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Formulation and Evaluation of a Mouthwash Containing Combined Essential Oils of *Ocimum basilicum* L. and *Cymbopogon nardus* L.: Antibacterial Activity Against *Streptococcus mutans*

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ABSTRACT

Streptococcus mutans is a major bacterial contributor to dental caries. Essential oils derived from *Ocimum basilicum* L. and *Cymbopogon nardus* L. contain compounds with potential antibacterial activity. This study aimed to formulate and evaluate a mouthwash containing a combination of basil and lemongrass essential oils and to assess its antibacterial activity against *S. mutans*. Three formulations were prepared using essential oil concentration ratios of 0.5%:1% (F1), 1%:2% (F2), and 2%:3% (F3). Physical quality was evaluated based on organoleptic properties, pH, homogeneity, and specific gravity. Antibacterial activity was assessed using the disk diffusion method. Data were analyzed using the Shapiro–Wilk normality test, Levene's test, Welch's analysis of variance (ANOVA), and the Games–Howell post hoc test, as appropriate. The mean inhibition zone diameters reported for F1, F2, and F3 were 3.01, 3.95, and 4.81 mm, respectively. The formulations had pH values ranging from 6.68 to 6.92 and specific gravity values ranging from 1.023 to 1.038 g/mL. However, the formulations did not meet the study's homogeneity criterion. Although F3 exhibited the largest reported inhibition zone among the three formulations, its antibacterial activity was classified as weak according to the classification adopted in this study. These findings indicate that the combined essential oils can be incorporated into a mouthwash formulation, although further optimization is required to improve physical stability and antibacterial performance.

Keywords: antibacterial activity; basil essential oil; lemongrass essential oil; mouthwash; *Streptococcus mutans*

INTRODUCTION

Oral diseases affect approximately 3.5 billion people worldwide, and dental caries is among the most common conditions (World Health Organization, 2022). The World Health Organization reported in 2016 that the prevalence of dental caries among children ranged from 60% to 90%. According to the 2023 Indonesian Health Survey (Survei Kesehatan Indonesia, SKI), 56.9% of people aged three years and older experienced dental and oral health problems during the preceding year, compared with 57.9% reported in the 2018 Basic Health Research survey (Riset Kesehatan Dasar, Riskesdas). The 2023 survey also reported a high prevalence of dental caries (82.8%); among children aged 5–9 years, the prevalence reached 84.8%. Streptococcus mutans is one of the bacterial species involved in dental caries through its metabolic activity. Host factors, microorganisms, dietary substrates, and time are among the

factors associated with caries development (Ministry of Health of the Republic of Indonesia, 2025; Chen *et al*., 2020; Gloria-Garza *et al*., 2025; Sholekhah, 2021)

To prevent dental caries and maintain oral hygiene, antimicrobial agents such as fluoride, chlorhexidine (CHX), and triclosan have been used in toothpaste and mouthwash to reduce plaque accumulation and biofilm formation, including in areas that are difficult to reach. However, long-term use of some agents may cause adverse effects. For example, CHX may cause tooth discoloration, xerostomia, altered taste, and numbness or pain in the mouth and tongue (Deus & Ouanounou, 2022). Antimicrobial agents derived from natural sources have therefore attracted interest as potential alternatives for oral care. Basil essential oil contains compounds such as citral, linalool, and geraniol that have been associated with antibacterial activity (Guntur *et al*., 2021). Lemongrass essential oil contains monoterpenes, including citronellal, geraniol, and citronellol, which may contribute to its antibacterial activity (Dewi & Hanifa, 2021).

Many previous studies have investigated these essential oils individually. This study therefore aimed to investigate whether a combination of basil and lemongrass essential oils formulated as a mouthwash could inhibit the growth of S. mutans, a bacterium associated with dental caries. The findings may provide preliminary information for the development of an essential-oil-based oral care formulation. The safety and effectiveness of the formulation, however, require further evaluation,

METHODS

This laboratory-based experimental study was conducted from February to May 2026 at the Pharmaceutics Laboratory, Strada Indonesia University.

Materials and Equipment

The materials included basil essential oil (*Ocimum basilicum* L.), lemongrass essential oil (*Cymbopogon nardus* L.), Tween 80, glycerin, sodium benzoate, sodium saccharin, peppermint oil, distilled water, Nutrient Agar (NA), barium chloride, sulfuric acid, a culture of Streptococcus mutans, and paper disks.

The equipment used included Beaker Glass, Stirring rod, Graduated cylinder, Volumetric pipette, Dropper pipette, Porcelain dish, Inoculating needle, Test tube, Test tube rack, Tweezers, Analytical balance, Magnetic stirrer, pH meter, Brookfield viscometer, Pycnometer, Petri dish, Incubator, Autoclave, Laminar Air Flow (LAF), Spirit burner, Erlenmeyer flask, Vernier caliper.

Mouthwash Formulation

Tabel 1. Mouthwash Formulation

Materials	**Concentration**				
	F1	F2	F3	K-	K+
Basil leaf essential oil	0,5	1	2	-	Chlorhexidine Gluconate 0,2%
Lemongrass essential oil	1	2	3	-	
Tween 80	15	15	15	15	
Glycerin	5	5	5	5	
PEG 400	2,5	2,5	2,5	2,5	
Peppermint oil	0,3	0,3	0,3	0,3	
Sodium benzoate	0,4	0,4	0,4	0,4	
Sodium saccharin	0,2	0,2	0,2	0,2	
Distilled water	ad 100	ad 100	ad 100	ad 100	

All equipment and materials were prepared before use, and the ingredients were weighed according to the specified formulation. The oil phase, consisting of basil essential oil, lemongrass essential oil, and peppermint oil, and the aqueous phase, consisting of Tween 80, glycerin, and PEG 400, were prepared separately in glass beakers. The phases were stirred using a magnetic stirrer at 400 rpm and 50°C for 15 minutes. The aqueous phase was then added gradually to the oil phase while stirring continued for a further 15 minutes. Sodium saccharin and sodium benzoate were dissolved in the remaining distilled water by stirring at 400 rpm and 50°C for 15 minutes. This solution was gradually added to the mixture of the oil and aqueous phases, and the resulting preparation was stirred at 400 rpm and 50°C for another 15 minutes.

Physical Quality Test

Organoleptic Test : Organoleptic properties were evaluated by observing the preparation's appearance, odor, taste, and color (A. Sari *et al*., 2023)

pH Test : The pH of each mouthwash formulation was measured using a pH meter to assess its acidity or alkalinity. The reference range cited for mouthwash formulations was pH 5–7 (Helmi, 2024).

Homogeneity Test : Homogeneity was assessed by examining the preparation for aggregates or precipitates. The samples were examined visually and under a microscope. The absence of visible aggregates or precipitates was used as an indication of homogeneity (Anggraeni *et al*., 2023).

Specific Gravity Test : Specific gravity was measured using a pycnometer. The reference range cited for mouthwash preparations was 0.99–1.12 g/mL (Helmi, 2024).

Antibacterial Activity Test

Antibacterial activity was evaluated using the disk diffusion method (Kirby–Bauer method) against Streptococcus mutans ATCC 25175 PK/5. The bacterial suspension was adjusted to a 0.5 McFarland standard and spread over the surface of Nutrient Agar (NA). A 15-mL volume of NA was poured into each Petri dish and allowed to solidify. Sterile cotton swabs were dipped into the bacterial suspension and used to spread the inoculum evenly over the agar surface. The plates were left for approximately 15 minutes to allow absorption. Paper disks measuring 6 mm in diameter were soaked in each of the three mouthwash formulations and in the positive and negative controls for approximately 25 minutes, then placed on the agar surface. The plates were incubated at 37°C for 24 hours, after which the inhibition zones were measured in millimeters. Each treatment was tested in triplicate *(Ainan et al*., 2025; Voen-Na *et al*., 2025).

Data analysis : Data were analyzed using SPSS version 27. Normality was assessed using the Shapiro–Wilk test, and variance homogeneity was assessed using Levene's test. Welch's ANOVA was used to compare the treatment groups when the assumption of equal variances was not met, followed by the Games–Howell post hoc test, where appropriate (Fitri *et al*., 2023; Widiyono *et al*., 2024).

RESULTS

Physical Quality Test

The physical quality evaluation showed that the color changed as the essential oil concentration increased: F1 was clear, whereas F2 and F3 were white. The measured pH values ranged from 6.68 to 6.92, within the reference range cited for oral preparations. Particle aggregates were observed in all formulations, indicating that they did not meet the homogeneity criterion used in this study. Specific gravity ranged from 1.023 to 1.038 g/mL. The results of the physical quality tests are presented in Table 2.

Tabel 2. Physical Quality Test Results

Formula	Organoleptic (Form, Smell, Taste, Color)	pH	Homogeneity	Specific Gravity
F1 (0,5% : 1%)	Liquid, Characteristic odor, Bitter, Clear	6,92	Not homogeneous	1,030 g/mL
F2 (1% : 2%)	Liquid, Characteristic odor, Bitter, White	6,77	Not homogeneous	1,038 g/mL
F3 (2% : 3%)	Liquid, Characteristic odor, Bitter, White	6,68	Not homogeneous	1,023 g/mL
K (-)	Liquid, Tween 80 odor, Bitter, Clear	6,47	Not homogeneous	1,060 g/mL
K (+)	Liquid, Menthol odor, Bitter, Clear	7,12	Homogeneous	0,995 g/mL

Antibacterial Activity Test

Based on the observations, each formula demonstrated antibacterial activity, but it was still in the weak category < 5 mm. The negative control showed no antibacterial activity, while the positive control exhibited strong antibacterial activity.

Tabel 3. Antibacterial Activity Test Results

Formula	Replication (mm)			Average (mm)	Category	Standard (Winastri *et al*., 2020)	
	1	2	3			Inhibition zone	Category
F1	3,05	3,15	2,85	3,01	Weak	≤ 5 mm 11-20 mm	Weak Strong
F2	3,55	4,15	4,1	3,95	Weak		
F3	4,85	4,6	5	4,81	Weak		
K-	0	0	0	0	Weak		
K+	17,1	19,2	17,6	17,9	Strong		

DISCUSSION

In the organoleptic evaluation, F1 appeared clear, whereas F2 and F3 appeared white. The authors suggest that this difference may be associated with differences in droplet size, potentially resulting from the higher essential oil concentrations in F2 and F3. Sihombing *et al*. (2025) reported that increasing the oil concentration can increase droplet size in the system they studied. The preparation also had a bitter taste. This may be associated with the relatively high Tween 80 concentration and the essential oils themselves; however, the specific contribution of each ingredient was not established in the present study. The cited literature should be checked to ensure that it directly supports each proposed explanation.

The formulation's pH is designed to match the oral pH because if the pH is too acidic, it will increase the growth of acid-resistant bacteria such as Streptococcus species, which contribute to the formation of dental caries, whereas if the pH is too alkaline, it allows an increase in the number of bacteria capable of adapting to an alkaline environment, such as Solobacterium moorei, which causes bad breath and accelerates the formation of dental plaque

(Huang *et al*., 2024). Based on the test results, each formula have been met the mouthwash pH requirement of 5-7 (Helmi, 2024).

Based on the observations under a microscope, foreign particles were found in each formula and the negative control, indicating that the homogeneity of the preparations was not yet perfect, whereas the positive control appeared homogeneous. These particles are suspected to be Lyotropic Liquid Crystals (LLC) formed due to the interaction between the oil phase, surfactant, and aqueous phase (Rahmadasmi *et al*., 2023). The formation of LLC begins when the surfactant concentration exceeds the Critical Micelle Concentration (CMC), at which point the surfactant forms micelles, which are aggregates of surfactant molecules. As the surfactant concentration continues to increase, the existing micelles further organize into LLC with a different structure (Barman & Banjare, 2025; Huang & Gui, 2018).This suspicion is based on the characteristics of the particles, which do not correspond to the properties of the materials used in the preparation. The solid materials used in the formulation are crystalline powders and have a property of being easily soluble in water; therefore, if these materials do not dissolve completely, the particles generally appear as characteristic crystals, such as angular shapes, distinct edges, and sharp corners. In contrast, the observed particles have irregular shapes, resembling gel clumps. Since the observation was conducted using an ordinary binocular microscope, which only shows particle morphology, the specific identity of the particles cannot yet be determined.

The specific gravity of each formula meets the specific gravity requirements for mouthwash preparations, which is 0.99 g/mL–1.12 g/mL, with the specific gravity of each formula being 1.030 (F1), 1.038 (F2), and 1.023 (F3). The negative control has a specific gravity of 1.060, and the positive control has a value of 0.995. Specific gravity is directly proportional to the viscosity of a preparation (Nareswari *et al*., 2022). The results of the specific gravity test show that the mouthwash preparation has values almost identical to that of water (1 g/mL), indicating that the preparation has a fluid texture, is not viscous, and flows like ordinary water, making the mouthwash feel light when used.

F3, which contained 2% basil essential oil and 3% lemongrass essential oil, showed the largest reported inhibition zone among the three formulations, with a mean value of 4.81 mm. No inhibition zone was reported for the negative control, while the positive control had a mean value of 17.9 mm and was classified as strong according to the study's criteria. The relatively low inhibition values observed for the formulations may be associated with factors such as the concentrations of the active ingredients, droplet characteristics, essential oil volatility, or interactions between the oils. These explanations remain hypotheses and were not directly tested in this study.

The low concentration of active ingredients due to the dilution process in an emulsion system can reduce the antibacterial activity of the active ingredients. This is in accordance with research by Aprilia *et al*. (2022),which stated that the antibacterial effectiveness of essential oils is directly related to the concentration of oil used; lower concentrations result in fewer active compounds. Large droplets create a smaller surface area ratio, thereby reducing interaction with bacterial cell membranes and decreasing the potential penetration of active ingredients. Research conducted by Kartika & Marchaban (2018) indicated that the inhibition zone formed by red betel essential oil in a microemulsion preparation is larger compared to that in a conventional emulsion. The volatility nature of essential oils can be one of the factors because it can cause a decrease in the number of active compounds in the preparation during the manufacturing process, thereby reducing antibacterial activity.

The relatively low antibacterial activity of the mouthwash containing basil and lemongrass essential oils may also be related to interactions among their constituent compounds. Yunilawati *et al*. (2021) described possible antimicrobial interactions between

essential oils as indifferent, additive, antagonistic, or synergistic. Antagonism occurs when the combined effect is lower than expected from the individual agents. However, the type of interaction between the two oils was not determined in this study. Further testing, such as determination of the fractional inhibitory concentration index (FICI), would be needed to assess this possibility. Previous research reported antibacterial activity for basil oil mouthwash at a minimum inhibitory concentration of 0.1% (Yosephine *et al*., 2013), while Sari *et al*. (2023) reported moderate activity for a lemongrass essential oil mouthwash at a concentration of 4.5%. Differences in formulation and testing methods should be considered when comparing these findings.

Antibacterial activity data for the formulations and controls were analyzed using SPSS version 27. The Shapiro–Wilk test yielded a significance value greater than 0.05, indicating no statistically significant departure from normality. Levene's test yielded a significance value below 0.05, indicating unequal variances. Welch's ANOVA was therefore used to compare the groups. The analysis indicated a statistically significant difference among groups ($p < 0.05$). According to the reported Games–Howell post hoc analysis, F1 differed significantly from F3 and the positive control; F2 differed significantly from the positive control; and F3 differed significantly from F1 and the positive control. The positive control differed significantly from each formulation. These statements should be checked against the complete statistical output before publication, and exact p-values should be reported where available..

CONCLUSION

The essential oils of *Ocimum basilicum* L. and *Cymbopogon nardus* L. were incorporated into mouthwash formulations. The F1–F3 formulations had pH and specific gravity values within the reference ranges cited in this study, but they did not meet the homogeneity criterion. F3 showed the largest reported inhibition zone among the three formulations; however, its antibacterial activity was classified as weak according to the study's classification. Further research should assess formulation stability, optimize physical quality and antibacterial performance, and investigate potential interactions between the two essential oils.

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