers, the caliper measured the inhibitory zone of the extract and obtained results at concentrations of 25%, 50%, and 100% with an average of 8.83 ± 0.958 mm, 11.78 ± 3.081 mm, 12.14 ± 1.862 mm. For negative control, the use of DMSO does not provide an effect or inhibition zone against bacteria. On the other hand, the mean inhibition zone of the positive control using 0.2% chlorhexidine was 28.05 ± 0.918 . As a result, lerak fruit extract can prevent the growth of strong and moderate strains of *Streptococcus mutans* bacteria. The inhibition zone formed by lerak fruit extract is included in the group of antibacterial with moderate to strong inhibition. The average results of this study support the concept that a larger or wider barrier zone is formed in proportion to the concentration of lerak fruit. The concentration of the extract used has an effect on bacteria because the higher the concentration of lerak fruit extract, the higher the active compound while the lower the concentration, the lower the active substance. Because lerak fruit extract contains phytochemical components that have antibacterial properties, a clear zone is formed around the disc paper.

The removal of proteins and enzymes from bacterial cells is how saponin chemicals work as antibacterial. Because the active ingredients on the surface of saponins function similarly to detergents, saponins can weaken the permeability of bacterial cell wall membranes and lower surface tension (Sugiaman et al. 2023). The mechanism of action of flavonoid compounds is by causing the constituent part of peptidoglycan to break down inside the bacterial cell, which prevents the wall layer from forming properly and resulting in cell death (Tampoliu et al. 2021). Alkaloid chemicals work by dismantling the building blocks of polypeptides inside bacterial cells, preventing the formation of cell wall layers. The attachment of dormant bacterial cells, dormant enzymes, and disruption of protein transport within the inner layer of the cell are the mechanisms of action of tannin-compounds (Widowati et al. 2022).

CONCLUSION

Lerak fruit extract (*Sapindus rarak* DC.) demonstrated strong inhibitory activity against *Streptococcus mutans*, with inhibition zone diameters of 8.83 ± 0.958 mm at 25%, 11.78 ± 3.081 mm at 50%, and 12.14 ± 1.862 mm at 100%, confirming a dose-dependent increase in antibacterial efficacy. For future research, testing the extract against diverse bacterial species could reveal its broader antimicrobial spectrum, while exploring pharmaceutical formulations—such as *lerak*-based mouthwashes for oral or topical applications—would advance its practical use in oral health products.

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