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Antibacterial Effects of Star Fruit Extract Against Streptococcus sanguinis

Member Reni Purba^{1*}, Gusbakti Rusip², Salsabilah³

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

²Department of Physiology, 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

rentinember 1000 agniati.com

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ABSTRACT

Streptococus sanguinis is a pioneer bacteria that colonize the oral cavity, plays a role in plaque formation, and contributes to the development of dental caries. Plaque control is needed to inhibit the growth of microorganisms that cause caries by using herbal plants such as starfruit. The aim of the research was to determine whether or not there was an antibacterial effect of starfruit extract (Averrahoa bilimbilin).) against the bacteria Streptococus sanguinis. This research includes laboratory experiments with the sample as a pure culture of Streptococus sanguinis obtained from the Microbiology Laboratory of North Sumatra University Hospital. There were 5 treatment groups (starfruit extract concentration 6.25%, 12.5%, 25%, 50%, 75%), positive control (chlorhexidine 0.2%), and negative control (DMSO). The antibacterial effect was tested using the disc diffusion method. Then, the data was analyzed using statistical tests, one-way ANOVA, and, after this, LSD. The research results were obtained mean, and standard deviation of the diameter of the inhibition zone of star fruit extract, concentrations of 12.5%, 25%, 50%, 75%, and positive control for bacteria Streptococus sanguinis were 7.05 ± 0.192 mm, 9.38 ± 0.171 mm, 13.38 ± 0.171 mm, 14.30 ± 0.216 mm, and 14.38 ± 0.096 mm, while the concentration of star fruit extract was 6.25% and the negative control no inhibition zone was found. Test results from one-way ANOVA revealed that there was a significant antibacterial effect of star fruit extract against the bacteria Streptococus sanguinis ($p \le 0.05$). Test results after this LSD found that the antibacterial effect of the 75% concentration extract was not significant with the positive control, and the 6.25% concentration was not significant with the negative control (p>0.05). From the research results, it can be concluded that the higher the concentration of the extract, the higher the antibacterial effect, with the most effective concentration being 75% and the MIC at a concentration of 12.5%.

1. Introduction

Streptococus sanguinis is one of the gram-positive cocci found in the oral cavity. Streptococus sanguinis acts as a pioneer bacteria, namely bacteria that first colonize the oral cavity. First, these bacteria colonize the tooth that has just fallen out through the toothpellicle. The existence of Streptococus sanguinis can make it easier for other bacteria to stick to the surface of the teeth, for example Streptococus mutans, Streptococcus gordonii, Actinomyces narslindii, Heamophilus parainfluenzae, Prevotella loescheii and *Eikenella corrodens.* This is because *Streptococus sanguinis* has enzyme-forming properties neuraminidase which plays a role in reducing the function of saliva to fight pathogenic bacteria so that plaque forms on the teeth. Plaque formation can be prevented by reducing the attachment of plaquecausing bacteria. Plaque control is one of the prevention measures carried out to reduce the accumulation of plaque that sticks to the surface of the teeth caused by food debris left in the oral cavity. The growth of bacteria that cause dental caries needs to be inhibited so that plaque does not occur. One way is by using herbal plants such as cloves, tea, and starfruit (*Averrahoa bilimbilin*).¹⁻⁵

Starfruit (Averrahoa bilimbilin) is a plant that is often used by Indonesian people. In Indonesia, starfruit is very abundant, usually used as a cooking ingredient in several areas such as Sumatra. This plant contains a lot of natural vitamin C, which is useful for maintaining the body's immune system and can provide protection from various diseases such as coughs, rheumatism, toothache, canker sores, bleeding gums, and others. Apart from that, starfruit also contains vitamins, namely riboflavin, niacin, vitamin B1, vitamin A, ascorbic acid, and carotene, and the minerals are phosphorus, iron, and calcium. Starfruit extract (Averrahoa bilimbilin) contains active compounds such as triterpenoids, saponins, tannins, flavonoids, sulfur, formic acid, and alkaloids. This compound has been proven to have pharmacological effects as an inhibitor of bacterial growth.⁶⁻¹⁰ This study aims to determine the effect of whether or not there is an antibacterial effect of star fruit extract (Averrahoa bilimbilin) against Streptococus sanguinis bacteria.

2. Methods

This study is an in vitro experimental research. Identification, culturing, and testing of bacterial samples *Streptococus sanguinis and* the inhibition zone were carried out at the Integrated Microbiology Laboratory, Faculty of Medicine, Universitas Sumatera Utara. This study uses bacterial cultures *Streptococus sanguinis* in a petri dish. Research subjects were grouped into 7 groups: group 1: negative control (DMSO), group 2: positive control (chlorhexidine 0.2%), groups 3-7: star fruit extract 6.25%-75%, where each group carried out replication of the treatment 4 times. This study has received approval from the medical and health research ethics committee of the Faculty of Medicine, Dentistry and Health Sciences, Universitas Prima Indonesia.

Making the extract consists of collecting, washing, weighing, and drying. 1000 grams of starfruit obtained

from Pancur Batu District were collected purposively. The starfruit is cleaned with running water, drained, and then cut into small pieces. The starfruit is then mashed using a blender and 1 liter of 70% ethanol until smooth, then put into a tightly closed container and stored. The extraction is done by maceration. The smooth starfruit fruit simplicia is left for the first 6 hours. Then let it sit in a jar with a lid and soak for 18 hours, stirring occasionally. After that, the soak is filtered using filter paper so that the filtrate and dregs are obtained and then collected.

The extraction process on the dregs was repeated using 500 ml of 70% ethanol for 24 hours while stirring occasionally and filtering again with filter paper and collecting. All filtrates obtained from the results of both macerations were combined and evaporated using a Rotavapor at a temperature of 40°C until a thick extract was obtained.

MHA (Himedia) powder was weighed using a balance or digital scale in the amount of 38 grams, put in an Erlenmeyer flask, and dissolved in 1 liter of distilled water. The media powder was stirred until a clear solution was formed, and then the Erlenmeyer flask was covered with aluminum foil. Carry out sterilization in an autoclave at 121°C for 15 minutes. The sterile media is poured into the petri dish after it reaches a temperature of 40°-45°C. MHB (Himedia) powder is weighed using a balance or digital scale, 21 grams of which are placed in an Erlenmeyer flask and dissolved in 1 liter of distilled water. The media powder was stirred until a clear solution was formed, and then the Erlenmeyer flask was covered with aluminum foil. Carry out sterilization in an autoclave at 121°C for 15 minutes. The sterile media is poured into the petri dish after it reaches a temperature of 40°-45°C. Inhibition zone measurements used the diffusion method on Mueller Hinton Agar media. The diameter of the clear zone (10-20 mm) has strong inhibition power, the diameter of the clear zone (5-10 mm) has moderate inhibition power, and the diameter of the clear zone (<5 mm) has weak inhibition power.

The data obtained in this research was processed and processed computerized using SPSS v.17.0 software. The statistical test used in the research was to obtain the inhibition zone value from repetition four times with the Descriptive Test, namely mean and standard deviation. To see the difference in the antibacterial effect of the 75% concentration group, 50%; 25%; 12.5%: 6.25%, chlorhexidine 0.2%, and DMSO using one way ANOVA test. As for seeing the difference in antibacterial effect between groups, 75%; 50%; 25%; 12.5%: 6.25%, chlorhexidine 0.2%, and DMSO using Post hoc assay (LSD).

3. Results and Discussion

The test results regarding the difference in antibacterial effects of star fruit extract (*Averrahoa bilimbilin*) against *Streptococus sanguinis* bacteria using the complete post hoc LSD statistical test can be seen in the following table (Table 1).

Table 1. Differences in antibacterial effects of star fruit extracts (*Averrahoa bilimbilin*) against bacteria *Streptococus* sanguinis.

Group		Mean difference	р
Starfruit 6.25%	Starfruit 12.5%	-7,05	0,000*
	Starfruit 25%	-9,38	0,000*
	Starfruit 50%	-13,38	0,000*
	Starfruit 75%	-14,30	0,000*
	Positive control	-14.38	0,000*
	Negative control	0	1,000
Starfruit 12.5%	Starfruit 25%	-2,33	0,000*
	Starfruit 50%	-6.33	0,000*
	Starfruit 75%	-7.25	0,000*
	Positive control	-7.33	0,000*
	Negative control	7,05	0,000*
Starfruit 25%	Starfruit 50%	-4,00	0,000*
	Starfruit 75%	-4,93	0,000*
	Positive control	-5,00	0,000*
	Negative control	9,38	0,000*
Starfruit 50%	Starfruit 75%	-0,93	0,000*
	Positive control	-1,00	0,000*
	Negative control	13,38	0,000*
Starfruit 75%	Positive control	-0,08	0,478
	Negative control	14,30	0,000*
Positive control	Negative control	14,38	0,000*

Information: Post hoc LSD *Significant.

Table 1 shows that there was no difference in the antibacterial effect between the 6.25% concentration of starfruit extract group and the negative control and between the 75% concentration of starfruit extract

group and the positive control (p>0.05), whereas between the other groups, there were differences in effect. significant antibacterial (p \leq 0.05).

The inhibition provided by starfruit extract is due to the compounds contained in it, which disrupt the integrity of cell membranes, inhibit the work of enzymes, interfere with protein and nucleic acid synthesis, and inhibit cell wall synthesis.¹¹⁻¹³ The active compounds contained in starfruit extract (*Averrhoa bilimbi*) are triterpenoids, saponins, tannins, flavonoids, and alkaloids.¹⁴⁻¹⁶ Flavonoid, tannin, and saponin compounds function as antibacterials that can bind proteins in the formation of bacterial cell walls, disrupt the formation of peptidoglycan in bacterial cells, damage the cytoplasmic membrane, which causes the permeability of the bacterial wall to be disturbed so that the bacterial cell wall is not formed completely and the bacteria are lysed.¹⁷⁻¹⁹

4. Conclusion

Starfruit extract (*Averrahoa bilimbilin*) 12.5%, 25%, 50%, and 75% can inhibit bacterial growth in *Streptococcus sanguinis*. From the results of this research, it was also found that a concentration of 75% was the most effective concentration, with a minimum inhibitory content (MIC) value at a concentration of 12.5%.

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