

ORIGINAL ARTICLE

Effectiveness of Mouthwash made from Sesewanua (*Clerodendrum Fragrans* Wild) to reduce *Streptococcus Mutans* Bacteria: Quasi-Experimental Study

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ABSTRACT

OBJECTIVE: To investigate the benefits of Sesewanua extract in reducing *Streptococcus mutans* bacteria in the mouth.

METHODOLOGY: This study used a quasi-experimental pre-post test design with a control group. A total of 100 female students who met the inclusion criteria were randomly selected and divided into four groups of 25 each: 1%, 2%, and 4% sesewanua extract mouthwash groups, and a control group (pure Water). Bacterial samples were taken from the cheek mucosa and tongue before and after the oral hygiene procedure. Data were analyzed using a paired t-test and a one-way ANOVA with a significance level of $p < 0.05$.

RESULTS: All three concentrations of sesewanua extract mouthwash were effective in significantly reducing the number of *Streptococcus mutans* bacteria. The 1% concentration reduced the average number of bacteria from 1592.0 to 1111.0 ($p=0.038$), the 2% concentration from 1025.8 to 918.7 ($p=0.034$), and the 4% concentration from 1319.4 to 441.0 ($p=0.012$). The control group did not show a significant decrease ($p=0.089$). Intergroup comparison analysis showed that the 4% concentration was significantly more effective than the 1% ($p=0.002$) and 2% ($p=0.001$) concentrations, while the comparison between the 1% and 2% concentrations did not differ significantly ($p=0.873$). The comparison between the 4% concentration and the control group did not show a significant difference ($p=0.201$).

CONCLUSION: Sesewanua (*Clerodendrum fragrans* Wild) extract mouthwash effectively reduced oral bacteria at all tested concentrations, with the highest concentration demonstrating the most optimal antibacterial activity. Higher extract concentrations yielded greater bacterial reduction, making the highest concentration the most recommended mouthwash formulation.

KEYWORDS: Herbal, Mouthwash, *Streptococcus mutans*, Oral Diseases, Hygiene

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INTRODUCTION

Clerodendrum fragrans has a long history of medicinal use in various cultures. This plant is a member of the Lamiaceae family. Previous research has shown that *Clerodendrum fragrans* leaves contain significant amounts of secondary metabolites. Some of these compounds include flavonoids, alkaloids, and saponins, which have antibacterial and anti-inflammatory properties¹.

Oral health is an important aspect of overall health, where pathogenic bacteria such as *Streptococcus mutans* play a major role in the etiology of dental caries². The use of chemical mouthwashes such as chlorhexidine has been proven effective as an antibacterial, but long-term use can cause side effects such as tooth discoloration, a metallic taste, and irritation of the oral mucosa³. Therefore, the exploration of natural ingredients as alternative mouthwashes has become increasingly relevant, especially during the COVID-19 pandemic, which has increased public awareness of oral hygiene⁴.

The use of herbal plant formulations as mouthwash has increased during the COVID-19 pandemic, as these formulations show positive antibacterial activity, thereby killing several pathogenic bacteria in the mouth⁵. Apart from that, it has also been proven that herbal mouthwash has fewer negative side effects than synthetic mouthwashes sold and widely distributed to the public⁶.

Clerodendrum fragrans (Vent.) Willd., also known as Sesewanua, belongs to the family Lamiaceae and contains diverse phytochemical constituents, including alkaloids, flavonoids, terpenoids, steroids, saponins, tannins, and phenolic compounds. Phytochemical investigations have identified specific bioactive compounds such as β -sitosterol, clerosterol, caffeic acid, kaempferol, acteoside, and leucoseptoside A in its leaves⁷. These secondary metabolites contribute to its documented pharmacological activities, which include antimicrobial, antioxidant, anti-inflammatory, analgesic, antipyretic, antidiabetic, and larvicidal effects⁸.

Ethanol is also known to enhance the extraction of bioactive compounds, thereby increasing the antibacterial properties of the formulation's ingredients. Previous research also shows that apart from the solvent, the extraction method also has a significant influence on the quality of the concentration of the bioactive compounds produced⁹. The extraction method employed constitutes a critical determinant of both the yield and the biological activity of the resulting plant extracts¹⁰. Research has demonstrated that modern extraction techniques such as ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) can significantly outperform conventional solvent extraction (CSE) in recovering bioactive compounds¹¹.

We investigated the benefits of Sesewanua extract (*Clerodendrum fragrans* Wild) in reducing *Streptococcus mutans* in the mouth. The novelty of this research is that no previous studies have examined sesewanua extract as a mouthwash. Therefore, this study provides new insights into the future use of mouthwash. We hope that this research will be a milestone in the use of sesewanua extract as a mouthwash and provide a basis for the mass production of this mouthwash.

METHODOLOGY

This study used a pre-post-test quasi-experimental design with a control group and was conducted in the female dormitory of the Ministry of Health Polytechnic of Manado, Indonesia. A total of 100 female students were randomly selected and met the inclusion criteria, namely, having good overall dental health, being willing to participate, being at least 17 years old, and not undergoing any therapy related to teeth or the mouth. This research started from June 2025 to February 2026.

After the samples were obtained, the participants were randomized using a computer system and divided into four groups of 25 female students each. The 4 groups of: Group 1, participants using 1% sesewanua extract mouthwash; Group 2, participants using 2% sesewanua extract mouthwash; Group 3, participants using 4% sesewanua extract mouthwash; and Group 4, participants using pure Water (placebo).

The selection of 1%, 2%, and 4% concentrations of *Clerodendrum fragrans* extract was based on several scientific considerations. First, the use of a doubling concentration series is a standard approach in dose-response studies to systematically evaluate the linear relationship between increasing doses and pharmacological effects. Second, a low concentration range (1–4%) was chosen because the mouthwash preparation comes into direct contact with the oral mucosa, so a concentration too high could irritate the soft tissues of the oral cavity. Third, previous studies on plant extracts as herbal mouthwashes generally used concentrations of 1–5%, which have been shown to be antimicrobially effective without significant cytotoxicity. Fourth, the three-level concentration design allows for the determination of the minimum effective concentration and evaluation of any statistically significant dose-dependent effects. A placebo group (pure Water) served as a negative control to distinguish the therapeutic effects of the extract from the purely mechanical effects of rinsing.

Sesewanua extract preparation was carried out strictly by collecting fresh leaves and undergoing quality control. The leaves were sorted to remove unusable leaves, then triturated and dried in a cool place to prevent phytochemical degradation from sunlight. This was done to maintain the physiological dryness of the leaves so that they were ready to be ground into powder.

The dried leaves were then milled using a mechanical grinder to obtain a fine powder. The extraction method was maceration, with 1000 g of leaf powder soaked in ethanol for 6 h. After soaking, the mixture was stirred slowly to increase the efficiency of compound extraction. The mixture from the previous process was left for 18 hours, then filtered to separate the liquid material from the plant extract, which remained in powder form. This process was repeated 3 times to ensure maximum phytochemical extraction. The combined filtrate was then evaporated using a low-pressure vacuum evaporator at a controlled temperature of 40°C, producing a concentrated sesewanua extract suitable for formulation into a mouthwash.

To ensure stability and effectiveness, the mouthwash ingredients must be carefully combined. The ingredients included methyl paraben as a preservative, sorbitol for sweetness, and sodium saccharin as a sweetener. The first step was to dissolve the ingredients in Water while gently heating the mixture to increase the solubility. To ensure uniform mixing, the concentrated sesewanua extract was added to the base solution.

Finally, distilled Water was added to the solution, and the desired concentration was obtained using a volumetric flask. The resulting mouthwash solution was rigorously filtered and exposed to ultraviolet (UV) light for sterilization, thereby minimizing the risk of microbial contamination before use in the study.

After providing informed consent, each of the 100 participants received a standard orientation covering the oral hygiene procedures essential to the study. The participants were instructed to brush their teeth for two–three minutes every morning using a predetermined brand of toothpaste. After brushing, the participants gargled for 60 s with 25 mL of the designated mouthwash formulation, following the protocol to ensure uniformity.

Sampling was performed by qualified personnel. Sampling was performed on the mucosal areas of the left and right cheeks and the back of the tongue immediately after oral hygiene procedures. The samples were transferred to a clean wooden spatula and stored at -80°C to maintain microbial viability until analysis.

The main microbiological content of *Streptococcus mutans* was evaluated in a microbiology laboratory. Specific cultures of *Streptococcus mutans* were used to ensure that the bacteria were properly isolated from the participants' mouths.

SPSS software was used. Descriptive analysis was used to summarize the participants' demographic characteristics. To test for significant differences in the number of oral bacteria between the pre- and post-test intervals, a paired t-test and one-way ANOVA were performed. The significance level was set at $p < 0.05$.

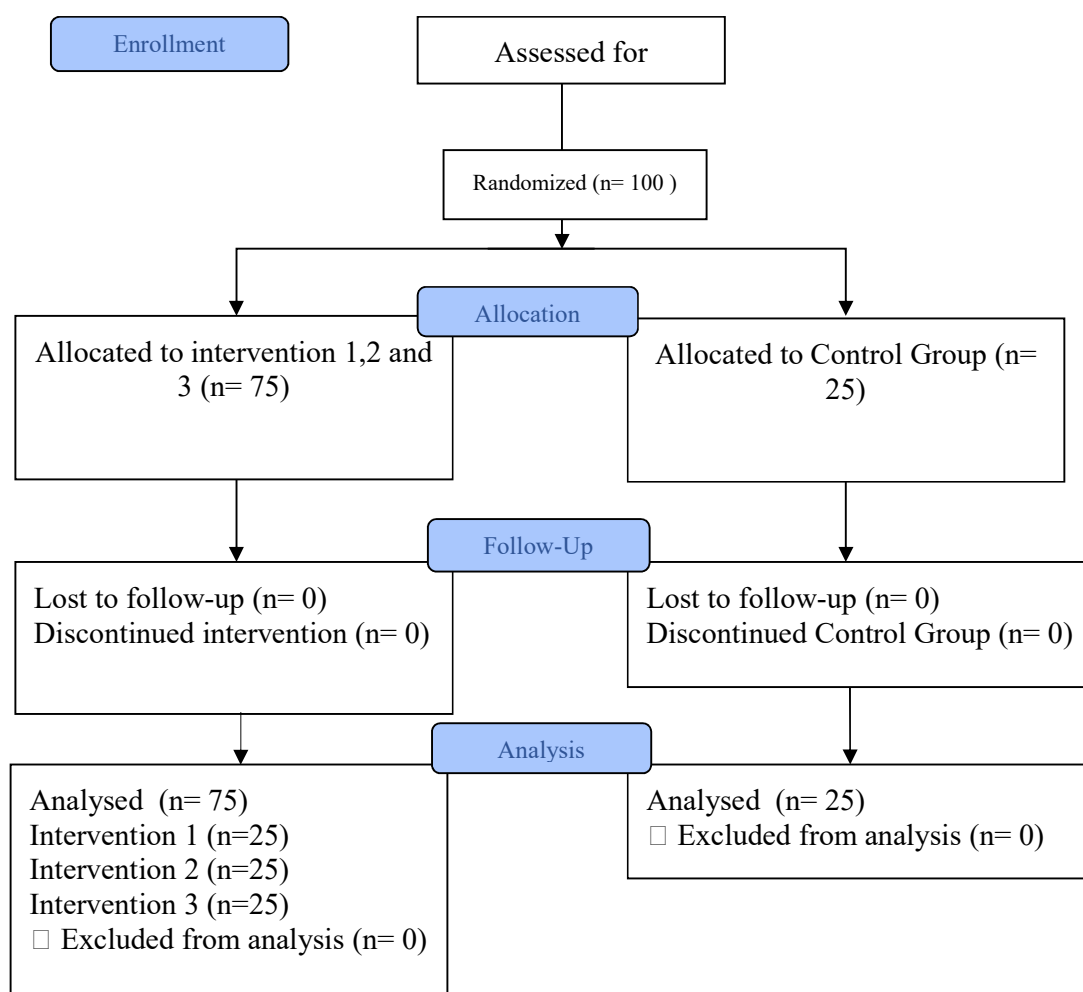


Figure 1: Consort Diagram

RESULTS

Table I shows the mouthwash formulations made from Sesewanua (*Clerodendrum fragrans Wild*) extract at varying concentrations of 1%, 2%, and 4% (weight/gram). Each variation in the concentration of the active ingredient (Sesewanua extract) was combined with other components that functioned as preservatives and flavor enhancers. Sorbitol, used as a sweetener, was set at 5 g for all concentrations, whereas saccharin sodium and methylparaben were added in fixed amounts of 0.1 and 0.2 g, respectively.

Table I: Sesewanua mouthwash formula

Substances	1% (w/g)	2% (w/g)	4% (w/g)	Purified Water (w/g)
Sesewanua extract	1	2	4	-
Sorbitol	5	5	5	5
Saccharin sodium	0.1	0.1	0.1	0.1
Methylparaben	0.2	0.2	0.2	0.2
Distilled Water	100	100	100	100

The results showed a significant reduction in bacterial counts at all concentrations of Sesewanua mouthwash (1%, 2%, and 4%) with p-values of 0.038, 0.034, and 0.012, respectively, all of which were below the threshold of $\alpha = 0.05$. This indicates that the use of this mouthwash was effective in reducing the number of oral bacteria. In contrast, the control group experienced an insignificant reduction ($p = 0.089$). **Table II**

Table II: Analysis of oral bacteria before and after oral hygiene

Sesewanua Mouthwashes Concentration	n	Pre-test (mean $\pm$ SD)	Post-test (mean $\pm$ SD)	p-value
1%	25	1592.0 $\pm$ 1278.42	1111.0 $\pm$ 1163.23	0.038
2%	25	1025.8 $\pm$ 530.03	918.7 $\pm$ 699.78	0.034
4%	25	1319.4 $\pm$ 816.10	441.0 $\pm$ 507.03	0.012
Control (purified Water)	25	1515.0 $\pm$ 1283.68	1416.0 $\pm$ 1131.37	0.089

Table III shows that the comparison of 1% and 4% concentrations differs significantly ($p = 0.002$), with the 4% mouthwash effectively reducing bacterial growth beyond the established limits. However, the 2% concentration also showed significance when compared to the 4% concentration ($p = 0.001$).

Table III: Effectiveness of Sesewanua Mouthwash to Reduce Oral Bacterial Growth

Respondent Group Concentration Type	Respondent Group Concentration Type	Mean Difference	Sig	95% CI Lower Bound	95% CI Upper Bound
1%	2%	66.31	0.873	-303.02	435.64
	4%	718.91	0.002	301.18	1136.64
	Purified Water	175.00	0.568	-870.55	1220.55
2%	1%	-66.31	0.873	-435.64	303.02
	4%	876.87	0.001	404.94	1348.80
	Purified Water	-107.10	0.636	-917.38	703.18
4%	1%	-718.91	0.002	-1136.64	-301.18
	2%	-876.87	0.001	-1348.80	-404.94
	Purified Water	497.4	0.201	-39.81	1034.61
Purified Water	1%	-175.00	0.568	-1220.55	870.55
	2%	107.10	0.636	-703.18	917.38
	4%	-497.4	0.201	-1034.61	39.81

DISCUSSION

The findings in **Table I** show that each formulation contained sodium saccharin (0.1 g), sorbitol (5 g), methyl paraben (0.2 g), and distilled Water, for a total weight of 100 g. The choice of sorbitol as a sweetener is supported by previous research showing improved taste and humectant properties that help maintain moisture in similar oral care products¹. This study used methylparaben because it is widely known as a preservative in various formulations. This is important because it can inhibit microbial growth¹³.

The decrease in bacterial concentration, as measured using Sesewanua mouthwash, was significant at all solution concentrations (p-value of < 0.05). In particular, Sesewanua at 2% concentration resulted in a decrease in the number of bacteria from an average of 1025.8 (± 530.03) to 441.0 (± 507.03). This significant decline is accompanied by several studies highlighting the antibacterial properties of various herbal extracts, which are effective in reducing oral pathogens^{14,15}.

The antibacterial efficacy of Sesewanua mouthwash can be attributed to the synergistic action of its bioactive phytochemical constituents, particularly flavonoids, tannins, and alkaloids, which exert antimicrobial effects through distinct yet complementary mechanisms. Flavonoids inhibit bacterial growth by forming complexes with extracellular and soluble proteins, suppressing bacterial enzymes, and causing energy exhaustion¹⁶. Tannins function by binding to proteins via hydrogen bonding and hydrophobic interactions, shrinking cell walls and disrupting membrane permeability¹⁷. Alkaloids exert antibacterial effects through multiple mechanisms, including disruption of cell membrane permeability, inhibition of cell wall synthesis by intercalating with DNA, suppression of nucleic acid and protein synthesis, respiratory inhibition, and interference with cell division via Z-ring perturbation¹⁸.

Mouthwash is known to effectively reduce the formation of biofilms and pathogens in the mouth¹⁹. This study underscores the importance of using Sesewanua, a sustainably produced mouthwash for dental and oral care. This is because herbal mouthwash has fewer side effects than synthetic mouthwashes²⁰.

Furthermore, the growing body of evidence supports the development of plant-based mouthwash formulations as viable alternatives to conventional synthetic products. Synthetic mouthwashes, particularly those containing chlorhexidine (CHX), although effective in reducing dental biofilm and controlling gingivitis, are associated with several adverse effects, including extrinsic tooth staining, taste alterations, oral mucosal irritation, and disruption of the oral microbiome toward a more acidic and potentially dysbiotic state²¹.

Long-term use of active ingredients such as cetylpyridinium chloride, chlorhexidine, and triclosan can cause taste changes and mouth staining²². Mouthwash formulations including sodium saccharin, sorbitol, and methylparaben have been associated with potential adverse reactions. Sodium saccharin can contribute to enamel demineralization²³. The selection of a low concentration range (1–4%) for the Sesewanua extract was intended to minimize the risk of soft-tissue irritation from direct mucosal contact.

Microbiological evaluation targeted *Streptococcus mutans*, a major cariogenic pathogen, using specific selective culture media to ensure proper isolation from participants' oral samples²⁴. Oral samples were collected from the mucosal surfaces of the left and right cheeks and the back of the tongue using a sterile wooden spatula and stored at -80°C to maintain microbial viability. Bacterial quantification was expressed as colony-forming units (CFU), a standard metric in oral microbiology for counting viable bacteria²⁵.

Studies have demonstrated that CHX can shift the oral microbiome composition, increasing Firmicutes and Proteobacteria while reducing beneficial nitrate-reducing bacteria, which may negatively impact both oral and systemic health²⁶. In contrast, herbal mouthwashes have shown comparable antimicrobial and antibiofilm efficacy with fewer reported side effects²⁷.

Plant-derived compounds exhibit significant antibacterial and antibiofilm activity against oral pathogens, such as *Streptococcus mutans*, offering a safer, long-term approach to oral hygiene²⁸. These findings reinforce the potential of naturally derived mouthwash formulations, such as Sesewanua-based products, as sustainable and effective alternatives for dental and oral care.

Compared with both higher concentrations and purified Water, the data in **Table III** show how well different Sesewanua mouthwash concentrations reduced oral bacterial growth. A p-value of 0.002 at the 4% concentration indicates a statistically significant decrease in bacterial growth compared to the 1% concentration, suggesting that higher concentrations produce more potent antibacterial effects²⁹. Studies demonstrating the superior efficacy of higher concentrations of active ingredients in mouthwashes support the concentration-dependent efficacy characteristic of antimicrobial agents³⁰.

The findings showed that concentrations of 2% and 4% significantly reduced bacterial levels in the mouth (p-value of 0.001). It is also known that chlorhexidine and cetylpyridinium chloride are commonly used in mouthwash products. Other findings show that these ingredients also show high efficacy in reducing bacteria³¹.

Chlorhexidine (CHX) and cetylpyridinium chloride (CPC) are two of the most widely studied antimicrobial active ingredients used in mouthwash formulations for controlling oral bacteria²⁹. Various studies have shown that these two ingredients produce a synergistic effect that enhances overall antimicrobial activity³². Mouthwash with a low concentration of 0.05% CHX combined with 0.05% CPC significantly reduced viable cells in oral biofilms, equivalent to a conventional 0.1% CHX mouthwash³³.

CPC itself, as a cationic quaternary ammonium compound, exhibits bactericidal activity against Gram-positive bacteria and, at higher concentrations, against Gram-negative bacteria³⁴. Specifically, a mouthwash containing CPC and sodium fluoride was shown to reduce *S. mutans* by up to 100% in biofilms, compared to only 16.82% with sodium fluoride alone³⁵. In addition, the use of CHX- and CPC-based mouthwashes consistently significantly reduced plaque index, gingival index, and bacterial colony count³⁵. These findings reinforce that CHX and CPC, either singly or in combination, are highly effective antibacterial agents in maintaining oral health.

A placebo group was administered distilled Water. In the control group, a p-value of >0.05 was obtained. This confirms the importance of using this herbal extract as part of oral and dental care³⁶. Distilled Water did not have a significant effect on the concentration of bacteria in the mouth, confirming the importance of using Sesewanua mouthwash to fight pathogenic bacteria³⁷.

Implication

The 4% concentration of Sesewanua mouthwash showed the strongest antibacterial effect, with significant differences compared to 1% (p=0.002) and 2% (p=0.001), and is therefore recommended as the optimal concentration for clinical application. This formulation has the potential to be used as an adjunct therapy in the management of gingivitis, reducing dental biofilm, and controlling cariogenic bacteria such as *Streptococcus mutans*. Considering that synthetic chlorhexidine-based mouthwashes are associated with side effects such as tooth staining and disruption of the oral microbiome, Sesewanua-based mouthwash offers a safer herbal alternative. Further research in the form of long-term clinical trials is needed to evaluate safety, compare efficacy directly with chlorhexidine, and optimize dosage for commercial development.

CONCLUSION

A mouthwash containing Sesewanua (*Clerodendrum fragrans* Wild) extract was shown to be effective in reducing the number of oral bacteria at all tested concentrations, while the control group using purified water did not show a statistically significant reduction. Among the three concentrations, the formula with the highest concentration showed the most optimal effectiveness in reducing the number of bacteria and was significantly different from the lower concentration. Meanwhile, the two lower concentrations did not show significant differences from each other. Therefore, it can be concluded that the higher the concentration of Sesewanua extract in the mouthwash formulation, the greater its effectiveness in reducing the growth of oral bacteria, with the highest concentration being the most recommended formulation.

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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Laoh JM: Conception, Design, Acquisition, Analysis, Interpretation of Data, Manuscript Writing & Approval

Fione VR: Design, Acquisition, Analysis, Interpretation of Data, Manuscript Writing & Approval

Purba RB: Design, Acquisition, Analysis, Interpretation of Data, Manuscript Writing & Approval

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