Decrease in the number of Streptococcus mutans and Staphylococcus aureus bacterial colonies after administration of sentul fruit peel extract gel (Sandoricum koetjape) in gingivitis model of white Wistar rats

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INTRODUCTION

Dental and oral diseases are among the most common diseases in Indonesia, with the percentage of the population experiencing dental and mouth problems in 2018 increasing by 57.6%, while in the province of Bali the proportion of dental and mouth diseases reached 41.6%. One of the most common dental and oral diseases in Indonesia is inflammation of the gums (gingivitis).

Gingivitis is inflammation of the gingiva which causes bleeding accompanied by swelling, redness, exudate and changes in the normal contour of the gingiva. Gingivitis is caused by the accumulation of bacteria in plaque, and plaque that accumulates in the mouth will experience mineralization to form tartar. Tartar is a medium for the growth and reproduction of bacteria that can cause gingivitis. The causes of gingivitis are divided into three groups, namely due to necrosis, not related to plaque, and accumulation of bacteria in plaque.

The most common treatments for gingivitis are scaling and root planing. Scaling is an effort to remove plaque, calculus and stains on the surface of the crown and root of the tooth. Root planing is cleaning and smoothing the tooth root's surface from necrotic tissue and residual bacteria and their products attached to the tooth's root surface.

Staphylococcus aureus and Streptococcus mutans bacteria, are one of the microorganisms that are in the mouth. Both of these bacteria can cause gingivitis. Staphylococcus aureus and Streptococcus mutans bacteria are normal inhabitants of the oral cavity, these bacteria can turn into pathogens if the living environment of these bacteria is favorable and there is an increase in population.

Sentul fruit can be eaten and is also used in traditional medicinal ingredients such as the root can treat diarrhea, the leaves can relieve fever, and the sawdust can be used as an anthelmintic. Several researchers have proven the efficacy of the sentul plant as a leucorrhoea, namely Warsinah et al. reported that the methanol extract of sentul stem bark could...
inhibit the growth of the *Candida albicans* fungus by 39.65%. In addition, the ethyl acetate extract of the leaves of the sentul lute plant also has anti-bacterial activity.\(^8\)

The results of the phytochemical screening examination of sentul fruit skin simplex powder showed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, antraquinone glycosides and steroids.\(^9\)

The results of research on sentul fruit peel extract from several phytochemical compounds, flavonoid compounds, saponins and tannins are aromatic hydroxyl groups that have antibacterial properties.\(^10\) The results of further research on sentul fruit peel extract (*Sandoricum koetjape*) have the ability to inhibit *Streptococcus mutans* and *Staphylococcus aureus* bacteria. The highest ability to inhibit bacterial growth was obtained at a concentration of 100%.\(^11\)

Sentul fruit peel extract is made in the form of nano gel so it is easy to apply in the treatment of gingivitis. Nanogels are very promising carriers compared to other drug delivery agents for therapy, macromolecular diagnostics and others.\(^12\) Nanogels are colloidal hydrogel nanoparticles that have three-dimensional physical and chemical bonds, contain a certain amount of water but are insoluble in distilled water. Nanoparticles in the gel matrix can increase the duration of contact and the therapeutic effect of topical drug administration.\(^13\) This nanocarrier system has unique properties, flexible nanoparticle size, large outer surface for multivalent conjugate binding, stability, biocompatibility and loading capacity. This system is capable of targeting specific cells and compartments.\(^14\)

Based on the above background, research was conducted on administering a nano gel of sentul fruit rind extract (*Sandoricum koetjape*) to reduce the number of bacterial colonies of *Streptococcus mutans* and *Staphylococcus aureus* in white gingivitis model of white Wistar rats.

**METHOD**

**Preparation of materials and tools**

The materials used in this study were sentul fruit skin samples, aluminum foil, ethanol, distilled water, filter paper, alcohol, mice, Muller Hinton Agar (MHA) media, isolates of *S. mutans* and *S. aureus* bacteria, feed (rat standard food and drinking water), propylene glycol, HPMC, metal paraben, propylparaben, crystal violet, lugol, arsenic/alcohol, and safranin.

The tools used in this study were beaker glass, glass funnel, Erlenmeyer, measuring cup, stir bar, spoon, dropper, rotary evaporator, digital scales, mortar, stamper, spatula, spatula, colony counter, petri dishes, analytical scales, laminar air flow, autoclaves, ovens, stirrers, glass objects, test tubes, cotton buttons, labels, and microscopes.

**Sentul Fruit Peel Extraction**

Sentul rind simplicia was extracted by maceration method with ethanol for 24 hours. The maceration method was chosen in this study because it is an easy method to carry out and uses simple tools, namely simply by immersing the sample in solvent. Then proceed with the filtering process and the filtrate is then evaporated with a vacuum rotary evaporator at 45°C, so that a thick extract is produced. The maceration process was repeated 2 times. After the extraction process then proceed with liquid-liquid fractionation using distilled water. The concentrated extract was put into a separatory funnel and added with distilled water. Then it was shaken and the distilled water fraction was taken followed by evaporation with a vacuum rotary evaporator at a temperature of 45°C, so that the ethanol extract aqua fractionation was produced.

**Manufacture of Nano Gel Extract from Sentul Fruit Peel**

Sentul fruit peel extract was then sonicated into extract nanoparticles with a temperature of 45°C and a frequency of 42 kHz for 30 minutes. Preparation of nano gel of Sentul fruit skin extract using HPMC basis. First, ± 30 ml of distilled water was heated so that it reached a temperature of ± 80°C, then removed and the HPMC was developed in it for 15 minutes, after the flowers were added methylparaben and propylparaben which had been dissolved in ethanol. Sentul fruit peel nanoparticle extract was added with propylene glycol little by little while continuing to grind until homogeneous, dipped in distilled water and stirred until homogeneous. The formula for nano gel extract of Sentul fruit skin with HPMC base. Sentul fruit skin nanoparticle extract 1 gram with the addition of ingredients in Table 1.

**In Vivo Testing Stage**

Selection of Experimental Animals

The experimental animals used were healthy male Wistar white rats and normal activities, with body weight between 180-200 g (adult male rat weight), aged 2-3 months, all experimental animals were kept in the same conditions. The experimental animals were adapted to the research environment for one week and before giving treatment the experimental animals were still given food and drink for 18 hours.

**Bacterial Induction**

Rats were induced by *S. mutans* and *S. aureus* bacteria in LPS (Lipopolysaccharide) preparations in the gingival sulcus of the first right lower incisor labial part for 7 days using a syringe of 0.01 ml. The research group was divided into 6 groups with 5 treatment groups which were induced by Pg-LPS.

**Experimental Animal Treatment**

The sample was divided into six groups, namely the negative control group (K-) which was the group without treatment and the positive control (K+) which was only given the penetration of *S. aureus* and *S. mutans* bacteria without being given nano gel of Sentul fruit peel extract, and the treatment group (KP) with the addition of 0.01 mL of HPMC of 1 gram and the addition of ingredients in Table 1. This group was then given 1 gram of HPMC as the base and 1 gram of Sentul fruit skin extract and the addition of ingredients in Table 1.
were each given 0.6% sentul fruit rind nano gel; 1.2%; 1.8%; and 2.4%. A total of 30 experimental animals that had been acclimatized for 7 days were induced by *S. aureus* and *S. mutans* bacteria in LPS preparations in the gingival sulcus of the lower right first incisor as much as 0.01 ml. A total of 30 experimental animals that had experienced inflammation were then treated with sentul fruit skin extract nanogel. On the fifth day after drug administration, gingival crevicular fluid (GCF) was taken using a cotton swab in the crevicular part of the anterior teeth of the rats.

**Antibacterial activity of sentul fruit peel extract in experimental animals due to LPS induction by the pour plate method**

Animals that had experienced inflammation were then treated with sentul rind extract nano gel for 4 days, on day 5 sampling was carried out in the sulcus gingiva to examine the number of microbial colonies. The number of bacteria present on the teeth was calculated using the Pour Plate method which was expressed as CFU/mL with a colony counter. The principle of the bacterial counting method is to grow living microorganism cells on agar media, so that the microorganisms will multiply and form colonies that can be seen directly and counted by eye without using a microscope. With this method, we can count bacterial cells that are still alive, determine the types of microbes that grow in the media and can isolate and identify the type of microbial colonies.

**Data analysis**

Data was collected based on the examination results from the Laboratory of the Faculty of Medicine, Universitas Udayana. Data analysis was performed using the SPSS program with a significance level of 0.05 \( (p = 0.05) \) and a 95% confidence level \( (\alpha = 0.05) \). The data obtained by the One-Way ANOVA test and if the effect is significant continue with the Duncan test.

**RESULT**

Analysis of the treatment effect was tested based on the average of *Streptococcus mutans* and *Staphylococcus aureus* bacteria between groups after being given the Sentul skin nano gel treatment. The results of the significance analysis with the One-Way ANOVA test are presented in Table 1 above. Significance analysis with the One Way Anova test in Table 1 shows that the number of *Staphylococcus Aureus* bacteria colonies has an \( F\)-value = 275.890 and \( p = 0.000 \). In contrast, the number of *Streptococcus mutans* bacteria colonies has an \( F\) value = 108.593 and a \( p = 0.000 \). This means that the mean of *Streptococcus mutans* and *Staphylococcus aureus* in the treatment group was significantly different \( (p<0.05) \) (Table 2).

In this study, to find out which groups were different, the Duncan test was carried out. The average value of the number of *Streptococcus mutans* and *Staphylococcus aureus* bacteria colonies after nano administration of Sentul fruit peel extract gel (*Sandoricum koetjape*) in gingivitis mouse model can be seen in Table 3. Treatment of 2.4% sentul skin peel extract nano gel resulted in the lowest number of *Streptococcus mutans* and *Staphylococcus aureus* bacteria colonies.

**DISCUSSION**

Treatment of 2.4% sentul skin peel extract nano gel resulted in the lowest number of *Streptococcus mutans* and *Staphylococcus aureus* bacteria colonies. This is because a higher extract concentration will certainly contain a higher concentration of phytochemical compounds as well. The higher the concentration of the extract, the higher the content of active compounds that are antibacterial so that the ability to inhibit bacterial growth is also greater. A decrease in concentration can cause a decrease in the ability of antibacterial activity, so that an increase in the inhibition of bacteria is directly proportional to the concentration of the extract. This is also in line with research conducted by Brooks et al., which stated that the ability of a material to inhibit bacterial growth is affected by the high concentration of antibacterial.

In *Staphylococcus aureus* bacteria, the number of colonies in experimental

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**Table 2.** ANOVA test *Streptococcus mutans* and *Staphylococcus aureus* colonies after administration of sentul fruit peel extract nano gel *Sandoricum koetjape* on gingvitis of white Wistar rats

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>( F )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> Between Groups</td>
<td>7135217.467</td>
<td>5</td>
<td>1427043.943</td>
<td>275.890</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Within Groups</td>
<td>124140.400</td>
<td>24</td>
<td>5172.517</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>729537.867</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. mutans</em> Between Groups</td>
<td>5745402.967</td>
<td>5</td>
<td>1149080.593</td>
<td>108.593</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Within Groups</td>
<td>253958.000</td>
<td>24</td>
<td>10581.583</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5999360.967</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.** The average value of the number of *Streptococcus mutans* and *Staphylococcus aureus* bacteria colonies after administration of sentul fruit peel extract (*Sandoricum koetjape*) nano gel on gingivitis mouse model

<table>
<thead>
<tr>
<th>No</th>
<th>Study group</th>
<th><em>S. aureus</em></th>
<th><em>S. mutans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative control</td>
<td>( 1.6 \times 10^{1.0} \pm 2.2 \times 10^{1.0} )</td>
<td>( 1.4 \times 10^{1.8} \pm 7.2 \times 10^{1.4} )</td>
</tr>
<tr>
<td>2</td>
<td>Positive control</td>
<td>( 3.3 \times 10^{3.6} \pm 6.8 \times 10^{3.9} )</td>
<td>( 3.4 \times 10^{2.8} \pm 6.1 \times 10^{2.9} )</td>
</tr>
<tr>
<td>3</td>
<td>Sentul fruit skin peel extract 0.6%</td>
<td>( 1.1 \times 10^{3.4} \pm 3.4 \times 10^{3.9} )</td>
<td>( 1.1 \times 10^{2.9} \pm 3.9 \times 10^{2.5} )</td>
</tr>
<tr>
<td>4</td>
<td>Sentul fruit skin peel extract 1.2%</td>
<td>( 7.1 \times 10^{1.8} \pm 1.8 \times 10^{1.8} )</td>
<td>( 6.9 \times 10^{1.5} \pm 5.2 \times 10^{1.5} )</td>
</tr>
<tr>
<td>5</td>
<td>Sentul fruit skin peel extract 1.8%</td>
<td>( 3.1 \times 10^{2.1} \pm 2.1 \times 10^{2.4} )</td>
<td>( 3.1 \times 10^{1.7} \pm 1.7 \times 10^{1.7} )</td>
</tr>
<tr>
<td>6</td>
<td>Sentul fruit skin peel extract 2.4%</td>
<td>( 2.2 \times 10^{1.9} \pm 1.9 \times 10^{1.9} )</td>
<td>( 2.6 \times 10^{1.2} \pm 1.2 \times 10^{1.2} )</td>
</tr>
</tbody>
</table>

Notes: The mean value followed by the same letter in the same column showed no significant difference on the 5% Duncan test; Standard deviation \( (n=5) \)
animals treated with 2.4% sentul peel extract nano gel was $2.2 \times 10^2 \pm 1.9 \times 10^1$ cfu, while the number of Streptococcus mutans bacteria colonies in experimental animals treated with Sentul skin extract nano gel 2.4 % of $2.6 \times 10^2 \pm 1.2 \times 10^1$ cfu. Administration of gel with the highest extract concentration reduced the number of bacterial colonies in experimental animals. The treatment of 2.4% sentul skin extract nano gel in experimental animals reduced the number of bacterial colonies equivalent to the positive control treatment. This shows that the sentul skin extract nano gel has antibacterial activity which can inhibit the growth of bacteria in the mouth.

Sentul fruit peel extract (Sandoricum koetjape) contains antibacterial compounds. Several secondary metabolite compounds contained in sentul fruit peel extract are flavonoids, alkaloids, triterpenoids, saponins, and polyphenols. These phytochemical compounds have antibacterial activity. Saponins are a class of terpenoid compounds that can inhibit the growth or kill bacteria by interfering with the process of cell wall formation, where the cell wall is not formed or is formed but imperfect. According to Nagappan et al. stated that flavonoids cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes caused by interactions between flavonoids and bacterial DNA. The lipophilic nature of flavonoids causes these compounds to damage bacterial cell membranes. In addition, the antibacterial activity of tannin compounds where tannins are thought to be related to their ability to inactivate microbial adhesins, enzymes, and transport proteins in cell membranes. Furthermore, other compounds, namely phenols, can kill bacterial cells, namely by denaturing bacterial cell proteins.

**CONCLUSION**

In the results of the study there was a decrease in the number of Streptococcus mutans and Staphylococcus aureus bacteria colonies after administration of Sentul fruit peel extract gel (Sandoricum koetjape) in gingivitis of White Wistar Rats. The best gel concentration was Sentul peel extract gel 2.4% with the smallest number of bacterial colonies, namely the number of Staphylococcus aureus colonies of 2.2 x 102 ± 1.9 x 101 CFU and the number of Streptococcus mutans colonies of 2.6 x 102 ± 1.2 x 101 CFU.

**CONFLICT OF INTEREST**

All author declares there is no conflict of interest regarding publication of the study.

**ETHICAL CONSIDERATION**

Ethical Committee Politeknik Kesehatan Denpasar has approved this study with ethical clearance reference number: LB.02.03/EA/KEPK/0334/2023.

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None.

**AUTHOR CONTRIBUTION**

All authors had contributed in manuscript writing and agreed for the final version of manuscript for publication.

**REFERENCES**