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Comparative effectiveness of angsana latex *(pterocarpus indiscus)* and *chlorhexidine* 0.2% against *streptococcus mutans* growth - an *in vitro* study

Abstract

Background: Angsana is one of the medicinal plants that can be used as a traditional medicine. Angsana has a scientific name *Pterocarpus indicus* from fabaceae family. Angsana contains secondary metabolites, which is thought to have antibacterial effects. The chemical constituents of the plant angsana are alkaloids, flavonoids, triterpenes, steroids, and phenolic. Researches had shown that angsana latex had antibacterial effect against *Streptococcus mutans* because it contains flavonoid. This day, the therapies that have been given to reduce the colonies of *Streptococcus mutans* in oral cavity, one of that is *chlorhexidine* 0.2%.

Objective: The aim of this research was to prove comparative effectiveness of angsana latex (*Pterocarpus indiscus*) and *chlorhexidine* 0.2% against *Streptococcus mutans* growth.

Material and Methods: This research was an experimental method laboratory (true experimental), with a post-test only design, using a completely randomized design, consisting of four treatments: angsana latex 40% w/v, angsana latex 80% w/v positive control (*chlorhexidine* 0.2%) and negative control (aquadest). Each treatment is repeated 6 times. The rated bactericidal effect of the inhibition zone formed on Muller Hinton media with diffusion method.

Result: One Way ANOVA test showed that inhibition zone had a significant difference, angsana latex 80% w/v had mean inhibiting zone higher than *chlorhexidine* 0.2% against *Streptococcus mutans*.

Conclusion: Based on this study, angsana latex 80% w/v had bactericidal effect higher than *chlorhexidine* 0.2% against *Streptococcus mutans*.

Keywords: comparative effectiveness, angsana latex, *chlorhexidine* 0.2%, *Streptococcus mutans*

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Introduction

Dental caries is the decay process that starts from enamel continues to dentin, dental caries is a disease associated with many factors. Data from the Ministry of Health of the Republic Indonesia in 2010 showed that the prevalence of dental caries in Indonesia reaches 60% up to 80% of the population and ranked sixth as a disease most suffered.[1] One of the main causes of dental caries is *Streptococcus mutans* producing enzymes glucosyltransferase (GTF).[2,3] There are many ways to prevent dental caries, one of them is antiseptic mouthwash. Mouthwash with an antiseptic can degrade the number of colonies of pathogenic bacteria in the oral cavity, reducing the occurrence of plaque, and dental caries.[4] One of the mouthwash recommended is *chlorhexidine gluconate* 0.2% as a standard mouthwash in dentistry, but *chlorhexidine* has side effects such as staining on teeth and reduce the power of taste when used in long term. So, we need other alternatives as a raw material for making mouthwash with minimal side effects, economical and efficacious. Alternative eligible are ingredients of the herbs.[5] One of medicinal plants which is widely used as a herbal medicine is the latex of angsana tree. Based on the results of the study, it was reported that the antimicrobial activity of angsana latex, support the use of traditional medicine associated with conditions of microorganisms in humans and as a result can fight multi-resistant microbes.[6] However, it is not known whether the latex of angsana tree have a bactericidal effect equivalent to mouthwash *chlorhexidine* 0.2% in killing the growth of *Streptococcus mutans*, so we need comparative studies on the bactericidal effect of angsana latex *(Pterocarpus indiscus)* and *chlorhexidine* 0.2% against *Streptococcus mutans*. This research is generally aimed to proving bactericidal effect of angsana latex equivalent to *chlorhexidine* 0.2% in inhibiting the *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. Caries is a pathological process involving demineralization of tooth because of organic materials due to the production of acid in the mouth tooth demineralization of organic materials due to the production of acid in the mouth. The sign is the demineralization of dental hard tissue followed by damage inflicted by 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) extending to the area of the pulp.[7] The main microorganisms in the mouth were associated with caries is *Streptococcus mutans* and *Lactobacilli acidophillus*. 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 a polysaccharide polymer glucose, causing dental plaque matrix has a consistency like gelatin, consequently helped bacteria to stick in teeth.[8]

Streptococcus mutans is a Gram-positive bacteria and includes in group varidians. *Streptococcus mutans* are facultative anaerobic, acidogenic that produce acid, which can stay in acidic environment, and produces a sticky polysaccharide called dextran. By the capabilities, *Streptococcus mutans* could supports other bacteria to attach the tooth enamel, supporting growth of acidoduric bacteria and making enamel soluble.[9] Thus resulting in soluble email.[9] *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 to have 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 the 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.[10] *Pterocarpus indiscus* is a type of plant deciduous plant tree with a height of 30-40 metres with a trunk diameter up to more than 2 meters. Usually poor form and 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 6-13cm. Flowers is bisexual, yellow bright and fragrant.[11]

Angsana plant has a large number of uses. Most communities often processe food (bark), latex (resin) and also leave. In some areas, shredded bark is boiled and the liquid is 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.[12] Chemical compounds contained in this plant shows the test positively to phenols, flavonoids, saponins, triterpenoids and tannins.[13] Flavonoid works as an antibacterial by inhibiting synthesis of nucleic acids bacteria and able to inhibit bacterial motility. Flavonoids 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, resulting in bacterial cell death. Meanwhile, the work of the reductase enzyme in bacterial electron transfer processes impaires bacterial growth.[14] *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 a cavity disinfection before placement of the restoration.[15] *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 inducing apoptosis or death of these cells due to cytotoxic effects.[16,17]

Material and methods

This research was a pure laboratory experimental (true experimental), with post-test only design, using a randomized design detailed consisting 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 be 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 sterile distilled water (aquades) and made concentration of 40% and 80%, concentration of 40% w/v created by inserting 2gram 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 5ml.

Sterilization

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

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 incubatation, *Streptococcus mutans* were detected with small form round colonies and the diameter is 1-2µm. Result of bacterial colonies growing for 24 hours suspended in 0.5ml 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 $3x10^8$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 $3x10^8$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, *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

Research has been conducted using 4 treatment, angsana latex 40% w/v, angsana 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 following figure. Figure 1 shows that there are variations in the inhibition zone formed from each treatment group angsana latex 40% w/v shows the average of inhibition zone is 14.6mm, angsana latex 80% w/v is 16.7mm, positive control (*chlorhexidine* 0.2%) had an average of inhibition zone is 16.0mm and negative control (aquades) is 0mm. 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 zones, so concentration of 80% is used as a benchmark to compare with positive and negative controls. Value of angsana latex inhibition zone diameters with concentration of 80%, *chlorhexidine* 0.2% and aquades can be seen in Table 1.

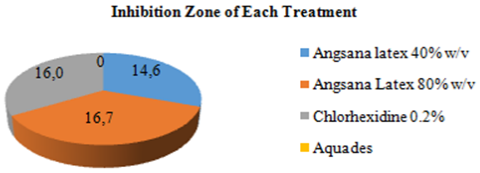

Figure 1 Inhibition zone (mm) of each treatment

Table 1 The average diameter zone of inhibition and standard deviation in each petri dish of angsana latex concentration of 80%, *chlorhexidine* 0.2% and aquades against bacteria *Streptococcus mutans*.

Concentration (%)	Mean (mm) ± SD
Angsana 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 angsana 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 16.667mm. To determine whether there is a significant difference between group, the performed statistical analysis using test One-way ANOVA whose results appear in Table 2. 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 24hours, then continued with Least Significant Difference (LSD) test to determine whether there is difference significantly between each group in the following table. 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.

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	0
Within groups	14.141	15	0.942		
Total	1087.725	17			

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

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

Treatment Groups	Treatment Groups	Mean Difference	Sig.
Chlorhexidine 0.2%	Angsana latex 80% w/v	0.583*	0
Chlorhexidine 0.2%	Aquades	16.083*	0
Angsana latex 80% w/v	Aquades	16.667*	0

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

Discussion

Streptococcus mutans with its ability is supporting other bacteria to stick in tooth enamel that causing dental caries.[18] Cavity cleanser is a material disenfectant 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*.[19] 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 16.667mm while *chlorhexidine* 0.2% had smaller inhibition zones 16.083mm. 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.[20]

Conclusion

Based on this study, it can be conclude that Angsana latex (*Pterocarpus indiscus*) 80%w/v has higher bactericidal effect than *chlorhexidine* 0.2% against bacterial growth of *Streptococcus mutans*. Further research is needed to determine a safe dose of Angsana latex (*Pterocarpus indiscus*) 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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Conflict of interest

The authors declare that there is no conflict of interest.

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