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Potential of Basil Leaves (*Ocimum x africanum* Lour) as a Herbal Mouthwash: Phytochemical Screening, Formulation, Physical Evaluation and Antibacterial Activity Againts *Streptococcus mutans*

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ABSTRACT
Oral diseases remain a major global public health problem, with dental caries being one of the most prevalent conditions primarily caused by Streptococcus mutans. Although commercial mouthwashes are effective in controlling oral microorganisms, their prolonged use may cause undesirable side effects, prompting the development of herbal alternatives. This study aimed to evaluate the potential of Ocimum × africanum Lour. leaves as an herbal mouthwash through phytochemical screening, formulation, physical quality evaluation, and antibacterial activity against Streptococcus mutans. Basil leaves were extracted by maceration using 96% ethanol and formulated into mouthwash preparations containing extract concentrations of 2.5% (F1), 5% (F2), and 10% (F3). Phytochemical screening was performed to identify the major secondary metabolites. The formulations were evaluated for organoleptic properties, pH, viscosity, specific gravity, and freeze–thaw stability. Antibacterial activity was determined using the paper disk diffusion method. The extract contained flavonoids, alkaloids, tannins, saponins, and steroids. All formulations exhibited acceptable physical characteristics and remained stable throughout the stability test. The antibacterial assay demonstrated inhibition zones of 7.15 mm, 7.06 mm, and 7.41 mm for F1, F2, and F3, respectively, indicating moderate antibacterial activity, with the 10% extract formulation showing the highest inhibitory effect. Statistical analysis demonstrated significant differences among treatment groups ($p < 0.05$), although post-hoc analysis indicated no significant difference among extract concentrations. These findings suggest that Ocimum × africanum leaf extract has promising potential as a natural antibacterial ingredient for herbal mouthwash formulations. Further studies involving minimum inhibitory concentration, long-term stability, and clinical evaluation are recommended.

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INTRODUCTION

Oral health is an essential component of general health and quality of life. Oral diseases remain among the most common non-communicable diseases worldwide, affecting individuals across all age groups. According to the World Health Organization (WHO), approximately 3.5 billion people suffer from oral diseases, with nearly 2 billion individuals affected by permanent dental caries and more than 500 million children experiencing caries in primary teeth. Dental caries remains a major public health concern because it causes pain, tooth loss, impaired mastication, and reduced quality of life when left untreated (Marlindayanti et al. 2017).

According to Basic Health Research (RISKESDAS) data, 53.2% of the Indonesian population suffered from active caries (cavities) in 2007 (Riskesdas, 2013). This data shows that caries or cavities are prevalent in several provinces, including Yogyakarta Special Region, East Java, Kalimantan, Sulawesi, Riau, Jambi, Sumatra, Bangka Belitung, and Maluku (Subekti et al. 2019). Poor oral hygiene leads to plaque buildup on teeth. Even after teeth are cleaned, plaque can still form at any time. Poor dental hygiene makes plaque stickier and more difficult to remove (Marlindayanti et al. 2017). Maintaining dental and oral hygiene is crucial. The condition of the mouth and teeth can change over time. Oral diseases can be caused by a lack of information about good oral hygiene. Common oral health conditions include canker sores, swollen gums, dental plaque, cavities, dental caries, and other oral problems (Dola et al. 2021). Dental caries is a multifactorial disease caused by the interaction between cariogenic microorganisms, dietary carbohydrates, susceptible tooth surfaces, and time. Among the microorganisms involved, *Streptococcus mutans* is considered the primary etiological agent due to its ability to adhere to tooth surfaces, produce extracellular polysaccharides, form biofilms, and generate organic acids that demineralize dental enamel. Therefore, reducing the population of *S. mutans* is an important strategy for preventing dental plaque formation and dental caries.

Several studies have demonstrated the antibacterial potential of basil leaf extracts against Streptococcus mutans. Dola et al. (2021) reported that ethyl acetate extract of basil formulated as a mouthwash exhibited antibacterial activity against *S. mutans,* while other investigators have shown inhibitory effects of basil extracts against *Staphylococcus aureus* and *Cutibacterium acnes.* Nevertheless, previous studies primarily focused on antibacterial activity alone or used different extraction solvents without comprehensively evaluating phytochemical composition, physicochemical characteristics of the formulation, and antibacterial performance within a single experimental design. Furthermore, studies investigating mouthwash formulations prepared from 96% ethanol extract of *Ocimum* × *africanum* Lour. remain limited. A concentration of 100% basil leaves can inhibit the growth of *Staphylococcus aureus* by 10.08 mm (Ariani, et al. 2020), at a concentration of 12% it can inhibit the growth of *Propionibacterium acne* by 0.1 cm (Septiandari, et al. 2015).

Basil leaves may also be beneficial in treating several diseases, as demonstrated by previous research. Basil can be used as an alternative treatment to prevent subclinical mastitis in dairy cows (Faradila et al. 2020), minimize hepatocyte damage in mice injected with uric acid (Wulandari et al. 2019), lower blood pressure in patients with stage one hypertension (Siagian et al. 2015), as first-line treatment for fever (Pratama et al. 2021), as an antiscabies agent for guinea pigs (Junita et al. 2020), as a chemopreventive agent for uterine cancer cells (Ismiyati and Nurhaeni, 2016), as an antidiabetic (Fardhani et al. 2023), and as an anti-inflammatory (Saputri et al. 2016).

Mouthwash is a liquid preparation containing extra antibacterial components that help fight oral germs, kill infections, cleanse, and act as an antiseptic. It also helps freshen breath and eliminate bad breath. Using mouthwash is essential for maintaining dental hygiene, treating pathogenic germs, and relieving symptoms of gingivitis and swollen gums (Litaay et al. 2023).

The stability of a solution during storage needs to be assessed in mouthwash preparations. Evaluation of mouthwash preparations can be performed using organoleptic tests, pH tests, viscosity tests, and clarity tests. The physical stability of a preparation will depend on the type and amount of additives added (Litaay et al., 2023). Unlike previous studies that mainly evaluated antibacterial activity, the present study integrates phytochemical screening, formulation development, physicochemical quality evaluation, stability testing, and antibacterial assessment of a mouthwash prepared from 96% ethanol extract of *Ocimum* × *africanum* Lour., thereby providing a more comprehensive evaluation of its potential as a herbal oral care product.

METHODS

1. Study Design

This experimental laboratory study consisted of four stages: (1) preparation and extraction of Ocimum × africanum Lour. leaves, (2) phytochemical screening, (3) formulation and physicochemical evaluation of mouthwash preparations, and (4) evaluation of antibacterial activity against Streptococcus mutans.

Fresh basil (*Ocimum* × *africanum* Lour.) leaves were collected from Situbondo, East Java, Indonesia, and taxonomically identified at the Tawangmangu Center for Research and Development of Medicinal Plants and Traditional Medicine, Central Java, Indonesia. The leaves were washed, air-dried at room temperature, pulverized, and sieved to obtain simplicia powder before extraction.

2. Extraction of Basil Leaves

Powdered basil leaves were extracted by maceration using 96% ethanol at room temperature for three consecutive 24-hour periods with occasional stirring. The filtrates were combined and concentrated under reduced pressure using a rotary evaporator to obtain a viscous extract.
The extract yield was calculated using the following equation:

$$Extract\ Yield\ (\%) = \frac{Weight\ of\ extract}{Weight\ of\ dried\ simplicia} \times 100 \quad (1)$$

3. Mouthwash Preparation

Formulation of Mouthwash

Three mouthwash formulations containing basil extract at concentrations of 2.5% (F1), 5% (F2), and 10% (F3) were prepared, as attached in Table 1. The extract was dispersed in glycerin until homogeneous. Sodium benzoate and sodium saccharin were subsequently incorporated, followed by Tween 80. Distilled water was gradually added while stirring until a homogeneous solution was obtained. Peppermint oil was added as the final ingredient before the preparation was transferred into sterile bottles.

4. Phytochemical Screening

Qualitative phytochemical screening was conducted to detect alkaloids, flavonoids, saponins, tannins, and steroids using standard phytochemical procedures. Alkaloids were identified using Wagner reagent. Flavonoids were detected using the magnesium–hydrochloric acid (Shinoda) reaction. Saponins were determined using the foam test. Tannins were identified using ferric chloride solution. Steroids were detected using the Liebermann–Burchard reaction.

a. Organoleptic Evaluation

Color, odor, and physical appearance were visually observed at room temperature.

b. pH Measurement

The pH of each formulation was measured using universal pH indicator paper

c. Viscosity Measurement

Viscosity was measured using a Brookfield viscometer equipped with spindle No. 62 at 100 rpm.

d. Specific Gravity

Specific gravity was determined using a calibrated pycnometer according to the following equation:

$$\rho = \frac{W_2 - W_0}{V} \qquad (2)$$

where:

- **ρ** = specific gravity (g/mL)
- $\mathbf{W_0}$ = weight of empty pycnometer (g)
- $\mathbf{W_2}$ = weight of pycnometer filled with sample (g)
- **V** = pycnometer volume (mL)

e. Freeze–Thaw Stability Test

Each formulation was subjected to six freeze–thaw cycles consisting of storage at 4 ± 2°C for 24 h followed by storage at 40 ± 2°C for another 24 h. Physical changes, including color, odor, and appearance, were evaluated after each cycle.

5. Antibacterial Activity

Sterile paper discs impregnated with each mouthwash formulation were placed on Mueller–Hinton Agar (MHA) plates inoculated with the bacterial suspension. The plates were incubated at 37°C for 24 h. The inhibition zone diameter was measured using a digital vernier caliper. The inhibition zone diameter was calculated using the following equation:

$$\text{Inhibition Zone} = \frac{(D_v - D_c) + (D_h - D_c)}{2} \qquad (3)$$

where:

- **Dv** = vertical diameter
- **Dh** = horizontal diameter
- **Dc** = disc diameter

6. Statistical Analysis

All experiments were performed in triplicate and expressed as mean ± standard deviation. Statistical analysis was carried out using IBM SPSS Statistics version 25. Data normality was assessed using the Shapiro–Wilk test. Differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by Least Significant Difference (LSD) post-hoc analysis when significant differences were observed. A value of $p < 0.05$ was considered statistically significant.

Table 1. Mouthwash Formula

Material		Formulation			
		K (-)	F1	F2	F3
Basil Leaf Extract	Bioactive	-	2,5%	5%	10%
Tween 80	Emulgator	3,75%	3,75%	3,75%	3,75%
Natrium Sakarin	Sweetener	1%	1%	1%	1%
Pappermint Oil	Fragrance	0,3%	0,3%	0,3%	0,3%
Natrium Benzoate	Preservative	0,4%	0,4%	0,4%	0,4%
Gliserin	Wetting Agent	5%	5%	5%	5%
Aquadest	Solvent	50 ml	50 ml	50 ml	50 ml

RESULTS

1. Extraction Yield of Basil Leaves

The maceration of *Ocimum* × *africanum* Lour. leaves using 96% ethanol produced a dark green viscous extract with a characteristic basil odor. From 400 g of dried simplicia, 64.21 g of extract was obtained, corresponding to an extraction yield of 16.05% (Table 2). The obtained yield exceeded the minimum requirement of 10% for herbal extracts (Lifiani et al., 2022).

Table 2. Extract Yield Results

Weight of Basil Leaf Simplicia	Extract Weight	Extract Yield	Extract Characteristics		
			Form	Color	Flavour
400 gram	64,21gram	16,05%	Thick	Deep green	Basil Specialty

2. Phytochemical Screening Screening Results

Qualitative phytochemical screening demonstrated the presence of alkaloids, flavonoids, saponins, tannins, and steroids in the basil leaf extract (Table 3). Several studies also indicate that basil leaves (Ocimum x africanum L.) contains alkaloids, anthocyanins, cardioglycosides, coumarins, flavonoids, glycosides, phenolics, quinones, saponins, steroids, terpenoids, and tannins (Timotius et al., 2021).

Table 3. Phytochemical Screening Results

Compound Screening	Results	Discoloration
Alkaloid Flavonoid Saponin	+	Brownish Yellow
Tannin	+	Brick Red
Steroid	+	There Is Blackish
	+	Green Foam
	+	Deep Green

3. Phytochemical Screening Screening Results

a. Organoleptic Evaluation

All formulations showed a homogeneous liquid appearance with a characteristic peppermint odor. Differences were observed only in color, which became darker as the concentration of basil extract increased (Table 4).

Tabel 4. Organoleptic test results

Test Sample	Color	Flavour	Form
F1	Deep green		
F2	Deep green		
F3		Peppermint	liquid
control (-)	Clear white		
control (+)	Clear blue		

b. pH Evaluation

All formulations showed a homogeneous liquid appearance with a characteristic peppermint odor. Differences were observed only in color, which became darker as the concentration of basil extract increased (Table 5).

Table 5. pH Test Results

Formulation	Average pH
F1	5
F2	6
F3	6
Control (-)	5.5
Control (+)	5

c. Viscosity Evaluation

Viscosity Test The viscosity test results showed F1 of 108, F2 of 152, F3 of 580, positive control of 143, and negative control of 84. The highest viscosity was shown in F3, while the lowest viscosity was in F1. The viscosity test results can be seen in Table 6.

Table 6. Viscosity Test Results

Formulation	Viscosity (cP)
F1	108
F2	152
F3	580
Control (-)	84
Control (+)	143

d. Gravity Evaluation

Specific Gravity Test The results of the specific gravity test were as follows: F1 was 1.03, F2 was 1.04, F3 was 1.10, the positive control was 1.03, and the negative control was 1.01. The highest specific gravity was shown in F3, while the lowest was in F1. The results of the specific gravity test on the basil leaf mouthwash preparation can be seen in Table 7.

Table 7. Specific Gravity Test Results

Formultion	Average value
F1	1. 03
F2	1.04
F3	1. 10
Control (-)	1,01
Control (+)	1.03

e. Freeze-Thaw Stability Evaluation

Freeze-thaw cycling test The stability test results showed that all formulations were liquid with a mint aroma and a deep green color. Stability testing was performed after the storage period. The results of the freeze-thaw cycling test are shown in Table 8.

Tabel 8. Freeze-Thaw Cycling Test Result

Formulation	After			Before		
	Shape	Color	Smell	Shape	Color	Smell
Control (-)	Liquid	Clear White			Clear White	
F1						
F2			Mint	Liquid		Mint
F3		Deep Green			Deep Green	

4. Antibacterial Activity

Streptococcus mutans Inhibition Test The inhibition test results for the control formulation (-) were 3.65 mm, F1 was 7.15 mm, F2 was 7.06 mm, F3 was 7.41 mm, and the control (+) was 3.38 mm. The largest inhibition zone is shown in F3. The inhibition zone results can be seen in Table 9 and Figure 1.

a b c

Figure 1. Streptococcus Mutans Inhibition Test: (a) First Repetition; (b) Second Repetition;(c) Third Repetition.

Table 9. Results of the Streptococcus mutans Inhibition Zone

Formulation	Average Inhibition Zona (mm)
F1	7,15[a]
F2	7,06[a]
F3	7,41[a]
Control (-)	3,65[b]
Control (+)	3,38[c]

The Shapiro–Wilk test confirmed that all data were normally distributed ($p > 0.05$). One-way ANOVA demonstrated a statistically significant difference among treatment groups ($p < 0.05$). Subsequent post-hoc analysis (LSD) indicated that all basil extract formulations differed significantly from the control groups, whereas no significant differences were observed among F1, F2, and F3 ($p > 0.05$). These findings suggest that increasing the basil extract concentration from 2.5% to 10% did not produce a statistically significant increase in antibacterial activity under the conditions of this study.

DISCUSSION

The inhibition zones obtained varied due to the varying concentrations of basil extract used in the formulations (Lifiani et al., 2022). The criteria for antibacterial inhibition strength are as follows: a zone diameter of 5 mm or less is considered weak, 5-10 mm is considered moderate, 10-20 mm is considered strong, and 20 mm or more is considered very strong (Nurmashita et al., 2015). These results indicate that F3 exhibited greater activity than the positive control.

The inhibitory value of the positive control was lower than all other formulations because Streptococcus mutans bacteria are sensitive to chlorhexidine and fluoride. The absence of chlorhexidine and fluoride additives in Listerine (K+) mouthwash makes Inhibitory activity was less than optimal (Sharma et al., 2018). Listerin is one of the most commonly used mouthwashes. Furthermore, Listerin contains eucalyptol, methyl salicylate, thymol, alcohol, and other additives, which have potential antimicrobial properties (Winastri et al., 2020). The inhibitory value of the negative control was due to the presence of peppermint oil in the mouthwash formulation. Peppermint oil contains essential oils that have the power to kill bacterial growth (Dola et al., 2022). The positive control used was Listerin mouthwash.

Similar results were obtained by a study conducted by Nuzula and Santoso (2017), which examined the effect of basil leaf extract on the inhibition of Streptococcus mutans bacteria. The

results of this study showed no significant difference despite differences in concentrations between 25%, 50%, 75%, and 100%. Furthermore, a study by Syahrul and Syahriel (2021) stated that there was no significant difference in the inhibition test for Streptococcus mutans when given basil leaf extract at several concentrations, namely 3.5% and 4%.

The compounds in basil leaves are very effective at damaging bacterial cell membranes and are lipophilic. The antibacterial activity of basil leaves is due to the presence of flavonoids and essential oils. The flavonoids and essential oils in basil leaves contain eugenol,a phenol derivative with bactericidal properties. Phenol kills bacteria by denaturing bacterial cell proteins and damaging the bacterial cell wall, thereby removing cations and macromolecules from the cell, thus disrupting bacterial growth. Inhibition also occurs by actively precipitating proteins and damaging lipids in the cell membrane (Syahrul and Syahriel, 2021). A 1% concentration of essential oil in basil leaves can inhibit bacterial growth by 87.50±3.33% (Ningrum and Wazna, 2018). Several studies also state that the essential oils in basil leaves can inhibit the growth of bacteria, fungi and mold (Justicia et al., 2017).

Streptococcus mutans is a gram-positive bacterium. Approximately 90-95% of gram-positive bacteria have a cell wall composed of peptidoglycan, bound to other molecules, such as telchoic acid and proteins. The structure of the gram-positive bacterial cell wall facilitates the penetration of flavonoid compounds, which have phenolic properties, making the hydrophobic flavonoid compounds easier to penetrate and act on the cell wall and within the cytoplasm (Nazzaro et al., 2013). Rahayu stated that phenolic compounds can damage and penetrate the cell wall and precipitate bacterial cell proteins. In large molecules, phenolic compounds can inactivate essential enzymes in bacterial cells, even at very low concentrations. Under many conditions, in the presence of sub-lethal antimicrobial compounds, microorganisms react by increasing the expression of stress response proteins to repair damaged proteins. However, at higher antimicrobial concentrations, this response is unable to prevent gram-positive bacteria from preventing cell death (Nazzaro et al., 2013).

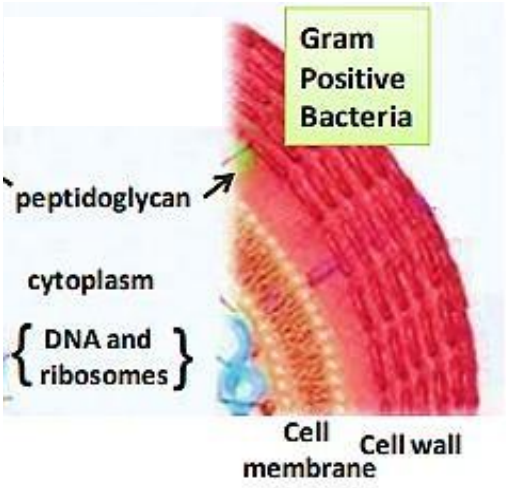

Figure 11. Schematic of The Membrane of Gram-Positive Bacteria (Nazzaro et al., 2013)

Flavonoids are secondary metabolites produced abundantly by plants, such as basil leaves. The combination of two basil flavonoid compounds, orientin and visenin, provides a synergistic antibacterial effect (Purnamaningsih and Supadmi, 2020). The mechanism of action of flavonoids

against bacteria involves disrupting the lipid bilayer, inducing bacterial membrane disruption and inhibiting several processes, such as biofilm formation, cell envelope synthesis, nucleic acid synthesis, the electron transport chain, and ATP synthesis (Yan et al., 2024). Flavonoids, particularly catechins, have been shown to disrupt bacterial membranes with lipid bilayers and inactivate or inhibit the synthesis of intracellular and extracellular enzymes (Gomial et al., 2019). The presence of peptidoglycan in gram-positive bacteria can facilitate catechin binding and absorption (Donadio et al., 2021). The binding of peptidoglycan by catechins disrupts bacterial cell wall biosynthesis (Gorniak et al., 2019). The antibacterial activity of flavonoids also involves different pathways, depending on the chemical characteristics of each compound. Flavonoids that are more hydrophobic prefer to interact with the phospholipid bilayer, disrupting membrane integrity, while flavonoid compounds with an increased number of hydroxyl groups on the phenol ring are associated with increased reactivity to proteins, enzyme inactivation, or receptor protection (Donalio et al., 2021). 2,4,60-trihydroxy-30-methylchalchone can cause Streptococcus mutans to leak intracellular substances such as proteins and ions (Gorniak et al., 2019).

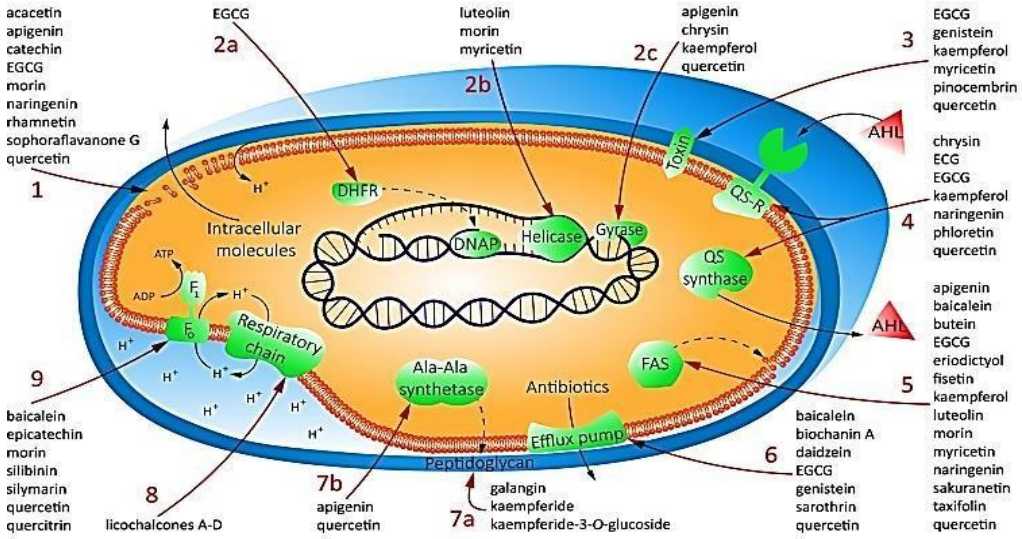

Figure 12. Diagrammatic Representation of Flavonoid Mechanisms (Gorniak et al., 2019)

Flavonoids work effectively to kill bacteria through various mechanisms. One is by disrupting membranes (1) and inhibiting nucleic acid synthesis (2a—through inhibition of dihydrofolate reductase (DHFR), 2b—through inhibition of helicase, 2c—through inhibition of gyrase/topoisomerase). Furthermore, flavonoids also inhibit bacterial virulence, such as toxins (3) and quorum sensing, which interferes with their ability to form biofilms (4). Antimicrobial action can also be achieved by inhibiting cell envelope synthesis, such as by inhibiting fatty acid synthase (FAS—5) and peptidoglycan synthesis (7a—through inhibition of Ala–Ala dipeptide synthesis, 7b—through inhibition of peptidoglycan cross-linking). Flavonoids can also inhibit efflux pumps, which can reverse antimicrobial resistance (6). Finally, flavonoids inhibit NADH-cytochrome c reductase activity in the bacterial respiratory chain (8) and inhibit ATP synthase (9) (Gorniak et al., 2019).

Alkaloid compounds can disrupt the peptidoglycan component of bacterial cell walls, causing damage to the bacterial cell wall and death. Saponins contain aglycone groups that can alter

and disrupt bacterial permeability (Lifiani et al., 2022). Flavonoid compounds form complexes with extracellular proteins in bacteria, which can damage the cytoplasmic membrane and cause lysis of intracellular compounds (Ariani et al., 2020). Tannins have antimicrobial properties by damaging and contracting the bacterial cell membrane, thereby disrupting bacterial cell permeability, leading to bacterial death (Winastri et al., 2020).

CONCLUSIONS

The findings of this study demonstrate that the 96% ethanol extract of *Ocimum* × *africanum* Lour. possesses promising potential as a natural antibacterial agent for herbal mouthwash formulations. The extract contained several bioactive secondary metabolites that contributed to its antibacterial activity against *Streptococcus mutans*. Among the tested formulations, the 10% extract (F3) exhibited the most favorable overall performance by combining the highest antibacterial activity with acceptable physicochemical characteristics and formulation stability. However, no statistically significant difference was observed among the tested extract concentrations. This study was limited to in vitro antibacterial evaluation using the disk diffusion method against a single bacterial species. Therefore, further studies involving minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), antibiofilm activity, long-term stability, safety evaluation, and clinical investigation are recommended to confirm the potential of this herbal mouthwash for practical application.

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