Potential Use of Angsana Latex (Pterocarpus Indicus) As an Alternative Mouthwash for Dental Cavities

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Abstract
Mouthwash is a chemical substance to inhibit plaque and caries formation. One of the most widely used mouthwashes is chlorhexidine but these mouthwashes have side effects on long-term use, for example changes of dental color, restorations, and mucous membranes, increased calculus formation, taste disorder, burning sensation, and mucosal irritation. Alternative mouthwash to replace chlorhexidine is a material that has antibacterial effect without side effects is angksana latex. The aim of the present review was to discuss potential use of Pterocarpus indicus's falconoid as an alternative anticaries mouthwash and looking at the possibility of its usage in mouthwash. Caries is a breakdown of teeth that is enamel, dentine, and cementum caused by the activity of oral flora bacteria present in a carbohydrate that can be distributed. Angsana latex (Pterocarpus indicus) has antibacterial effect because it contains phenol, flavonoids, saponins, triterpenoids and tannin alkaloids. Flavonoids work as antibacterial by inhibiting the synthesis of bacterial nucleic acids and are able to inhibit bacterial motility. Angsana latex is easy to find, easy to manufacture and it is a mild antibacterial that is beneficial for long-term use. Regarding its beneficial effect and safety of angksana latex, it could be proposed as an ideal long term use mouthwash.

Key Words: Angsana Latex; Alternative Mouthwash; Caries.

Introduction
Caries is a pathological process tooth demineralization of organic materials due to the production of acid in the mouth. The sign is followed by damage the organic material resulting in bacterial invasion and death of the pulp. Dental caries are characterized by tissue damage, starting from the tooth surface (pits, fissure and interproximal areas) extends to the area of the pulp. The main microorganisms in the mouth were associated with caries are Streptococcus mutans and Lactobacilli. Bacterial plaque dominant in dental caries is Streptococcus mutans. These bacteria are cariogenic being able to immediately make acid from carbohydrates that can be fermented. The bacteria also thrive in acidic conditions and attached to the tooth surface because of their ability to make an external polysaccharide very sticky on teeth. It consists of polysaccharide polymer glucose; causing dental plaque matrix has a consistency like gelatin, consequently helped bacteria to stick on teeth [1]. Streptococcus mutans is a Gram-positive bacterium and includes in group varidians.

Streptococcus mutans is an anaerobic, acidogenic that produce acid, which can stay in acidic environment, and produces a sticky polysaccharide called dextran. By the capabilities, Streptococcus mutans could support other bacteria to attach the tooth enamel, support growth acidoduric other bacteria. Thus resulting in soluble enamel [2]. Streptococcus mutans is the cariogenic bacteria because it can make acid form of carbohydrates that can be fermented. The bacteria can thrive in acidic conditions and can stick to the surface of tooth because of their ability to make extracellular polysaccharides. Extracellular polysaccharide, this consists of a matrix polymer of glucose that causes plaque has gelatin-like consistency, so that bacteria can easily stick to the teeth and attached to one another. Plaque will be thicker in longer time. Salivary function can be inhibited because of the activity of bacteria. Ability of Streptococcus mutans to exploit some extra and intracellular storage compounds have ecological benefits in addition and increase amount of acid and level of acidity in oral cavity. These acids cause environmental resistance of bacteria and...
flourish in an environment with a low pH in the matrix of the plaque in demineralized enamel, thus beginning the process of dental caries [3].

Pterocarpus indiscus is a type of plant deciduous trees with a height of 30-40 meters with a trunk diameter up to more than 2 meters. Usually have short buttresses. Wood issued exudate or dark red latex called “kino” or “dragon’s blood”. Compound leaves with 5-11 leaflets and hairy. Flower with a length of 6-13 cm. Flowers are bisexual, yellow bright and fragrant. Angsana plant has a large number of uses. Most communities often processed food (bark), latex (resin) and also leave. In some areas, shredded bark is boiled and the liquid is taken too used orally for treat dysentery and diarrhea. Angsana latex has health benefits too, among others to stop diarrhea, lowering fever, accelerate aging and accelerate wound healing in particular for burns. Chemical compounds contained in this plant show the test positively to phenols, flavonoids, saponins, triterpenoids and tannins [4]. Flavonoid works as an antibacterial by inhibiting synthesis of nucleic acids bacteria and able to inhibit bacterial motility. Flavonoids works by interfere with the binding of hydrogen in the nucleic acid so that the synthesis process DNA-RNA inhibited. In addition flavonoids, can also prevent the growth bacteria by disrupting the stability of the cell membrane and energy metabolism bacteria. This resulted in bacterial cell death. Meanwhile, the work of the reductase enzyme in bacterial electron transfer processes lead impaired bacterial growth [5].

Chlorhexidine is typically used to remove contaminants bacteria. Chlorhexidine is also effective in reducing the growth of Streptococcus mutans mutants found on the exposed root surface caries. Therefore antibacterial, chlorhexidine is also recommended as cavity disinfection before placement of the restoration [6]. Chlorhexidine has cytotoxic effects on odontoblast cells for cell odontoblast cell layer lining the pulp and cell periperi first to affect by chemicals that reach the pulp chamber by diffusion. Odontoblasts are specialized cells that have an important role in the process pulp healing and the formation of mineralized tissue barrier. The presence of substances the chemical can interfere with odontoblasts can damage pulp dentinal directly induce apoptosis or death of these cells due to cytotoxic effects [7]. This research is generally aimed to proving bactericidal effect of angsana latex equivalent to chlorhexidine 0.2% to the growth Streptococcus mutans. The specific objective of this study was to measure the inhibition zone of angsana latex (Pterocarpus indiscus) concentration of 40% w/v, angsana latex (Pterocarpus indiscus) concentration of 80% w/v and inhibition zone chlorhexidine 0.2% against growth of Streptococcus mutans. Results of this research are expected to provide scientific evidence on the bactericidal effect of angsana latex (Pterocarpus indiscus) 40% or 80% w/v equivalent to chlorhexidine 0.2% in inhibiting the growth of Streptococcus mutans, which can be used as one of the basic further research to produce an oral antiseptic with herbal ingredients angsana latex.

Details Experimental

This research was a pure laboratory experimental (true experimental), with post-test only design, using a randomized design detailed consists of 4 treatments, among others: Angsana latex 40% w/v, Angsana latex 80% w/v, positive control (chlorhexidine 0.2%) and negative controls (aquades). Each treatment is repeated 6 times repetition. The number of repetitions for each treatment group, obtained from the results of the calculation with Federer formula.

Material and Equipments

The materials used in this research are Angsana latex 40% w/v, Angsana latex 80% w/v, chlorhexidine 0.2%, aquades, Streptococcus mutans bacteria, Muller Hinton Agar (MHA), Brain Heart Infusion media (BHI). Equipments used in this study are an analytical balance, a petri dish, bunsen lamp, ose sterile, sterile cotton stick, funnel, glass beaker, a small test tube, rack test tube, pipette, micropipette, autoclave, incubator and stems glass stirrer.

Dilution of Angsana Latex

Angsana latex diluted with distilled water (aquades) and made concentration of 40% and 80%, concentration of 40% w/v created by inserting 2 gram of angsana latex and added aquades until volume reach 5 ml. Concentration of 80% w/v is made by inserting 4 grams of Angsana latex in tube and added aquades until volume reach 5 ml.

Sterilization

The next stages are sterilization procedure. The equipments that necessary washed then dried and sterilized in autoclave at 121°C for 15 minutes.

Preparation of Bacteria

Preparation of bacteria by scraping Streptococcus mutans to the blood agar media and then allowed to stand in an incubator with temperature 37°C for 24 hours. After incubated, Streptococcus mutans will detected with small round colonies and the diameter is 1-2 µm. Result of bacterial colonies growing for 24 hours suspended in 0.5 ml of BHI liquid and carried incubation for 5-8 hours at 37°C. Do the addition of sterile distilled water (aquades) to the suspension of bacteria at BHI, so the turbidity according to the standard concentration of bacteria Mc Farland I as big as 3x108 cfu/ml.

Antibacterial Test

Bactericidal effect test of angsana latex (Pterocarpus indiscus) 40% w/v and 80% w/v is done by taking a standardized bacterial suspension with Mc Farland I of 3x108 cfu/ml with sterile cotton stick and smeared on Muller Hinton Agar medium. Then prepare 24 pieces pitting, which each divided into four groups for Angsana latex 40% w/v, Angsana latex 80% w/v, chlorhexidine 0.2% and aquades. Into each petri dish placed six dics and treated for each petri dish comprising a solution of Angsana latex.
40% w/v, Angsana latex 80% w/v, and chlorhexidine 0.2% and as much as one drop of aquades. The next step is incubated at 37°C for 24 hours. Tests conducted bactericidal effect with observations made after 24 hours of incubation. Observations of bactericidal effect done by measuring the diameter of inhibition zone around pitting. To determine the measurements of inhibition zone in sample is look at the inhibition area on the surface of nutrient agar medium that clear around pitting using caliper.

Results and Discussion

Research has been conducted using treatment, angasana latex 40% w/v, angasana latex 80% w/v, chlorhexidine 0.2% and aquades. Each treatment was tested using sinks and performed in 6 repetitions. Inhibition zone measurement results from each treatment to Streptococcus mutans can be seen in (Figure 1).

![Inhibition Zone of Each Treatment](image)

**Figure 1.** Inhibition Zone of angasana latex concentration of 40% (A) angasana latex concentration of 80% (B), aquades (C) and chlorhexidine 0.2% (D) against bacteria Streptococcus mutans

![Inhibition Zone (mm) of each treatment](image)

**Figure 2.** Inhibition Zone (mm) of each treatment

(Figure 2) shows that there are variations in the inhibition zone formed from each treatment group angasana latex 40% w/v shows the average of inhibition zone is 14.6 mm, angasana latex 80% w/v is 16.7 mm, positive control (chlorhexidine 0.2%) had an average of inhibition zone is 16.0 mm and negative control (aquades) is 0 mm. The measurement results is the average of diameter inhibition zone on each group after 24-hour incubation seen that the diameter zone of inhibition at a concentration of 40% was able to inhibit the growth of Streptococcus mutans colonies [8]. However, having small inhibition zones, so concentration of 80% is used as a benchmark to compare with positive and negative controls. Value of angasana latex inhibition zone diameters with concentration of 80%, chlorhexidine 0.2% and aquades can be seen in the following table.

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Mean (mm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angsana latex 80% w/v</td>
<td>16.667±0.8164</td>
</tr>
<tr>
<td>Chlorhexidine 0.2%</td>
<td>16.083±1.4702</td>
</tr>
<tr>
<td>Aquades</td>
<td>0.0000±0.0000</td>
</tr>
</tbody>
</table>

Based on the table above, it appears that the average diameter of inhibition zone angasana latex 80% w/v has the greatest inhibition zone compared with chlorhexidine 0.2% and aquades on bacterial growth of Streptococcus mutans with an average 16.667 mm. To determine whether there is a significant difference between groups, then performed statistical analysis using test One-way ANOVA whose results appear in (Table 2).

**Table 2. Test Results Statistics One Way ANOVA**

<table>
<thead>
<tr>
<th>Sum of squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>1073.583</td>
<td>2</td>
<td>536.791</td>
<td>569.372</td>
</tr>
<tr>
<td>Within groups</td>
<td>14.141</td>
<td>15</td>
<td>.942</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1087.725</td>
<td>17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on the table above shows test results of One-way ANOVA, the inhibition zone diameter difference was significant (p <0.000) among all group after an incubation period of 24 hours, then continued with Least Significant Difference (LSD) test to determine whether there is difference significantly between each group in the following table.

**Table 3. Results of LSD on the difference between the diameter of inhibition zone Angsana Latex 80% w / v chlorhexidine 0.2%, and aquades.**

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Treatment Groups</th>
<th>Mean Difference</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine 0.2%</td>
<td>Angsana latex 80% w/v</td>
<td>0.583*</td>
<td>.000</td>
</tr>
<tr>
<td>Chlorhexidine 0.2%</td>
<td>Aquades</td>
<td>16.083*</td>
<td>.000</td>
</tr>
<tr>
<td>Angsana latex 80% w/v</td>
<td>Aquades</td>
<td>16.667*</td>
<td>.000</td>
</tr>
</tbody>
</table>

Note: *Least Significant Difference (LSD) test:*p<0.05; significant

Significant differences can be seen when the value of (p <0.05) in value significance. From (Table 3), the results of LSD seen that all groups’
treatment had a significant inhibitory zone differences. *Streptococcus mutans* with its ability is support other bacteria to stick in tooth enamel that causing dental caries.8 Cavity cleanser is a material disinfection cavity to remove smear layer after tooth prepared. Cavity cleanser commonly used is chlorhexidine 0.2%. Chlorhexidine has a wide spectrum of antibacterial activity namely Gram-positive bacteria, especially *Streptococcus mutans* [9].

Based on the results of research, Angsana latex 40% w/v, Angsana latex 80% w/v and chlorhexidine 0.2% can inhibit the growth of *Streptococcus mutans*. Angsana latex 80% w/v is effective when compared with chlorhexidine 0.2% in inhibiting the growth of *Streptococcus mutans* bacteria as a material of cavity cleanser. Angsana latex 80% w/v derived from herbal ingredients can be used as an alternative material cavity cleanser because it has an inhibition zone was higher than chlorhexidine 0.2% as significant. The average zone of inhibition Angsana latex 80% w/v is 16.667 mm while chlorhexidine 0.2% had smaller inhibition zones 16.083 mm. In this study angsana latex may inhibit *Streptococcus mutans* because angsana latex contains flavonoids as an antibacterial. Flavonoids causing damage to the permeability of the bacterial cell wall, microsomes and lysosomes as a result of interaction between flavonoids with DNA bacteria.

Based on this study, it can be conclude that Angsana latex (*Pterocarpus indicus*) 80% w/v has an effect bactericidal higher than chlorhexidine 0.2% against bacterial growth of *Streptococcus mutans*. Further research is needed to determine a safe dose of Angsana latex (*Pterocarpus indicus*) 80% w/v equivalent with chlorhexidine 0.2% in inhibiting the growth of bacteria *Streptococcus mutans* as a solution cavity cleanser in the field of dentistry.

References