Alfa mangostin extract (garcinia mangostana l) effectiveness on the biofilm thickness growth of streptococcus sanguinis

Rahmawati Sri Praptiningsih*, Rosa Pratiwi*, Tazkia Yustisiani Nurafifah***

ABSTRACT

Background: Periodontal disease is caused by the accumulation of bacteria from biofilms in the periodontal tissues. Streptococcus sanguinis is one of bacteria lives in biofilm. One of the herbal ingredients that can be used as an antibacterial is the mangosteen fruit (Garcinia mangostana L.), especially the rind of the mangosteen fruit. The main compound in the mangosteen rind is known as alfa-mangostin, the compound alfa-mangostin has anti-bacterial benefits. The purpose of this research is to determine the effectiveness usage of alpha mangostin toward Streptococcus sanguinis biofilm thickness growth.

Method: Experimental research design, with post-test only control group design with a sample size of 27. The treatment group consisted of alpha mangostin 3.125, 6.25, and 12.5 g/ml and the control group were distilled water. Measurement of Streptococcus sanguinis biofilm thickness was carried out by ELISA-reader. The statistical test was carried out using the One Way Anova test.

Results: The results showed the lowest average thickness of Streptococcus sanguinis with the addition of -mangostin 12.5 g/ml was 0.371, while the thickness of Streptococcus sanguinis with the addition of chlorhexidine was the highest at 1.860. One Way Anova test obtained a significance number of 0.001 (p <0.05) so it can be concluded that there are significant differences in the 4 groups of Streptococcus sanguinis.

Conclusion: Alpha mangostin has antibacterial properties that can reduce the thickness of the Streptococcus sanguinis biofilm.

Keywords: Alpha mangostin; biofilm thickness; Streptococcus sanguinis
INTRODUCTION

Periodontal diseases are diseases that penetrate the periodontal tissues, cementum, gingiva, periodontal ligament, and alveolar bone. Periodontal diseases can occur due to the accumulation of microorganisms that are in the periodontal tissue. If a disease is not properly treated right away, it may require tooth extraction in the future. The prevalence of periodontitis in residents aged ≥ 15 years based on Basic Health Research (Riskesdas) data in 2018 was 67.8%, this means that out of 10 Indonesian people, 7 individuals experience periodontitis. Periodontitis is caused by microorganisms, one of which is Streptococcus sanguinis.

Streptococcus sanguinis are bacteria that lead to the colonization of other bacteria, creating plaque. Streptococcus sanguinis has the ability to aggregate against the surrounding bacteria so that it has an important role in the maturation activity of plaque on the tooth surface. The process of plaque formation begins with the initial bacteria that contaminate the pellicle, Streptococcus sanguinis which becomes an inducer in the activity of plaque formation. Streptococcus sanguinis binds directly to the tooth surface pellicle using various mechanisms, one of which is the prolinerich method. Prolinerich is a bonding process between sanguinis and salivary proteins. Streptococcus sanguinis facilitates other bacteria so that they can form a colony on the tooth surface and form a plaque. Streptococcus sanguinis was selected in this study because sanguinis is a gram-positive bacterium. In periodontitis, gram-positive bacteria play a greater role because the percentage of gram-positive bacteria in periodontitis is greater, 56%, while gram-negative bacteria are smaller, 44%.

Streptococcus sanguinis is one of the bacteria that causes dental plaque, dental plaque can be prevented by using mouthwash with ingredients chlorhexidine. Chlorhexidine mouthwash can minimize plaque formation and prevent the onset of periodontal diseases. Chlorhexidine mouthwash has bacteriostatic and bactericidal properties on various bacteria, especially the bacteria present in dental plaque. The use of chlorhexidine for a long time, for example more than 2 weeks, has the effect of causing burning mouth syndrome, disturbance of the sense of taste, erosion of the oral mucosa, staining of teeth, and xerostomia.

S. sanguinis is also a natural member of the resident oral biofilm community, and was the only species identified to be significantly associated with dental health. The α-mangostin compound is one of the bioactive compounds possessed by mangosteen rind, the alpha-mangostin compound is a yellow dye that is insoluble in water, however, the alpha-mangostin can dissolve in methanol, ether, ethanol, acetone and chloroform. In this study the aim was to look at the absorbance or thickness of the Streptococcus sanguinis bacterial biofilm, when given treatment in the form of alpha mangostin, with each content.

MATERIALS AND METHODS

This research is included in the experimental research method with the post-test only group design and has passed the ethical test number 371/B.1-KEPK/SAFKG/V/2022. The sample size calculated based on the Federer formula obtained the number of 6 samples plus 10% reserve sample, in the three treatment groups. In this study the samples were divided into 4 groups, 3 α-mangostin groups with various contents of 3.125, 6.25 and 12.5 µg/ml and the control group were positive (+) chlorhexidine with the total number of samples is 27 samples.

The first method of this research was the preparation of Streptococcus sanguinis bacterial
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Preparation of Streptococcus sanguinis bacterial suspension

Streptococcus sanguinis bacterial suspension was prepared using Brain Heart Infusion (BHI) media. After that, the saliva and pure isolate of Streptococcus sanguinis were added to the Brain Heart Infusion (BHI) liquid medium. The growth of Streptococcus sanguinis bacteria was adjusted to Mc Farland's turbidity standard of 0.5 so that the resulting bacterial suspension was 1.5 x 108 CFU/ml. Bacteria will be incubated at 37°C for 24 hours.

Biofilm Thickness Measurement

Pellicles were prepared on 4 well plates, after which 200 µl of saliva was added which had been filtered with a 0.22 µl minisart syringe filter in each well plate. Then incubated for 15 minutes at 37°C. Remove saliva by sucking and then rinsing with 200 µl Phosphate Buffer Saline (PBS). A-mangostin treatment group and the Chlorhexidine control group

Streptococcus sanguinis solution and artificial saliva that have been made can be divided into 4 on the well plate media, the solution is put in each well of the well plate as much as 200 µl. Each solution that has been added to the well plate media will be tested with α-mangostin compounds containing 3.125, 6.25 and 12.5 µg/ml and 0.2% Chlorhexidine. The test solution was put into the well plate media using a micropipette of 100 µl, then the sample was incubated for 24 hours at 37°C, then the remaining planktonic cells were removed and rinsed using sterile PBS 3 times and dried. The well plate media was dripped with 1% gentian violet crystals as much as 150 µl for 20 minutes and rinsed with PBS solution 3 times then dried for 5 minutes. Then the results will be read, by measuring using OD (Optical density) with a wavelength of around 540 nm using an ELISA-reader

RESULTS

The result of the Streptococcus sanguinis thickness test were carried out using an ELISA-reader and in the study showed the average thickness of Streptococcus sanguinis in 3 treatment groups given α-mangostin containing 3.125 µg/ml, 6.25 µg/ml, 12.5 µg/ml and the positive control treatment group by administering 0.2% Chlorhexidine, can be seen in table 1.

<table>
<thead>
<tr>
<th>No</th>
<th>Grup</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-mangostin 3.125 µg/ml</td>
<td>0.469 ± 0.081</td>
</tr>
<tr>
<td>2</td>
<td>α-mangostin 6.25 µg/ml</td>
<td>0.421 ± 0.088</td>
</tr>
<tr>
<td>3</td>
<td>α-mangostin 12.5 µg/ml</td>
<td>0.371 ± 0.045</td>
</tr>
<tr>
<td>4</td>
<td>Chlorhexidine 0.2%</td>
<td>1.860 ± 0.213</td>
</tr>
</tbody>
</table>

Based on table 1, the average thickness of Streptococcus sanguinis biofilm with the addition of 12.5 µg/ml α-mangostin was the lowest at 0.371, while the thickness of Streptococcus sanguinis with the addition of chlorhexidine was the highest at 1.860. Then proceed with the Shapiro-Wilk test (number of samples n <50), to determine normality. The results of the normality test for all normally distributed data (p ≥0.05). The data was then tested for homogeneity using the Levene Statistical test. Homogeneity test obtained a value of 0.006 (p
<0.05) which indicates that the data for all groups is not homogeneous.

If the data is normally distributed and not homogeneous, the next step is a nonparametric test, the Kruskal Wallis test to find out whether there is a difference in the thickness of Streptococcus sanguinis in each group. The results of the Kruskal Wallis test can be seen in the following table.

### Table 2. Kruskal Wallis hypothetical test

<table>
<thead>
<tr>
<th>Between Groups</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.001</td>
</tr>
</tbody>
</table>

The significance number shown in the table is 0.001 (p<0.05) showed that there is a significant difference in the 4 groups of Streptococcus sanguinis thickness test. To find out which group has the most significant difference, the Mann Whitney test was carried out. The test results can be seen in the following table.

### Table 3. Mann Whitney test

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Between Groups</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-mangostin 3.125</td>
<td>α-mangostin 6.25 μg/ml</td>
<td>0.150</td>
</tr>
<tr>
<td></td>
<td>α-mangostin 12.5 μg/ml</td>
<td>0.025*</td>
</tr>
<tr>
<td></td>
<td>Chlorhexidine 0.2%</td>
<td>0.004*</td>
</tr>
<tr>
<td>A-mangostin 6.25</td>
<td>α-mangostin 12.5 μg/ml</td>
<td>0.229</td>
</tr>
<tr>
<td></td>
<td>Chlorhexidine 0.2%</td>
<td>0.004*</td>
</tr>
<tr>
<td>A-mangostin 12.5</td>
<td>Chlorhexidine 0.2%</td>
<td>0.004*</td>
</tr>
</tbody>
</table>

* = for results that have significant differences.

Based on the Mann Whitney test, it showed that there were significant differences in the thickness of Streptococcus sanguinis (p <0.05) in several groups. The 3.125 μg/ml α-mangostin group was significantly different from the 12.5 μg/ml α-mangostin and 0.2% chlorhexidine group. The 6.25 μg/ml α-mangostin group had a significant difference to 0.2% chlorhexidine. The 12.5 μg/ml α-mangostin group also had a significant difference to 0.2% chlorhexidine.

### DISCUSSION

This research was conducted to determine the difference in the thickness of Streptococcus sanguinis which was treated with α-mangostin. The treatment group was divided into 3, the α-mangostin group 3.125 μg/ml, 6.25 μg/ml, and 12.5 μg/ml as well as the control group in the form of chlorhexidine 0.2%. The average thickness of the Streptococcus sanguinis biofilm in the α-mangostin addition group was 3.125 μg/ml, 6.25 μg/ml, 12.5 μg/ml, and chlorhexidine 0.2% respectively are 0.421, 0.469, 0.371 and 1.860.

Previous research conducted by Soegiharto et al., (2022) regarding the effectiveness of alpha mangostin Garinia...
Mangostana L (mangosteen peel extract) where the concentration is 12.5 µg/ml it is found that this concentration is effective in slowing down the growth and development of Staphylococcus aureus. This situation explains that the high concentration makes the inhibition zone formed even bigger so that it can minimize the growth and development of Staphylococcus aureus so that the biofilm that forms is thinner.

The results of statistical analysis obtained significant differences between each group. These conditions explained that there was a significant difference between the treatment groups α-mangostin 3.125; 6.25; and 12.5 µg/ml and the control group chlorhexidine 0.2%. In the Mann Whitney follow-up test, there was a difference in biofilm thickness value in the 12.5 µg/ml α-mangostin group and 0.2% chlorhexidine. Alpha-mangostin is an organic molecule that has potential and fast bactericidal in targeting the inner membrane of induced bacterial cells and can reduce the tendency of bacterial mutations.

Research by Nguyen et al., (2014) stated that α-mangosteen is effective on Streptococcus biofilm thickness mutant growth. This happens because of the α-mangostin exhibits anti-biofilm properties against planktonic Streptococcus mutans through several activities. This activity by reducing the production of acid by damaging the membranes of these organisms. The anti-biofilm activity of α-mangostin is bactericidal with a mechanism of action that destroys the bacterial cytoplasmic membrane. Damage to the cytoplasmic membrane causes intracellular components in bacteria to occur in bacteria. α-mangostin is the abundance of elements in mangosteen peel extract and has a variety of bioactivities, in the form of antibacterial.

Table 4.1 shows that the alpha mangostin group was more effective than the chlorhexidine positive control group. Chlorhexidine has a bactericidal and bacteriostatic effect on all types of bacteria, both gram positive (+) and gram negative (-) bacteria. (Soegiharto et al., 2022). Chlorhexidine is more effective on Gram (+) bacteria than Gram (-) bacteria, chlorhexidine is an antiseptic where absorption is carried out on the tooth surface and has anti-bacterial abilities on organisms that try to stick to the teeth. Chlorhexidine has antibacterial properties, but not as effective as α-mangostin because α-mangostin has high antibacterial activity. The mechanism of chlorhexidine as an antibacterial is that its inhibition is carried out by the synthesis of macromolecules in bacterial cells and causes damage to the bacterial cell membrane because it is lipophilic.

In addition, the use of chlorhexidine for more than 2 weeks period has an impact that can cause burning in the oral mucosa, disruption of the sense of taste, erosion of the oral mucosa, staining of the teeth, and xerostomia. Therefore α-mangostin can be used as an alternative to the use of chlorhexidine.

In this study, crystal violet was used for the activity of painting the Streptococcus sanguinis bacterial biofilm. Crystal violet is one technique for testing biofilms. Staining using crystal violet can color live and dead cells and their components are made into a biofilm matrix so that they are suitable for staining biofilms on Streptococcus sanguinis bacteria. Crystal violet is a protein stain which usually binds to each other and colors the surface using molecules with a negative charge in the form of an exopolysaccharide matrix as a biofilm matrix structure.
In this study, incubation was carried out for 24 hours. Since 24 hours is the harvest time, the logarithmic or exponential phase with the highest number of cells, reaching 10 to 15 billion bacterial cells per milliliter. Suggestions for researchers who will carry out further research should incubate for 2-4 hours, because Streptococcus sanguinis is the initial colonizing bacteria that has been formed in 2-4 hours.

The limitation in this study is that $\alpha$-mangostin as a treatment group is not easy to find on the market. $\alpha$-mangostin because it is obtained from the pre-order process and requires a long time of 1 month.

To be more precise, artificial saliva rather than natural saliva should be used in the study because artificial saliva does not contain any additional bacteria, whereas natural saliva is more likely to do so. So that it is more accurate, researchers advise using fake saliva. But you must first sterilize if you intend to use natural saliva.

**CONCLUSION**

Based on the results of the research and discussion can be concluded that, Alfa mangostin has anti-bacterial properties that can reduce the thickness of Streptococcus sanguinis biofilms. There is the best effectiveness in the alpha mangostin group with a content of 12.5μg/ml. Alpha mangostin is more effective in reducing the thickness of the Streptococcus sanguinis biofilm compared to chlorhexidine 0.2%.

The author’s recommendations are based on the results of the research result, it is necessary to conduct further research on the other content of mangosteen (Garcinia mangostana L.) on the growth of Streptococcus sanguinis biofilm thickness. Researchers recommend the results from a subsequent study will be more accurate if artificial saliva is used rather than natural saliva because natural saliva is more likely to mix with other bacteria. However, you should first sterilize it if you still intend to use natural saliva.

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**REFERENCES**

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