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Formulation of Moringa and Eggshell-Based Mouthwash as Antibacterial Against Streptococcus mutans

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Abstract

Background: Mouthwash is a potential solution to reduce oral health problems, particularly dental caries caused by Streptococcus mutans. However, many commercial mouthwash products contain high alcohol content, which may increase the risk of oral cancer.

Objective: This study aimed to formulate a natural mouthwash combining Moringa Oleifera leaf extract and Eggshell powder and evaluate its antibacterial activity against Streptococcus mutans and the total calcium carbonate content

Methods: The research was conducted through laboratory experiments using maceration extraction with 96% ethanol. The extract was formulated into three mouthwash concentrations: 5%, 10%, and 15%. Phytochemical screening was carried out to identify bioactive compounds, and the formulations were evaluated for organoleptic properties, pH, viscosity, and antibacterial activity using the agar well diffusion method.

Results: The mouthwash formulations demonstrated physical stability and contained flavonoids, saponins, terpenoids, and tannins. Antibacterial tests showed no inhibition at 5%, moderate inhibition at 10% (6.15 mm), and 15% (7.21 mm), while the positive control showed potent inhibition (24.06 mm).

Conclusions: The combination of Moringa Oleifera extract and Eggshell powder has potential as a natural antibacterial mouthwash against Streptococcus mutans, particularly at higher concentrations, and fulfills physical evaluation criteria.

Cite this Article

INTRODUCTION

Maintaining general physical health depends on oral and dental hygiene. Oral and dental diseases such as periodontitis, gingivitis, and dental caries can induce bad breath and other symptoms. Brushing your teeth twice daily and flossing your teeth will help you maintain oral health. Targeting interproximal locations prone to periodontal and dental disease is less effective using this method. More cleaning methods are needed to enhance the effectiveness of dental care (1). According to The Global Burden of Disease Study 2016, dental caries affects about half of the world's population (3.58 billion people), while periodontal disease is considered the 11th most common worldwide. The Ministry of Health of the Republic of Indonesia (2018) reported that the frequency of periodontitis in Indonesia is 74.1%. Periodontal disease can accelerate tooth decay and cause significant infection problems (2).

Dental caries is one of the most common oral health issues caused by Streptococcus mutans, a bacterium capable of fermenting carbohydrates and producing acid that demineralizes tooth enamel (3). One way to prevent dental health problems is with mouthwash. Some mouthwashes, however, have alcohol, synthetic sweeteners, and other chemicals that could be dangerous for a person's health. Hence, natural ingredients are essential in mouthwash formulations to reduce the adverse consequences. Found in the Moringa tree's (*Moringa oleifera L.*) leaves are secondary metabolite chemicals with antibacterial characteristics—flavonoids, terpenoids, tannins, and saponins. According to research, Moringa Oleifera leaves have an inhibitory effect on *Streptococcus mutans*, with an 80% ethanol extract of 14. 02 mm (strong category), a 60% concentration of 12. 03 mm (strong category), and a 40% concentration of 9 00 mm (moderate category) (4).

Eggshells rich in calcium carbonate ($CaCO_3$) can be used as an additive in dental care products. Calcium carbonate makes up 94% of Eggshells, magnesium carbonate 1%, calcium phosphate 1%, and 4% other organic components. In the manufacture of toothpaste, this calcium carbonate content can help remove food particles and stains from teeth (5). Additionally, eggshell powder assists in forming apatite minerals, which are vital for tooth structure, and has antiseptic and antibacterial properties (6).

Researchers hope to create a mouthwash that can stop the spread of *Streptococcus mutans*, a significant cause of tooth decay, by combining calcium carbonate from Eggshells with moringa leaf extract. The product is expected to have dual consumer benefits and a few side effects.

MATERIALS AND METHODS

Ethanol Extraction of Moringa Leaves

Extraction is done by maceration. One of the extraction techniques that produces secondary metabolites by soaking is maceration. Easy and practical, this method is suitable for extracting compounds sensitive to heat because it does not require heating. A total of 1,500 grams of Moringa leaf powder is weighed carefully. Furthermore, 1,500 grams of Moringa leaf powder is soaked in 3.5 liters of 96% ethanol, stirred for 24 hours to be extracted by the maceration method, then filtered until the filtrate is obtained, which is then re-macerated at least three times. The separated filtrate is then concentrated using a rotary evaporator between 50 and 60 degrees Celsius. Then the extract is heated in a water bath at a temperature of 60 degrees Celsius until a thick extract is obtained.

Egg Shell Analysis

Eggshells come from unused household waste. With EDTA titration, analysis of CaCO3 levels is complemented by Proximate and XRF analysis of other Eggshell concentrations.

Evaluation of Mouthwash Formulation

In the first step of mouthwash production, the water-soluble elements form the first phase (water phase). Once these ingredients form Indonesia foodgrades—sodium benzoatec(koepoe koepoe), sorbitol (Tropicana slim), tween 80, and glycerin—have been combined in a mortar, the water-insoluble constituents like peppermint oil, Eggshells, and Moringa leaf extract are introduced. The two phases are mixed and blended until smooth, distilled water is introduced, then filtered and housed in a transparent container. Using negative and positive controls with commercial items free from alcohol, (7) researched this mouthwash composition comprising a range of concentrations (5%, 10%, 15%)(7,8).

Organoleptic Test

Evaluation of mouthwash preparations is done by observing the preparation in terms of shape, color, taste, and aroma. This examination is done at room temperature.

Viscosity Test

Viscosity of the mouthwash composition was measured using an Ostwald viscometer. Up to 5 mL of preparation size was seen in the sample. The viscosity value influences the viscosity of the mouthwash combination. The more pleasant and easy it is to use for gargling, the closer the mouthwash formula's viscosity is to that of water. Whereas the viscosity of pure water is roughly one mPas, or one cP, most commercial mouth rinses have a viscosity under 7.25 (9).

pH Test

The pH of the produced mouthwash has to be based on the quality requirements of herbal mouthwash, which is 4.5–10.5 according to (SNI 12 3524-1995). pH meter (Luton PH 22,Taiwan) is used to conduct this testing.

Antibacterial Test of Mouthwash Preparations

The antibacterial efficacy of the combined mouthwash made from Moringa leaves and Eggshells was evaluated against S. mutans bacteria after a 24-hour incubation period using the well method, which was carried out three times. The inhibition zone was considered very strong if it exceeded 20 mm, strong between 10-20 mm, moderate between 5-10 mm, and low below 5 mm.

RESULTS AND DISCUSSIONS

The results of this study indicate that eggshells have a calcium carbonate content found in the eggshell analysis, which was 93.93%. Calcium carbonate (CaCO3) is a scrubbing agent needed in toothpaste (Table 1).

Table 1. Egg Shell Analysis Results

No	Content	(% Weight)
1	Water	1,01
2	Protein	3,31
3	Pure Fat	0,04
4	Calcium Carbonate (CaCO3)	93,93
5	Phosphorus	0,45
No	**Content**	**(% Weight)**

6	Magnesium	0,08
7	Potassium	0,04
8	Ferro	0,03
9	Molybdenum	1

Remarks: (12)

Solid maceration extract is made by weighing 150 grams with a yield of 10%. It is dark green and has a distinctive aroma of Moringa leaves (Table 2). 96% ethanol was chosen as the solvent for isolating active compounds from selected plants because of its effectiveness, availability, capacity to inhibit microbial development, and ability to attract active chemicals. Furthermore, ethanol acts as a preservative by inhibiting the growth of fungus, bacteria, and mold. This solvent liquid lets the active compounds dissolve because it can pass through cell walls and enter the interior cells where active compounds are stored (10).

Table 2. Ethanol Extract Yield Results of Moringa Leaves

No	Material	Material Weight of Simplex (g)	Weight of Extract (g)	Yield (%)
1	Moringa Leaves	1,500	150	10

Formulations with percentages of 5%, 10%, and 15% produce liquid preparations that have color variations in each mouthwash formula. The color variations seen are influenced by the concentration level of the extract in each formula (Table 3) and (Figure 1).

Table 3. Mouthwash Formula Combination of Moringa Leaf Extract and Egg Shell

No	Material	Formula (%)				Function
		K(-)	FI	FII	FIII	
1	Ethanol extract of Moringa leaves	-	5	10	15	Active Substance
2	Egg shells	-	5	10	15	Active Substance
3	Tween 80	5	5	5	5	Surfactant
4	Glycerin	5	5	5	5	Humectant
5	Sorbitol	3	3	3	3	Sweetener
6	Peppermint oil	0,15	0,15	0,15	0,15	Flavoring
7	Sodium Benzoate	0,15	0,15	0,15	0,15	Preservative
8	Adhesive water	100ml	100ml	100ml	100ml	Solvent

Figure 1. Formula mouthwash preparation of Moringa Leaf and Eggshell extract, K(-) = Negative control, F1 = extract at 5% concentration, F2 = extract at 10% concentration, F3 = extract at 15% concentration.

The organoleptic test results show that the prepared mouthwash composition matched the stated requirement of being thin rather than thick. The extraction of its active ingredients gives the product its vivid colors; the darker the preparation gets, the higher the concentration of active ingredients. Mint aroma comes from the inclusion of peppermint oil, which offers its perfume. Sorbitol's sweetener adds a sweet flavor; peppermint oil gives it a minty taste. The data show that the material used performed as intended and produced the desired effects. The deep and dark tint of the product causes the color of the preparation made using this formula to match the results of Suryani's 2019 study (11). The color is a product of adding a concentrated, dark-colored plant extract acquired through maceration. At 10%, the resulting color is also brownish yellow; the 15% recipe results in a dark yellow. This is so because the more concentrated the formula, the stronger the color produced. The 5%, 10%, and 15% formulas have a unique menthol, and Moringa leaf fragrance, and the glycerin imparts a sweet flavor; the Moringa leaf extract provides a somewhat bitter and peppery flavor without mouthwash formulation sediments (Table 4).

Table 4. Organoleptic Test Results of Mouthwash Combination of Moringa Leaf Extract and Egg Shell

No	Concentration	Shape	Color	Aroma	Taste
1	5%	Solution	Slightly dark brownish yellow	Typical of moringa leaves and menthol	Sweet, cold
2	10%	Solution	Dark brownish yellow	Typical of moringa leaves and menthol	Sweet, cold
3	15%	Solution	Darker brownish yellow	Typical of moringa leaves and menthol	Sweet cold

The viscosity greatly influences how thick a mouthwash solution is when gargled in the mouth. A mouthwash with a consistency similar to water will be easier and more palatable when gargling. Based on the viscosity measurement of mouthwash containing Moringa leaf extract and Eggshell, the results showed that the viscosity of the mouthwash formula with concentrations of 5%, 10%, and 15% meets the criteria. (Table 5).

Table 5. Viscosity Test Results of Mouthwash Combination of Moringa Leaf Extract and Egg Shell

No	Concentration	Time	Viscosity	According to the Ministry of Health of the Republic of Indonesia (1979)	Information
1	5%	17,93	0,591	<7,25	MC
2	10%	21,75	0,581	<7,25	MC
3	15%	23,21	0,513	<7,25	MC

Description: MC = Meets Criteria

The pH test is conducted to determine the pH level of the product that is safe and meets the pH standards as an oral antibacterial. The pH level of the mouthwash product significantly affects the types of bacteria that can grow. The results of the pH test of the mixture of moringa leaf extract and egg shells can be seen in Table 6.

Table 6. Results of pH Test of Mouthwash Combination of Moringa Leaf Extract and Egg Shell

No	Concentration	pH Value	Acid/Base	pH Limits According to (SNI 12-3524-1995)	Information
1	5%	4,3	Acid	4,5-10,5	DNMC
2	10%	4,5	Acid	4,5-10,5	MC
3	15%	4,5	Acid	4,5-10,5	MC

Description: MC = Meets Criteria, DNMC = Does Not Meet Criteria

The observation result found several degrees of variation, ranging from none to moderate. The biggest inhibition zone was shown at a concentration of 15%; at 5%, the least. Antibacterial tests of moringa leaf extract against germs causing dental caries were previously conducted; however, they were done with a different approach and not in the manner of a mouthwash (Table 7).

Table 7. Antibacterial Test Results Diameter of Bacterial Growth Inhibition Zone (mm)

No	Formula	Test Bacteria	Repetition			Average Diameter of Inhibition Zone ±	Information
			1	2	3		
1	K(-)	*Staphylococcus Mutans*	-	-	-	-	None
2	K(+)		23.36	25.25	23.57	±24.06	Very strong
3	F1		0.00	0.00	0.00	-	None
4	F2		6.24	6.12	6.08	±6.15	Moderate
5	F3		7.20	7.25	7.18	±7.21	Moderate

The results were obtained from observing the antibacterial effectiveness of mouthwash made from Moringa leaf extract against S. mutans bacteria(Figure 2).

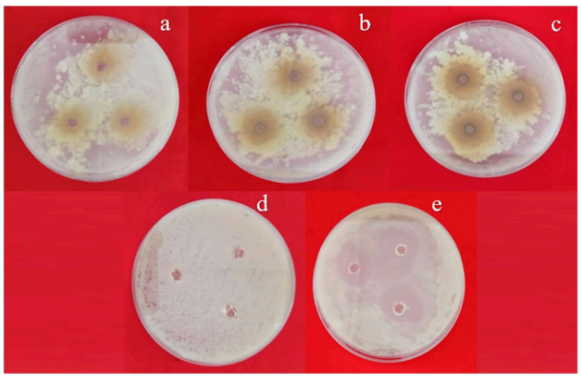

Figure 2. were obtained after an incubation period of 24 hours using the well method carried out 3 times (a) Formula 5%, (b) Formula 10%, (c) Formula 15%, (d) Control -, (e) Control +.

The study showed that combining Moringa oleifera L. leaf extract and egg shells in mouthwash preparations with 5%, 10%, and 15% showed activity against bacteria. Still, no activity was detected in the formula with a concentration of 5%. This may be due to the low concentration of the extract and the possibility of contamination during the manufacture of mouthwash. Research by Tarigan (2020) suggests that moringa leaf extract can kill S. mutans bacteria with a notable inhibition zone. However, a remarkable inhibition zone of 15 was found. 875 mm; 11. 875 mm; and 10. 25 mm at concentrations of 80%, 40%, and 10% (13).

CLINICAL IMPLICATION

This study suggests combining Moringa Oleifera leaf extract and Eggshell powder may offer a safer, natural alternative to alcohol-based mouthwashes. The formulation could be particularly beneficial for populations sensitive to alcohol, such as children or elderly individuals. With further development and validation, this herbal-based mouthwash has the potential to contribute to better preventive dental care and reduce the incidence of *Streptococcus mutans*-related caries.

LIMITATIONS

This study has several limitations. Lastly, no long-term stability tests were conducted to assess the formulation's shelf life before human application. Despite these limitations, this study provides a valuable basis for further research on developing natural antimicrobial mouthwashes.

CONCLUSIONS

The formulated natural mouthwash containing Moringa Oleifera leaf extract and Eggshell-derived calcium carbonate demonstrated stability in physical characteristics (organoleptic, pH, and viscosity tests). Phytochemical screening confirmed the presence of bioactive secondary metabolites such as flavonoids, tannins, saponins, and terpenoids. The antibacterial assay showed inhibitory effects against *Streptococcus mutans* at 10% and 15% concentrations, with inhibition zones of 6.15 mm and 7.21 mm, respectively. These results indicate the potential of this herbal mouthwash as a safer alternative to alcohol-based products. Further research is needed to enhance efficacy and explore effects on other oral pathogens.

CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTOR CONTRIBUTIONS

The experiments and initial manuscript draft, which also provided necessary resources, were completed by Pande Bagus Mahendra Putra Parmanantha. Ni Luh Putu Eka Kartika Sari designed and supervised the experiments and analyzed the antibacterial data. I, Putu Dhananjaya Dharsila Gosa, contributed to the formatting and proofreading of the manuscript.

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