

Effectiveness of Wuluh Starfruit Leaf (*Averrhoa bilimbi* Linn) Extract on The Growth *Streptococcus mutans* In Vitro

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ABSTRACT

Background: Starfruit (*Averrhoa bilimbi* Linn) contains vitamin C, flavonoids, tannins, and triterpenoids. This active compound is reported to prevent the growth of bacteria, including *Streptococcus mutans*. **Objective:** This study aims to determine the effectiveness of starfruit leaf extract (*Averrhoa bilimbi* Linn) in inhibiting *Streptococcus mutans*. **Method:** This type of research is true experimental with a post-test-only control design, where this research uses the Kirby-Baur diffusion method with samples of *Streptococcus mutans* bacteria and uses wuluh leaf extract with concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%. And DMSO solution as a negative control. **Results:** The formation of a clear zone shows the ability of starfruit leaf extract to inhibit the growth of *Streptococcus mutans*, with an average inhibitory diameter of 12.05 ± 0.7188 , 9.50 ± 0.3464 , 65 ± 0.2380 , 7.80 ± 0.4546 , 6.78 ± 0.8016 . **Conclusion:** Based on the research results, it can be concluded that *Averrhoa bilimbi* Linn can inhibit *Streptococcus mutans*.

Keywords: *Averrhoa bilimbi* Linn, Flavonoids, *Streptococcus mutans*

ABSTRAK

Latar Belakang: Belimbing wuluh (*Averrhoa bilimbi* Linn) banyak mengandung vitamin C, flavonoid, tanin, dan triterpenoid. senyawa aktif tersebut dilaporkan dapat mencegah perkembangan bakteri, termasuk *Streptococcus mutans*. **Tujuan:** Penelitian ini bertujuan untuk mengetahui efektivitas ekstrak daun belimbing wuluh (*Averrhoa bilimbi* Linn) dalam menghambat *Streptococcus mutans*. Metode: Jenis penelitian ini adalah true eksperimental dengan desain penelitian post test only control design, dimana penelitian ini menggunakan metode difusi Kirby-Baur dengan sampel bakteri *Streptococcus mutans* dan menggunakan ekstrak daun wuluh dengan konsentrasi 50%, 25%, 12,5%, 6,25%, 3,125%. Dan larutan DMSO sebagai kontrol negatif. **Hasil:** Terbentuknya zona bening menunjukkan kemampuan ekstrak daun belimbing wuluh dalam menghambat pertumbuhan *Streptococcus mutans*, dengan rerata diameter hambat sebesar $12,05 \pm 0,7188$, $9,50 \pm 0,3464$, $65 \pm 0,2380$, $7,80 \pm 0,4546$, $6,78 \pm 0,8016$. **Kesimpulan:** Berdasarkan hasil penelitian dapat disimpulkan bahwa *Averrhoa bilimbi* Linn dapat menghambat *Streptococcus mutans*.

Kata Kunci: *Averrhoa bilimbi* Linn, flavonoid, *Streptococcus mutans*

1. Introduction

Wuluh starfruit (*Averrhoa bilimbi* Linn) is often used in traditional medicine.¹ The WHO survey revealed that about 80% of the world's population's health problems can be treated with medicinal plants. In Indonesia, the preferred treatment and treatment with medicinal plants is currently being promoted in dentistry. One of them is the use of wuluh starfruit leaves in medicine.² Wuluh starfruit leaves are a traditional medicine because active substances, including tannins, sulfur, formic acid, essential oils, and flavonoids, can inhibit bacterial growth.³ Flavonoids are the largest group of phenolic compounds that are effective at inhibiting the growth of viruses, bacteria, and fungi.⁴

One of the main bacteria causing dental caries is *Streptococcus mutans*. These bacteria have a virulence factor that allows them to colonize, forming biofilms that produce acids that destroy dental minerals.⁵ When caries is not treated, it can lead to edentulous and odontogenic infections.^{6,7} Polymicrobials cause odontogenic infections. The pathogenesis of this infection depends on the synergistic relationship between aerobic and anaerobic bacteria. Most odontogenic infections occur

as a result of pulp necrosis. The involvement of bacteria in the periapical tissue, of course, invites an inflammatory response. However, because the host condition is not very good and the virulence is high enough, it will create an abscess.⁸

Handayani's research on "Antibacterial Effectiveness of Wuluh Starfruit Leaves Powder (*Averrhoa bilimbi* Linn). Against the Growth of *Streptococcus mutans* Bacteria" results showed that starfruit leaf powder can inhibit the growth of *Streptococcus mutans* bacteria.³ According to Smullen, this antibacterial activity can also be caused by the tannins and triterpenoids contained therein, which can damage the bacterial cell membrane and porin.⁸ Based on the description above, it can be seen that starfruit leaves have chemical properties that can be used as antibacterials.¹¹ This makes researchers interested in further researching the effectiveness of wuluh starfruit leaf extract (*Averrhoa bilimbi* Linn) on the growth of *Streptococcus mutans* in vitro.

2. Material and Methods

This type of research is experimental with a post-test-only control group design. The sample used was pure bacterial culture *Streptococcus mutans*. Wuluh starfruit leaves were collected purposively from Pancur Batu. Wuluh starfruit leaf extract has been prepared and stored in the Laboratory of Traditional Medicine at the USU Faculty of Pharmacy. The simplicia powder was weighed as much as 100g and then put into a closed container. Add 96% ethanol as much as 1 liter, then stir for the first 6 hours. Let stand for 18 hours while occasionally stirred. Then, filter it using cotton filter paper and reservoir filtrate (Maserat I).

Repeat the extraction process on the dregs using ethanol 96% as much as 0.5 liters to obtain macerate II. Join the two maserates. Steam the mass using a Rotavapor tool at a temperature of 40 °C. Wuluh starfruit leaf extract has been prepared and stored in the laboratory. The extract was made dense by the maceration method. Then various concentrations of extract dilutions were made, namely concentrations of 50%, 25%, 12.5%, 6.25%, and 3.125%. It weighed the extract needed for each last concentration diluted with DMSO to a volume of 10 ml. Antibacterial activity tests carried out several stages: 1) Sterilization of tools, making bacterial media, making media skew, making bacterial culture stock, preparing bacterial inoculums, inhibitory tests, and inhibition zone measurements. The data that has been obtained is then analyzed with one-way ANOVA and LSD tests.

3. Result and Discussion

Wuluh starfruit leaves extracts 50%, 25%, 12.5%, 6.25%, and 3.125% were tested for their antibacterial activity against *Streptococcus mutans* bacteria. Every three discs are placed in Petri dishes for each group. Following is the result after incubating the petri dishes for 24 hours (Table 1).

Table 1. The inhibition zone diameter of wuluh starfruit leaves against *Streptococcus mutans*

Wuluh Starfruit Leaf	Inhibition zone diameter (mm)				
	U1	U2	U3	U4	Mean±SD
50%	13,0	12,2	11,6	11,4	12,05±0,7188
25%	10,0	9,4	9,4	9,2	9,50±0,3464
12,5%	9,7	8,6	8,5	8,5	8,65±0,2380
6,25%	8,4	7,8	7,3	7,7	7,80±0,4546
3,125%	7,9	6,2	6,2	6,8	6,78±0,8016
DMSO	0,0	0,0	0,0	0,0	0,00±0,000

The results showed that the wuluh starfruit leaves extract 50%, 25%, 12.5%, 6.25%, and 3.125% clear around the disc paper in NA medium, indicating the presence of a zone inhibit against the growth of *Streptococcus mutans* bacteria with a high amount different for each replication are, while DMSO was not found the diameter of the inhibition zone. The mean value of the inhibition zone diameter of wuluh starfruit leaves extract 50%, 25%, 12.5%, 6.25%, and 3.125% are 12.05±0.7188; 9.50±0.3464; 8.65±0.2380; 7.80±0.4546; and 6.78±0.8016.

Oneway ANOVA test was used to see the antibacterial effect of wuluh starfruit leaves extract 50%, 25%, 12.5%, 6.25%, and 3.125% and DMSO in the growth treatment group *Streptococcus mutans* bacteria. Oneway ANOVA statistical test results obtained p value = 0.000 (p <0.05). This value indicates that the wuluh starfruit leaves extract 50%, 25%, 12.5%, 6.25%, and 3.125%, and DMSO has

an antibacterial effect on bacterial growth *Streptococcus mutans*. The results of the one-way ANOVA test are presented below (Table 2).

Table 2. One-way ANOVA test results antibacterial effect of wuluh starfruit leaves

Concentration	Mean	SD	p-value
50%	12,05	0,7188	0,000
25%	9,50	0,3464	
12,5%	8,80	0,2380	
6,25%	7,74	0,4546	
3,125%	6,64	0,8016	
DMSO	0,00	0,0000	

The follow-up test was carried out after the one-way ANOVA statistical test. Namely the posthoc test with LSD, which aims to see whether it is there are differences in antibacterial effects between the two treatment groups. The following are the results of the post hoc test LSD. The results of the posthoc test with LSD showed all groups of wuluh starfruit leaves 50%, 25%, 12.5%, 6.25%, 3.125% and DMSO obtained $p < 0.05$ means that there is a significant difference

There are two methods to measure the effectiveness of antibacterial, namely the disk diffusion method and the conventional dilution method. In this study, researchers used the Kirby Bauer diffusion method, which is a method to determine antibacterial activity by the formation of the *Streptococcus mutans* inhibition zone produced by extracts of wuluh starfruit leaves whose inhibitory diameter was measured using a caliper in mm units. The zone of inhibition is an indicator of inhibition against bacteria by forming areas where microorganisms are not grown on the agar medium. The inhibition zone's diameter indicates the bacteria's sensitivity to antibacterial substances. The wider the diameter of the inhibition zone, the more sensitive the bacteria will be.⁹

Davis Stout stated the provisions of the antibacterial strength, namely: resistance of 20 mm or more means very strong, 10-20 mm (strong), 5-10 mm (moderate), and 5 mm or less means weak.¹⁰ Wuluh starfruit leaf extract with a concentration of 3.125% has the lowest inhibitory power in inhibiting the growth of *Streptococcus mutans* bacteria with an average of 6.78 ± 0.8016 mm, categorized as medium potency. In contrast, 50% of starfruit leaf extract has the highest inhibitory power in inhibiting the growth of *Streptococcus mutans* bacteria with a mean of 12.05 ± 0.7188 has a strong potential. At a concentration of 25%, it has a mean of 9.50 ± 0.3464 , which has moderate potential. At a concentration of 12.5%, it has a mean of 8.65 ± 0.2389 , medium potential, and a concentration of 6.25%, which has a mean of 7.80 ± 0.4546 , a moderate potential.

In the repetition results, there was a decrease in the diameter of the inhibition zone. The difference in the inhibition zone is probably caused by the organism's sensitivity, the culture medium, the incubation conditions, and the agar diffusion rate. The rate of agar diffusion is influenced by the concentration of microorganisms, media composition, incubation temperature, and incubation time.¹¹

The results showed that the wuluh starfruit leaves 50%, 25%, 12.5%, 6.25%, and 3.125% showed an inhibition zone around the disc paper in the NA medium against the growth of *Streptococcus mutans*. Different for each replication, whereas DMSO found no inhibition zone diameters. This shows that wuluh starfruit leaves have an antibacterial effect against *Streptococcus mutans*. In line with previous research on the effect of wuluh starfruit leaves on *Streptococcus mutans*, the extract concentrations in blood media were 10.5%, 11%, and 12% to show no bacterial growth. From the description above, it is known that wuluh starfruit leaves have chemical properties that can be used as antibacterial agents.⁹ The results of this study show that the smaller the concentration, the smaller the diameter of the inhibition, and vice versa. This follows the research of Sukandar et al. (2010), who found that the higher the concentration, the more antibacterial active substances are contained, so the antibacterial potential is greater. The concentration affects the antibacterial action of the test material.²

The data from the research results have been tested normality with Shapiro-Wilk and are normally distributed. The results of the oneway Anova statistical test showed that the $p = 0.000$ ($p < 0.05$) that the wuluh starfruit leaves was 50%, 25%, 12.5%, 6.25%, and 3.125%, and DMSO has an

antibacterial effect against *Streptococcus mutans*. Besides being an antibacterial, wuluh starfruit leaves are also used to heal wounds, thaw blood clots, analgesics, diuretics, and treat sore throat.¹² There is an antibacterial effect on wuluh starfruit leaves because some of its active compounds include alkaloids, flavonoids, saponins, tannins, steroids or triterpenoids, and essential oils.¹³ Essential oil is one of the secondary metabolites produced by higher plants and has a vital role for the plants themselves and for human life.¹⁴ Flavonoids and tannins are the most active compounds that act as antibacterials.¹⁵

The mechanism of action of flavonoids as antibacterials is divided into three mechanisms: inhibiting nucleic acid synthesis, cell cytoplasmic membrane function, and energy metabolism. The antibacterial mechanism of flavonoids inhibits nucleic acid synthesis through intercalation or hydrogen bonding by accumulating nucleic acid bases, inhibiting the formation of DNA and RNA.¹⁶ The mechanism of flavonoids inhibiting the function of the cell cytoplasmic membrane is to form complex compounds with extracellular proteins so that they can directly damage the bacterial cell membrane, followed by the release of intracellular compounds and potassium loss.¹⁶ The ability of tannins as antibacterials is related to their ability to deactivate bacterial adhesins, inhibit enzyme action and inhibit protein transport to the inner layer of cells. Tannins also damage the polypeptide components of the cell wall so that the formation of the cell wall is less than perfect. This causes bacterial cells to become lysed due to osmotic and physical pressure so that the bacterial cells will die.¹⁶

Streptococcus mutans is a facultative anaerobic bacteria that can still live in aerobic conditions. If you live in aerobic conditions, tannin compounds will disturb the bacteria. Xie's research showed that tannins affected inhibiting the growth of *Streptococcus mutans* mutans.¹⁷ In this research, the whole wuluh starfruit leaves extract antibacterial test against *Streptococcus mutans* without separating the active compounds. Therefore, it is necessary to carry out further in-vivo research so that this extract can be used clinically as an antibacterial *Streptococcus mutans*

4. Conclusion

The Wuluh starfruit leaf extract (*Averrhoa bilimbi* Linn) has an antibacterial effect on the growth of *Streptococcus mutans* bacteria in vitro.

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Authors Contribution

Contribution	Riza A	Oes A	Siregar IB	Hayati F
Concepts or ideas	√	√	√	√
Design	√	√	√	√
Definition of intellectual content	√			
Literature search	√	√	√	√
Experimental studies	√			
Data acquisition	√	√		√
Data analysis	√	√		√
Statistical analysis	√		√	√
Manuscript preparation	√		√	√
Manuscript editing	√	√		√
Manuscript review	√	√		√



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