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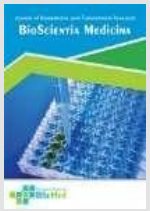

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Guava Leaf Extract: A Promising Alternative to Chlorhexidine for Reducing *Streptococcus mutans* Colonization on Orthodontic Appliances

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A B S T R A C T

Background: *Streptococcus mutans* is a major contributor to the formation of dental plaque and the initiation of caries. Orthodontic appliances, particularly removable ones, can create favorable conditions for *S. mutans* colonization, increasing the risk of caries and other oral health issues. Chlorhexidine is a commonly used antimicrobial agent in dentistry, but it can have side effects like tooth staining and altered taste. Guava leaf extract has shown promising antibacterial properties due to its rich content of flavonoids, tannins, and other bioactive compounds. This study aimed to compare the effectiveness of guava leaf extract and chlorhexidine in reducing *S. mutans* colonization on acrylic-based removable orthodontic appliances. **Methods:** This in vitro study used 25 acrylic plates, which were divided into five groups: guava leaf extract at concentrations of 75%, 80%, and 90%, chlorhexidine gluconate 0.2% (positive control), and aquades (negative control). The acrylic plates were first contaminated with *S. mutans* and then immersed in the respective solutions for 10 minutes. The number of *S. mutans* colonies was then counted using a colony counter. **Results:** The mean number of *S. mutans* colonies was significantly lower in the chlorhexidine group (27.8 ± 6.6 CFU/ml) and the guava leaf extract groups (9.4 ± 3.3 CFU/ml for 90%, 42 ± 7.8 CFU/ml for 80%, and 381 ± 81.1 CFU/ml for 75%) compared to the aquades group (1461.2 ± 274.5 CFU/ml). There was no significant difference between the chlorhexidine group and the 90% and 80% guava leaf extract groups. **Conclusion:** Guava leaf extract, particularly at concentrations of 90% and 80%, is as effective as chlorhexidine in reducing *S. mutans* colonization on orthodontic appliances. Guava leaf extract may be a promising natural alternative to chlorhexidine for maintaining oral hygiene in orthodontic patients, especially those with concerns about chlorhexidine's side effects.

1. Introduction

Malocclusion, a condition characterized by the misalignment of teeth and jaws, presents a significant oral health concern worldwide, affecting millions of individuals across the globe. This prevalent issue can have far-reaching consequences, impacting not only the aesthetics of one's smile but also essential functions such as chewing, speech, and overall facial harmony. The World Health Organization (WHO) recognizes the gravity of malocclusion, ranking it as the third most common oral health problem globally, underscoring the urgent need for effective orthodontic interventions. Orthodontic treatment, encompassing a range of techniques and appliances, aims to correct malocclusion and restore proper oral function and aesthetics. While traditional fixed appliances, commonly known as braces, have long been the mainstay of orthodontic treatment, removable appliances have gained significant popularity in recent years. These appliances offer patients greater

flexibility and convenience, allowing for easier removal during eating, cleaning, and special occasions. However, the use of removable appliances, particularly those made from acrylic-based materials, introduces unique challenges in maintaining optimal oral hygiene.[1-3]

The oral cavity harbors a complex and dynamic ecosystem of microorganisms, collectively known as the oral microbiome. While many of these microorganisms play beneficial roles in maintaining oral health, certain species, particularly *Streptococcus mutans*, are recognized as primary contributors to dental caries, the most common chronic disease affecting humans. *S. mutans* thrives in the presence of dietary sugars, producing acids that erode tooth enamel, leading to the formation of cavities and other oral health complications. Removable orthodontic appliances, with their porous acrylic surfaces, provide an ideal environment for bacterial adhesion and biofilm formation. Biofilms are complex communities of microorganisms embedded in a self-produced extracellular matrix, offering protection from environmental stressors and antimicrobial agents. *S. mutans*, a key player in oral biofilm formation, readily colonizes these appliances, increasing the risk of caries development, especially in orthodontic patients. Chlorhexidine gluconate, a broad-spectrum antimicrobial agent, has long been a cornerstone of oral hygiene protocols in dentistry. Its widespread use stems from its effectiveness in controlling the growth of various oral pathogens, including *S. mutans*. However, despite its antimicrobial prowess, chlorhexidine is not without its drawbacks. Concerns have been raised regarding its potential side effects, such as tooth staining, taste alteration, and the emergence of bacterial resistance, prompting the search for alternative antimicrobial strategies. In the quest for safer and more sustainable antimicrobial solutions, natural plant extracts have garnered significant attention. Guava (*Psidium guajava* L.), a tropical fruit widely consumed for its nutritional value, has also emerged as a promising source of natural antimicrobial agents. Guava leaves, traditionally used in various folk remedies, have been found to possess potent antibacterial properties, attributed to their rich phytochemical composition.[4-7]

Guava leaves harbor a diverse array of bioactive compounds, including flavonoids, tannins, and terpenoids, which contribute to their remarkable antimicrobial activity. Flavonoids, particularly quercetin, have demonstrated strong antibacterial effects against *S. mutans* and other oral pathogens. These compounds disrupt bacterial cell wall integrity, inhibit bacterial enzymes, and interfere with bacterial adhesion, effectively hindering their growth and colonization. Tannins, another important class of phytochemicals found in guava leaves, also play a crucial role in combating bacterial proliferation. Tannins exert their antimicrobial action by precipitating bacterial proteins and inhibiting bacterial enzymes, further compromising their survival and virulence. The potential of guava leaf extract as a natural antimicrobial alternative to chlorhexidine has sparked considerable interest in the field of dentistry. Its effectiveness in reducing *S. mutans* colonization, coupled with its favorable safety profile, makes it an attractive candidate for oral hygiene applications, particularly in orthodontic patients.[8-10] This study aimed to investigate the antimicrobial efficacy of guava leaf extract against *S. mutans* colonization on acrylic-based removable orthodontic appliances.

2. Methods

This in vitro study employed a meticulous and comprehensive methodological approach to investigate the antimicrobial efficacy of guava leaf extract against *Streptococcus mutans* colonization on acrylic-based removable orthodontic appliances. The study design, sample preparation, extract preparation, phytochemical screening, bacterial culture and contamination, treatment procedures, colony count determination, and statistical analysis are described in detail below.

A post-test only control group design was utilized to assess the antimicrobial activity of guava leaf

extract and chlorhexidine against *S. mutans*. Twenty-five acrylic plates, each measuring 10 x 10 x 2 mm, were fabricated from cold-cured acrylic resin, a commonly used material for removable orthodontic appliances. These plates were randomly assigned to five distinct groups, with five plates in each group, ensuring a balanced representation of experimental conditions. The five groups were as follows; K+ (Positive Control): This group served as the positive control and consisted of acrylic plates treated with 0.2% chlorhexidine gluconate, a standard antimicrobial agent used in dentistry; K- (Negative Control): This group acted as the negative control and involved acrylic plates treated with aquades (sterile distilled water), providing a baseline for bacterial growth without any antimicrobial intervention; 75%: In this group, acrylic plates were treated with a 75% concentration of guava leaf extract, allowing for the evaluation of its antimicrobial activity at a lower concentration; 80%: This group comprised acrylic plates treated with an 80% concentration of guava leaf extract, enabling the assessment of its efficacy at a higher concentration; 90%: In this group, acrylic plates were treated with a 90% concentration of guava leaf extract, representing the highest concentration tested to determine its maximum antimicrobial potential.

Guava leaves were carefully collected, ensuring their freshness and quality, and then subjected to a series of processing steps to obtain the desired extract. The leaves were thoroughly washed to remove any debris or contaminants and then dried in a shaded area to preserve their bioactive compounds. The dried leaves were ground into a fine powder using a suitable grinding apparatus and sieved through a 60-mesh sieve to ensure uniform particle size. The powdered guava leaves were then subjected to maceration, a process of soaking the plant material in a solvent to extract its soluble constituents. In this study, 70% ethanol was used as the solvent due to its ability to effectively extract a wide range of bioactive compounds from plant materials. The maceration process was carried out for 72 hours with occasional stirring to facilitate thorough extraction. After maceration, the extract was carefully filtered to remove any solid residues, resulting in a clear liquid containing the extracted bioactive compounds. To concentrate the extract, a rotary evaporator was employed, which utilizes reduced pressure and gentle heating to remove the solvent, leaving behind a more concentrated solution. The concentrated guava leaf extract was then diluted with dimethyl sulfoxide (DMSO), a versatile solvent commonly used in biological research, to achieve the desired concentrations of 75%, 80%, and 90%.

To gain a deeper understanding of the chemical constituents responsible for the antimicrobial activity of guava leaf extract, a comprehensive phytochemical screening was conducted. This screening involved a series of tests designed to detect the presence of various bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and triterpenoids/steroids. Alkaloids, a diverse group of nitrogen-containing compounds, were detected using three different tests: Mayer's test, Wagner's test, and Dragendorff's test. Each test relies on the reaction of alkaloids with specific reagents, resulting in the formation of characteristic precipitates or color changes. Flavonoids, a large class of polyphenolic compounds, were detected using the Shinoda test. This test involves the addition of magnesium and hydrochloric acid to the extract, leading to the formation of an orange-red color in the presence of flavonoids. Tannins, a group of astringent, polyphenolic compounds, were detected using the Ferric chloride test. This test relies on the reaction of tannins with ferric chloride, resulting in the formation of a bluish-black or greenish-black color. Saponins, a group of glycosides with soap-like properties, were detected using the Foam test. This test involves shaking the extract solution vigorously, leading to the formation of persistent foam in the presence of saponins. Triterpenoids and steroids, a group of lipid-soluble compounds, were detected using the Liebermann-Burchard test. This test involves the addition of specific reagents to the extract, resulting in

the formation of a reddish-brown color for triterpenoids and a green color for steroids.

Streptococcus mutans ATCC 25175, a standard strain used in oral microbiology research, was obtained from a certified microbiology laboratory. The bacteria were carefully cultured in Brain Heart Infusion Broth (BHI-B), a nutrient-rich medium that supports the growth of a wide range of microorganisms. The culture was incubated at 37°C for 24 hours, allowing the bacteria to multiply and reach a suitable concentration for the experiment. To ensure consistency in bacterial concentration across all experimental groups, the turbidity of the *S. mutans* suspension was adjusted to match McFarland standard 0.5, which corresponds to approximately 1 x 10^8 CFU/ml. This standardization is crucial for accurate and reliable comparison of bacterial colonization among different treatment groups.

Prior to bacterial contamination and treatment, all acrylic plates were sterilized in an autoclave at 121°C for 15 minutes to eliminate any potential microbial contaminants. This sterilization step ensures that any observed bacterial growth is solely due to the experimental conditions and not due to pre-existing contamination. To mimic the oral environment and facilitate bacterial attachment, the sterilized acrylic plates were immersed in artificial saliva for one hour. Artificial saliva provides a more realistic simulation of the oral cavity compared to using a simple buffer solution. After saliva immersion, the plates were gently rinsed with Phosphate Buffer Saline (PBS) to remove any excess saliva or loosely attached bacteria. Each acrylic plate was then immersed in 10 ml of the standardized *S. mutans* suspension and incubated at 37°C for 24 hours, allowing the bacteria to adhere to the acrylic surface and form biofilms. This contamination step simulates the colonization of orthodontic appliances by *S. mutans* in the oral cavity. Following contamination, the plates were carefully removed from the bacterial suspension, rinsed with PBS to remove any non-adherent bacteria, and transferred to their respective treatment solutions for 10 minutes. This treatment duration was chosen based on previous studies and pilot experiments to ensure adequate exposure to the antimicrobial agents without causing damage to the acrylic material.

After the 10-minute treatment period, the acrylic plates were again rinsed with PBS to remove any residual treatment solution or loosely attached bacteria. Each plate was then placed in a separate tube containing BHI-B, providing a nutrient-rich environment for the dislodged bacteria to grow. The tubes were vigorously vortexed for 30 seconds to dislodge the bacteria from the acrylic surface and ensure a homogenous suspension. To quantify the number of viable bacteria, serial dilutions up to 10^-2 were prepared for each tube. This dilution step reduces the bacterial concentration to a manageable level for accurate counting. A 0.1 ml aliquot of the 10^-2 dilution was then spread evenly on Plate Count Agar (PCA), a general-purpose medium that supports the growth of a wide range of bacteria. The plates were incubated at 37°C for 24 hours, allowing the bacteria to grow and form visible colonies. After incubation, the number of *S. mutans* colonies on each plate was carefully counted using a colony counter, a device that aids in accurate and efficient enumeration of bacterial colonies. The colony counts were then used to calculate the mean colony count and standard deviation for each treatment group, providing a quantitative measure of the antimicrobial efficacy of guava leaf extract and chlorhexidine.

The data obtained from the colony counts were meticulously analyzed using SPSS software, a powerful statistical analysis tool widely used in research. To ensure the validity of statistical tests, the normality of data distribution was assessed using the Shapiro-Wilk test, which evaluates whether the data follows a normal distribution. Additionally, the homogeneity of variance, which assumes that the variances of different groups are equal, was checked using Levene's test. One-Way ANOVA, a statistical test used to compare the means of three or more groups, was employed to determine if there were any significant differences in the mean number of *S. mutans* colonies among the five treatment groups. If a

significant difference was found, post hoc Tukey's HSD test, a multiple comparison test, was used to identify specific pairwise comparisons that were significantly different. This post hoc analysis allows for a more detailed understanding of the antimicrobial effects of different concentrations of guava leaf extract compared to chlorhexidine and the negative control. The results of the statistical analysis were carefully interpreted to draw meaningful conclusions about the antimicrobial efficacy of guava leaf extract and its potential as a natural alternative to chlorhexidine in orthodontic practice.

3. Results

Table 1 presents the results of the phytochemical screening conducted on the guava leaf extract. This screening aimed to identify the presence of various bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and triterpenoids/steroids, which are known to contribute to the medicinal properties of plants. The table provides information on the detection methods used, the results obtained (positive or negative), observations made during the tests, and the potential role of each phytochemical in antibacterial activity; Alkaloids: The extract tested positive for alkaloids using Mayer's, Wagner's, and Dragendorff's tests. These tests indicated the presence of alkaloids by the formation of characteristic precipitates. Alkaloids are known to disrupt bacterial cell wall synthesis and function, contributing to their antibacterial activity; Flavonoids: The Shinoda test yielded a positive result for flavonoids, indicated by the formation of an orange-red color. Flavonoids are recognized for their ability to disrupt bacterial cell wall integrity, inhibit bacterial enzymes, and interfere with bacterial adhesion; Tannins: The presence of tannins was confirmed by the Ferric Chloride test, which produced a bluish-black or greenish-black color. Tannins are known to precipitate bacterial proteins, inhibit bacterial enzymes, and reduce bacterial adhesion, contributing to their antibacterial effects; Saponins: The Foam test indicated the presence of saponins by the formation of persistent foam after shaking the extract solution. Saponins are known to disrupt bacterial cell wall integrity and function, playing a role in antibacterial activity; Triterpenoids/Steroids: The Liebermann-Burchard test yielded a positive result for triterpenoids/steroids, indicated by the formation of a reddish-brown color for triterpenoids and a green color for steroids. These compounds are also known to disrupt bacterial cell wall integrity and function, contributing to antibacterial effects.

Table 2 presents the results of the antibacterial activity of guava leaf extract and chlorhexidine against *Streptococcus mutans*. The table displays the mean colony count (x 10^2 CFU/ml) ± standard deviation (SD) for each treatment group, along with the corresponding p-value obtained from one-way ANOVA. The lower the mean colony count, the higher the antibacterial activity; Chlorhexidine (K+): The chlorhexidine group (positive control) showed a significantly lower mean colony count (27.8 ± 6.6 CFU/ml) compared to the aquades group (K-) (1461.2 ± 274.5 CFU/ml), indicating strong antibacterial activity against *S. mutans*; Guava Leaf Extract: All three guava leaf extract groups (90%, 80%, and 75%) demonstrated significantly lower mean colony counts compared to the aquades group (K-), suggesting that guava leaf extract possesses antibacterial activity against *S. mutans*. The 90% guava leaf extract group exhibited the lowest mean colony count (9.4 ± 3.3 CFU/ml) among all groups, even lower than the chlorhexidine group, indicating the highest antibacterial activity. The 80% guava leaf extract group also showed a low mean colony count (42 ± 7.8 CFU/ml), comparable to the chlorhexidine group. The 75% guava leaf extract group had a higher mean colony count (381 ± 81.1 CFU/ml) compared to the 90% and 80% groups, but still significantly lower than the aquades group; p-value: The p-value of 0.000 obtained from one-way ANOVA indicates a statistically significant difference in mean colony counts among the treatment groups. This suggests that the different treatments had varying effects on the growth of *S. mutans*.

Table 1. Phytochemical screening of guava leaf extract.

Phytochemical	Detection method	Result	Observations	Role in antibacterial activity
Alkaloids	Mayer's Test	Positive	Formation of a white or cream precipitate	Disrupt bacterial cell wall synthesis and function
	Wagner's Test	Positive	Formation of a reddish-brown precipitate	-
	Dragendorff's Test	Positive	Formation of an orange precipitate	-
Flavonoids	Shinoda Test	Positive	Formation of an orange-red color after the addition of magnesium and hydrochloric acid	Disrupt bacterial cell wall integrity, inhibit bacterial enzymes, and interfere with bacterial adhesion
Tannins	Ferric Chloride Test	Positive	Formation of a bluish-black or greenish-black color	Precipitate bacterial proteins, inhibit bacterial enzymes and reduce bacterial adhesion
Saponins	Foam Test	Positive	Formation of persistent foam after shaking the extract solution	Disrupt bacterial cell wall integrity and function
Triterpenoids/ Steroids	Liebermann-Burchard Test	Positive	Formation of a reddish-brown color for triterpenoids and a green color for steroids	Disrupt bacterial cell wall integrity and function

Table 2. Antibacterial activity of guava leaf extract and chlorhexidine against *S. mutans*.

Group	Treatment	Mean Colony Count (x 102 CFU/ml) ± SD	p-value *
1	Chlorhexidine gluconate 0.2% (K+)	27.8 ± 6.6	
2	Guava leaf extract 90%	9.4 ± 3.3	
3	Guava leaf extract 80%	42 ± 7.8	
4	Guava leaf extract 75%	381 ± 81.1	
5	Aquades (K-)	1461.2 ± 274.5	
			0.000

*p<0.05 one-way ANOVA.

Table 3 presents the results of the post hoc LSD (Least Significant Difference) test, which was conducted to perform pairwise comparisons between the different treatment groups after a significant difference was found in the overall ANOVA analysis. This test helps to identify specific groups that differ significantly from each other in terms of their mean colony counts, indicating varying levels of antibacterial activity; Chlorhexidine vs. Guava Extracts: There was no significant difference between the chlorhexidine group and the 90% and 80% guava leaf extract groups. This suggests that the antibacterial activity of guava leaf extract at these concentrations is comparable to that of chlorhexidine.

However, there was a significant difference between the chlorhexidine group and the 75% guava leaf extract group, indicating that the lower concentration of guava leaf extract was less effective than chlorhexidine; Chlorhexidine vs. Aquades: There was a significant difference between the chlorhexidine group and the aquades group, confirming the strong antibacterial activity of chlorhexidine; Guava Extracts vs. Each Other: There was no significant difference between the 90% and 80% guava leaf extract groups, suggesting that increasing the concentration from 80% to 90% did not result in a significant improvement in antibacterial activity. However, there were significant differences between the 90% and 75% groups, as well as between the 80% and 75% groups, indicating that the higher concentrations of guava leaf extract were more effective than the lower concentration; Guava Extracts vs. Aquades: There were significant differences between all guava leaf extract groups (90%, 80%, and 75%) and the aquades group, confirming the antibacterial activity of guava leaf extract at all tested concentrations.

Table 3. Post Hoc LSD test for multiple comparisons of antibacterial activity.

Comparison	Mean Difference (x 102 CFU/ml)	p-value*	Interpretation
Chlorhexidine vs. Guava 90%	18.4	0.823	Not significant
Chlorhexidine vs. Guava 80%	-14.2	0.863	Not significant
Chlorhexidine vs. Guava 75%	-353.2	0.000	Significant
Chlorhexidine vs. Aquades	-1433.4	0.000	Significant
Guava 90% vs. Guava 80%	-32.6	0.692	Not significant
Guava 90% vs. Guava 75%	-371.6	0.000	Significant
Guava 90% vs. Aquades	-1451.8	0.000	Significant
Guava 80% vs. Guava 75%	-339.0	0.000	Significant
Guava 80% vs. Aquades	-1419.2	0.000	Significant
Guava 75% vs. Aquades	-1080.2	0.000	Significant

*p<0.05 post hoc LSD.

4. Discussion

The results of this study unequivocally demonstrate the remarkable antibacterial activity of guava leaf extract against *Streptococcus mutans*, a primary cariogenic bacterium implicated in the development of dental caries. This finding holds significant implications for the field of dentistry, particularly in the realm of orthodontics, where the risk of caries development is elevated due to the presence of appliances that can create favorable conditions for bacterial colonization and biofilm formation. *Streptococcus mutans* is a Gram-positive bacterium that plays a crucial role in the initiation and progression of dental caries, a prevalent oral health issue affecting individuals of all ages. This bacterium thrives in the oral cavity, particularly on tooth surfaces and orthodontic appliances, where it forms biofilms, complex communities of microorganisms embedded in a self-produced extracellular matrix. The cariogenic nature of *S. mutans* stems from its ability to metabolize dietary sugars, producing acids that erode tooth enamel, the outermost protective layer of teeth. This acid-induced demineralization of enamel leads to the formation of cavities, commonly known as dental caries, which can cause pain, discomfort, and tooth loss if left untreated. *S. mutans* possesses several virulence factors that contribute to its ability to cause dental caries. *S. mutans* can adhere to tooth

surfaces and orthodontic appliances through the production of adhesins, which are cell-surface proteins that bind to specific receptors on the tooth surface. This adhesion allows *S. mutans* to colonize the oral cavity and form biofilms. *S. mutans* is highly acidogenic, meaning it can produce large amounts of acid as a byproduct of sugar metabolism. This acid production is responsible for the demineralization of tooth enamel and the formation of cavities. *S. mutans* is also acid-tolerant, meaning it can survive and thrive in acidic environments that would be lethal to other bacteria. This acid tolerance allows *S. mutans* to persist in the oral cavity and continue to produce acid, even after the pH of the oral environment has dropped. Orthodontic appliances, whether fixed or removable, are used to correct malocclusion, a misalignment of teeth and jaws. While these appliances offer significant benefits in terms of improving oral health and aesthetics, they can also inadvertently increase the risk of caries development. This is because orthodontic appliances, particularly those made from acrylic-based materials, provide additional surfaces for bacterial adhesion and biofilm formation, creating a more conducive environment for *S. mutans* to thrive and produce acids. Maintaining optimal oral hygiene with orthodontic appliances can be challenging, especially for patients who are not accustomed to the additional cleaning routines required. Fixed appliances, such as braces, consist of brackets and wires that are bonded to the teeth, making it difficult to thoroughly clean the areas around the brackets and under the wires. Removable appliances, while offering greater flexibility in terms of cleaning, can still be challenging to clean effectively, especially if they have intricate designs or are made from porous materials that can harbor bacteria. The search for safe and effective antimicrobial agents to combat *S. mutans* and reduce the risk of caries development has led to the exploration of natural plant extracts. Guava leaf extract, derived from the leaves of the guava plant (*Psidium guajava* L.), has emerged as a promising candidate due to its rich content of bioactive compounds, such as flavonoids, tannins, and terpenoids. These compounds have been shown to possess potent antibacterial properties, disrupting bacterial cell walls, inhibiting bacterial enzymes, and interfering with bacterial protein synthesis. The bioactive compounds in guava leaf extract can disrupt the integrity of the bacterial cell wall, leading to cell lysis and death. Guava leaf extract can inhibit the activity of bacterial enzymes, such as glucosyltransferase, which is involved in the production of extracellular polysaccharides that contribute to biofilm formation. Guava leaf extract can interfere with bacterial protein synthesis, preventing the bacteria from producing essential proteins needed for growth and survival. Notably, the study revealed that guava leaf extract, at concentrations of 90% and 80%, exhibits comparable efficacy to 0.2% chlorhexidine in reducing *S. mutans* colonization on acrylic-based orthodontic appliances. Chlorhexidine, a widely used antimicrobial agent in dentistry, has long been considered the gold standard in oral antimicrobial care due to its broad-spectrum activity against various oral pathogens. However, concerns regarding its potential side effects, such as tooth staining and taste alteration, have prompted the search for alternative antimicrobial agents. The observation that guava leaf extract, a natural product derived from the leaves of the guava plant (*Psidium guajava* L.), can match the antimicrobial efficacy of chlorhexidine is particularly encouraging. This finding suggests that guava leaf extract could serve as a safe and effective alternative to chlorhexidine, especially for patients who are concerned about the potential side effects of chlorhexidine or who prefer natural oral hygiene solutions. The results of this study are consistent with previous research that has highlighted the antimicrobial potential of guava leaf extract against various oral pathogens, including *S. mutans*. These studies have attributed the antibacterial activity of guava leaf extract to its rich content of bioactive compounds, such as flavonoids, tannins, and terpenoids. These compounds have been shown to disrupt bacterial cell walls, inhibit bacterial enzymes, and interfere with bacterial protein synthesis,

effectively hindering their growth and colonization. The findings of this study have significant implications for the management of oral health in orthodontic patients. The use of guava leaf extract as an alternative to chlorhexidine could lead to improved oral health outcomes, reduced risk of caries development, and greater patient satisfaction. Additionally, the use of natural antimicrobials like guava leaf extract may reduce the risk of developing bacterial resistance, a growing concern with the widespread use of synthetic antimicrobials.[11-13]

The remarkable antibacterial activity exhibited by guava leaf extract against *Streptococcus mutans* can be primarily attributed to its rich and diverse phytochemical profile. This profile encompasses a wide array of bioactive compounds, each with its unique chemical structure and mechanism of action, working synergistically to combat bacterial growth and colonization. Among these compounds, flavonoids, tannins, and terpenoids stand out as key players in the antimicrobial arsenal of guava leaf extract. Flavonoids, a large and diverse group of polyphenolic compounds, are ubiquitously present in the plant kingdom and are renowned for their antioxidant, anti-inflammatory, and antimicrobial properties. In the context of guava leaf extract, flavonoids, particularly quercetin, have been identified as potent antibacterial agents against *S. mutans*. Quercetin's antibacterial activity stems from its ability to disrupt bacterial cell wall integrity and interfere with bacterial adhesion to surfaces. The bacterial cell wall, a complex and rigid structure composed of peptidoglycans, plays a crucial role in maintaining cell shape, protecting against osmotic stress, and facilitating interactions with the environment. Quercetin, by disrupting the cell wall's structural integrity, compromises the bacterium's ability to survive and proliferate. The bacterial cell wall is a complex and dynamic structure that serves as the first line of defense against environmental insults. It is composed primarily of peptidoglycans, which are long chains of alternating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) residues cross-linked by peptide bridges. This intricate network of peptidoglycans provides the cell wall with its rigidity and strength, allowing it to withstand osmotic pressure and maintain cell shape. Quercetin, a flavonoid with potent antibacterial properties, has been shown to disrupt the integrity of the bacterial cell wall by interfering with the synthesis and assembly of peptidoglycans. Specifically, quercetin inhibits the activity of enzymes involved in the cross-linking of peptidoglycans, such as transpeptidases and penicillin-binding proteins (PBPs). These enzymes are essential for the formation of the peptide bridges that connect the NAG and NAM residues, providing the cell wall with its structural integrity. By inhibiting the activity of these enzymes, quercetin weakens the cell wall, making it more susceptible to osmotic stress and lysis. This disruption of cell wall integrity can lead to bacterial cell death, effectively controlling bacterial growth and proliferation. Bacterial adhesion, the process by which bacteria attach to surfaces, is a critical step in the colonization process. Bacteria, including *S. mutans*, adhere to surfaces, such as tooth enamel and orthodontic appliances, through the production of adhesins, which are cell-surface proteins that bind to specific receptors on the surface. This adhesion allows bacteria to establish a foothold and form biofilms, complex communities of microorganisms embedded in a self-produced extracellular matrix. Quercetin has been shown to interfere with bacterial adhesion by binding to bacterial adhesins, preventing them from interacting with their corresponding receptors on the surface. This inhibition of adhesion can disrupt biofilm formation and colonization, effectively hindering the growth and proliferation of *S. mutans*. The presence of quercetin in guava leaf extract underscores its potential as a natural alternative to synthetic antimicrobial agents, such as chlorhexidine. Quercetin's multifaceted antibacterial action, coupled with its safety profile, makes it an attractive candidate for oral health applications, particularly in the prevention and management of dental caries in orthodontic patients. Tannins, another important

group of compounds found in guava leaf extract, also contribute significantly to its antibacterial activity. These polyphenolic compounds are known for their astringent properties and their ability to interact with proteins, forming insoluble complexes. Tannins exert their antibacterial effects by precipitating bacterial proteins and inhibiting bacterial enzymes. Bacterial proteins play crucial roles in various cellular processes, including metabolism, growth, and virulence. By precipitating these proteins, tannins disrupt bacterial structure and function, compromising their ability to survive and cause disease. Tannins have a high affinity for proteins, and their interaction with bacterial proteins can lead to the formation of insoluble complexes. These complexes can disrupt the structure and function of bacterial proteins, impairing their ability to carry out essential cellular processes. For example, tannins can precipitate enzymes involved in bacterial metabolism, hindering their ability to catalyze biochemical reactions and generate energy for the cell. In addition to precipitating bacterial proteins, tannins can also directly inhibit the activity of bacterial enzymes. Enzymes are essential for bacterial metabolism and survival, catalyzing various biochemical reactions that are necessary for growth, reproduction, and virulence. Tannins can bind to the active sites of enzymes, preventing them from interacting with their substrates and catalyzing reactions. This inhibition of enzyme activity can disrupt critical cellular processes, leading to bacterial growth arrest and death. The presence of tannins in guava leaf extract further enhances its antibacterial potential. Tannins' ability to disrupt bacterial structure and function, coupled with their protein-binding properties, makes them valuable components in the fight against oral pathogens, such as *S. mutans*. The antibacterial activity of guava leaf extract is not solely attributed to the individual actions of flavonoids and tannins. Rather, it is the synergistic interaction of these and other bioactive compounds that contributes to the overall efficacy of guava leaf extract in combating *S. mutans* colonization.[14-16]

The use of guava leaf extract as an alternative to chlorhexidine for oral hygiene in orthodontic patients presents several potential advantages. Guava leaf extract is a natural product with minimal side effects compared to chlorhexidine, which can cause tooth staining and taste alteration. This makes guava leaf extract a more appealing option for patients seeking natural and less disruptive oral hygiene solutions. Chlorhexidine gluconate, while recognized for its broad-spectrum antimicrobial activity against various oral pathogens, has been associated with several side effects that can impact patient compliance and overall oral health. One of the most common side effects of chlorhexidine is tooth staining. Chlorhexidine can bind to tannins and other chromogenic compounds found in food and beverages, leading to the formation of extrinsic stains on tooth surfaces. These stains can range in color from yellow to brown and can be difficult to remove with regular brushing. Chlorhexidine can also cause taste alteration, often described as a bitter or metallic taste. This can affect patients' enjoyment of food and beverages, potentially leading to decreased appetite and nutritional deficiencies. In some cases, chlorhexidine can cause oral mucosal irritation, dryness, and burning sensations. These side effects can be uncomfortable and may discourage patients from using chlorhexidine as directed. The side effects associated with chlorhexidine can significantly impact patient compliance with oral hygiene regimens. Patients who experience tooth staining or taste alteration may be less likely to use chlorhexidine as directed, leading to suboptimal oral hygiene and an increased risk of caries development. Additionally, the discomfort caused by oral mucosal irritation, dryness, and burning sensations can further discourage patients from using chlorhexidine, compromising their oral health. In contrast to chlorhexidine, guava leaf extract has a favorable safety profile, with minimal reported side effects. This makes it a more appealing option for patients seeking natural and less disruptive oral hygiene solutions. The minimal side effects of guava leaf extract can be attributed to its natural origin and the absence of harsh chemicals or synthetic

ingredients. Guava leaf extract is derived from the leaves of the guava plant (*Psidium guajava* L.), a tropical fruit tree native to Central America and widely cultivated in tropical and subtropical regions around the world. Guava leaves have a long history of traditional medicinal use, and their safety and efficacy have been supported by numerous scientific studies. Guava leaf extract is typically prepared using water or ethanol extraction methods, which do not involve the use of harsh chemicals or synthetic ingredients. This ensures that the final product is free from potentially harmful substances that could cause side effects. In recent years, there has been a growing trend towards natural and holistic approaches to healthcare. Patients are increasingly seeking natural alternatives to synthetic medications and oral hygiene products, driven by concerns about potential side effects and a desire for more sustainable and environmentally friendly options. Guava leaf extract, as a natural product derived from a plant source, aligns with this trend and offers patients a safe and effective alternative to chlorhexidine. The demand for natural oral hygiene products has been steadily increasing in recent years, reflecting a growing awareness of the potential risks associated with synthetic ingredients and a desire for more sustainable and environmentally friendly options. Guava leaf extract, with its natural origin and minimal side effects, is well-positioned to meet this growing demand. The minimal side effects of guava leaf extract can lead to improved patient compliance with oral hygiene regimens. Patients are more likely to adhere to oral hygiene practices that are comfortable and do not cause unpleasant side effects. This improved compliance can contribute to better oral health outcomes and a reduced risk of caries development. Patient compliance with oral hygiene regimens is essential for maintaining optimal oral health and preventing dental caries. However, compliance can be challenging, especially for patients who are undergoing orthodontic treatment, which requires additional cleaning routines and may involve the use of antimicrobial agents that can cause side effects. Guava leaf extract, with its favorable safety profile and minimal side effects, has the potential to enhance patient compliance with oral hygiene regimens. By offering a comfortable and effective alternative to chlorhexidine, guava leaf extract can encourage patients to adhere to their oral hygiene practices, leading to better oral health outcomes and a reduced risk of caries development. The use of natural antimicrobials like guava leaf extract may reduce the risk of developing bacterial resistance, a growing concern with the widespread use of synthetic antimicrobials. This potential advantage could contribute to the long-term sustainability of oral health management strategies. Bacterial resistance to antimicrobial agents is a global health crisis that threatens the effective treatment of infectious diseases. The overuse and misuse of antibiotics and other antimicrobial agents have led to the emergence and spread of resistant strains of bacteria, making it increasingly difficult to treat infections. Bacteria can produce enzymes that inactivate antimicrobial agents, rendering them ineffective. Bacteria can modify the target site of antimicrobial agents, preventing them from binding and exerting their effects. Bacteria can develop efflux pumps, which are membrane proteins that actively pump antimicrobial agents out of the cell. Bacteria can reduce their permeability to antimicrobial agents, preventing them from entering the cell. In the oral cavity, the development of bacterial resistance to chlorhexidine and other synthetic antimicrobial agents is a growing concern. The widespread use of these agents in oral hygiene products and dental procedures has created selective pressure, favoring the survival and proliferation of resistant strains of bacteria. These resistant strains can be more difficult to control, leading to persistent infections and increased risk of caries development. Natural antimicrobials, such as guava leaf extract, offer a potential solution to the growing threat of bacterial resistance. These agents often have multiple mechanisms of action, making it more difficult for bacteria to develop resistance. Additionally, natural antimicrobials are often derived from plant sources that have been used for centuries in traditional

medicine, suggesting that they may be less likely to promote resistance development compared to synthetic agents. Guava leaf extract exerts its antibacterial activity through multiple mechanisms, including the disruption of bacterial cell walls, inhibition of bacterial enzymes, and interference with bacterial protein synthesis. This multifaceted action makes it more difficult for bacteria to develop resistance compared to agents that have a single mechanism of action. The use of guava leaf extract as an alternative to chlorhexidine could contribute to the long-term sustainability of oral health management strategies. By reducing the risk of bacterial resistance development, guava leaf extract can help preserve the effectiveness of antimicrobial agents and ensure that they remain available for future generations.[17-20]

5. Conclusion

This in vitro study investigated the antimicrobial efficacy of guava leaf extract against Streptococcus mutans colonization on acrylic-based removable orthodontic appliances. The results demonstrated that guava leaf extract, particularly at concentrations of 90% and 80%, exhibits comparable efficacy to 0.2% chlorhexidine in reducing *S. mutans* colonization on acrylic-based orthodontic appliances. Guava leaf extract, with its rich content of flavonoids, tannins, and other bioactive compounds, has emerged as a promising natural alternative to chlorhexidine for maintaining oral hygiene in orthodontic patients. The findings of this study have significant implications for the field of dentistry, particularly in the realm of orthodontics, where the risk of caries development is elevated due to the presence of appliances that can create favorable conditions for bacterial colonization and biofilm formation. Guava leaf extract, with its favorable safety profile and minimal side effects, has the potential to enhance patient compliance with oral hygiene regimens and contribute to the long-term sustainability of oral health management strategies. Further research, including in vivo studies and clinical trials, is warranted to fully elucidate the clinical efficacy and safety of guava leaf extract as an alternative to chlorhexidine for oral hygiene in orthodontic patients. The potential advantages of guava leaf extract, including its natural origin, minimal side effects, and potential to reduce the risk of bacterial resistance development, make it an attractive candidate for further investigation and potential integration into oral hygiene protocols for orthodontic patients.

6. References

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