ANTIBACTERIAL ACTIVITY OF RAMBAI LEAF EXTRACT (Sonneratia caseolaris) CONCENTRATION 25%, 50%, 75%, AND 100% AGAINST Streptococcus sanguinis

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ABSTRACT
Background: Dental plaque is a soft layer formed by collection of bacteria that is firmly attached to the tooth surface which if left unchecked can cause tooth decay, periodontal disease and dental caries. Streptococcus sanguinis is the first bacteria to form colonization in plaque formation. Plaque can be diminished by using chlorhexidine gluconate 0.2% mouthwash, but in long-term use it can cause side effects, so an alternative herbal-based mouthwash that has minimal side effects is needed. Rambai leaf extract can be used as an alternative mouthwash because it contains antibacterial compounds, namely, flavonoids, tannins, phenols, triterpenoids, and steroids. Purpose: The purpose of this study was to determine the antibacterial activity of rambai leaf extract at concentrations of 25%, 50%, 75% and 100% against Streptococcus sanguinis bacteria. Methods: This study used true experimental research with post-test only with a control group design using rambai leaf extract 25%, 50%, 75%, 100%, chlorhexidine gluconate 0.2%, and aquadest. The study used 6 groups with 4 repetitions with a total of 24 samples. Each group was incubated for 24 hours at 37°C, then the diameter of the inhibition zone was measured using a calliper. Results: The results of the Mann Whitney test showed that there was a significant difference between each treatment group and there was no significant difference between 100% rambai leaf extract and chlorhexidine gluconate 0.2%. The average diameter of the inhibition zone of rambai leaf extract was 25% by 9.75 mm, 50% by 13.37 mm, 75% by 16.12 mm, 100% by 19.5 mm, chlorhexidine gluconate 0.2% by 19.12 mm and aquadest 0 mm. Conclusion: Rambai leaf extract with concentrations of 25%, 50%, 75%, and 100% had antibacterial activity against Streptococcus sanguinis.

Keywords: Antibacterial Activities, chlorhexidine gluconate 0.2%, rambai leaves extracts, Streptococcus sanguinis

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INTRODUCTION
Dental plaque is a soft layer formed by collection of bacteria that proliferate and adhere firmly to the tooth surface.\(^1\)\(^2\) Bacteria that accumulate in plaque are caused by poor oral hygiene, which can cause periodontal disease and dental caries.\(^1\)\(^3\)\(^4\) Periodontal disease is a disease characterized by destruction of the supporting tissues of the teeth such as gums, periodontal ligament, cementum, and alveolar bone. Periodontal disease is a disease of the oral cavity that often occurs in Indonesia. The 2018 RISKESDAS data states that periodontal disease has a fairly high prevalence, namely gingivitis as much as 96.58%.\(^5\)

Gingivitis is a disease that occurs due to inflammation of the gums which is characterized by swelling, discoloration, bleeding, and lesions on the gums. Gingivitis results from an inflammatory response to bacteria and the formation of plaque around the gingival margin. Plaque occurs on the rough surface of the teeth and is often found on one third of the gingival surface.\(^5\)\(^6\) Plaque is clinically similar in color to teeth, so it cannot be seen clearly and is difficult to distinguish from the color of tooth enamel.\(^1\)\(^4\) The pellicle that coats the tooth surface is the initial stage of dental plaque formation, followed by the adhesion of the pioneer bacterium Streptococcus sanguinis.\(^7\)

Streptococcus sanguinis the first bacterium to colonize in the formation of dental plaque. These bacteria are facultative anaerobes and a gram-positive bacteria.\(^8\) Streptococcus sanguinis has pili that can bind to saliva, so it can stick to the tooth surface.\(^9\) Streptococcus sanguinis can penetrate salivary glycoproteins and facilitate nutrition for other bacteria, resulting in bacterial colonization on the tooth surface in the process of plaque formation.\(^5\)\(^10\)

Plaque formation on the tooth surface can be inhibited by mechanical action, chemical action or combination of the two. Plaque control with mechanical
action can be done by brushing teeth properly and correctly, but it is not optimal in inhibiting plaque formation, so it can be combined with chemical action. Plaque control is chemically carried out using mouthwash to reduce the attachment of bacteria to the tooth surface. Chlorhexidine gluconate 0.2% mouthwash is the gold standard that can inhibit and kill bacteria. Chlorhexidine gluconate 0.2% contains phenol and chlorinated hydrocarbons which can affect the amount of bacterial attachment to plaque and has a high level of disinfectant properties because these substances are very active on all bacteria and other types of microbes. Usage of Mouthwash with chlorhexidine gluconate 0.2% can result in staining of the teeth and tongue, loss of taste sensation in the sense of taste, mucosal irritation, and increased calculus formation. The side effects of chlorhexidine gluconate 0.2% are quite a lot, so alternative ingredients are needed in the manufacture of mouthwash. The use of herbal plants is widely chosen by the people of Indonesia because it has minimal side effects.

Rambai is one of the herbal plants that can be used as traditional medicine. Rambai is a type of mangrove plant that grows on the banks of river mouths. The rambai plant is one of the plants found in South Kalimantan which is trusted by the community as herbal medicine. This is because the leaves on the rambai plant have many benefits, namely as an analgesic, antibacterial, and have antiinflammatory properties. Rambai leaves (Sonneratia caseolaris) contain secondary metabolites that act as antibacterial, such as flavonoids, tannins, phenols, triterpenoids, and steroids.

Sogandi’s research (2017) revealed that 96% ethanol extract of rambai leaves was able to inhibit the growth of Escherichia coli bacteria. Held’s research (2020) revealed that 90% concentration of rambai leaf extract had the largest inhibition zone diameter of 14.2 mm in inhibiting the growth of Streptococcus mutans. From this description, the antibacterial activity test of rambai leaf extract has the potential to inhibit the growth of Streptococcus sanguinis.

MATERIALS AND METHODS

This research has received ethical approval from the Ethics Committee of the Faculty of Dentistry, University of Lambung Mangkurat No. 028/KEPKG-FKGULM/EC/IV/2022. This study used a true experimental design with post-test only with control group design consisting of 6 treatments using rambai leaf extract 25%, 50%, 75%, 100%, positive control chlorhexidine gluconate 0.2%, and negative control aquadest. Each treatment was repeated 4 times so that the total sample was 24 pieces.

The process of making rambai leaf extract was carried out at the Biomedical Laboratory of the Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin. Rambai leaves are taken from 1-3 leaves from the leaf shoots collected as much as one kilogram, then the rambai leaf extract is made using the maceration method using 96% ethanol as solvent. The results of rambai leaf extract were diluted with distilled water and divided into concentrations of 25%, 50%, 75%, and 100%.

Preparation of Streptococcus sanguinis (ATCC® 10556™) obtained from the MBRO Biotekindo Laboratory. Antibacterial activity was tested by the diffusion method which was carried out by applying Streptococcus sanguinis with sterile cotton swabs on Mueller Hinton Agar (MHA) media, then paper disc was soaked in rambai leaf extract with concentrations of 25%, 50%, 75%, 100%, chlorhexidine gluconate 0.2%, and distilled water for 3 hours, then put a paper disc on MHA media containing Streptococcus sanguinis bacteria. MHA media were incubated for 24 hours at 37°C, after which the diameter of the inhibition zone was measured using a caliper.

RESULTS

The results of the average value and standard deviation of the diameter of the inhibition zone in each treatment can be seen as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± Std. Deviation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDR 25%</td>
<td>9.75 ± 0.50</td>
</tr>
<tr>
<td>EDR 50%</td>
<td>13.37 ± 0.48</td>
</tr>
<tr>
<td>EDR 75%</td>
<td>16.12 ± 0.63</td>
</tr>
<tr>
<td>EDR 100%</td>
<td>19.50 ± 0.58</td>
</tr>
<tr>
<td>CHX 0.2%</td>
<td>19.12 ± 0.63</td>
</tr>
<tr>
<td>Aquadest</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

The results of the average diameter of the inhibition zone of rambai leaf extract at a concentration of 25% was 9.75 mm, a concentration of 50% had an inhibition zone diameter of 13.37 mm, a concentration of 75% had an inhibition zone diameter of 16.12 mm, a concentration of 100% had an inhibition zone diameter of 19.50 mm, chlorhexidine gluconate 0.2% had an inhibition zone diameter of 19.12 mm, and there was no inhibition zone diameter in aquadest. The highest average diameter of the inhibition zone was 100% rambai leaf extract and the lowest average diameter of the inhibition zone was aquadest negative control. These results indicate that there are differences in the variation of the inhibition zone of each treatment.

Figure 1. Inhibition zone of rambai leaf
extract on the growth of *Streptococcus sanguinis*

The data that has been collected from each treatment was tested for normality using Sapiro-wilk. non-parametric analysis test was carried out by Kruskal Wallis analysis Kruskal Wallis showed that the significant value of $p = 0.000 < 0.05$, meaning that there was a significant difference in the diameter of the inhibition zone for each treatment group, then the Mann Whitney to determine which group gave a significant difference.

**Table 2. Mann Whitney test results antibacterial activity of rambai leaf extract (Sonneratia caseolaris)** chlorhexidine gluconate 0.2% and aquades on the growth of *Streptococcus sanguinis.*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EDR 25%</th>
<th>EDR 50%</th>
<th>EDR 75%</th>
<th>EDR 100%</th>
<th>CHX 0.2%</th>
<th>Aquades</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDR 25%</td>
<td>0.017*</td>
<td>0.017*</td>
<td>0.017*</td>
<td>0.017*</td>
<td>0.011*</td>
<td></td>
</tr>
<tr>
<td>EDR 50%</td>
<td>0.019*</td>
<td>0.019*</td>
<td>0.019*</td>
<td>0.013*</td>
<td>0.013*</td>
<td></td>
</tr>
<tr>
<td>EDR 75%</td>
<td>0.019*</td>
<td>0.019*</td>
<td>0.019*</td>
<td>0.013*</td>
<td>0.343</td>
<td>0.013*</td>
</tr>
<tr>
<td>EDR 100%</td>
<td>0.019*</td>
<td>0.019*</td>
<td>0.019*</td>
<td>0.013*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHX 0.2%</td>
<td>0.019*</td>
<td>0.019*</td>
<td>0.019*</td>
<td>0.013*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aquades</td>
<td>0.343</td>
<td>0.013*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on the table above, the treatment with a significant value $<0.05$ had a significant difference. While the comparison between rambai leaf extract at a concentration of 100% and chlorhexidine gluconate 0.2% has a significance value of $>0.05$ so there is no significant difference and it can be concluded that the 100% concentration of rambai leaf extract (*Sonneratia caseolaris*) has an average inhibition zone, which is equivalent to chlorhexidine gluconate 0.2%.

**DISCUSSION**

The results of the antibacterial activity of rambai leaf extract (*Sonneratia caseolaris*) at concentrations of 25%, 50%, 75%, and 100% on the growth of *Streptococcus sanguinis* using the diffusion test method showed that each treatment of rambai leaf extract (*Sonneratia caseolaris*) has antibacterial activity in inhibiting the growth of *Streptococcus sanguinis*. The results of this study that 100% concentration of rambai leaf extract had the strongest antibacterial activity against the growth of *Streptococcus sanguinis,* because it had an average inhibition zone diameter of 19.5 mm which exceeded that of rambai leaf extract at a concentration of 25%, namely 9.75 mm, 50% that is 13.37 mm, 75% is 16.5 mm, and chlorhexidine gluconate 0.2% is 19.12 mm.

Each paper disc shows an increase in the diameter of the inhibition zone starting from a concentration of 25% to 100%. Increasing the concentration of rambai leaf extract, the number of secondary metabolite compounds also increases so that it facilitates the penetration of these compounds into bacterial cells and the antibacterial that is released will be greater.\textsuperscript{12,14}

The category of antibacterial activity measurement can be classified by category by Davis and Stout who state that the diameter of the inhibition zone consists of 4 categories, namely the weak category if the inhibition zone formed is 5 mm, the medium category if the inhibition zone formed is 5-10 mm, the strong category if the inhibition zone formed is 10-20 mm, and the category is very strong if the inhibition zone formed is 20 mm. Based on this classification, the antibacterial activity of Based on this classification, the antibacterial activity of rambai leaf extract at a concentration of 25% had a moderate antibacterial effect against *Streptococcus sanguinis,* while concentrations of 50%, 75%, 100% and chlorhexidine gluconate 0.2% had a strong antibacterial effect against *Streptococcus sanguinis.*\textsuperscript{15}

This study used the maceration method as the process of extracting rambai leaves with 96% ethanol as solvent. The solvent used is 96% ethanol because it has a high level of polarity so the withdrawal of polar and semipolar compounds is easier to draw and non-toxic.\textsuperscript{12,16} This research was conducted with an ethanol-free test to obtain a 100% extract so as to avoid bias in the study.

The content of secondary metabolite compounds contained in rambai leaf extract can inhibit the growth of bacteria such as flavonoids, tannins, phenols, triterpenoids, and steroids. Rambi leaves used in this research are leaf shoots that have a higher phenolic, flavonoid, and tannin content compared to old rambai leaves.\textsuperscript{17} Flavonoid compounds can inhibit bacterial growth by disrupting cell membrane permeability and inhibiting the binding of the ATPase/adenosine triphosphatase so that it can cause bacterial DNA to fail to replicate.\textsuperscript{18,19} Flavonoids have an antibacterial mechanism by entering the cell and damaging the protein structure due to the coagulation of proteins in the cell membrane. The cytoplasmic membrane and cell wall become unstable so that the active transport function, selective permeability function, and protein composition are disrupted. It causes the bacterial cell to lose its shape and lysis.\textsuperscript{18,20,21}

Tannins are antibacterial compounds that can inactivate enzymes and interfere with protein transport in the bacterial cell layer, resulting in bacterial lysis.\textsuperscript{14,20} Astringent in tannins also act as antibacterial by attaching to the bacterial cell wall and then the cells undergo morphological changes. This will make the cells become brittle and eventually die.\textsuperscript{22} The mechanism of tannins as antibacterial can also by inhibition of cell wall synthesis by forming irreversible-rich proteins.\textsuperscript{23} Tannins can form hydrogen bonds with proteins contained in bacterial cells. The formation of hydrogen bonds between tannins and proteins causes a change in the shape of the protein molecule so that the
protein is denatured and will reduce the biochemical activity of bacteria. 24

Phenol is an antibacterial compound because it has the ability to damage cell membranes and denature cell proteins. Phenol can form bonds with proteins in the bacterial cell wall through hydrogen bonds, causing damage to the protein structure. Phenol can also change the permeability of cell walls and cytoplasmic membranes so that there is an imbalance of macromolecules and ions in the cell and causes lysis of bacterial cells. 20,25

The secondary metabolites of rambai leaves are triterpenoids and steroids. The mechanism of triterpenoids as antibacterial is by reacting with porins on the outer membrane of the bacterial cell wall. This causes the permeability of the bacterial cell wall to decrease and eventually bacterial lysis occurs.26,27 Steroids have specific antibacterial activity because they are associated with lipid membranes and cause leakage of liposomes. 23 Steroids can interact with cell phospholipid membranes that are permeable to lipophilic compounds, causing decreased membrane integrity and changes in the morphology of the membrane causing cell lysis.14,25 Similarly, 0.2% chlorhexidine gluconate has an antibacterial mechanism by damaging the cytoplasmic membrane of bacteria by binding to cytoplasmic membrane phospholipids and causing leakage of cell components and cell death. 18,28

Secondary metabolite compounds contained in rambai leaf extract are flavonoids, tannins, phenols, triterpenoids, and steroids that can work as antibacterial causing damage to the structure of Streptococcus sanguinis bacteria so that bacteria lyses and causes the death of bacteria. The death of Streptococcus sanguinis bacteria as the first bacteria to colonize in the formation of dental plaque causes a reduction in the formation of plaque accumulation in the oral cavity to reduce the potential for gingivitis and caries.18

The results of this study indicate that rambai leaf extract (Sonneratia caseolaris) at each concentration has antibacterial activity that can potentially be used as an herbal-based mouthwash because it is able to inhibit Streptococcus sanguinis, which is a pioneer bacterium in the formation of plaque colonization in the oral cavity. In this study, the optimal concentration of rambai leaf extract in inhibiting Streptococcus sanguinis was 100% concentration because it had the same inhibitory power as chlorhexidine gluconate 0.2%. There was antibacterial activity in the extract of rambai (Sonneratia caseolaris) leaves at concentrations of 25%, 50%, 75% and 100% against the growth of Streptococcus sanguinis.

REFERENCES


