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ORIGINAL ARTICLE

Comparison of bacang mango leaf extract concentrations (25%, 50%, and 75%) in inhibiting Streptococcus mutans in vitro

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ABSTRACT

Observations in Pancur Batu District, Deli Serdang Regency, revealed an abundant growth of Bacang mango trees (*Mangifera foetida*), yet their potential benefits remain underutilized by the local community. This study aimed to investigate and provide insight into the antibacterial properties of Bacang mango leaf extract. An experimental laboratory study was conducted using a randomized posttest-only control group design. The results demonstrated that Bacang mango leaf extract effectively inhibited the growth of *Streptococcus mutans* in vitro, surpassing the efficacy of 0.2% chlorhexidine digluconate used as a positive control. Measurement of the inhibition zone diameters indicated a proportional relationship between extract concentration and antibacterial efficacy. At 25% concentration, the mean inhibition zone diameter was 13.15 ± 0.49 mm. Increasing the concentration to 50% yielded a larger diameter of 15.75 ± 0.66 mm, while the highest antibacterial activity was observed at 75% concentration, with an inhibition zone diameter of 16.73 ± 0.55 mm. In comparison, the 0.2% chlorhexidine digluconate positive control produced an inhibition zone diameter of only 12.50 ± 0.26 mm. These findings suggest that Bacang mango leaf extract at 75% concentration is the most effective in inhibiting *Streptococcus mutans* growth. Its significantly greater inhibitory effect relative to lower concentrations and the positive control highlights its potential as a natural antibacterial agent.

Keywords: Bacang mango leaf extract, dental caries, *Streptococcus mutans*

Introduction

Streptococcus mutans is a primary pathogenic agent in the development of dental caries.[1,2] This Gram-positive bacterium ferments carbohydrates into acids, leading to demineralization of the dental enamel.[3,4] Extracts from the leaves of Mangifera foetida lour (commonly known as mangga bacang) have long been recognized for their potential antibacterial properties. However, their specific efficacy in inhibiting the growth of *S. mutans* requires further elucidation through in-depth research. The antibacterial activity is hypothesized to derive from bioactive compounds present in the leaves, such as mangiferin.[5]

Several previous studies have demonstrated that mangga bacang leaf extracts possess inhibitory effects against various bacterial species. Aseng[6] reported that a combined infusion of mangga bacang leaves and aloe vera exhibited significant *in vitro* antibacterial activity against *Escherichia coli*. Furthermore, Lestari and Pambudi[5] found that disinfectant formulations based on mangga bacang leaf extract were effective against *Staphylococcus aureus*. Additionally, Setiawan et al.[7] confirmed the antibacterial potential of

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methanolic extracts of mangga bacang leaves against *Enterobacter aerogenes*. The pathogenic similarities among these bacteria and *S. mutans* suggest that comparable antibacterial effects may be observed with the extract.

Despite considerable research on the antibacterial properties of mangga bacang leaf extracts, investigations specifically focusing on the inhibitory effects against *S. mutans* remain limited. Therefore, this study aims to comprehensively evaluate the impact of mangga bacang leaf extract on the growth of *S. mutans*. This research is imperative given that dental caries continues to pose a significant global health challenge, and there is an ongoing need to develop safe and effective natural antimicrobial therapies. In particular, this study will investigate the mechanism of action of active compounds such as mangiferin, with the expectation of providing novel insights pertinent to preventive dentistry.

This study holds substantial relevance and potential contribution to the field. Beyond addressing gaps in scientific knowledge, the findings are anticipated to serve as a foundation for the development of more effective caries prevention products. By elucidating the mechanisms through which mangga bacang leaf extracts exert their effects, this research may facilitate the utilization of natural agents in oral care formulations, ultimately contributing to the improvement of public oral health.

Method

Study Design

This study employed a laboratory experimental design using a randomized posttest-only control group design. The extraction of Bacang mango leaf was conducted at the Phytochemical Laboratory, Faculty of Pharmacy, University of Sumatera Utara. The antibacterial efficacy testing of the extract against *Streptococcus mutans* was performed at the Microbiology Laboratory, Faculty of Pharmacy, University of Sumatera Utara. The entire research was carried out from February 2025 to March 2025.

Sampling

The population consisted of pure cultures of *Streptococcus mutans*. Samples comprised bacterial cultures obtained from the Microbiology and Phytochemical Laboratories, Faculty of Pharmacy, University of Sumatera Utara. Samples were divided into five treatment groups: Group I received 25% extract concentration, Group II 50%, and Group III 75%. Group IV, treated with 2% chlorhexidine digluconate, served as the positive control, and Group V, treated with DMSO, served as the negative control. Sample replication was determined using the Federer formula: $(t - 1)(n - 1) \geq 15$, where t is the number of treatment groups and n is the number of replications. With five groups ($t = 5$), the calculation $(5 - 1)(n - 1) \geq 15$ simplifies to $4n - 4 \geq 15$, yielding $n \geq 4.75$. Therefore, each group was replicated six times to minimize bias, resulting in a total sample size of 30 (5 groups × 6 replications). The primary material used was Bacang mango leaf. Inclusion criteria required leaves to be fresh and intact, whereas exclusion criteria encompassed leaves that were old, wilted, decayed, or physically damaged.

Variables

The independent variable was the treatment concentration of Bacang mango leaf extract at 25%, 50%, and 75%. The dependent variable was the diameter of the inhibition zone formed around the disk, measured to evaluate antibacterial activity against *Streptococcus mutans*. Controlled variables—maintained to avoid confounding effects—included the growth medium, incubation temperature and duration (37°C for 24 hours), sterilization of instruments and materials, time of observation, geographic origin of the Bacang mango leaves, and standardization of the extraction process.

Equipment and Materials

Equipment utilized included separatory funnels, autoclaves, ovens, electronic scales, thermometers, timers, face shields, petri dishes, test tube racks, incubators, vials, gloves, cotton swabs, calipers, Bunsen burners, forceps, parchment paper, blenders, stirring rods, filter paper, Erlenmeyer flasks, beakers, rotary evaporators, porcelain dishes, mortar and pestle, reagent bottles, micropipettes, and measuring cylinders. Materials comprised Bacang mango leaves, pure cultures of *Streptococcus mutans*, 70% ethanol, distilled water, DMSO (dimethyl sulfoxide), methanol (spiritus), Nutrient Agar (NA) media, and 2% chlorhexidine digluconate solution as the positive control.

Procedures

Bacang mango leaves were purposively sampled from Meliala Plantation, Deli Serdang, North Sumatera. Collected leaves were sorted, washed, and dried. A total of 250 grams of dry leaves was ground into a fine powder using a blender and stored in sterile containers. The leaf powder was macerated with 2 liters of 70% ethanol in a closed container for 24 hours, protected from sunlight. The filtrate was separated from the residue, which was subjected to a second maceration with 1 liter of 70% ethanol to maximize yield. Combined filtrates were concentrated with a rotary evaporator at 50°C until a viscous extract was obtained. The concentrated extract was diluted with 10% DMSO to produce a stock solution, which was further diluted to final treatment concentrations of 25%, 50%, and 75%. Glassware was sterilized in an oven at 170°C for 90 minutes, and culture media were sterilized in an autoclave at 121°C for 15 minutes. Nutrient Agar (NA) slants were prepared by dissolving 2 grams of NA powder in 100 ml distilled water, heated until homogeneous, and sterilized. Pure *Streptococcus mutans* cultures were aseptically inoculated onto NA slants and incubated at 37°C for 24 hours for renewal. Bacterial suspensions were prepared by suspending colonies in 0.9% NaCl solution to match the McFarland 0.5 standard, corresponding to approximately 1.5 × 10^8 CFU/ml. Antibacterial activity was assessed using the Kirby-Bauer disk diffusion method. Bacterial suspensions were evenly swabbed onto NA plates, and sterile paper disks impregnated with each extract concentration (25%, 50%, 75%), 2% chlorhexidine (positive control), and DMSO (negative control) were placed on the agar surface. Plates were incubated at 37°C for 24 hours, after which inhibition zone diameters around each disk were measured.

Data Analysis

Quantitative data, represented by inhibition zone diameters, will be tabulated and graphed. Statistical significance of differences in antibacterial efficacy among treatment groups will be analyzed using one-way Analysis of Variance (ANOVA) with SPSS software version 20.

Results

Inhibition Zone Diameter Test Results

The diameter of the inhibition zones produced by mango bacang leaf extract (*Mangifera foetida* Lour.) at various concentrations (25%, 50%, and 75%), as well as 2% chlorhexidine digluconate (CHX) and dimethyl sulfoxide (DMSO), on *Streptococcus mutans* growth was measured using the disc diffusion method. The clear zones formed around the paper discs were measured with a caliper, and the results are presented in Table 1.

Table 1. Inhibition zone diameter of mango bacang leaf extract (*Mangifera foetida* Lour.) against *Streptococcus mutans*

Group	Diameter of Inhibition Zone (mm)						Mean ± SD
	Replicate						
	1	2	3	4	5	6	
Mango Bacang Leaf Extract 25%	13,6	13,8	13	13,2	12,5	12,8	13,15 ± 0,49
Mango Bacang Leaf Extract 50%	16,9	16,1	15,6	15,2	15,1	15,6	15,75 ± 0,66
Mango Bacang Leaf Extract 75%	17,3	17,3	17	16,1	16,1	16,6	16,73 ± 0,55
Chlorhexidine Digluconate 2% (CHX)	12,4	12,5	12,9	12,6	12,1	12,5	12,50 ± 0,26
DMSO (Negative Control)	0	0	0	0	0	0	0,00 ± 0,00

Table 1 shows that mango bacang leaf extract exhibited inhibitory activity against *Streptococcus mutans* at concentrations of 25%, 50%, and 75%, with mean inhibition zone diameters of 13.15 ± 0.49 mm, 15.75 ± 0.66 mm, and 16.73 ± 0.55 mm, respectively. The 2% chlorhexidine digluconate control demonstrated a mean inhibition zone diameter of 12.50 ± 0.26 mm, while the negative control (DMSO) showed no inhibitory effect. Notably, the 75% concentration of mango bacang leaf extract produced the largest mean inhibition zone against *S. mutans*.

Normality Test Results

For sample sizes less than 50, the Shapiro-Wilk test is appropriate for assessing data normality. The Shapiro-Wilk test results indicated that the inhibition zone diameter data for mango bacang leaf extract against *Streptococcus mutans* were normally distributed, as evidenced by p-values greater than 0.05 (Table 2).

Table 2. Normality Test Results (Shapiro-Wilk)

	Group	p value (Shapiro Wilk)
Inhibition Zone Diameters	Mango Bacang Leaf Extract 25%	0,91
	Mango Bacang Leaf Extract 50%	0,36
	Mango Bacang Leaf Extract 75%	0,17
	Chlorhexidine Digluconate 2% (CHX)	0,80

One-Way ANOVA Test

A one-way ANOVA was conducted to determine whether there were statistically significant differences in the antibacterial efficacy against Streptococcus mutans among the tested groups. The analysis yielded a p-value of 0.000 ($p < 0.05$), indicating significant differences in inhibition zone diameters among the groups, as shown in Table 3.

Table 3. One-Way ANOVA Test

	Group	n	Mean	*p-value*
Inhibition Zone Diameters	Mango Bacang Leaf Extract 25%	6	13,15	0,000
	Mango Bacang Leaf Extract 50%	6	15,75	
	Mango Bacang Leaf Extract 75%	6	16,73	
	Chlorhexidine Digluconate 2% (CHX)	6	12,50	
	DMSO	6	0	

Differences in Inhibitory Effect of Mango Bacang Leaf Extract Against Streptococcus mutans

Post hoc analysis using the Least Significant Difference (LSD) test was performed to examine differences in inhibition zones between pairs of groups. The results are summarized in Table 4.

Table 4. LSD Post Hoc Comparison Between Groups

	Group	*Mean diff*	p
Mango Bacang Leaf Extract 25%	Mango Bacang Leaf Extract 50%	-2,60*	0,00
	Mango Bacang Leaf Extract 75%	-3,58*	0,00
	Chlorhexidine Digluconate 2% (CHX)	0,65*	0,02
	DMSO	13,15*	0,00
Mango Bacang Leaf Extract 50%	Mango Bacang Leaf Extract 75%	-0,98*	0,001
	CHX 2%	3,25*	0,00
	DMSO	15,75*	0,00
Mango Bacang Leaf Extract &5%	CHX 2%	4,23*	0,00
	DMSO	16,73*	0,00
CHX 2%	DMSO	12,50*	0,00

**Significant difference ($p < 0.05$)*

The LSD post hoc test revealed significant differences in inhibition zone diameters against Streptococcus mutans among all groups ($p < 0.05$). The 25% mango bacang leaf extract showed significant differences when compared to the 50% and 75% concentrations, as well as when compared to the 2% chlorhexidine digluconate and DMSO controls.

Discussion

The extract of bacang mango leaves contains various phytochemicals, including saponins, terpenoids, phenols, and flavonoids, which play critical roles as antibacterial agents.[5] Each compound exerts its antibacterial effect via distinct mechanisms. Saponins, for instance, disrupt the bacterial cytoplasmic membrane, compromising cellular homeostasis and ultimately causing cell death.[8] Terpenoids exhibit lipophilic properties, enabling interactions with transmembrane proteins (porins), thereby inhibiting the uptake of essential nutrients, which leads to bacterial cell death.[9] Phenols act by oxidizing and damaging the bacterial cell wall, depleting metabolic substrates, and inactivating critical enzymes. Phenolic compounds can also bind adhesin proteins, hindering bacterial adherence.[10] Lastly, flavonoids induce damage to the cytoplasmic membrane through protein denaturation and coagulation, disrupting vital cellular functions and resulting in bacterial cell death.[11]

This study evaluated the antibacterial efficacy of bacang mango leaf extract against *Streptococcus mutans* at three concentrations: 25%, 50%, and 75%. Chlorhexidine digluconate 0.2% served as the positive control, while dimethyl sulfoxide (DMSO) was used as the negative control. Antibacterial activity was

assessed using the disc diffusion method, where the presence of a clear inhibition zone around the paper disc indicates antibacterial efficacy. The diameter of the inhibition zone correlates positively with antibacterial potency. Inhibition strength was classified according to criteria established by Davis and Stout, where an inhibition diameter of 10 to 20 mm is considered strong.[12]

Results demonstrated that all concentrations of the mango leaf extract produced inhibition zones classified as strong. The mean inhibition zone diameters were 13.15 ± 0.49 mm at 25%, 15.75 ± 0.66 mm at 50%, and 16.73 ± 0.55 mm at 75% concentration. The positive control, chlorhexidine digluconate 0.2%, also exhibited strong antibacterial activity with a mean inhibition zone diameter of 12.50 ± 0.26 mm. In contrast, the negative control (DMSO) showed no antibacterial activity.

Statistical analysis using one-way ANOVA revealed a significant difference among treatment groups and controls ($p = 0.000$, $p < 0.05$), confirming the notable antibacterial activity of bacang mango leaf extract against *S. mutans* in vitro. These findings align with previous studies by Irfan[13] and Munadiyah[14], which reported increased antibacterial potency with higher extract concentrations. Variations between studies may be attributable to external factors, such as geographic and temporal differences in sample collection and the type of solvents employed during extraction.

In conclusion, the bacang mango leaf extract effectively inhibits the growth of *S. mutans*, showing greater efficacy at a 75% concentration compared to chlorhexidine digluconate 0.2%. These results support its potential application as a natural agent for dental caries prevention.

Conclusion

In conclusion, the ethanolic extract of *Mangifera foetida Lour.* (mangga bacang) leaves demonstrates significant, concentration-dependent antibacterial activity against the cariogenic bacterium *Streptococcus mutans*. All tested concentrations (25%, 50%, and 75%) exhibited strong inhibitory effects, with the 75% concentration proving to be the most potent. Notably, the antibacterial efficacy of all extract concentrations was statistically superior to that of the 2% Chlorhexidine digluconate positive control. These findings strongly suggest that the bioactive compounds within *M. foetida* leaf extract are highly effective at inhibiting *S. mutans* growth. Therefore, this extract represents a promising natural alternative for incorporation into oral hygiene products aimed at the prevention and control of dental caries.

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