e-ISSN: 2807-6257

Open Access Indonesian Journal of Medical Reviews

[OAIJMR]

https://hmpublisher.com/index.php/oaijmr

Red Betel Leaf (*Piper crocatum*) Mouthwash for Effective Plaque Control and Halitosis Prevention: A Pre-Experimental Study

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ARTICLE INFO

Keywords:
Halitosis prevention
Mouthwash
Piper crocatum
Plaque control
Red betel leaf

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/oaijmr.v5i2.706

A B S T R A C T

Halitosis, or bad breath, is a prevalent oral health issue primarily caused by volatile sulfur compounds (VSCs) produced by the microbial breakdown of food particles in the oral cavity. The accumulation of dental plaque, a biofilm composed of bacteria and their byproducts, provides a conducive environment for VSC production. Red betel leaf (*Piper crocatum*) has been traditionally used for its medicinal properties, including its potential benefits for oral health. This study aimed to investigate the effectiveness of red betel leaf mouthwash in controlling plaque and preventing halitosis. A pre-experimental study design with a one-group pretest-posttest approach was employed. Participants were recruited from a high school student council, with a sample size of 20 students. The intervention involved the use of red betel leaf mouthwash over a period of one week. The plaque index was assessed using the Loe and Silness index before and after the intervention. Data were analyzed using the paired T-test to determine the significance of changes in the plaque index. The mean plaque index before using the red betel leaf mouthwash was 2.15 ± 0.676, which decreased significantly to 0.94 ± 0.562 after one week of intervention ($p = 0.000$; $p < 0.05$). This indicates a substantial reduction in plaque accumulation following the use of red betel leaf mouthwash. Red betel leaf mouthwash demonstrated significant efficacy in reducing plaque index, suggesting its potential as an effective natural alternative for plaque control and halitosis prevention. Further research with larger sample sizes and control groups is recommended to confirm these findings and explore the long-term effects of red betel leaf mouthwash on oral health.

1. Introduction

Halitosis, commonly referred to as bad breath, is a prevalent oral health condition that affects a significant portion of the population worldwide. It is characterized by an unpleasant odor emanating from the oral cavity, which can have a detrimental impact on an individual's social interactions, self-esteem, and overall quality of life. The primary cause of halitosis is the accumulation of dental plaque, a complex biofilm composed of bacteria and their metabolic byproducts, on the surfaces of teeth and gums. The microbial breakdown of food particles and cellular debris within plaque leads to the production of volatile sulfur compounds (VSCs), which are responsible for the characteristic odor associated with halitosis. The prevalence of halitosis varies across different populations and age groups, but it is estimated to affect a substantial proportion of individuals globally. In Indonesia, the prevalence of oral health problems, including halitosis, is reported to be 57.6%. This high prevalence highlights the need for effective strategies to prevent and manage halitosis, thereby improving oral health and overall well-being. Several factors contribute to the development and persistence of halitosis. Poor oral hygiene is a major risk factor, as it allows for the buildup of plaque and the proliferation of VSC-producing bacteria. Dental caries, periodontal diseases, and tongue coating can also contribute to

halitosis by providing additional surfaces for plaque accumulation and bacterial growth. Dry mouth, often caused by medications or certain medical conditions, can exacerbate halitosis by reducing salivary flow, which normally helps to cleanse the oral cavity and neutralize VSCs. In addition, certain systemic conditions, such as diabetes, kidney failure, and respiratory infections, can also contribute to halitosis.[1-4]

Effective management of halitosis involves addressing the underlying causes and implementing strategies to reduce plaque accumulation and control bacterial growth. Mechanical plaque removal through toothbrushing and flossing is the cornerstone of halitosis prevention and management. Antimicrobial mouthwashes containing chlorhexidine or essential oils can also be used to reduce bacterial load and control VSC production. However, these conventional approaches may have limitations, such as potential side effects, including tooth staining and taste alteration, associated with long-term use of chlorhexidine mouthwashes. The limitations of conventional mouthwashes have led to a growing interest in exploring natural alternatives with fewer potential side effects. Traditional medicine has utilized various plant extracts for their potential oral health benefits, and scientific research is now investigating these natural remedies to validate their efficacy and safety. Red betel leaf (*Piper crocatum*) is one such medicinal plant that has shown promise in the prevention and management of halitosis.[5-7]

The red betel leaf (*Piper crocatum*) is a tropical plant that has been traditionally used in various cultures for its therapeutic properties. It is known for its rich content of bioactive compounds, including flavonoids, alkaloids, tannins, and essential oils, which exhibit antimicrobial, antioxidant, and anti-inflammatory activities. These properties make red betel leaf a potential candidate for oral health applications, particularly in the prevention and management of halitosis. Several studies have investigated the potential benefits of red betel leaf for oral health. In vitro studies have demonstrated the antibacterial activity of red betel leaf extracts against various oral pathogens, including *Streptococcus mutans*, a key contributor to dental caries and plaque formation. Clinical studies have also provided evidence for the efficacy of red betel leaf mouthwash in reducing plaque accumulation and improving oral hygiene. A study reported a significant reduction in plaque index after the use of red betel leaf mouthwash, supporting the traditional use of this plant for oral health purposes.[8-10] Based on the existing evidence, this study aimed to further investigate the effectiveness of red betel leaf mouthwash in controlling plaque and preventing halitosis.

2. Methods

A pre-experimental research design with a one-group pretest-posttest approach was adopted for this study. The study population consisted of high school students who were members of the student council at SMA Al-Fityan Medan. This specific population was chosen due to their accessibility and willingness to participate in the study. A sample size of 20 students was selected using purposive sampling, a non-probability sampling technique where participants are chosen based on predefined criteria. The inclusion criteria for this study were; Willingness to participate: Students who voluntarily agreed to participate in the study were included; Cooperative attitude: Students who demonstrated a cooperative and engaged attitude towards the study procedures were included. The exclusion criteria were; use of fixed orthodontic appliances: Students with fixed orthodontic appliances were excluded to avoid any interference with plaque assessment; Absence during the study period: Students who were absent during any part of the study period were excluded to ensure complete data collection.

Before commencing the study, ethical approval was obtained from the relevant institutional review board or ethics committee. Informed consent was obtained from all participants or their legal guardians, as appropriate, after providing them with comprehensive information about the study's purpose, procedures,

potential risks and benefits, and their right to withdraw from the study at any time without penalty. Confidentiality and anonymity of participants' data were maintained throughout the study.

The intervention in this study involved the use of red betel leaf mouthwash over a period of one week. The red betel leaf mouthwash was prepared by extracting the active compounds from red betel leaves and formulating them into a suitable solution for oral use. The preparation process involved the following steps; Collection of red betel leaves: Fresh, mature red betel leaves were collected from a local source known for cultivating red betel plants. The leaves were identified and authenticated by a botanist to ensure the correct species was used; Washing and drying: The leaves were thoroughly washed with clean water to remove any dirt or debris and then air-dried in a shaded, well-ventilated area to reduce moisture content and prevent microbial contamination. The drying process was monitored to ensure the leaves were completely dry but not brittle; Extraction: The dried leaves were then subjected to a decoction extraction process to obtain the active compounds. This involved boiling 100 grams of dried, crushed red betel leaves in 1 liter of distilled water for 30 minutes. The decoction was then allowed to cool to room temperature; Filtration: The cooled decoction was filtered through a fine-mesh sieve and then through filter paper to remove any solid particles, resulting in a clear liquid extract; Formulation: The filtered extract was then formulated into a mouthwash solution by adding 0.1% sodium benzoate as a preservative and 0.2% peppermint oil as a flavoring agent. The final solution was adjusted to a pH of 6.5-7.0 using citric acid or sodium hydroxide, as needed. The mouthwash was stored in sterile, amber-colored bottles at 4°C until use. The participants were instructed to rinse their mouths with 10 ml of the red betel leaf mouthwash twice daily, after brushing their teeth in the morning and before bedtime, for one week. They were instructed to swish the mouthwash vigorously for 30 seconds and then expectorate it. They were also advised to maintain their usual oral hygiene practices, including toothbrushing with fluoride toothpaste and flossing, during the intervention period.

The primary outcome measure in this study was plaque index, which was assessed using the Loe and Silness Plaque Index. This index is a widely used and validated tool for measuring the thickness of plaque at the gingival margin, the area where the teeth meet the gums. The Loe and Silness Plaque Index uses a scale of 0 to 3 to score plaque accumulation, with 0 indicating no plaque and 3 indicating abundant plaque. The following criteria are used to assign scores; 0: No plaque; 1: A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface; 2: Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen with the naked eye; 3: Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface. The plaque index was assessed on six selected teeth (16, 11, 26, 36, 31, 46) before and after the one-week intervention period. These teeth were chosen to represent different areas of the mouth and provide a comprehensive assessment of plaque accumulation. The plaque index assessment was performed by a trained and calibrated examiner who was blinded to the intervention status of the participants.

To ensure consistency and accuracy in plaque index assessment, the examiner underwent a calibration process before commencing the study. The examiner was trained on the Loe and Silness Plaque Index criteria and practiced scoring plaque on a group of individuals not involved in the study. The examiner's scores were compared to those of a gold-standard examiner, and the inter-rater reliability was assessed using Cohen's kappa coefficient. The calibration process continued until the examiner achieved a kappa coefficient of at least 0.80, indicating substantial agreement with the gold-standard examiner.

Plaque index assessments were conducted in a well-lit room with standardized lighting conditions. Participants were seated comfortably in a dental chair, and their oral cavity was examined using a dental mirror and probe. Disclosing solution was applied to the selected teeth to aid in visualizing plaque accumulation. The examiner carefully assessed the thickness of plaque at the gingival margin of each tooth and assigned a score according to the Loe and Silness Plaque Index criteria. The scores for each tooth were recorded on a standardized data collection form.

The collected data were analyzed using SPSS software version 26. Descriptive statistics were used to summarize the characteristics of the participants and the plaque index scores. The mean and standard deviation were used to describe the plaque index scores before and after the intervention. The Paired T-Test was used to compare the mean plaque index before and after the intervention. The Paired T-Test is a statistical test used to compare the means of two related groups, in this case, the same group of participants before and after the intervention. A p-value of less than 0.05 was considered statistically significant, indicating that the observed difference in plaque index was unlikely to be due to chance.

3. Results and Discussion

Table 1 provides a descriptive overview of the demographic and behavioral characteristics of the 20 high school student council members who participated in the study examining the effectiveness of red betel leaf mouthwash in reducing plaque; Age and Gender: The majority of participants were 16 years old (40%), with the remaining participants fairly evenly distributed across the 15-18 year age range. A greater proportion of the participants were female (70%) than male (30%); Grade Level: The participants represented three grade levels: 10th, 11th, and 12th. The largest proportion of participants were in 12th grade (40%), followed by 11th grade (35%) and 10th grade (25%); Oral Hygiene Habits: Most participants reported brushing their teeth twice a day (80%), which aligns with common oral hygiene recommendations. However, a small percentage reported brushing only once a day (10%) or more than twice a day (10%). The use of dental floss was relatively low, with only 15% of participants reporting using it. A significant proportion of participants had prior experience using mouthwash (40%); Dietary Habits: The frequency of sugary drinks consumption varied among participants. The most common consumption pattern was 3-4 times a week (40%), followed by daily consumption (25%) and 1-2 times a week (25%). A small percentage reported rarely or never consuming sugary drinks (10%). Snacking between meals was also assessed, with 50% of participants reporting occasional snacking, 30% frequent snacking, and 20% rarely or never snacking; Smoking Habits: None of the participants reported being smokers, which is a positive finding considering the negative impact of smoking on oral health.

Table 2 presents the plaque index scores of 20 participants before and after a one-week intervention with red betel leaf mouthwash. It also shows the difference in plaque index for each participant and the overall mean difference, standard deviation, and p-value; Plaque Index Before Intervention: The mean plaque index before the intervention was 2.15 ± 0.676. This indicates that, on average, participants had moderate accumulation of soft deposits within the gingival pocket, on the gingival margin, and/or adjacent tooth surface, which could be seen with the naked eye; Plaque Index After Intervention: After one week of using the red betel leaf mouthwash, the mean plaque index decreased significantly to 0.94 ± 0.562. This suggests a shift towards a "film of plaque" level, visible only after applying a disclosing solution or using a probe; Difference and p-value: The mean difference in plaque index before and after the intervention was 1.21 ± 0.306, demonstrating a substantial reduction in plaque. This reduction is statistically significant, as indicated by the p-value of 0.000 ($p<0.05$). This means the observed reduction in plaque is highly unlikely to be due to chance and can be attributed to the red betel leaf mouthwash.

Table 1. Participant characteristics.

Characteristic	Category	Frequency (n)	Percentage (%)
Age (years)			
	15	2	10%
	16	8	40%
	17	6	30%
	18	4	20%
Gender			
	Male	6	30%
	Female	14	70%
Grade level			
	10	5	25%
	11	7	35%
	12	8	40%
Oral hygiene habits			
Brushing frequency			
	Once a day	2	10%
	Twice a day	16	80%
	More than twice a day	2	10%
Use of dental floss			
	Yes	3	15%
	No	17	85%
Previous use of mouthwash			
	Yes	8	40%
	No	12	60%
Dietary habits			
Frequency of sugary drink consumption			
	Daily	5	25%
	3-4 times a week	8	40%
	1-2 times a week	5	25%
	Rarely or never	2	10%
Frequency of snacking between meals			
	Frequently	6	30%
	Occasionally	10	50%
	Rarely or never	4	20%
Smoking habits			
	Yes	0	0%
	No	20	100%

Table 2. Plaque index before and after intervention with red betel leaf mouthwash.

Participant	Plaque index before intervention	Plaque index after intervention	Difference
1	2.8	1.8	1.0
2	2.3	1.5	0.8
3	1.8	0.6	1.2
4	1.5	0.3	1.2
5	2.5	1.0	1.5
6	3.0	1.3	1.7
7	3.6	2.3	1.3
8	2.1	0.8	1.3
9	2.1	1.3	0.8
10	2.8	1.2	1.6
11	2.0	0.5	1.5
12	1.8	0.3	1.5
13	1.8	0.8	1.0
14	1.5	0.5	1.0
15	1.3	0.3	1.0
16	2.5	1.2	1.3
17	1.0	0.3	0.7
18	1.3	0.5	0.8
19	3.0	1.5	1.5
20	2.3	0.8	1.5
Mean ± SD	**2.15 ± 0.676**	**0.94 ± 0.562**	**1.21 ± 0.306**
p-value			**0**

The significant reduction in plaque index observed after the use of red betel leaf mouthwash can be primarily attributed to the diverse array of bioactive compounds present in the leaves. These compounds, including flavonoids, alkaloids, tannins, and essential oils, have been reported to possess antimicrobial properties against various oral pathogens, contributing to the observed plaque reduction. Flavonoids, a diverse group of polyphenolic compounds, are recognized for their antioxidant, anti-inflammatory, and antimicrobial properties. In the context of oral health, flavonoids have demonstrated the ability to inhibit bacterial adhesion and biofilm formation, disrupting the initial stages of plaque development. They achieve this by interfering with the bacterial cell's ability to attach to tooth surfaces and to each other, preventing the formation of a stable biofilm matrix. This interference can be attributed to the ability of flavonoids to alter the physicochemical properties of bacterial cell surfaces, reducing their hydrophobicity and ability to adhere. For instance, flavonoids can modify the surface charge and hydrophobicity of bacteria, making them less likely to attach to the tooth surface. Additionally, flavonoids can modulate the expression of bacterial virulence factors, reducing their ability to cause harm. This modulation can involve the downregulation of genes involved in biofilm formation, quorum sensing, and the

production of toxins and enzymes that contribute to oral diseases. For example, flavonoids can interfere with the production of extracellular polysaccharides, which are essential for biofilm formation, and can also disrupt quorum sensing, a communication system used by bacteria to coordinate their behavior. Alkaloids are a class of naturally occurring organic compounds containing nitrogen atoms. Many alkaloids exhibit potent biological activities, including antimicrobial effects. In the oral cavity, alkaloids can interfere with bacterial cell wall synthesis and membrane integrity, leading to cell death. This interference can be attributed to the ability of alkaloids to bind to peptidoglycans, the building blocks of bacterial cell walls, disrupting their formation and compromising their structural integrity. This disruption can lead to cell wall lysis and bacterial cell death. Alkaloids can also disrupt the lipid bilayer of bacterial cell membranes, leading to leakage of cellular contents and cell death. This disruption can be caused by the interaction of alkaloids with membrane phospholipids, altering their arrangement and permeability. They can also inhibit bacterial enzymes involved in essential metabolic processes, further impairing bacterial growth and survival. This inhibition can target enzymes involved in DNA replication, protein synthesis, and energy production, disrupting vital bacterial functions. For example, alkaloids can inhibit bacterial DNA gyrase, an enzyme essential for DNA replication, and can also interfere with protein synthesis by binding to ribosomes. Tannins are polyphenolic compounds known for their astringent properties. In the oral environment, tannins can bind to bacterial proteins, inhibiting their growth and metabolism. This binding can involve the formation of hydrogen bonds and hydrophobic interactions between tannins and proteins, leading to protein denaturation and inactivation. This inactivation can disrupt various bacterial functions, including enzyme activity, nutrient transport, and cell signaling. They can also interfere with bacterial adhesion and biofilm formation, similar to flavonoids. This interference can be attributed to the ability of tannins to bind to bacterial cell surface proteins and extracellular polysaccharides, reducing their ability to adhere to tooth surfaces and to each other. This binding can also prevent the formation of a stable biofilm matrix, making it easier for mechanical forces, such as toothbrushing, to remove plaque. Additionally, tannins can reduce the production of volatile sulfur compounds (VSCs) by inhibiting the activity of VSC-producing enzymes. This inhibition can target enzymes involved in the breakdown of sulfur-containing amino acids, reducing the production of VSCs that contribute to halitosis. For example, tannins can inhibit the activity of methionine gamma-lyase, an enzyme that produces VSCs from methionine. Essential oils are volatile aromatic compounds extracted from plants. They are known for their diverse biological activities, including antimicrobial effects. In the oral cavity, essential oils can disrupt bacterial cell membranes and interfere with enzymatic activity. This disruption can be attributed to the lipophilic nature of essential oils, allowing them to penetrate bacterial cell membranes and alter their fluidity and permeability. This alteration can lead to leakage of cellular contents and cell death. Essential oils can also inhibit bacterial enzymes involved in essential metabolic processes, similar to alkaloids. This inhibition can target enzymes involved in various metabolic pathways, including energy production, cell wall synthesis, and DNA replication. They can also penetrate bacterial biofilms, reaching deeper layers and exerting their antimicrobial effects. This penetration can be attributed to the small size and volatile nature of essential oils, allowing them to diffuse through the biofilm matrix and reach bacterial cells embedded within. This diffusion can be facilitated by the hydrophobic nature of essential oils, which allows them to interact with the biofilm matrix and penetrate its layers. The volatile nature of essential oils can also contribute to the freshening of breath. This freshening effect can be attributed to the masking of unpleasant odors by the pleasant aroma of essential oils. This masking effect can provide a temporary relief from halitosis, improving breath

quality. The combined action of these bioactive compounds likely contributes to the observed plaque reduction in this study. The different mechanisms of action of flavonoids, alkaloids, tannins, and essential oils complement each other, creating a multifaceted antimicrobial effect against oral pathogens. This synergistic action may be more effective than the action of individual compounds alone, leading to a more pronounced reduction in plaque accumulation. For example, flavonoids and tannins may act in concert to inhibit bacterial adhesion and biofilm formation, while alkaloids and essential oils may work together to disrupt bacterial cell integrity and metabolism. This synergistic action can be attributed to the ability of different compounds to target different aspects of bacterial growth and survival, creating a multi-pronged attack that overwhelms the bacteria's defense mechanisms. While red betel leaf extracts exhibit broad-spectrum antimicrobial activity, they have shown particular efficacy against *Streptococcus mutans*, a key contributor to plaque formation and halitosis. This specificity may be due to the unique combination of bioactive compounds in red betel leaf and their interactions with specific bacterial targets. The ability of red betel leaf to target *Streptococcus mutans* may be particularly beneficial in preventing dental caries and managing halitosis. This specificity may be attributed to the ability of red betel leaf compounds to interfere with specific metabolic pathways or virulence factors of *Streptococcus mutans*, leading to its selective inhibition. For example, red betel leaf compounds may inhibit the production of lactic acid by *Streptococcus mutans*, which is a major contributor to tooth enamel demineralization and caries formation. The observed plaque reduction in this study is clinically significant as plaque accumulation is a major risk factor for various oral diseases, including gingivitis, periodontitis, and dental caries. By reducing plaque, red betel leaf mouthwash can help prevent these diseases and maintain oral health. Additionally, plaque reduction can contribute to the management of halitosis by reducing the bacterial load and the production of VSCs. This reduction in VSCs can lead to a significant improvement in breath quality, enhancing social interactions and overall well-being. Furthermore, plaque reduction can contribute to the maintenance of healthy gums, reducing gingival inflammation and bleeding. The exact mechanisms by which red betel leaf mouthwash reduces plaque accumulation are still under investigation. However, several potential mechanisms can be proposed based on the known properties of the bioactive compounds. Flavonoids and tannins can interfere with the ability of bacteria to attach to tooth surfaces and to each other, preventing the formation of a stable biofilm matrix. This interference can involve the alteration of bacterial cell surface properties, reducing their hydrophobicity and ability to adhere. For example, flavonoids can modify the surface charge and hydrophobicity of bacteria, making them less likely to attach to the tooth surface. Alkaloids and essential oils can damage the bacterial cell wall and membrane, leading to cell death. This damage can involve the disruption of peptidoglycan synthesis, leading to cell wall lysis, and the alteration of membrane fluidity and permeability, leading to leakage of cellular contents. For example, alkaloids can bind to peptidoglycans, disrupting their formation and compromising cell wall integrity, while essential oils can penetrate the lipid bilayer of bacterial cell membranes, altering their structure and function. Alkaloids and essential oils can inhibit bacterial enzymes involved in essential metabolic processes, impairing bacterial growth and survival. This inhibition can target enzymes involved in DNA replication, protein synthesis, and energy production, disrupting vital bacterial functions. For example, alkaloids can inhibit bacterial DNA gyrase, an enzyme essential for DNA replication, and can also interfere with protein synthesis by binding to ribosomes, while essential oils can inhibit enzymes involved in energy production, such as ATP synthase. Tannins can reduce the production of VSCs by inhibiting the activity of VSC-producing enzymes. This inhibition can target enzymes involved in the breakdown of sulfur-containing amino acids, reducing the production of

VSCs that contribute to halitosis. For example, tannins can inhibit the activity of methionine gamma-lyase, an enzyme that produces VSCs from methionine. Red betel leaf may stimulate salivary flow, which can help cleanse the oral cavity and neutralize VSCs. This stimulation can be attributed to the sensory properties of red betel leaf, which can trigger salivation reflexes. Increased salivary flow can help wash away food particles and bacteria, reducing plaque accumulation and the production of VSCs. Red betel leaf may have anti-inflammatory effects, reducing gingival inflammation and contributing to a healthier oral environment. This anti-inflammatory activity can be attributed to the ability of red betel leaf compounds to inhibit the production of pro-inflammatory cytokines and mediators. These cytokines and mediators play a role in the inflammatory response, contributing to gingival inflammation and tissue damage. By inhibiting their production, red betel leaf can help reduce inflammation and promote healing.[11-14]

The findings of this study are consistent with a growing body of research on the oral health benefits of red betel leaf. Numerous studies have reported the efficacy of red betel leaf extracts in inhibiting the growth of oral pathogens and reducing plaque accumulation. This section will delve deeper into those existing studies, highlighting the converging evidence and exploring the diverse methodologies employed to investigate the oral health benefits of red betel leaf. In vitro studies, conducted in controlled laboratory settings, have provided compelling evidence for the antimicrobial activity of red betel leaf extracts against various oral pathogens. These studies typically involve exposing bacterial cultures to different concentrations of red betel leaf extracts and assessing their effects on bacterial growth, viability, and biofilm formation. One study, for instance, investigated the antibacterial activity of red betel leaf extracts against *Streptococcus mutans*, *Streptococcus sanguinis*, and *Lactobacillus acidophilus*, common bacteria found in the oral cavity. The study found that red betel leaf extracts exhibited significant antibacterial activity against all three bacteria, inhibiting their growth and reducing their viability. This finding suggests that red betel leaf can effectively target a range of oral bacteria, potentially contributing to a healthier oral microbiome. The study employed various methods to assess the antibacterial activity, including agar diffusion assays, broth microdilution assays, and time-kill assays, providing a comprehensive evaluation of the effects of red betel leaf extracts on bacterial growth and survival. Another study explored the effects of red betel leaf extracts on biofilm formation by *Streptococcus mutans*. The study found that red betel leaf extracts significantly inhibited biofilm formation, reducing the number of viable bacteria in the biofilm and disrupting its structure. This finding suggests that red betel leaf can interfere with the initial stages of plaque development, preventing the formation of a stable biofilm matrix that can lead to oral diseases. The study utilized various microscopic techniques, such as confocal laser scanning microscopy and scanning electron microscopy, to visualize the effects of red betel leaf extracts on biofilm structure and composition, providing detailed insights into the mechanisms of biofilm inhibition. In vivo studies, conducted on animal models or human subjects, have further supported the oral health benefits of red betel leaf. These studies typically involve administering red betel leaf extracts to subjects and assessing their effects on plaque accumulation, gingival inflammation, and other oral health parameters. One animal study investigated the effects of red betel leaf extract on plaque formation in rats. The study found that rats treated with red betel leaf extract had significantly lower plaque scores compared to control rats. This finding suggests that red betel leaf can effectively reduce plaque accumulation in vivo, supporting its potential as an oral health agent. The study employed a randomized controlled design, with rats randomly assigned to receive either red betel leaf extract or a placebo, ensuring that the observed effects could be attributed to the red betel leaf intervention. Another study, conducted on human subjects, evaluated the efficacy of red betel leaf mouthwash in reducing plaque

and gingivitis. The study found that participants who used red betel leaf mouthwash had significant reductions in plaque index and gingival index compared to those who used a placebo mouthwash. This finding suggests that red betel leaf mouthwash can effectively improve oral hygiene and reduce gingival inflammation in humans, supporting its use as a natural alternative for oral health care. The study employed a double-blind, randomized controlled design, with participants and examiners blinded to the intervention status, minimizing bias and ensuring the reliability of the results. Clinical studies, conducted on human subjects in a clinical setting, have provided further evidence for the efficacy of red betel leaf in oral health applications. These studies typically involve comparing the effects of red betel leaf interventions with those of conventional treatments or placebos. One clinical trial compared the efficacy of red betel leaf mouthwash with chlorhexidine mouthwash in reducing plaque and gingivitis. The study found that both mouthwashes were effective in reducing plaque and gingivitis, but red betel leaf mouthwash was associated with fewer side effects, such as tooth staining and taste alteration. This finding suggests that red betel leaf mouthwash can be a safe and effective alternative to chlorhexidine mouthwash, particularly for long-term use. The study employed a crossover design, with participants using both mouthwashes in a randomized order, allowing for a within-subject comparison and minimizing the influence of individual variability. Another clinical trial investigated the effects of red betel leaf extract on halitosis. The study found that participants who chewed red betel leaf had significant reductions in VSC levels compared to those who chewed a placebo. This finding suggests that red betel leaf can effectively manage halitosis by reducing the production of VSCs, supporting its traditional use for breath freshening. The study utilized gas chromatography to measure VSC levels in participants' breath, providing objective data on the effects of red betel leaf on halitosis. The use of red betel leaf for oral health purposes is deeply rooted in traditional medicine practices in various cultures. In Indonesia, red betel leaf has been traditionally used for its antiseptic and anti-inflammatory properties, and it is often chewed to maintain oral hygiene. The traditional use of red betel leaf for oral health has been passed down through generations, reflecting its long-standing recognition as a valuable natural remedy. In some cultures, red betel leaf is also used in combination with other herbs and spices, such as cloves and cardamom, to enhance its oral health benefits. The scientific evidence supporting the efficacy of red betel leaf in plaque control validates its traditional use and highlights its potential as a valuable natural remedy for oral health. The convergence of traditional knowledge and scientific evidence strengthens the case for the use of red betel leaf in oral health promotion and disease prevention. This convergence also emphasizes the importance of integrating traditional medicine practices with modern scientific research to develop effective and culturally appropriate oral health interventions.[15-17]

The findings of this study have several implications for oral health practice, suggesting that red betel leaf mouthwash can be a valuable addition to existing oral hygiene protocols. Its efficacy in reducing plaque, natural origin, minimal side effects, potential for halitosis prevention, and feasibility in resource-constrained settings make it a promising candidate for integration into various oral health promotion and disease prevention strategies. Red betel leaf mouthwash can be considered as a potential adjunct to mechanical plaque control methods, such as toothbrushing and flossing. While mechanical plaque removal remains the cornerstone of oral hygiene, red betel leaf mouthwash can complement these methods by providing additional antimicrobial action against oral pathogens. The bioactive compounds in red betel leaf can reach areas that may be difficult to access with mechanical cleaning alone, such as interdental spaces and subgingival areas, further reducing plaque accumulation and promoting oral health. The use of red betel leaf mouthwash as an adjunct to mechanical plaque control can be particularly beneficial for individuals who may have difficulty with proper

brushing and flossing techniques, such as children, older adults, and individuals with disabilities. The mouthwash can provide an additional layer of protection against plaque buildup, reducing the risk of oral diseases. The use of red betel leaf mouthwash can be promoted as a preventive measure against halitosis. By reducing plaque accumulation, the mouthwash can help control the growth of VSC-producing bacteria, thereby mitigating the development of bad breath. This is particularly relevant in populations with a high prevalence of halitosis, such as Indonesia, where oral health awareness and access to dental care may be limited. Red betel leaf mouthwash can be incorporated into daily oral hygiene routines as a preventive measure against halitosis. Its pleasant taste and natural origin make it a suitable option for regular use, encouraging individuals to maintain good oral hygiene practices and prevent the development of bad breath. Red betel leaf mouthwash can also be considered as a potential adjunctive therapy in the management of oral diseases, such as gingivitis and periodontitis. The anti-inflammatory and antimicrobial properties of red betel leaf can help reduce gingival inflammation and control bacterial growth, contributing to the healing process and preventing further disease progression. Incorporating red betel leaf mouthwash into the treatment plan for gingivitis and periodontitis can complement conventional therapies, such as scaling and root planing, by providing additional antimicrobial and anti-inflammatory action. This can lead to improved treatment outcomes and a better prognosis for patients with oral diseases. The accessibility and affordability of red betel leaf make it a feasible option for oral health promotion in resource-constrained settings. The plant is readily available in many tropical regions, and its simple preparation method allows for easy integration into community-based oral health programs. This can empower communities to take control of their oral health and utilize locally available resources for disease prevention. Red betel leaf mouthwash can be promoted as a cost-effective and sustainable oral health intervention in resource-constrained settings. Its use can be integrated into existing community health programs, such as school health programs and maternal and child health programs, providing accessible and affordable oral health care to underserved populations. The use of red betel leaf mouthwash should be accompanied by comprehensive oral health education to ensure its proper and effective use. Oral health professionals, such as dentists and dental hygienists, can play a crucial role in educating patients about the benefits of red betel leaf mouthwash, its proper preparation and use, and its integration into daily oral hygiene routines. Oral health education should also emphasize the importance of maintaining good oral hygiene practices, including proper brushing and flossing techniques, regular dental checkups, and a healthy diet, to complement the use of red betel leaf mouthwash and achieve optimal oral health outcomes.[18-20]

4. Conclusion

The study demonstrated that red betel leaf mouthwash significantly reduced plaque index in a group of high school students. This supports the traditional use of red betel leaf for oral health and suggests its potential as a natural alternative for plaque control and halitosis prevention. The observed plaque reduction can be attributed to the bioactive compounds in red betel leaf, including flavonoids, alkaloids, tannins, and essential oils, which have antimicrobial properties. While the findings are promising, the study has limitations. The pre-experimental design with a one-group pretest-posttest approach limits the ability to draw definitive conclusions about the cause-and-effect relationship between red betel leaf mouthwash and plaque reduction. The small sample size and the specific population of high school students limit the generalizability of the findings to other populations. Further research with larger sample sizes, control groups, and randomized controlled trials is recommended to confirm these findings and explore the long-term effects of red betel leaf mouthwash on oral health. Future studies should also investigate the

specific mechanisms of action of red betel leaf mouthwash and its potential use in combination with other oral health interventions. The findings of this study have implications for oral health practice, suggesting that red betel leaf mouthwash can be a valuable addition to existing oral hygiene protocols. Its efficacy in reducing plaque, natural origin, minimal side effects, potential for halitosis prevention, and feasibility in resource-constrained settings make it a promising candidate for integration into various oral health promotion and disease prevention strategies.

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