RESEARCH
www.commprac.com
ISSN 1462 2815

EFFICACY OF *SESEWANUA (CLERODENDRUM FRAGRANS WILD)* MOUTHWASH ON ORAL BACTERIA GROWTH: A QUASI-EXPERIMENT STUDY

Joice Mermy Laoh [1], Marlisa [2], Yos Banne [3], Vega Roosa Fione [4], Rudolf Boyke Purba [5] and Baithesda [6]

[1,2] Nursing Department, Health Polytechnic of the Ministry of Health Manado, North Sulawesi, Indonesia.
[3] Pharmacy Department, Health Polytechnic of the Ministry of Health Manado, North Sulawesi, Indonesia.
[4] Dental Health Department, Health Polytechnic of the Ministry of Health Manado, North Sulawesi, Indonesia.
[5] Nutrition Department, Health Polytechnic of the Ministry of Health Manado, North Sulawesi, Indonesia.
[6] Nursing and Health Sciences, Sari Putra Indonesia University Tomohon, Tomohon City, North Sulawesi, Indonesia.

DOI: 10.5281/zenodo.13739738

Abstract

Introduction: Oral diseases have been treated using natural ingredients because of their anti-inflammatory, antioxidant, and antibacterial properties, also found in herbs. Still, only a few studies have examined using herbal-based mouthwashes as antibacterials in the mouth. This study examined the efficacy of a mouthwash containing *sesewanua* (Clerodendrum Fragrans Wild) on oral bacteria growth. **Material and Methods:** This quasi-experiment was conducted with one hundred female students. Participants were categorized into four groups of 25 each. Before and after oral hygiene, samples of a swab from oral mucosa and tongue dorsum were examined to count the streptococcus mutans. The outcome measures were analysed using a paired t-test and One-way ANOVA; a p-value of less than 0.05 is defined as statistical significance. **Results:** We found oral hygiene using 1%, 2%, and 4% *sesewanua* mouthwash significantly reduced the growth of oral bacteria ($p<0.05$). The 2% *sesewanua* mouthwash concentration more effectively reduced oral bacterial growth ($p<0.05$). There was a decrease in the average amount of bacteria in the oral cavity. **Conclusions:** The 2% concentration of *sesewanua* mouthwash works best when lessening oral bacterial growth. Practising regular oral hygiene using the right antibacterial mouthwash is critical due to the rising prevalence of oral diseases globally. The search for alternative products is still significant since herbal remedies made from plants utilized in traditional medicine have shown to be an excellent alternative to chemical medication. The *sesewanua* mouthwash may be used as routine oral hygiene to prevent and treat oral diseases in both clinical and community settings.

Keywords: Herbal, Mouthwash, Oral Bacteria, Oral Diseases, Oral Hygiene.

INTRODUCTION

Oral diseases are a prevalent health issue that affects many people worldwide. According to the WHO Global Oral Health Status Report (1), around 3.5 billion people globally suffer from oral diseases, with middle-income countries accounting for three out of every four cases. Primary tooth decay affects 514 million children globally, whereas caries of permanent teeth afflict around 2 billion adults worldwide. Periodontal disease is an oral disease and one of the most common inflammatory conditions affecting adults. It is estimated that over 19% of adults globally suffer from severe periodontal disorders, with over 1 billion cases worldwide. In Indonesia, 74.1% of the population suffers from periodontitis (2).

Scientific studies have discovered a link between specific bacteria and dental health. For instance, there is a positive correlation between *Veillonella* (3) and *Capnocytophaga ochracea* (4). In healthy individuals who do not suffer from bad breath, *Streptococcus salivarius* is often found on the tongue's top surface (5) A thorough investigation of the typical oral microbiome revealed that the most common species in healthy individuals include *Granulicatella* advances, *Gemella hemolysis*, and *Streptococcus mutans* (6). While there is still no agreement on the exact bacterial species that serve as indicators of dental health, there is increasing evidence of the most prevalent bacterial species present in healthy individuals.

Periodontitis is a chronic condition that affects adults. It is caused by a particular bacterial ecology that inflames the host and destroys periodontal tissue, which leads to the formation of pockets and causes teeth to fall out (7). Non-plaque-associated bacteria, viruses, and fungi can also cause gingival lesions linked to herpes and candidiasis (8). In addition, a specific microecosystem defines malignant tissue in cases of oral cancer. It's interesting to note that oral microbes may also be linked to health conditions outside of the mouth.

There are suggestions of links between oral microbes and gastrointestinal cancers (9), immunological (10), cardiometabolic (11), and respiratory disorders (12), as well as complications during pregnancy (13). Many oral diseases and disorders share modifiable risk factors for the four most common non-communicable diseases (NCDs): diabetes, cancer, chronic respiratory illnesses, and tobacco use. Furthermore, there is a reciprocal relationship between the onset and advancement of periodontal disease and diabetes (14,15). Dental cavities are also caused by high sugar consumption, and there is a causative connection between the two (15).

Access to dental care is often a luxury that many low-income individuals cannot afford, leaving them in need of a more effective and efficient alternative to dental prevention and treatment. Various agents such as biguanide antiseptics, quaternary ammonium antiseptics, phenolic antiseptics, and oxygenating agents are used to treat oral infections (16). However, several of these medications have adverse effects, including tooth discoloration, vomiting, and diarrhea.

Furthermore, bacteria have become resistant to many antibiotics commonly used to treat oral infections, such as tetracycline, penicillin, cephalosporins, and erythromycin (17). As a result, natural medicines extracted from plants used in traditional treatments have proven to be a viable alternative to chemical cures, and the search for alternative treatments remains a significant concern (18).

Ongoing research is being carried out to find alternative long-term safe and effective medicine that has a lower side effect rate than synthetic materials. Antimicrobial substances, which have potential health benefits, are being studied, especially for their effects on the oral cavity (18). These substances are primarily derived from metal salts, phenolic compounds, and herbal extracts, all of which have shown positive outcomes in clinical and laboratory investigations (19). Since herbal extracts are non-synthetic and non-chemical and have been used for a long time in traditional medicine, extensive research has been done on them (20). Oral diseases have been treated using natural components because of their anti-inflammatory, antioxidant, and antibacterial properties, which are also present in herbs (21)

The tropical forests of Indonesia are home to native people who possess exceptional knowledge about plants that are believed to have medicinal properties. Traditional

healing is the primary source of healthcare in many regions. In North Sulawesi, for instance, people have been using traditional medicine to treat various ailments such as body pain, sprains, and swallowing pain (22). *Clerodendrum Fragrans Wild*, commonly known as *sesewanua*, is an antipyretic (23) and is used in traditional spas in North Sulawesi (24,25). It contains 13.47% quercetin, a flavonoid chemical that has anti-inflammatory and mildly anticancer properties (26–28).

Sesewanua contains antibacterial and anti-inflammatory properties, which makes it a suitable active ingredient for use in mouthwash. However, there is no prior research on the effectiveness of *sesewanua* extract as a mouthwash. Thus, this study aims to investigate the impact of varying concentrations of *sesewanua* extract on the growth of oral bacteria and to determine the optimum concentration of sesewanua for reducing oral bacteria. The results of this study can be beneficial in preventing and treating oral diseases, both in clinical and community settings.

MATERIALS AND METHODS

Study Design

This research was conducted at the Manado Health Polytechnic female dormitory as a pre-post-test with a control group design. This quasi-experiment study began after obtaining the necessary regulatory permits. Participants were randomly assigned to receive 4%, 2%, or 1% of *sesewanua* extract, or purified water. Each group had an equal number of participants. The primary objective was to identify differences in the overall effectiveness of these four interventions. Participants were treated similarly throughout the study. Participants in each parallel group were required to avoid accidentally contaminating the other group through unexpected co-interventions.

Participants

A total of one hundred female students were randomly selected and divided into four groups based on certain eligibility criteria. The criteria included willingness to participate, good dental and overall health, being at least 17 years old, and not receiving any medication therapy. The sample size was determined based on a previous study conducted by Jain and Jain in 2016 (27).

Preparation of sesewanua extract

The leaves collected for this research are first sorted and washed under running water. They are then drained and dried in open air, away from direct sunlight. The drying process continues until the leaves reach a state of physiological dryness, which means that they can be broken up by hand. After this, the leaves are sorted once again and ground into a powder.

For this research, the maceration extraction method was used. This involved soaking 1000 grams of *sesewanua* leaf powder in a 70% ethanol solvent for 6 hours, stirring occasionally, and then leaving it for another 18 hours. The macerate was then separated by filtration. This process was repeated twice using the same solvent. All the macerate was collected, and the solvent was evaporated at 40°C using a low-pressure vacuum evaporator until a thick extract was obtained.

To make the mouthwash, sorbitol, saccharin natrium, and methyl pabaren were each dissolved in water and heated. The *sesewanua* extract was then dissolved in sorbitol. All solutions were combined in a measuring flask, and distilled water was added to

reach the mark. The solution was then filtered and sterilized using a UV lamp.

Procedures

All 100 participants who provided their informed consent were randomly assigned to one of four groups, each consisting of 25 students. The intervention group used a mouthwash containing 1%, 2%, and 4% *sesewanua*, while the other group received a placebo mouthwash made of purified water. Before starting the study, all participants received instructions on basic oral hygiene practices. During the study, participants were instructed to brush their teeth with the same toothpaste for two to three minutes in the morning and gargle with 25 ml of mouthwash for 60 seconds. Before and after oral hygiene, qualified personnel took a swab from the left and right buccal oral mucosa and tongue dorsum with a sterile wooden spatula immediately after oral hygiene procedure. The swab was then transferred to a clean wooden spatula that was kept at -80°C until it was processed further in the same microbiological laboratory. The aim was to count the number of *streptococci mutans*. The students were free to withdraw from the study at any time during the study period.

Ethical Consideration

The study has been given ethical approval and permission has been granted from Health Research Ethics Committee Poltekkes Kemenkes Manado, under the number KEPK.01/10/409/2023. Each participant has provided informed consent, and the data has been de-identified after the data processing protocol. Students have the right to withdraw from the study at any point during the study period.

Statistical Analysis

The study used a paired t-test and One-way ANOVA test to analyse the count of oral bacteria for significant differences between pre-test and post-test scores. Statistical significance was defined as a p-value of less than 0.05 and SPSS v.17 software was used for the analysis.

RESULTS

One hundred healthy female students were surveyed, and Tables 2 and 3 present the number of oral bacteria collected for the intervention and placebo groups and the effectiveness of *sesewanua* mouthwash in reducing oral bacteria growth.

The study involved 25 participants, and the results showed that administering a 1% concentration of the mouthwash reduced bacterial growth in the mouth by an average of 481.0 (from 1592.0 to 1111.0). Similarly, administering a 2% concentration of the mouthwash reduced bacterial growth by an average of 584.8 (from 1025.8 to 441.0), while a 4% concentration reduced it by an average of 400.7 (from 1319.4 to 918.7). Additionally, using purified water for oral hygiene reduced bacterial growth in the mouth by an average of 268.4 (from 1515.0 to 1246.6).

When comparing the average growth of oral bacteria between the 1% and 2% concentrations, while there is a difference of -99.8, it is not statistically significant ($p = 0.322$). Similarly, there is no significant difference in the average growth of bacteria between the 1% and 4% concentrations ($p = 0.461$). However, the 1% concentration did show a significant difference compared to the control group ($p = 0.003$). On the other hand, there is a significant difference ($p = 0.010$) between the 2% and 4% concentrations. Additionally, when compared to the control (water), the 2%

concentration also showed a significant difference ($p < 0.001$). Finally, compared to the control group, there was no significant difference ($p = 0.149$) in the effectiveness of the 4% concentration of sesewanua mouthwash.

DISCUSSION

The purpose of this study was to evaluate the effectiveness of *sesewanua* mouthwash in reducing the number of oral bacteria that can lead to oral diseases. The study involved 100 participants aged between 17-22 years who followed the trial protocol. They observed a significant decrease in the number of harmful bacteria in their mouths after using *sesewanua* (Clerodendrum Fragrans Wild) mouthwash with concentrations of 1%, 2%, and 4%. This improvement in oral hygiene was achieved using *sesewanua* mouthwash.

The study's results indicate that using *sesewanua* and purified water in varying concentrations for oral hygiene at the post-test significantly reduces the number of oral bacteria. Previous studies have shown that *sesewanua* leaves combined with ethanol extract have the potential to be antibacterial (29). *Sesewanua* plants also exhibit antimicrobial activity, including antiplasmodial and antiprotozoal (30,31). In addition to its antimicrobial activity, *sesewanua* has anti-cancer properties that can suppress cancer cell growth while inhibiting bacterial growth (26). The methanol fraction of *sesewanua* leaves can inhibit bacterial growth with the largest inhibition zone (32).

An experiment was conducted to determine the ideal concentration of s*esewanua* for reducing oral bacteria. The trial involved using 1%, 2%, and 4% sesewanua mouthwash, and the participants observed a decrease in oral bacteria growth. The data indicated a significant drop immediately after oral hygiene, and it was found that a 2% concentration of *sesewanua* mouthwash is a great substitute for oral hygiene. This can be seen from the difference in the mean number of oral bacteria compared to 4% and purified water. This result differs from a previous study that found *sesewanua* leaf ethanol extract to be a potential antibacterial agent against Staphylococcus aureus, Escherichia coli, and Salmonella typhi, at concentrations of 20%, 40%, and 60% (29). The extract was included in the solid group for inhibiting Staphylococcus aureus bacteria, while at concentrations of 40% and 60%, it was included in the strong group to inhibit Escherichia coli and Salmonella typhi bacteria. This is in line with the theory that the larger the extract concentration, the larger the diameter of the inhibitory area. Therefore, the greater the concentration of the antimicrobial compound being tested, the greater the antimicrobial activity of the compound (33). The difference in results is possible because the research subjects had good oral and dental health, and further antibacterial activity against gram-positive and gram-negative bacteria could not be detected.

This study had limitations, such as its relatively short trial period and in the same population. More clinical studies with a diverse subject population and a more extended trial period are required to investigate its potential as a therapeutic agent in the management of daily oral hygiene.

CONCLUSIONS

There was a reduction in the average quantity of bacteria in the oral cavity after applying *sesewanua* mouthwash as part of oral hygiene. The 2% concentration of *sesewanua* mouthwash is more effective in reducing oral bacterial growth. The

findings of this study may be applied to the prevention and treatment of oral disorders in both clinical and community settings. Hence, future research is needed to evaluate the efficacy of *sesewanua* mouthwash as a formula for oral hygiene among critical illness patients in the hospital.

Acknowledgments

This work is supported by DIPA Politeknik Kesehatan Kementrian Kesehatan Manado Grants for the 2023 budget year.

References

1) WHO. Global oral health status report: towards universal health coverage for oral health by 2030: summary of the WHO European Region. Vol. 57, Dental Abstracts 2022.
2) kementerian Kesehatan RI. Kementrian Kesehatan RI. 2018. Profil Kesehatan Indonesia 2018. Jakarta: Kemenkes RI. Diakses pada tanggal 31 Januari 2019 dari http://www.depkes.go.id/resources/download/pusdatin/profil-kesehatanindonesia/Profil-Kesehatan-Indonesia-tahun-2018.pdf. Jurnal Ilmiah Kesehatan Masyarakat. 2018;11 edisi 2.
3) Kuboniwa M, Tribble GD, James CE, Kilic AO, Tao L, Herzberg MC, et al. Streptococcus gordonii utilizes several distinct gene functions to recruit Porphyromonas gingivalis into a mixed community. Mol Microbiol. 2006;60(1). DOI: 10.1111/j.1365-2958.2006.05099.x
4) Sakamoto M, Huang Y, Ohnishi M, Umeda M, Ishikawa I, Benno Y. Changes in oral microbial profiles after periodontal treatment as determined by molecular analysis of 16S rRNA genes. J Med Microbiol. 2004;53(6). DOI: 10.1099/jmm.0.45576-0
5) Kazor CE, Mitchell PM, Lee AM, Stokes LN, Loesche WJ, Dewhirst FE, et al. Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy patients. J Clin Microbiol. 2003;41(2). DOI: 10.1128/JCM.41.2.558-563.2003
6) Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol. 2005;43(11). DOI: 10.1128/JCM.43.11.5721-5732.2005
7) Tegelberg P, Tervonen T, Knuuttila M, Jokelainen J, Keinänen-Kiukaanniemi S, Auvinen J, et al. Long-term metabolic syndrome is associated with periodontal pockets and alveolar bone loss. J Clin Periodontol. 2019;46(8). DOI: 10.1111/jcpe.13154
8) Holmstrup P, Plemons J, Meyle J. Non–plaque-induced gingival diseases. J Clin Periodontol. 2018;45. DOI: 10.1111/jcpe.12938
9) Schmidt BL, Kuczynski J, Bhattacharya A, Huey B, Corby PM, Queiroz ELS, et al. Changes in abundance of oral microbiota associated with oral cancer. PLoS One. 2014;9(6). DOI: 10.1371/journal.pone.0098741
10) Şenel S. An overview of physical, microbiological and immune barriers of oral mucosa. Vol. 22, International Journal of Molecular Sciences 2021.
11) Li Y, Zhu M, Liu Y, Luo B, Cui J, Huang L, et al. The oral microbiota and cardiometabolic health: A comprehensive review and emerging insights. Vol. 13, Frontiers in Immunology 2022.
12) Dong J, Li W, Wang Q, Chen J, Zu Y, Zhou X, et al. Relationships Between Oral Microecosystem and Respiratory Diseases. Vol. 8, Frontiers in Molecular Biosciences 2022.
13) Wen X, Fu X, Zhao C, Yang L, Huang R. The bidirectional relationship between periodontal disease and pregnancy via the interaction of oral microorganisms, hormone and immune response. Vol. 14, Frontiers in Microbiology 2023.
14) Kebede TG, Holtfreter B, Kocher T, Meisel P, Dietrich T, Biffar R, et al. Association of Periodontal Destruction and Diabetes with Mortality. J Dent Res. 2017;96(1). DOI: 10.1177/0022034516668839
15) Moreira ARO, Batista RFL, Ladeira LLC, Thomaz EBAF, Alves CMC, Saraiva MC, et al. Higher sugar intake is associated with periodontal disease in adolescents. Clin Oral Investig. 2021;25(3). DOI: 10.1007/s00784-020-03387-1

16) Addy M. Chlorhexidine compared with other locally delivered antimicrobials: A short review. J Clin Periodontol. 1986;13(10). DOI: 10.1111/j.1600-051X.1986.tb01434.x

17) Bidault P, Chandad F, Grenier D. Risk of bacterial resistance associated with systemic antibiotic therapy in periodontology. Vol. 73, Journal of the Canadian Dental Association 2007.

18) Prabu GR, Gnanamani A, Sadulla S. Guaijaverin - A plant flavonoid as potential antiplaque agent against Streptococcus mutans. J Appl Microbiol. 2006;101(2). DOI: 10.1111/j.1365-2672.2006.02912.x

19) Salehi B, Jornet PL, López EPF, Calina D, Sharifi-Rad M, Ramírez-Alarcón K, et al. Plant-derived bioactives in oral mucosal lesions: A key emphasis to Curcumin, Lycopene, chamomile, aloe Vera, green tea and coffee properties. Vol. 9, Biomolecules 2019.

20) Kaur R, Tirth A, Ravishankar T, … SK-J of M, 2017 U. Dental herbalism: A review. Journal of Medicinal Plants Studies. 2017;5(5).

21) Kalaskar AR, Bhowate RR, Kalaskar RR, Ghonmode S. Novel neem leaves extract mouthwash therapy for oral lichen planus. J Herb Med. 2021;26. DOI: 10.1016/j.hermed.2020.100408

22) Singh Dhaliwal J, Singh Gambhir R, Kaur Sodhi S, Shaheed G, Nurolaini Binti Pg Haji Muhammad Kifli D, Assistant Professor S, et al. Herbs and their use in oral care Herbs and their use in oral care: A Review. Vol. 7, Brunei Darussalam Journal of Health 2017.

23) Tuuk KPA, Koamesah SMJ, Lidia K. Uji efek antipiretik ekstrak etanol bawang merah (Allium ascalonium L.) pada tikus galur sprague-dawley (Rattus norvegicus) yang diinduksi vaksin DPT-HB. Cendana Medical Journal. 2020;19(1).

24) Arini DID, Kinho J. Diversity of Medicinal Plants on Coastal Forest in Tangkoko Natural Reserve. Jurnal Wasian. 2015;2(1). DOI: 10.20886/jwas.v2i1.863

25) Nonke Sherly Kaunang E, Yermia Semuel M, Eva Nonke Sherly Kaunang C. Botanical and phytochemical constituents of several medicinal plants from mount Klabat north Minahasa. ~ 29 ~ Journal of Medicinal Plants Studies. 2017;5(2).

26) Yudistira A. Uji Aktivitas Anti Kanker Payudara Ekstrak Daun Sesewanua (Clerodendron squmatum vahl.) terhadap Sel Kanker Payudara T47D. Pharmacon Jurnal Ilmiah Farmasi. 2017;6(2).

27) Jain I, Jain P. Comparative evaluation of antimicrobial efficacy of three different formulations of mouth rinses with multi-herbal mouth rinse. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2016;34(4). DOI: 10.4103/0970-4388.191409

28) Sapiun Z, Pangalo P, Imran AK, Wicita PS, Daud RPA. Determination of total flavonoid levels of ethanol extract Sesewanua leaf (Clerodendrum fragrans Wild) with maceration method using UV-vis spectrofotometry. Pharmacognosy Journal. 2020;12(2). DOI: 10.5530/pj.2020.12.56

29) Kumakauw VV, Simbala HEI, Mansauda KLR. Aktivitas Antibakteri Ekstrak Etanol Daun Sesewanua (Clerodendron Squamatum Vahl.) terhadap Bakteri Staphylococcus aureus Escherichia coli dan Salmonella typhi. Jurnal MIPA. 2020;9(2). DOI: 10.35799/jmuo.9.2.2020.28946

30) Abouzid SF, Wahba HM, Elshamy A, Cos P, Maes L, Apers S, et al. Antimicrobial activity of some Clerodendrum species from Egypt. Nat Prod Res. 2013;27(11). DOI: 10.1080/14786419.2012.686905

31) Simorangkir M, Maha AP. Antibacterial Activity And Phytochemical Screening From Chromatography Fraction Of Ethanol Extract Of Sarang Banua (Clerodendrum fragrans Vent Willd) Against Salmonella enterica. Indonesian Journal of Chemical Science and Technology (IJCST). 2020;3(2). DOI: 10.24114/ijcst.v3i2.19525

32) Kadir FN, Runtuwene MRJ, Kamu VS. Uji Aktivitas Antibakteri Fraksi Daun Sesewanua (Clerodendron squamatum Vahl.) Terhadap Bakteri Streptococcus mutans dan Escherichia coli. Jurnal MIPA. 2021;10(2). DOI: 10.35799/jmuo.10.2.2021.33592

33) Masriadi, Sukmawati, Hasta Handayani Idrus. Formulation Herbal Mouthwash Combination Extract of Ginger and Lemongrass as Antibacterial Causes of Halitosis in Diabetes Mellitus Patients. Indian Journal of Forensic Medicine & Toxicology. 2021;15(4). DOI: 10.37506/ijfmt.v15i4.16963