

ANTIBACTERIAL EFFECT OF AVERRHOA BILIMBI EXTRACT ON LACTOBACILLUS ACIDOPHILUS ATCC 4356

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ABSTRACT

Introduction: *Lactobacillus acidophilus* is one of the dominant bacteria that plays a significant role in the development of dental caries. This bacterium produces extracellular polysaccharides that facilitate bacterial adhesion to dental plaque. One of the approaches to preventing dental caries is the use of antibacterial agents to inhibit bacterial growth in dental plaque. *Averrhoa bilimbi* (cucumber tree fruit) leaves have the potential to act as antibacterial agents due to the presence of active compounds that can inhibit bacterial growth. **Aim:** To determine the effect of *Averrhoa bilimbi* (cucumber tree fruit) leaf extract on inhibiting the growth of *Lactobacillus acidophilus* ATCC 4356. **Methods:** This study was an experimental laboratory with a post-test only control group design. The concentrations of *Averrhoa bilimbi* leaf extract used were 6.25%, 12.5%, 25%, and 50%, and a negative control of aquadest (sterile water). **Results:** The analysis with One Way ANNOVA showed p -value < 0.05 , indicating a significant effect of *Averrhoa bilimbi* leaf extract in inhibiting the growth of *Lactobacillus acidophilus* ATCC 4356. **Conclusion:** *Averrhoa bilimbi* (cucumber tree fruit) leaf extract has an effect on inhibiting the growth of *Lactobacillus acidophilus* ATCC 4356. These findings suggest the potential of *Averrhoa bilimbi* leaf extract as a natural antibacterial agent

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INTRODUCTION

Dental caries is a common oral disease worldwide.¹ The Global Burden of Disease Study

estimates that the incidence of dental caries has increased from 1990 to 2019.² According to the World Health Organization's (WHO) Oral Health Status Report in 2023, approximately two billion individuals suffer from permanent dental caries, and 514 million children experience dental caries in their primary teeth. These statistics indicate that dental caries remains a significant global oral health issue.² In Indonesia, dental caries ranks as the sixth most prevalent disease affecting the population.³

Dental caries begins with the formation of plaque on the tooth surface.⁴ Plaque consists of a collection of bacteria that develop within a matrix known as a biofilm.⁵ The dominant bacteria involved in the development of dental caries are *Streptococcus mutans* and *Lactobacillus acidophilus*. These bacteria convert carbohydrates in food into acids through the fermentation process. Continuous exposure to carbohydrates gradually acidifies the oral cavity, leading to demineralization of the teeth.⁶

This bacterium accelerates the demineralization process, leading to the formation of dental caries.⁷ *Lactobacillus acidophilus* is found in dental plaque, and its colony count has a positive correlation with individuals who have active dental caries.⁸ This is linked to the bacterium's ability to thrive in acidic environments, resulting in an increased colony count during the progression of dental caries. Additionally, *Lactobacillus acidophilus* is capable of producing extracellular polysaccharides that facilitate bacterial adhesion to dental plaque.⁹

Dental caries can be prevented by controlling plaque.¹⁰ Plaque control inhibits the growth and metabolism of bacterial colonization within the biofilm.¹⁰ Antibacterial compounds used for chemical plaque control can be derived from both natural and synthetic sources.¹¹ Long-term use of artificial substances is not recommended, as it may trigger side effects in the oral cavity. These side effects include discomfort, tooth discoloration, and desquamation of the oral mucosa.¹² This underscores the need for

alternative antibacterial agents derived from natural sources.¹³

One of Indonesia's native plants recognized for its medicinal properties is *cucumber tree fruit* (*Averrhoa bilimbi* L.).¹⁴ This plant is effective in treating various ailments, including itching, gum inflammation, mouth ulcers, skin rashes, mumps, rheumatism, hypertension, diabetes, and digestive disorders.¹⁵ *Cucumber tree fruit* (*Averrhoa bilimbi* L.) leaves are a safe, affordable, and environmentally friendly traditional medicine.¹⁶ These leaves contain flavonoids, saponins, tannins, and alkaloids, which can inhibit the growth of viruses, bacteria, and fungi.^{15,17} These compounds are capable of inhibiting carbohydrate fermentation activity in bacteria, making them useful for preventing plaque formation on teeth.¹⁸ According to existing research, the author is interested in investigating the effect of *cucumber tree fruit* leaf extract on inhibiting the growth of *Lactobacillus acidophilus* ATCC 4356 at concentrations of 6.25%, 12.5%, 25%, and 50%.

METHODS

This research was a laboratory-based experimental study (true experimental) using a posttest-only control group design. The study was conducted at the Organic Chemistry of Natural Materials Laboratory and the Microbiology Laboratory Universitas Andalas (UNAND). The preparation of *cucumber tree fruit* (*Averrhoa bilimbi* L.) leaf extract began with plant identification at the Herbarium Laboratory Unand. The sample used in this study was a pure culture of *Lactobacillus acidophilus* ATCC 4356 obtained

from the Microbiology Laboratory UNAND. This research has been approved by the UNAND Ethics Committee with number 166/UN.16.2/KEP-FK/2024.

This study consisted of six treatment groups: *cucumber tree fruit* (*Averrhoa bilimbi* L.) leaf extract at concentrations of 6.25%, 12.5%, 25%, and 50%, a negative control with aquadest. The *cucumber tree fruit* (*Averrhoa bilimbi* L.) leaf extract was prepared using the maceration method, in which 500 grams of *cucumber tree fruit* (*Averrhoa bilimbi* L.) leaves were weighed, placed in a maceration vessel, and soaked in 70% ethanol solvent. The extraction process was conducted over three days, with stirring for five minutes each day. A concentrated *cucumber tree fruit* leaf extract was obtained by evaporating the solvent using a rotary evaporator.

The inhibitory effect of *cucumber tree fruit* (*Averrhoa bilimbi* L.) leaf extract on the growth of *Lactobacillus acidophilus* ATCC 4356 was tested using the Kirby-Bauer disc diffusion method with MRS-A as the test medium. The bacterial suspension was prepared using an MRS-B medium, with the suspension standardized to a 0.5 McFarland turbidity. The paper discs were soaked for 15 minutes in each treatment and control group solution, then placed on MRS-A medium inoculated with *Lactobacillus acidophilus* ATCC 4356, followed by incubation for 48 hours at 37°C. The inhibition zones formed around the paper discs were measured using calipers. The data obtained from these measurements were processed and analyzed using One-Way ANOVA and Post Hoc LSD (Least Significant Differences) tests.

RESULTS

The results of this study indicate that *cucumber tree fruit* (*Averrhoa bilimbi* L.) leaf extract at concentrations of 6.25%, 12.5%, 25%, and 50% can inhibit the growth of *Lactobacillus acidophilus* ATCC 4356, whereas distilled water has no inhibitory effect on the growth of *Lactobacillus acidophilus* ATCC 4356. Distilled water, as a negative control, is a neutral substance, which accounts for its lack of effect on the growth of *Lactobacillus acidophilus* ATCC 4356.

Table 1. Average Inhibition Zone Values for Treatment and Control Groups

Treatment Group	Sample	Mean ± Standard Deviation
Leaf extract of cucumber tree fruit 6,25%	6	0,994 ± 0,199
Leaf extract of cucumber tree fruit 12,5%	6	1,320 ± 0,311
Leaf extract of cucumber tree fruit 25%	6	1,610 ± 0,243
Leaf extract of cucumber tree fruit 50%	6	2,062 ± 0,288
Aquadest	6	0,000 ± 0,000

Table 1 shows that the inhibition zones formed by the *cucumber tree fruit* (*Averrhoa bilimbi* L.) leaf extract treatment groups were classified as having weak inhibitory power, with the largest inhibition zone observed at the 50% concentration and the smallest at the 6.25% concentration.

Table 2. Post Hoc LSD Test Results

Treatment Group	12,5%	25%	50%	Aquadest
Ekstrak 6,25%	0,037*	0,000*	0,000*	0,000*
Ekstrak 12,5%		0,061	0,000*	0,000*
Ekstrak 25%			0,005*	0,000*
Ekstrak 50%				0,000*

The Post Hoc LSD Test in Table 2 shows a p-value of <0.05, indicating a significant effect among *cucumber tree fruit* (*Averrhoa bilimbi* L.)

leaf extract at concentrations of 6.25%, 12.5%, 25%, and 50% and distilled water as the negative control, in inhibiting the growth of *Lactobacillus acidophilus* ATCC 4356.

DISCUSSION

The inhibition zones observed in the *cucumber tree fruit* (*Averrhoa bilimbi* L.) leaf extract treatment groups are attributed to the presence of active compounds that act as antibacterial agents. Studies by Yanti and Vera (2019), Hasim et al. (2019), and Panjaitan et al. (2017) have shown that *Averrhoa bilimbi* L. leaf extract contains flavonoids, saponins, tannins, and alkaloids.^{19–21} Flavonoids interfere with enzyme activity and bacterial cell wall synthesis, leading to cell lysis and the release of intracellular materials. Saponins reduce the surface tension of cell membranes, facilitating the penetration of antibacterial compounds into the cell and causing damage to the bacterial cytoplasm. Tannins can inactivate bacterial adhesins, resulting in membrane disruption. Alkaloids form bonds with DNA and react with amino acids to alter the structure and composition of bacterial amino acids, thereby inducing lysis in the bacterial cell nucleus.^{21,22}

This study demonstrates an increase in the inhibition zone values with higher concentrations of *cucumber tree fruit* (*Averrhoa bilimbi* L.) leaf extract. These results are consistent with Saputera's study in 2018, which examined the effect of *Averrhoa bilimbi* L. leaf extract on the growth of *Streptococcus mutans*.¹² The study found that as the concentration of *cucumber tree*

fruit leaf extract increased, the resulting inhibition zone also grew larger.¹² Several previous studies have explained that the concentration level influences the quantity of active compounds extracted. This occurs because higher concentrations of the extract contain greater amounts of active compounds, thereby enhancing its antibacterial activity. The concentration of the extract also affects the rate of diffusion, where higher concentrations facilitate faster diffusion, thus resulting in larger inhibition zones.^{21,23}

The One-Way ANOVA statistical analysis revealed a p-value of <0.05, indicating a significant effect of *Averrhoa bilimbi* L. leaf extract at concentrations of 6.25%, 12.5%, 25%, and 50%, in inhibiting the growth of *Lactobacillus acidophilus* ATCC 4356. A study conducted by Saputera et al. in 2018 reported that the methanol extract of *Averrhoa bilimbi* L. leaves at concentrations of 6.25%, 12.5%, 25%, 50%, and 100% exhibited an inhibitory effect on the growth of *Streptococcus mutans*. The findings of this study align with the present research, which also demonstrates antibacterial activity in *Averrhoa bilimbi* L. leaf extract, although there are differences in the size of the inhibition zones produced. Saputera et al. (2018) found that the smallest inhibition zone was formed at a concentration of 6.25%, measuring 10.08 mm, while the largest inhibition zone occurred at a concentration of 100%, measuring 19.30 mm.¹² These differences may be attributed to the variation in solvents used for extracting *Averrhoa bilimbi* L. leaves. Each solvent type has distinct abilities and characteristics in extracting

antibacterial compounds from natural sources, depending on the polarity of the solvent and the compounds being extracted. This aligns with the principle of "like dissolves like," which explains that a compound tends to dissolve in a solvent with similar chemical properties.^{24,25}

CONCLUSION

This study demonstrated that *Averrhoa bilimbi* (cucumber tree fruit) leaf extract exhibits antibacterial activity against *Lactobacillus acidophilus* ATCC 4356. Inhibitory effects were observed at concentrations of 6.25%, 12.5%, 25%, and 50%, indicating a concentration-dependent relationship. These findings suggest the potential of *Averrhoa bilimbi* leaf extract as a natural antibacterial agent.

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