

Potential Use of Angsana Latex (*Pterocarpus Indiscus*) 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 has 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 angsana latex. The aim of the present review was to discuss potential use of *Pterocarpus indiscus*'s flavonoid 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 indiscus*) has antibacterial effect because it contains phenol, flavonoids, saponins, triterpenoids and tanninalkaloids. 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 angsana latex, it could be proposed as an ideal long term use mouthwash.

Keywords: 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 bacteria 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 thrives in acidic conditions and can stick to the surface of tooth because of their ability to make extra-cellular 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 has 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 leaves. In some areas, shredded bark is boiled and the liquid is taken to 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 shows 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* 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 peripheri first to affected 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 pulpodentinal directly induce apoptosis or death of these cells due to cytotoxic effects [7].

This research is generally aimed to proving bactericidal effect of angšana latex equivalent to *chlorhexidine* 0.2% to the growth *Streptococcus mutans*. The specific objective of this study was to measure the inhibition zone of angšana latex (*Pterocarpus indiscus*) concentration of 40% w/v, angšana 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 angšana 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 angšana 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: Angšana latex 40% w/v, Angšana 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.

Materials and Equipments

The materials used in this research are Angšana latex 40% w/v, Angšana 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 Angšana Latex

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

Sterilization

The next stage is 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 37oC for 24 hours. After incubated, *Streptococcus*

mutans will detected with small form 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 angšana 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 Angšana latex 40% w/v, Angšana latex 80% w/v, *chlorhexidine* 0.2% and aquades. Into each petri dish placed six discs and treated for each petri dish comprising a solution of Angšana latex 40% w/v, Angšana latex 80% w/v, *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

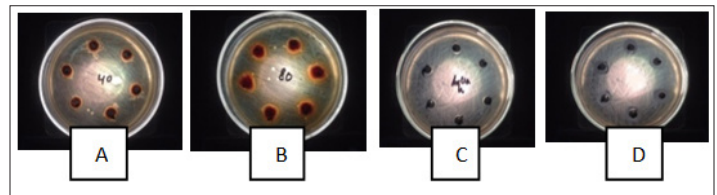


Figure 1: Inhibition Zone of angšana latex concentration of 40% (A) angšana latex concentration of 80% (B), aquades (C) and *chlorhexidine* 0.2% (D) against bacteria *Streptococcus mutans*

Research has been conducted using 4 treatment, angšana latex 40% w/v, angšana 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 2.

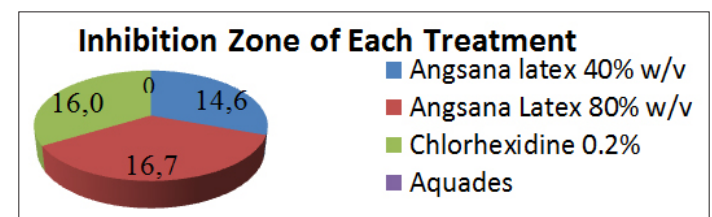


Figure 2: Inhibition Zone (mm) of each treatment

Figure 2 shows that there are variations in the inhibition zone formed from each treatment group angšana latex 40% w/v shows the average of inhibition zone is 14.6 mm, angšana 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 has performed 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. However,

having a small inhibition zone, so concentration of 80% is used as a benchmark to compare with positive and negative controls. Value of angšana latex inhibition zone diameters with concentration of 80%, *chlorhexidine* 0.2% and aquades can be seen in the following table.

Table 1: The Average Diameter Zone of Inhibition and Standard Deviation in Each Petri Dish of Angšana Latex Concentration of 80%, Chlorhexidine 0.2% and Aquades against Bacteria Streptococcus Mutans

Concentration (%)	Mean (mm) ± SD
Angšana latex 80% w/v	16.667±0.8164
Chlorhexidine 0.2%	16.083±1.4702
Aquades	.00000±.00000

Based on the table above, it appears that the average diameter of inhibition zone angšana 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 group, then performed statistical analysis using test One-way ANOVA whose results appear in Table 2.

Table 2: Test Results Statistics One Way ANOVA

	Sum of squares	Df	Mean Square	F	Sig.
Between groups	1073.583	2	536.791	569.372	.000
Within groups	14.141	15	0.942		
Total	1087.725	17			

Note: Analysis of variance (ANOVA) test: P < 0.05; significant

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 Angšana Latex 80% w / v chlorhexidine 0.2%, and aquades

Treatment Groups	Treatment Groups	Mean Difference	Sig.
Chlorhexidine 0.2%	Angšana latex 80% w/v	0.583*	.000
Chlorhexidine 0.2%	Aquades	16.083*	.000
Angšana latex 80% w/v	Aquades	16.667*	.000

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, Angšana latex 40% w/v, Angšana latex 80% w/v and *chlorhexidine* 0.2% can inhibit the growth of *Streptococcus mutans*. Angšana 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. Angšana 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 Angšana latex 80% w/v is 16.667 mm while *chlorhexidine* 0.2% had smaller inhibition zones 16.083 mm. In this study angšana latex may inhibit *Streptococcus mutans* because angšana 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 Angšana 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 Angšana 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.

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