Aloha International Journal of Health Advancement (AIJHA)
ISSN 2621-8224
Volume 6 Number 8, August 2023
http://journal.aloha.academy/index.php/aijha
RESEARCH
DOI: http://dx.doi.org/10.33846/aijha60803

Testing the Effectiveness of Herbal Mouthwash Made from Betel Leaves and Kalamansi Orange

Iwan Iskandar[1], Hevi Horiza[2] (Corresponding Author), Mutia Yuhesti[3]
[1]Sanitation Study Program, Department of Environmental Health, Poltekkes Kemenkes Tanjungpinang, Riau Islands, Indonesia
[2]Sanitation Study Program, Department of Environmental Health, Poltekkes Kemenkes Tanjungpinang, Riau Islands, Indonesia; hevi220987@gmail.com
[3]Sanitation Study Program, Department of Environmental Health, Poltekkes Kemenkes Tanjungpinang, Riau Islands, Indonesia

Submitted: July 31, 2023 -Revised: August 24, 2023 -Accepted: August 25, 2023 -Published: August 31, 2023

ABSTRACT

The oral cavity is the most complex and easily accessible site for microbial colonization in the human body. Teeth, gingiva, tongue, and buccal mucosa have different surfaces for microbial colonization. To reduce microbes in the oral cavity, medicinal plants can be used. Medicinal plants that can be utilized include Betel Leaves and Kalamansi orange. Betel leaves are well-known in Indonesian society not only for their traditional consumption but also for their widespread availability throughout Indonesia. The study aimed to develop a new mouthwash candidate using organic materials without the addition of alcohol compounds. This research was a laboratory experimental study, involving the preparation of betel leaf extract, the production of Betel Kalamansi Mouthwash (Sirkala), and testing the Betel Kalamansi Mouthwash (Sirkala) against two types of bacteria, *Streptococcus mutans* and Bacillus *cereus*, compared to the patented Listerine mouthwash. The stages include organoleptic testing and data processing. The expected outcome of this research is the development of an alcohol-free mouthwash made from organic materials. Microbiological effectiveness test results show that the herbal mouthwash formula Sirkala can effectively eliminate the target bacteria Streptococcus mutans, with a killing ability of up to 99.999% at contact times of 10, 15, and 20 seconds. This is comparable to the results of testing Listerine mouthwash widely available in the market. However, based on testing against Bacillus cereus, the herbal mouthwash formulation Sirkala is unable to eliminate the target bacteria like Listerine mouthwash.
Keywords: efficacy testing; herbal mouthwash; betel leaf; calamansi orange

INTRODUCTION

The oral cavity is the most complex and easily accessible site for microbes to colonize in the human body. Teeth, gingiva, tongue, and buccal mucosa have different surfaces for microbial colonization. The constant production of saliva and the availability of sugar and amino acids from consumed food provide nutrients for microbial growth. Bacteria are the most commonly found microbes in the human oral cavity. The human oral cavity is home to approximately 700 identified species of bacteria.

Oral bacteria are distributed in specific locations; for example, some species of Streptococcus, including normal flora in the mouth, use sugar to produce lactic acid and are found in various areas of the mouth. Streptococcus salivarius inhabits the surface of the tongue, Streptococcus mitis occupies much of the cheek mucosa, and Streptococcus sanguis resides on the tooth surface. Additionally, bacteria that cause oral and dental diseases are also found, such as mutans Streptococcus, which causes tooth decay, as well as some species of Actinomyces, Nocardia, Corynebacterium,[1-5] Veilonella, and Fusobacterium, which cause periodontitis.[6-11]

Natural ingredients around us can also be utilized to create mouthwash. Among these ingredients are betel leaves, kalamansi oranges, and several other natural substances. As a solvent, we use non-alcoholic distilled water. Several research findings indicate the ability of betel leaves and kalamansi oranges in their antibacterial activities. The antibacterial properties of betel leaf extract have been demonstrated.[12-16] Betel leaf extract with ethanol as a solvent can inhibit the growth of Staphylococcus aureus bacteria using the disc diffusion method, with the smallest concentration of betel leaf extract being 106 ppm in the strong resistance category. Ibrahim (2013) proved in their research that betel leaf extract can inhibit the growth of Streptococcus viridans bacteria using the disc diffusion method with concentrations of 20% and 30% betel leaf extract, resulting in average inhibition zones of 11.67 mm and 14 mm in the weak resistance category. Meanwhile, concentrations of 50%, 75% found average inhibition zones of 17.67 mm and 19 mm in the inhibition category. At a concentration of 100% betel leaf extract, the average inhibition zone was 21.33 mm in the strong resistance category.[17]

For dental and oral health, we can also make use of kalamansi lime, commonly known as kalamansi oranges. Kalamansi oranges also contain numerous chemical compounds that can inhibit the development of

Aloha International Journal of Health Advancement (AIJHA)
ISSN 2621-8224
Volume 6 Number 8, August 2023
RESEARCH
http://journal.aloha.academy/index.php/aijha DOI: http://dx.doi.org/10.33846/aijha60803

microorganisms. The compounds in the peel of kalamansi oranges, the main components of their essential oil, include β-sitopenia, β-pinene, and D-limonene. The fruit contains organic acids such as ascorbic acid, citric acid, malic acid, tartaric acid, and benzoic acid. The leaves produce limonoids and transfer them to the fruit and seeds. Kalamansi oranges are rich in chemical compounds, allowing them to be used as anti-acne, antimicrobial, antitussive, anti-inflammatory, laxative, detoxifier, antioxidant, and anxiolytic agents. A study conducted by Oktasila et al.[18] on the antibacterial activity test of kalamansi orange leaves (*Citrofortunella microcarpa*) against *Staphylococcus aureus* and *Escherichia coli* showed that the ethanol extract of kalamansi orange leaves has antibacterial activity against *S. aureus* and *E. coli* bacteria. The inhibition zone diameters were moderate, measuring 7.20 and 5.73 mm at a concentration of 40%. Meanwhile, the antibacterial activity of kalamansi orange leaf essential oil falls into the strong category, with inhibition zone diameters of 14.83 and 13.00 mm at a concentration of 20%. Based on previous research findings, the researchers were interested in creating a mouthwash formulation from natural ingredients available in our surroundings, using a solvent and distilled water that are safe for oral health.

METHODS

The research stages are outlined as follows: 1) petel leaf extraction, 2) production of betel kalamansi mouthwash (sirkala), 3) testing betel kalamansi mouthwash (sirkala) against 2 bacterial strains, *Streptococcus mutans* and *Bacillus cereus*, compared to patented listerine mouthwash, 4) product testing by the national agency of drug and food control (BPOM) according to listerine standards as a benchmark, 5) mouthwash viscosity assessment, 6) preference testing, 7) data processing and analysis.

This study was a laboratory experimental research with a control using listerine patented mouthwash containing methyl salicylate. The study included 30 panelists who meet the criteria and were willing to participate as research subjects. Materials used were betel leaf extract, kalamansi juice, 70% ethanol, stevia powder, cardamom powder, mint leaves - reagents for phytochemical testing of betel leaf extracts. Equipment used were extraction tools set, erlenmeyer flask, measuring glass, porcelain dish, sample bottles.

Green Betel Leaf Extraction Process

To obtain a concentrated extract of Green Betel Leaves, several steps are carried out:

1) Three kilograms of Green Betel Leaves are thoroughly washed and dried at room temperature for 1-2 days.
2) The dried Betel Leaves are ground into a powder to obtain Green Betel Leaf powder.
3) This Green Betel Leaf powder is then macerated for 3-5 days using 70% alcohol.
4) After 5 days, the maceration result is separated between the residue and the filtrate.
5) The obtained filtrate is introduced into a Rotary Evaporator to evaporate the residual alcohol solvent, yielding the concentrated extract.

The concentrated extract undergoes qualitative and quantitative testing of its compound content. For the extraction process, qualitative and quantitative tests of the Green Betel Leaf are conducted in the Chemistry Laboratory at Universitas Negeri Padang.

Mouthwash Production Process

1) Prepare all the necessary ingredients.
2) Weigh all the ingredients according to the Listerine Patent specifications: betel leaf extract 1.56 grams as a substitute for Eucalyptol and Thymol in Listerine; mint leaves 0.42 grams as a substitute for Menthol in Listerine; 0.6 grams of cardamom powder as a substitute for Methyl Salicylate in Listerine; 2.5 grams of kalamansi juice as a substitute for Benzoic Acid and Polycamer 407 in Listerine, and distilled water as a solvent to achieve a total mixture volume of 1 liter.
3) After all ingredients are weighed, combine them and heat the mixture until fully homogeneous.
4) Filter the mouthwash mixture using filter paper.
5) Sterilize the sample bottles before filling them with the mouthwash.

Procedure for Analysis

Qualitative and quantitative tests for betel leaf extract are conducted at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang. Qualitative analysis aims to detect the presence

Aloha International Journal of Health Advancement (AIJHA)
ISSN 2621-8224
Volume 6 Number 8, August 2023
http://journal.aloha.academy/index.php/aijha
RESEARCH
DOI: http://dx.doi.org/10.33846/aijha60803

of saponins, tannins, and flavonoids. Quantitative analysis is performed to determine the concentration of saponins, total phenols, flavonoids, tannins, and antioxidants.

Microbiological Testing of SIRKALA Mouthwash compared to LISTERINE for Streptococcus mutans and Bacillus cereus based on SNI EN 1040:2005. The SIRKALA mouthwash, which has been sterilized using an autoclave at 121°C, is sent to the IPBCC Laboratory in Bogor for effectiveness testing against Streptococcus mutans and Bacillus cereus according to SNI EN 1040:2005 standards.

RESULTS

The following details are the composition used in the organic mouthwash based on the 1999 Listerine Patent. However, as the substances used in the Listerine Patent are inorganic and potentially hazardous to health, we have replaced these materials with organic counterparts that serve the same function.

Table 1. Listerine patent and substitute ingredients

Listerin (patent)	Functions	Substitute ingredients:
Ethanol	Solvent and antibacterial	Water
Thymol	Antibacterial	Green betel leaf extract
Eucalyptol	Antibacterial	Green betel leaf extract
Mentol	Refreshment	Mint leaves
Metil Salicilat	Warm Sensation	Cardamom
Benzoic Acid	Calamansi	Calamansi
Caramel	Sweetener	Stevia
Poloxamer 407	Surfactant	Calamansi

Table 2. Test results of Listerine and 1 herbal mouthwash formula "Sirkala" based on SNI EN 1040:2005

Sample	Targeted bacteria	Contact time (seconds)	Contact time (seconds)	Contact time (seconds)	Contact time (seconds)	Final log concentration	Log reduction	Conclusion of each treatment
109 (Listerine Patent)	*S. mutan*	10	$1,6 \times 10^8$	<14	8,20	<2.15	>6.05	Capable to eliminate 99.999% of the targeted bacteria.
		15	$1,6 \times 10^8$	<14	8,20	<2.15	>6.05	Capable to eliminate 99.999% of the targeted bacteria.
		20	$1,6 \times 10^8$	<14	8,20	<2.15	>6.05	Capable to eliminate 99.999% of the targeted bacteria.
	B. cereus	10	7.5×10^7	<14	7,87	<2.15	>5,72	Capable to eliminate 99.999% of the targeted bacteria.
		15	7.5×10^7	<14	7,87	<2.15	>5,72	Capable to eliminate 99.999% of the targeted bacteria.
		20	7.5×10^7	<14	7,87	<2.15	>5,72	Capable to eliminate 99.999% of the targeted bacteria.
171 ("Sirkala" mouthwash Formula based on Listerine Compotition)	*S. mutan*	10	1,6 x 108	<14	8,20	<2.15	>6.05	Capable to eliminate 99.999% of the targeted bacteria.
		15	1,6 x 108	<14	8,20	<2.15	>6.05	Capable to eliminate 99.999% of the targeted bacteria.
		20	1,6 x 108	<14	8,20	<2.15	>6.05	Capable to eliminate 99.999% of the targeted bacteria.
	B. cereus	10	7.5 x 106	369,5	6,87	3,57	3,31	Unable to eliminate 99.999% of the targeted bacteria.
		15	7.5 x 106	339,5	6,87	3,53	3,34	Unable to eliminate 99.999% of the targeted bacteria.
		20	7.5 x 106	316	6,87	3,50	3,38	Unable to eliminate 99.999% of the targeted bacteria.

Aloha International Journal of Health Advancement (AIJHA)
ISSN 2621-8224
Volume 6 Number 8, August 2023
RESEARCH
http://journal.aloha.academy/index.php/aijha DOI: http://dx.doi.org/10.33846/aijha60803

Based on the microbiological test results conducted at the IPBCC Laboratory in Bogor, it was found that the SIRKALA mouthwash demonstrated the ability to eliminate 99.99% of Streptococcus mutans bacteria, known as the plaque or dental caries-causing bacteria. However, it did not exhibit the capacity to eradicate *Bacillus cereus* bacteria, which is responsible for acute diarrhea.

Based on the laboratory test findings, the herbal mouthwash formula of Sirkala exhibits the capability to eliminate the targeted *Streptococcus mutans* bacteria with a killing efficacy of 99.999% within contact times of 10, 15, and 20 seconds. This aligns with the effectiveness observed in the testing of the widely circulated Listerine mouthwash. However, concerning the *Bacillus cereus* bacteria, the herbal mouthwash formula of Sirkala did not demonstrate the ability to eliminate the targeted bacteria, unlike the Listerine mouthwash. These results indicate that the herbal mouthwash formulation of Sirkala is not yet effective in combating or eradicating the bacteria responsible for causing diarrhea within the oral cavity.

DISCUSSION

The findings indicate that, according to SNI 1040:2005 standards, the herbal mouthwash combination of green betel leaf and kalamansi can effectively eliminate *Streptococcus mutans* bacteria. *Streptococcus mutans* is known to trigger dental caries. In Indonesia, dental caries stands as the sixth most prevalent ailment among the populace. Awareness regarding oral and dental health remains relatively low among Indonesian communities. The estimated data from the Data and Information Center (Pusdatin) regarding individuals aged 15 and above amounts to 176,689,336. When converted at a rate of 53.2%, this signifies that approximately 93,998,727 individuals in Indonesia suffer from active dental caries. This prevalence rate of 53.2% translates to a national DMF-T index (Decay Missing Filling-Tooth) of 4.85. This indicates an average of 5 damaged teeth per person in the Indonesian population. The DMF-T index serves as an indicator of dental health status, summing the D-T, M-T, and F-T indices, reflecting instances of tooth decay (D), missing teeth (M), and filled teeth (F) experienced by an individual.[19]

Indonesia is estimated to harbor 30,000 plant species out of the 40,000 species spread worldwide. Approximately 9,600 of these are reported as medicinal plants, with about 300 plant species already in use as traditional remedies.[20] Among these, the green betel leaf and kalamansi are beneficial and serve in addressing dental caries concerns. In traditional Indian medicine, the betel leaf is renowned for its aromatic properties, possessing warmth, antiseptic traits, and even enhancing sexual arousal. The tannin content in betel leaves is believed to reduce vaginal secretions, safeguard liver functions, and prevent diarrhea. Betel leaves also contain arecoline throughout the plant, stimulating the central nervous system and cognition, enhancing peristaltic movements, and relieving snoring. The eugenol content in betel leaves has the ability to eliminate *Candida albicans* fungus, prevent premature ejaculation, and act as an analgesic. Furthermore, betel leaves are frequently utilized by communities to combat bad breath, treat wounds, stop bleeding gums, manage mouth ulcers, and eliminate body odor. Kalamansi, on the other hand, is utilized in food and beverages ingredient (imparting acidity to foods, or used for juices or marmalades) and as a medicinal/vitamin ingredient, serving as an anti-acne, antimicrobial, antitussive, anti-inflammatory, anticonstipation, antidote, antioxidant, and anti-anxiety agent.

CONCLUSION

The microbiological effectiveness test results demonstrate that the herbal mouthwash formula of Sirkala is capable of eliminating the targeted Streptococcus mutans bacteria, with a killing efficacy of 99.999% within contact times of 10, 15, and 20 seconds. This aligns with the testing outcomes of the widely available Listerine mouthwash in the market. However, based on the Bacillus cereus testing results, the herbal formulation of Sirkala mouthwash fails to eradicate the targeted bacteria, unlike the performance of Listerine mouthwash.

REFERENCES

1. Yumoto H, Hirota K, Hirao K, Ninomiya M, Murakami K, Fujii H, Miyake Y. The pathogenic factors from oral Streptococci for systemic diseases. Int J Mol Sci. 2019 Sep 15;20(18):4571. DOI: 10.3390/ijms20184571.
2. Abranches J, Zeng L, Kajfasz JK, Palmer SR, Chakraborty B, Wen ZT, Richards VP, Brady LJ, Lemos JA. Biology of oral streptococci. Microbiol Spectr. 2018 Oct;6(5):10.1128/microbiolspec.GPP3-0042-2018. DOI: 10.1128/microbiolspec.GPP3-0042-2018.

3. Zhang Y, Fang J, Yang J, Gao X, Dong L, Zheng X, Sun L, Xia B, Zhao N, Ma Z, Wang Y. *Streptococcus mutans*-associated bacteria in dental plaque of severe early childhood caries. Journal of Oral Microbiology. 2022;14:1. DOI: 10.1080/20002297.2022.2046309
4. Dinis M, Agnello M, Cen L, Shokeen B, He X, Shi W, Wong DTW, Lux R, Tran NC. Oral microbiome: *Streptococcus mutans*/caries concordant-discordant children. Front Microbiol. 2022 Feb 17;13:782825. DOI: 10.3389/fmicb.2022.782825.
5. Peng X, Cheng L, You Y, et al. Oral microbiota in human systematic diseases. Int J Oral Sci. 2022;14(14). DOI: 10.1038/s41368-022-00163-7
6. Zhou P, Manoil D, Belibasakis GN, Kotsakis GA. Veillonellae: beyond bridging species in oral biofilm ecology. Front Oral Health. 2021;Oct 29;2:774115. DOI: 10.3389/froh.2021.774115.
7. Read E, Curtis MA, Neves JF. The role of oral bacteria in inflammatory bowel disease. Nat Rev Gastroenterol Hepatol. 2021;18:731-742. DOI: 10.1038/s41575-021-00488-4
8. Signat B, Roques C, Poulet P, Duffaut D. *Fusobacterium nucleatum* in periodontal health and disease. Curr Issues Mol Biol. 2011;13(2):25-36.
9. Gheorghe DN, Camen A, Popescu DM, Sincar C, Pitru A, Ionele CM, Nicolae FM, Danilescu CM, Roman A, Florescu C. Periodontitis, Metabolic and gastrointestinal tract diseases: current perspectives on possible pathogenic connections. J Pers Med. 2022 Feb 24;12(3):341. DOI: 10.3390/jpm12030341.
10. Chen Y, Huang Z, Tang Z, Huang Y, Huang M, Liu H, Ziebolz D, Schmalz G, Jia B and Zhao J. More than just a periodontal pathogen –the research progress on *Fusobacterium nucleatum*. Front. Cell. Infect. Microbiol. 2022;12:815318. DOI: 10.3389/fcimb.2022.815318
11. Lenartova M, Tesinska B, Janatova T, Hrebicek O, Mysak J, Janata J and Najmanova L. The oral microbiome in periodontal health. Front. Cell. Infect. Microbiol. 2021;11:629723. DOI: 10.3389/fcimb.2021.629723
12. Lubis RR, Marlisa, Wahyuni DD. Antibacterial activity of betle leaf (*Piper betle* l.) extract on inhibiting *Staphylococcus aureus* in conjunctivitis patient. Am J Clin Exp Immunol. 2020 Feb 25;9(1):1-5.
13. Nayaka NMDMW, Sasadara MMV, Sanjaya DA, Yuda PESK, Dewi NLKAA, Cahyaningsih E, Hartati R. *Piper betle* (L): recent review of antibacterial and antifungal properties, safety profiles, and commercial applications. Molecules. 2021 Apr 16;26(8):2321. DOI: 10.3390/molecules26082321.
14. Phensri P, Thummasema K, Sukatta U, Morand S, Pruksakorn C. In vitro antimicrobial activity of *Piper betle* leaf extract and some topical agents against methicillin-resistant and methicillin-susceptible *Staphylococcus* strains from canine pyoderma. Animals (Basel). 2022;Nov 18;12(22):3203. DOI: 10.3390/ani12223203.
15. Sarma C, Rasane P, Kaur S, Singh J, Singh J, Gat Y, Garba U, Kaur D, Dhawan K. Antioxidant and antimicrobial potential of selected varieties of *Piper betle L.* (Betel leaf). An Acad Bras Cienc. 2018; Oct-Dec;90(4):3871-3878. DOI: 10.1590/0001-3765201820180285.
16. Heliawati L, Lestari S, Hasanah U, Ajiati D, Kurnia D. Phytochemical profile of antibacterial agents from red betel leaf (*Piper crocatum Ruiz and Pav*) against bacteria in dental caries. Molecules. 2022;27(9):2861. DOI: 10.3390/molecules27092861
17. Ibrahim AM. Uji efektitifitas ekstrak daun sirih hijau (*Piper betle Linn*) terhadap pertumbuhan bakteri *Streptococcus viridans* dengan metode disc diffusion. Skripsi. Jakarta: Universitas Islam Negeri Syarif Hidayatullah; 2013.
18. Oktasila D, Nurhamidah, Handayani D. Uji aktivitas antibakteri daun jeruk kalamansi (*Citrofortunella microcarpa*) terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*. ALOTROP, Jurnal Pendidikan Dan Ilmu Kimia. 2019;3(2):158-169.
19. Purnakarya I, Ramayanti S. Peran makanan terhadap kejadian karies gigi. Jurnal Kesehatan Masyarakat. 2013;7(2).
20. Roslizawati, Ramadhan NY, Fakrurrazi, Hernialtian. Antibacterial activity of ethanol extract and stew of antplant (*Myrmecodia sp.*) against Bacteri *E. coli*. Medika Valeninaria. 2013;7(2):0853-1915.