

## ORIGINAL ARTICLE

# Antibacterial activity of mangosteen (*Garcinia mangostana* Linn.) leaf ethanol extract against oral pathogenic bacteria: a laboratory experimental study

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## KEYWORDS

Antibacterial, mangosteen, *porphyromonas gingivalis*, *aggregatibacter actinomycetemcomitans*, *streptococcus sanguinis*

## ABSTRACT

**Introduction:** Nature-based medicines are multi-component agents that work in a multi-targeted manner with minimal side effects. Mangosteen leaves (*Garcinia mangostana* L.) contain flavonoids, tannins, and saponins that serve as antibacterial agents. This research aimed to analyze alternative agents to chlorhexidine mouthwash derived from natural ingredients. **Methods:** This laboratory experimental study observed the inhibition zone diameters of mangosteen leaf ethanol extract at concentrations ranging from 3.125% to 100% against *P. gingivalis*, *A. actinomycetemcomitans*, and *S. sanguinis* using the disc diffusion method. One-Way ANOVA followed by Tukey HSD tests were used to analyze the statistical differences among the bacterial groups across all concentrations. **Results:** The 100% concentration exhibited the largest inhibitory zones:  $14.78 \pm 0.60$  mm for *P. gingivalis*,  $11.08 \pm 0.02$  mm for *A. actinomycetemcomitans*, and  $10.55 \pm 0.48$  mm for *S. sanguinis*. One-Way ANOVA and Tukey HSD tests showed a p-value of 0.000, indicating a significant antibacterial effect against *P. gingivalis*, *A. actinomycetemcomitans*, and *S. sanguinis*. **Conclusion:** Ethanol extract of mangosteen leaves (*Garcinia mangostana* L.) exhibits significant antibacterial activity, with the highest inhibitory effect observed at a 100% concentration.

## INTRODUCTION

Periodontal disease is a significant dental and oral health problem in Indonesia, with a fairly high prevalence ranging from 60-74.1%. Periodontal disease affects about 20-50% of the world's population. According to the World Health Organization (WHO), the prevalence of periodontal disease in adults ranges from 36-63% in developing countries and 14-47% in developed countries.<sup>1</sup> Globally, approximately 11% of the world population may have severe

periodontitis, affecting 743 million individuals.<sup>2</sup> The main cause of periodontal disease is bacteria in the accumulation of plaque and calculus. Poor oral hygiene promotes the accumulation of plaque and calculus on the teeth and gingival surfaces, which is strongly associated with increased disease prevalence and severity.<sup>3</sup>

Plaque is a complex of bacterial biofilms on the surface of teeth, including pathogenic microorganisms that invade periodontal tissue. Calculus or tartar is a calcification of plaque that can accumulate in the supragingival and subgingival areas. Periodontal disease is the result of the interaction between pathogens and the immune system. Damage to periodontal tissue can occur if bacterial pathogenicity is more dominant to the immune response.<sup>4</sup>

The most dominant bacteria in causing periodontal disease is *Porphyromonas gingivalis*. This bacterium is a type of anaerobic gram-negative bacteria that lives in the subgingival area and causes the accumulation of pro-inflammatory prostaglandins and cytokines that result in periodontal tissue damage. In this regard, *Porphyromonas gingivalis* (*P. gingivalis*) is a key pathogen in the oral microbiome and a Gram-negative oral anaerobic bacterium that plays a key role in the pathogenesis of periodontitis.<sup>5,6</sup> In addition, *Aggregatibacter actinomycetemcomitans* is a Gram-negative oral pathobiont that is associated with severe form of periodontitis. The leukotoxin of *Aggregatibacter actinomycetemcomitans* is one of the main virulence factors of this bacterium which can destroy host immune tissues.<sup>7</sup> *Streptococcus sanguinis* is a pioneering colonizer, aiding in the attachment of succeeding organisms, and a key player in oral biofilm development. As a facultative anaerobic species, *Streptococcus sanguinis* is abundant in both supragingival and subgingival plaque.<sup>8</sup> Chlorhexidine is an antimicrobial agent.<sup>9</sup>

It is commercially available in the forms of mouth rinse, gel, varnish, toothpastes, sprays, and sugar free chewing gum.<sup>10-12</sup> It acts on the inner cytoplasmic membrane; hence, it is a membrane active type of substance. It is dicationic at pH levels above 3.5. It prevents plaque accumulation, hence it is an antiplaque and antigingivitis agent and reduces the adherence of *porphyromonas gingivalis* to epithelial cells. It can be bacteriostatic or bactericidal depending on the dose. It acts against a wide array of bacteria including gram-positive and gram-negative bacteria, dermatophytes, and lipolytic viruses.<sup>10,11</sup> Chlorhexidine gluconate at a concentration of 0.2% is regarded as the gold standard in the reduction of plaque formation and as a local bacterial compound due to its broad-spectrum antimicrobial action and high substantivity.<sup>10,13</sup>

However, chlorhexidine use may be associated with several adverse effects, including taste alteration, tooth and tongue staining, oral mucosal irritation or discomfort, and xerostomia, which may limit its long-term acceptability.<sup>14</sup> In addition, the oral cavity has been recognized as a reservoir of antimicrobial resistance, further supporting the need to explore alternative plant-derived antibacterial agents for oral health applications.<sup>15</sup> Research needs to be done to find alternative agents to chlorhexidine mouthwash derived from natural ingredients.<sup>16</sup> Antibacterial agents that are expected to be used as alternatives, one of which is mangosteen (*Garcinia mangostana* L.).<sup>17,18</sup>

*Garcinia mangostana* L. has attracted considerable attention due to the presence of bioactive compounds such as xanthenes, flavonoids, tannins, and other phenolic compounds, which exhibit antioxidant, anti-inflammatory, and antimicrobial activities.<sup>19</sup> Previous studies on mangosteen have predominantly focused on the fruit pericarp rather than the leaves and have reported antibacterial activity against several pathogenic bacteria.<sup>20</sup> In contrast, mangosteen leaves also contain flavonoids, tannins, and saponins, which may contribute to their antibacterial potential.

Mangosteen (*Garcinia mangostana* L.) is highly beneficial for the health of the body because it contains xanthenes which can function as antioxidants, anti-inflammatory and antimicrobials.<sup>21</sup> The part used in this study is the mangosteen

leaf (*Garcinia mangostana* L.) which will be determined and maximized. The solvent used in the manufacture of mangosteen leaf ethanol extract (*Garcinia mangostana* L.) is 96% ethanol, based on the level of safety and ease when evaporated and its properties that are able to dissolve almost all substances, both polar, semipolar, and nonpolar and can attract flavonoid compounds optimally.<sup>22</sup>

Previous studies on *Garcinia mangostana* have predominantly focused on the fruit pericarp, particularly because it contains high levels of xanthenes and other bioactive compounds with antibacterial properties.<sup>23</sup> However, other parts of the plant, including the leaves, remain relatively underexplored despite containing phytochemical compounds such as flavonoids, tannins, and saponins that may contribute to antibacterial activity. Compared to the fruit peel, mangosteen leaves are more readily available and renewable plant material, making them a potential alternative source of natural antibacterial agents.

Therefore, this study investigated the antibacterial activity of mangosteen leaf ethanol extract against three oral bacterial species, namely *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus sanguinis*. These bacteria are important oral microorganisms associated with periodontal disease and dental biofilm formation. Evaluating the antibacterial effects against these three bacteria simultaneously provides a broader assessment of the antibacterial potential of mangosteen leaves and highlights their possible application as a natural ingredient for mouthwash formulations as an alternative to chlorhexidine.

## METHODS

This study was a laboratory experimental study using a post-test only control group design. The observed variable was the diameter of the inhibition zone produced by the ethanol extract of mangosteen leaves (*Garcinia mangostana* L.) at concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, 75%, and 100% against three oral bacteria. The inhibition zone diameter was measured using a caliper in millimeters (mm).

In this study, 0.2% chlorhexidine was used as the positive control, while 10% dimethyl sulfoxide (DMSO) served as the negative control. Mangosteen leaves were collected from Puspahiang Village, Puspahiang District, Tasikmalaya Regency, West Java Province, Indonesia. Plant identification (determination) was performed at the Biosystematics and Molecular Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University.

The extract was prepared using the maceration extraction method. Maceration is a technique that involves soaking plant material in a suitable solvent to dissolve bioactive compounds, usually at room temperature and without high heating.<sup>24</sup> In this study, 96% ethanol was used as the extraction solvent because it can dissolve both polar and semi-polar phytochemical compounds. Distillation was used to remove excess water from ethanol with high moisture content. Phytochemical screening was conducted to identify the presence of major secondary metabolites including alkaloids, flavonoids, tannins, saponins, and terpenoids using standard qualitative phytochemical tests. Alkaloids were detected using Dragendorff's reagent, flavonoids using the Shinoda test, tannins using the ferric chloride test, saponins using the foam test, and terpenoids using the Liebermann–Burchard reaction. These tests were performed to confirm the presence of bioactive compounds in the mangosteen leaf extract.

The mangosteen leaf ethanol extract was diluted using 10% DMSO to obtain concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, 75%, and 100%. Antibacterial activity was evaluated using the disc diffusion (Kirby–Bauer) method against *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus sanguinis*. These bacteria were selected because they are important oral microorganisms associated with dental plaque formation and periodontal

disease, and *P. gingivalis* and *A. actinomycetemcomitans* are widely recognized as major periodontal pathogens.<sup>25</sup>

Bacterial colonies previously cultured on Mueller–Hinton Agar (MHA) were inoculated into Mueller–Hinton Broth (MHB) to obtain active bacterial cultures. The bacterial suspension was homogenized using a vortex mixer, and its turbidity was adjusted to match the 0.5 McFarland standard, corresponding to approximately  $1.5 \times 10^8$  CFU/mL. The bacterial suspension was then evenly spread onto the surface of MHA plates using a sterile cotton swab.

Sterile paper discs impregnated with the extract solutions were placed on the inoculated agar surface, and the plates were incubated at 37 °C for 18–24 h. All procedures followed the Clinical and Laboratory Standards Institute guidelines for antimicrobial susceptibility testing. Antibacterial activity was determined by measuring the diameter of the inhibition zones around each disc.<sup>26</sup> The antibacterial activity was interpreted based on inhibition zone diameter categories: <5 mm indicates no antibacterial activity, 5–10 mm indicates moderate activity, 10–20 mm indicates strong activity, and >20 mm indicates very strong antibacterial activity.

All experiments were performed in triplicate, and the results were expressed as mean  $\pm$  standard deviation (SD). The number of replications was determined using the Federer experimental design formula  $(t-1)(r-1) \geq 15$ . Based on this criterion, three replications were considered sufficient for the number of treatment groups used in this study. Data normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene’s test. Since the data were normally distributed and homogeneous ( $p > 0.05$ ), differences in inhibition zone diameters among treatment groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc test. A  $p$ -value  $< 0.05$  was considered statistically significant.

## RESULTS

Plant identification was first conducted at the Biosystematics and Molecular Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University. The identification results confirmed that the mangosteen leaves obtained from Puspahiang Village belong to the species *Garcinia mangostana* L. (Table 1).

**Table 1. Mangosteen Leaf Determination Results (*Garcinia mangostana* L.)**

Information	Identification Results
Scientific Name	<i>Garcinia mangostana</i> L.
Synonym	<i>Mangostana</i> <i>Garcinia</i> Gaertn.
Local Name	Mangosteen
Family	<i>Clusiaceae</i>

A total of 600 g of mangosteen leaves were submitted to the Aretna Medika Utama Laboratory for extraction. The resulting extract was subsequently subjected to phytochemical screening, and the results are presented in Table 2. The antibacterial activity of the mangosteen leaf ethanol extract was evaluated against *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus sanguinis*. The mean inhibition zone diameters  $\pm$  standard deviation (SD) for each bacterial species at different extract concentrations are shown in Table 3. The results showed varying levels of antibacterial activity among the tested bacterial species. Overall, the extract demonstrated inhibitory effects against all three oral pathogens, although the magnitude of inhibition differed among the bacteria. These findings indicate that the antibacterial efficacy of mangosteen leaf ethanol extract depends on the bacterial species tested.

**Table 2 Phytochemical Testing of Mangosteen Leaf Extract Concentration.**

Active Biological Components of Mangosteen Leaf Extract	Phytochemical Test Results
Phenolic	++++
Tannins	++++
Flavonoids	++++
Saponins	++++
Triterpenoids	++++
Terpenoids	++++
Alkaloids	++++

Information:  
 +++++: very high content  
 ++++: High content  
 ++ : medium content  
 + : low content  
 -: Negative/does not contain

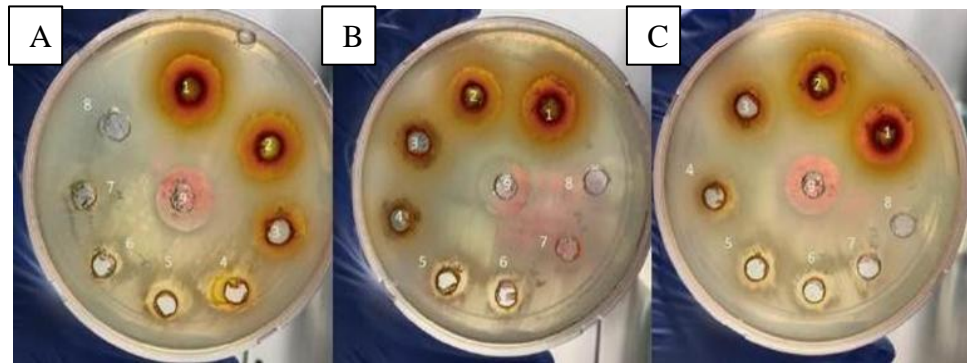
**Table 3. Mean inhibition zone diameters (mm) of mangosteen (*Garcinia mangostana* L.) leaf ethanol extract against *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus sanguinis***

Treatment	<i>Porphyromonas gingivalis</i>	<i>Aggregatibacter actinomycetemcomitans</i>	<i>Streptococcus sanguinis</i>
Positive Control ( <i>Chlorhexidine</i> 0,2%)	16.69 ± 0.60 <sup>a</sup>	34.15 ± 0.04 <sup>a</sup>	18.10 ± 0.06 <sup>a</sup>
Negative Control (DMSO 10%)	0.00 ± 0.00 <sup>g</sup>	0.00 ± 0.00 <sup>g</sup>	0.00 ± 0.00 <sup>g</sup>
Mangosteen Leaf Extract 100%	14.78 ± 0.60 <sup>ab</sup>	11.08 ± 0.02 <sup>b</sup>	10.55 ± 0.48 <sup>b</sup>
Mangosteen Leaf Extract 75%	14.02 ± 0.95 <sup>bc</sup>	8.67 ± 0.45 <sup>c</sup>	9.41 ± 0.46 <sup>c</sup>
Mangosteen Leaf Extract 50%	13.53 ± 0.53 <sup>bcd</sup>	7.48 ± 0.35 <sup>d</sup>	6.09 ± 0.07 <sup>d</sup>
Mangosteen Leaf Extract 25%	12.16 ± 0.21 <sup>cd</sup>	3.06 ± 0.07 <sup>e</sup>	4.66 ± 0.64 <sup>e</sup>
Mangosteen Leaf Extract 12.5%	11.48 ± 1.43 <sup>de</sup>	1.13 ± 0.09 <sup>f</sup>	1.09 ± 0.10 <sup>f</sup>
Mangosteen Leaf Extract 6.25%	9.79 ± 0.34 <sup>e</sup>	0.00 ± 0.00 <sup>g</sup>	0.00 ± 0.00 <sup>g</sup>
Mangosteen Leaf Extract 3.125%	6.47 ± 0.90 <sup>f</sup>	0.00 ± 0.00 <sup>g</sup>	0.00 ± 0.00 <sup>g</sup>

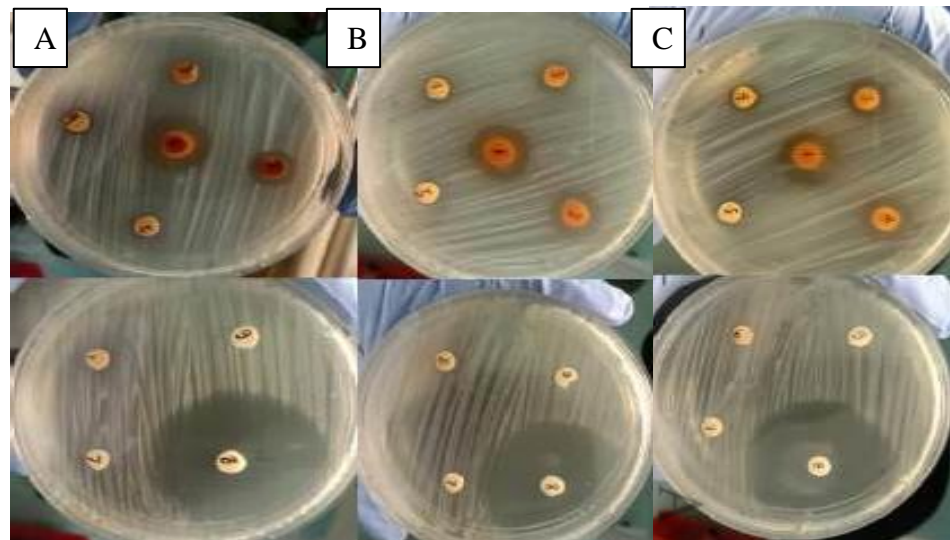
Values are presented as mean ± standard deviation (SD) from three independent replicates. Different superscript letters within the same column indicate statistically significant differences among treatment groups for each bacterial species ( $p < 0.05$ , Tukey HSD test). CHX = chlorhexidine; DMSO = dimethyl sulfoxide.

The antibacterial activity of mangosteen leaf ethanol extract against *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus sanguinis* is presented in Table 3. The results indicate that the extract exhibited inhibitory effects against all tested bacteria, with the largest inhibition zones generally observed at higher extract concentrations. The positive control (0.2% chlorhexidine) produced the largest inhibition zones for all bacterial species, while the negative control (10% DMSO) showed no inhibitory activity.

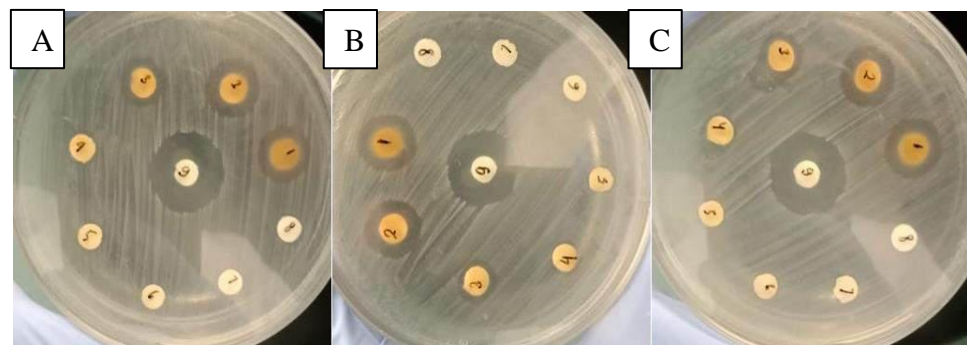
Overall, *P. gingivalis* showed greater susceptibility to the extract compared with *A. actinomycetemcomitans* and *S. sanguinis*. Statistical analysis using one-way ANOVA showed significant differences in inhibition zone diameters among treatment groups ( $p < 0.05$ ). The Tukey HSD post-hoc test further revealed significant differences between several extract concentrations, as indicated by different superscript letters in Table 3. Higher concentrations of mangosteen leaf extract generally produced significantly larger inhibition zones compared with lower concentrations, indicating a concentration-dependent antibacterial effect.



**Figure 1.** Inhibition Zone of Mangosteen Leaf Ethanol Extract (*Garcinia mangostana L.*) against the bacterium *Porphyromonas gingivalis*. (A) first replication. (B) Second replication. (C) Third replication. Group 1 mangosteen leaf extract 100%; Group 2 mangosteen leaf extract 75%; Group 3 mangosteen leaf extract 50%; Group 4 mangosteen leaf extract 25%; Group 5 mangosteen leaf extract 12,5%; Group 6 mangosteen leaf extract 6,25%; Group 7 mangosteen leaf extract 3,125%; Group 8 Negative Control DMSO10%; Group 9 positive control chlorhexidine 0,2%.



**Figure 2.** Inhibition Zone of Mangosteen Leaf Ethanol Extract (*Garcinia mangostana Linn.*) against bacterium *Aggregatibacter Actinomycetemcomitans*. First Replication (A), Second Replication (B), and Third Replication (C), Group 1 mangosteen leaf extract 100%; Group 2 mangosteen leaf extract 75%; Group 3 mangosteen leaf extract 50%; Group 4 mangosteen leaf extract 25%; Group 5 mangosteen leaf extract 12,5%; Group 6 mangosteen leaf extract 6,25% ; Group 7 mangosteen leaf extract 3,125%; Group 8 positive control chlorhexidine 0,2%. Group 9 Negative Control DMSO10%.



**Figure 3.** Results of observation of inhibitory zone at various concentrations of mangosteen leaf ethanol extract against *Streptococcus sanguinis* bacteria. (A) first replication. (B) Second replication. (C) Third replication. Group 1 mangosteen leaf extract 100%; Group 2 mangosteen leaf extract 75%; Group 3 mangosteen leaf extract 50%; Group 4 mangosteen leaf extract 25%; Group 5 mangosteen leaf extract 12,5%; Group 6 mangosteen leaf extract 6,25%; Group 7 mangosteen leaf extract 3,125%; Group 8 Negative Control DMSO10%; Group 9 positive control chlorhexidine 0,2%.

The inhibition zone diameters produced by mangosteen leaf ethanol extract against *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus sanguinis* are presented in Table 3. In general, higher extract concentrations produced larger inhibition zones, indicating stronger antibacterial activity. Among the tested bacteria, *P. gingivalis* showed the highest susceptibility to the extract, as indicated by the largest inhibition zone diameters across most concentrations. In contrast, *A. actinomycetemcomitans* and *S. sanguinis* demonstrated lower susceptibility, particularly at lower extract concentrations where no inhibition zones were observed. The positive control (0.2% chlorhexidine) produced the largest inhibition zones for all tested bacteria, while the negative control (10% DMSO) showed no antibacterial activity. These results indicate that the antibacterial activity of mangosteen leaf ethanol extract is concentration-dependent and varies among bacterial species.

**Table 4. Classification of Inhibition Zone against *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus sanguinis*.**

Treatment	<i>Porphyromonas gingivalis</i> ,	<i>Aggregatibacter actinomycetemcomitans</i>	<i>Streptococcus sanguinis</i>
Mangosteen Leaf Extract 100%	Strong	Strong	Strong
Mangosteen Leaf Extract 75%	Strong	Moderate	Moderate
Mangosteen Leaf Extract 50%	Strong	Moderate	Moderate
Mangosteen Leaf Extract 25%	Strong	-	-
Mangosteen Leaf Extract 12.5%	Strong	-	-
Mangosteen Leaf Extract 6.25%	Moderate	-	-
Mangosteen Leaf Extract 3.125%	Moderate	-	-

Based on the inhibition zone classification presented in Table 4, the antibacterial activity of mangosteen leaf ethanol extract varied depending on the extract concentration and bacterial species. At higher concentrations (100%, 75%, and 50%), the extract generally showed strong antibacterial activity against *Porphyromonas gingivalis*. In contrast, the antibacterial effect against *Aggregatibacter actinomycetemcomitans* and *Streptococcus sanguinis* was mostly moderate at similar concentrations.

Among the tested bacteria, *Porphyromonas gingivalis* exhibited the highest sensitivity to the mangosteen leaf extract, as indicated by the larger inhibition zone diameters observed at several concentrations. Conversely, *Aggregatibacter actinomycetemcomitans* showed the highest resistance, with smaller inhibition zones and no inhibitory effect observed at lower extract concentrations. *Streptococcus sanguinis* demonstrated intermediate susceptibility compared with the other tested bacteria.

The normality test results showed that the data were normally distributed ( $p > 0.05$ ). The homogeneity test indicated that the variance among groups was homogeneous ( $p = 0.084$ ). Therefore, a one-way ANOVA was conducted to analyze the differences in inhibition zone diameters among treatment groups. The analysis revealed a statistically significant difference among the groups ( $p < 0.05$ ) with an F value of 142.136, indicating that the treatments had significantly different antibacterial effects.

**Table 5. One Way ANOVA Result.**

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	620.109	8	77.154	142.136	0
Within Groups	9.816	18	0.545		
Total	629.925	26			

The Tukey Honestly Significant Difference (HSD) test was conducted as a post hoc analysis following the one-way ANOVA to identify specific pairwise differences among treatment groups against *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus sanguinis* at a significance level of  $\alpha = 0.05$ . The table presents the adjusted p-values for multiple comparisons between the positive control (chlorhexidine 0.2%), negative control (DMSO 10%), and various concentrations of mangosteen leaf extract [Table 6].

The results indicate that the negative control group showed significant differences compared with almost all treatment groups ( $p < 0.05$ ), confirming the absence of antibacterial activity in the solvent control. In contrast, the positive control demonstrated statistically significant differences when compared with most extract concentrations, except for certain lower concentrations where no significant difference was observed ( $p > 0.05$ ), suggesting comparable inhibitory effects.

## DISCUSSION

The higher the concentration of ethanol extract of mangosteen leaves (*Garcinia mangostana* L.) then the greater the inhibition against the growth of the bacteria produced. The strongest inhibitory force is at a concentration of 100% against all three bacteria. At concentrations of 50% and 75%, the inhibition was strong against *Porphyromonas gingivalis* [Table 4], while against *Aggregatibacter actinomycetemcomitans* and *Streptococcus sanguinis*, it had moderate inhibition.

The differences in antibacterial susceptibility among the tested bacteria may be related to variations in their cell wall structure, metabolic characteristics, and ecological roles in oral biofilms. *Porphyromonas gingivalis* is an obligate anaerobic Gram-negative bacterium that possesses a relatively permeable outer membrane, which may facilitate the penetration of bioactive phytochemical compounds such as flavonoids, tannins, and saponins present in mangosteen leaf extract. These compounds can disrupt bacterial membranes and interfere with enzymatic and metabolic processes, resulting in stronger antibacterial effects.

In contrast, *Streptococcus sanguinis* is a facultative anaerobic early colonizer of dental biofilms and has different physiological characteristics that may influence its tolerance to certain antimicrobial compounds. The structural composition of its cell envelope and its adaptive metabolic pathways may reduce its susceptibility to certain plant-derived antibacterial agents. Consequently, the inhibitory effect observed against *S. sanguinis* was lower than that against *P. gingivalis* in this study. The results of the study using the One Way ANOVA and Tukey HSD tests showed that the p-value was  $< 0.05$ , so  $H_0$  was rejected, which means that there is an antibacterial effect between ethanol extract of mangosteen leaves (*Garcinia mangostana* L.) on the bacteria *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus sanguinis*. The higher the concentration of mangosteen leaf ethanol extract (*Garcinia mangostana* L.), the greater the inhibition against the growth of the three bacteria.

These findings are consistent with previous studies investigating the antibacterial properties of *Garcinia mangostana* L extract inhibiting the growth of *S. mutans* and *P. gingivalis* in biofilms significantly compared to the negative control ( $P < 0.05$ ). The most effective concentration and incubation time for inhibiting biofilm growth was 100% in 6 h for *Streptococcus mutans* and 100% in

24 h for *Porphyromonas gingivalis*. Based on the results of this study, the authors also observed that achieving maximum antimicrobial activity from mangosteen peel extracts required longer incubation periods and higher concentrations. Previous studies have reported that xanthone-containing mangosteen peel extract exhibits significant antimicrobial activity against oral microorganisms, including *Candida albicans* and *Streptococcus mutans*, in in vitro experiments.<sup>23,27</sup> Oral biofilms are formed through several successive stages, starting with the formation of an acquired pellicle in the form of a thin layer of salivary glycoproteins attached to the tooth surface. Then, the primary bacteria begin to form colonies that can change the surrounding conditions to be more suitable for the growth of obligate anaerobic bacteria.<sup>28</sup>

A recent systematic review and meta-analysis reported that *Garcinia mangostana* extracts exhibit significant antibacterial activity against various Gram-positive and Gram-negative bacteria.<sup>28</sup> The findings of this study have important implications for the development of alternative antibacterial agents. The increasing prevalence of antibiotic-resistant pathogens has become a major global health concern, highlighting the urgent need to explore natural products as alternative antimicrobial agents. The effectiveness of *G. mangostana* extracts against resistant strains of bacteria could provide a complementary approach to traditional antibiotics, potentially reducing reliance on synthetic antimicrobial agents.<sup>29</sup>

Mangosteen, a tropical plant native to Indonesia, has a long-standing tradition of use in herbal medicine for various health conditions, including cancer, malaria, immune modulation, cardiovascular diseases, atherosclerosis, hypertension, and thrombosis. The therapeutic properties of mangosteen are primarily attributed to its rich content of secondary metabolites, particularly xanthones, which constitute approximately 40% of the peel. Xanthones are polyphenolic cyclic ketone compounds known for their pharmacological activities, including antioxidant, anti-proliferative, anti-inflammatory, and antimicrobial effects.<sup>30</sup> These compounds exhibit a range of mechanisms that target bacterial cells, making them effective against both Gram-positive and Gram-negative bacteria.<sup>29</sup>

Mangosteen contains various bioactive phytochemical compounds such as flavonoids, phenolic compounds, tannins, saponins, alkaloids, and triterpenoids, which have been reported to exhibit antibacterial activity. These compounds can disrupt bacterial cell membranes, interfere with enzyme activity, and inhibit bacterial metabolic processes, thereby suppressing bacterial growth, including oral pathogens such as *Porphyromonas gingivalis*.<sup>23,31</sup> Flavonoids exert antibacterial activity by disrupting bacterial cell membrane integrity and interfering with cellular metabolic processes. These compounds can form complexes with extracellular and soluble proteins, leading to membrane damage and inhibition of bacterial energy metabolism.<sup>32–34</sup> Saponins caused severe damage to bacteria through disruption of the cytoplasmic membrane and membrane proteins, resulting in leakage of cell contents.<sup>34,35</sup> This disruption results in membrane damage and the subsequent release of essential cellular components, including proteins, nucleic acids, and nucleotides, ultimately leading to bacterial cell lysis.<sup>35–37</sup>

Tannins can interact with proteins through hydrogen bonding and hydrophobic interactions, forming tannin–protein complexes that may disrupt bacterial cell membrane proteins and affect membrane permeability.<sup>38,39</sup> Tannins exhibit antibacterial activity by interacting with bacterial cell wall components, particularly peptidoglycan structures, which disrupt cell wall integrity and may lead to bacterial cell lysis.<sup>40–42</sup> Tannins also possess the ability to inactivate bacterial enzymes and interfere with protein processes within the cell.<sup>43,44</sup> Steroids induce leakage in bacterial liposomes and reduce membrane integrity as well as cell membrane morphology. Terpenoids bind to transmembrane proteins, resulting in altered cell wall permeability. Alkaloids disrupt the formation of peptidoglycan in bacterial cell walls.<sup>43</sup>

The antibacterial activity of *Garcinia mangostana* is primarily attributed to its rich content of bioactive compounds, particularly xanthenes, which are polyphenolic compounds known for their diverse pharmacological effects. Research indicates that the xanthone content in mangosteen peel can reach up to 40%, contributing to its antioxidant, anti-inflammatory, and antimicrobial properties.<sup>29</sup> Xanthone, saponins, terpenoids, tannins, and flavonoid compounds in mangosteen peel have antimicrobial activity. Xanthone can retard cell replication. Saponin can increase surface tension of bacteria cell walls so it will be strained very strongly and cause cell membrane destruction since the most important components such as protein, nucleic acid, and nucleotide serve as bacteria survival, out of the cell. The lipophilic terpenoid can destroy cell membrane, and then tannin can inactivate bacteria cell adhesion (attached molecule at host cell) existing in cell surface which is able to inhibit protein transport enzyme through cell membrane, while flavonoid has very active property to slow down bacteria.<sup>30</sup>

*Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus sanguinis* are categorized as gram-negative bacteria, a classification that can be attributed to differences in their cell wall thickness. Gram-negative bacteria possess a relatively thick peptidoglycan layer (ranging from 2 to 7 nm) situated between an inner and an outer membrane. The outer membrane, which measures approximately 7 to 8 nm in thickness, is composed of lipids, proteins, and lipopolysaccharides. The primary structural component of the bacterial cell wall, peptidoglycan (also known as murein), provides rigidity and is essential for maintaining cell integrity and shape.<sup>25,31-33</sup>

In gram-negative bacteria, such as *Escherichia coli*, the structure includes a double membrane system, where the plasma membrane is enveloped by a permeable outer membrane. In contrast, the cell wall of gram-positive bacteria consists of a single layer that contains 1 to 4% lipids, whereas the cell wall of gram-negative bacteria is characterized by three layers, which include lipoproteins, a phospholipid outer membrane, and lipopolysaccharides.<sup>25,31-33</sup>

The extract used in this study was a crude ethanol extract, and the specific active compounds responsible for the antibacterial effects were not isolated or quantified. Although phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, tannins, saponins, and alkaloids, variations in compound concentration and potential synergistic or antagonistic interactions were not explored.

Clinically, these findings suggest that mangosteen leaf extract may serve as a potential alternative or adjunct to chlorhexidine, particularly for patients who experience adverse effects associated with long-term chlorhexidine use. The moderate to strong antibacterial activity observed at higher extract concentrations indicates possible application in the formulation of herbal mouthwashes, gels, or local delivery systems aimed at reducing oral bacterial load while minimizing side effects.

Several limitations should be considered when interpreting the findings of this study. First, the antibacterial activity was evaluated using the disc diffusion method, which provides preliminary information on antimicrobial effects but does not determine minimum inhibitory concentration (MIC) values or bactericidal mechanisms. Second, the extract used in this study was a crude ethanol extract, and the specific active compounds responsible for the antibacterial activity were not isolated or quantified. Third, the experimental design was limited to in vitro conditions, which may not fully reflect the complex environment of the oral cavity.

Future research should focus on isolating and characterizing the bioactive compounds responsible for the antibacterial activity of mangosteen leaves. Additional studies using MIC and MBC assays, biofilm models, and in vivo experiments involving more oral microorganisms are needed to further evaluate the therapeutic potential of mangosteen leaf extract in oral health care.

## CONCLUSION

The ethanol extract of mangosteen leaves (*Garcinia mangostana* L.) exhibited significant, concentration-dependent antibacterial activity against *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus sanguinis*, with the highest inhibition observed at a 100% concentration. Implication of this research shows promise for incorporation into oral healthcare products such as mouthwashes or gels. Further studies, including MIC, biofilm, and in vivo evaluations, are required to support its clinical application and product development.

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