



Antibacterial Effect of Starfruit Extract (*Averrhoa blimbi* L.) against *Streptococcus mutans*

Member Reni Purba^{1*}, Dian Soraya Tanjung², Ilma Al Halifa Hasibuan³

¹Department of Dental Public Health, Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, Medan, Indonesia

²Department of Public Dental Health Sciences, Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, Medan, Indonesia

³Dentistry Study Program, Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia, Medan, Indonesia

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*Corresponding author:

Member Reni Purba

E-mail address:

renimember1060@gmail.com

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ABSTRACT

Dental caries is caused by gram-positive bacteria *Streptococcus mutans*, which is part of the normal flora of the mouth. Herbal medicines have powerful antibacterial compounds that can be used to limit the growth of germs that cause infections. The aim of the research was to determine whether starfruit extract (*Averrhoa blimbi* L.) has antibacterial properties against *Streptococcus mutans*. This type of research uses a posttest only control group design in the laboratory. *Streptococcus mutans* in pure culture were used as the research sample. This study had two control groups, one with four repetitions of DMSO as a negative control and one with a positive control (0.2% chlorhexidine), and four treatment groups with starfruit extract concentrations (20%, 40%, 60%, and 80 %). The disc diffusion method was used to test the antibacterial effect. One-way ANOVA and post hoc LSD statistical tests were then used to test the results. The results showed that, for *Streptococcus mutans* bacteria, the mean and standard deviation of the diameter of the inhibition zone of starfruit extract at concentrations of 20%, 40%, 60%, and 80% were 12.03 ± 0.33 mm, 16.70 ± 0.48 mm, 19.98 ± 0.13 mm, 22.25 ± 0.26 mm, and 19.25 ± 0.91 mm in the positive control, but no zone of inhibition was observed in the negative control. The width of the inhibition zone in starfruit extract (*Averrhoa blimbi* L.) at doses of 20%, 40%, 60%, 80%, positive control, and negative control was significantly different, according to the results of the one-way ANOVA test ($p=0.000$; $p \leq 0.05$). Antibacterial efficacy against *Streptococcus mutans* varied significantly ($p \leq 0.05$) among all star fruit extract groups and positive and negative controls, based on LSD post hoc test findings. Based on the research results, it can be said that star fruit extract at the most effective concentration, namely 80%, has antibacterial properties against *Streptococcus mutans*.

1. Introduction

Dental caries is a condition that affects the hard tissues of the oral cavity due to demineralization of dentin and enamel, closely related to eating foods high in cariogenic components. The unique capacity of deep bacteria is that they consume sucrose to produce a sticky extracellular substance called dextran, which causes the caries process. The enzyme dextransucrase (hexocyltransferase), which relies on polysaccharides,

allows these bacteria to produce plaque. *Streptococcus mutans* is a typical oral flora that includes gram-positive bacteria. They are round in shape, facultative anaerobic, and grouped in chains. These bacteria contribute to the fermentation of carbohydrates, and then they become acidic, which causes demineralization of teeth and oral cavity infections. Antibacterials are compounds that have the ability to stop the growth of bacteria and eradicate

microorganisms that cause infection. The gold standard in dentistry for preventing dental plaque is the antibacterial agent chlorhexidine gluconate 0.2%. Long-term use of chlorhexidine can cause a number of local side effects, including discoloration of teeth, tooth restoration and dorsum of the tongue; ulceration of the oral mucosa and paresthesia; unilateral or bilateral parotid gland swelling; and increased supragingival calculus formation.¹⁻⁵

Utilization of active antibacterial compounds found in medicinal plants is one alternative that can be done. Flavonoids, saponins, and tannins found in natural ingredients such as starfruit have antibacterial properties. This plant, the name starfruit or *Averrhoa bilimbi*, is found throughout Indonesia. Starfruit is widely used in herbal medicine to cure various diseases caused by bacteria. This plant has a high natural vitamin C content, which helps strengthen the body's defenses against disease. The fruit and leaves of the plant are the parts used as medicine.⁶⁻¹⁰ This study aims to determine the antibacterial effect of starfruit extract (*Averrhoa bilimbi* L.) against *Streptococcus mutans*.

2. Methods

This study is experimental research in vitro. This study was carried out using pure cultures of bacteria *Streptococcus mutans*. This study was carried out in six groups, where each group carried out four repetitions. Treatment groups (20%, 40%, 60%, and 80%) starfruit extract, positive control (0.2% chlorhexidine), and negative control (DMSO). This study has received approval from the medical and health research ethics committee of Universitas Prima Indonesia. 500 grams of fresh star fruit are cleaned under running water, drained, then cut into pieces. The star fruit is then blended until smooth. Next, in a closed glass jar, the smooth star fruit is soaked in 3.75 liters of 96% ethanol for five days while shaking once or twice during this time. The results of soaking for five days are filtered to produce filtrate and dregs. Once obtained, the filtrate is collected and stored in a closed

bottle. The remainder was then soaked again for two days while stirring periodically in 1.25 liters of 96% ethanol.

Using a sterile needle and a test tube filled with 5 ml of 0.9% NaCl solution, the test bacteria (*Streptococcus mutans*) were extracted. The bacteria were then diluted and homogenized for 10 minutes using a vortex. Next, compare the turbidity with the McFarland turbidity criteria of 1.5×10^8 cfu/mL. The bacterial suspension showed turbidity when compared with the turbidity threshold of 0.5. Colonies were added to the suspension if they were less turbid, and nutrient broth (NB) was added if they were more turbid. 38g of NA medium, each dissolved in 1 liter of distilled water, boiled to ensure complete dissolution, then in autoclave for 15 minutes at 121°C to sterilize. Transfer into a 5-millimeter thick petri dish. The agar medium can be used after it has cooled. Bacterial colonies were spread evenly with a sterile cotton bud onto NA agar media and Brain Heart Infusion Agar (BHIB) at a concentration determined by the McFarland standard, namely 100 μ . After that, at concentrations of 20%, 40%, and 80%, the blind disk was dipped in starfruit extract. Then, we have to immerse the blind disk into the negative and positive controls. Then, put the agar media along with the paper discs into the incubator and set the temperature to 37°C for a 24-hour cycle. Next, use a digital caliper to measure the width of the drag zone. The diameter of the inhibition zone of the clean area around the paper disk where microorganisms cannot grow is used to calculate the inhibition power. Data analysis was carried out using SPSS version 25 software. Univariate and bivariate analysis was carried out to determine differences in inhibition zone diameter between groups, with $p < 0.05$.

3. Results and Discussion

Table 1 shows the results of a comprehensive one-way ANOVA statistical test to determine the antibacterial action of starfruit extract (*Averrhoa bilimbi* L.) against *Streptococcus mutans*.

Table 1. Antibacterial effect of star fruit extract (*Averrhoa blimbi* L.) against *Streptococcus mutans*.

Group	Inhibition zone diameter	p
	Mean±SD	
Starfruit 20%	12,03±0,33	0,000*
Starfruit 40%	16,70±0,48	
Starfruit 60%	19,98±0,13	
Starfruit 80%	22,25±0,26	
Positive control	19,25±0,91	
Negative control	0	

* One-way ANOVA, $p < 0.05$.

Based on the findings of the one-way ANOVA statistical test, the width of the inhibition zone in starfruit extract (*Averrhoa blimbi* L.) at doses of 20%, 40%, 60%, 80%, positive control, and negative control was significantly different ($p=0.000$; $p \leq 0.05$). When compared to several concentrations of star fruit extract, the best concentration to prevent the growth of *Streptococcus mutans* is 80% (Table 1).

The active ingredients in star fruit extract, including flavonoids, provide antibacterial qualities. Flavonoid compounds as antibacterials can kill bacteria directly and reduce dangerous bacteria. The potential of flavonoids to change the nature of protein bonds in cell membranes and lysis occurs so that chemicals reach the nucleus and change permeability, which can cause stunted development or even death. Phenolic chemicals or macromolecules with some subgroups such as flavonoids, phenolic acids, stilbenes, lignans, tannins, alkaloids, saponins, cardiac glycosides, sterols, and terpenoids are also found in star fruit. By interacting with lipids and membrane proteins, the antibacterial properties of phenolics can reduce membrane contact, increase membrane instability, and increase cytoplasmic membrane hyperpolarization. The result is membrane dysfunction, which results in bacterial cell death.¹¹⁻¹⁶

4. Conclusion

Starfruit extract has antibacterial properties against *Streptococcus mutans* at doses of 20%, 40%, 60% and 80%.

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