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ORIGINAL ARTICLE

Nano-based mouthwash containing white basil essential oil a potent alternative for oral hygiene

Nguyen Phuoc Vinh[1,2*], Nguyen Vu Giang Bac[3], Nguyen Du Thien[1], Do Tuong Dinh Minh[4], Dao Tien Trung[1]

[1]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Health Sciences, Vietnam National University Ho Chi Minh City, Ho Chi Minh City, Vietnam
[2]Department of Pharmaceutics, Research Center Of Discovery and Development Of Healthcare Products, Vietnam National University Ho Chi Minh City, Vietnam
[3]Department of Microbiology and Parasitology, School of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam
[4]Department of Science, John F. Kennedy Catholic High School, Washington, U.S.A

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ABSTRACT

Background and Objectives: White basil (*Ocimum gratissimum* L. Lamiaceae) essential oil exhibits potent antibacterial effects but its aqueous insolubility and high volatility restrict its applications. This study aimed to develop a nanodispersed mouthwash containing white basil essential oil, optimizing of surfactant/co-surfactant type and ratio, assess its physicochemical stability and antibacterial efficacy against *Streptococcus mutans* ATCC 25175.

Materials and Methods: Formulations combining white basil essential oil, Tween-80, isopropanol, Labrasol and water were prepared. Particle size, zeta potential, and pH were measured. Stability was evaluated under accelerated (40°C, 75% RH) and stressed (60°C, 75% RH) conditions over three months. Antimicrobial efficacy was assessed via minimum bactericidal concentration (MBC) after 30-second exposure.

Results: Characterization of the optimized formulation revealed an average particle size of 128 nm, neutral zeta potential, pH 3.42. Stability testing demonstrated thermodynamic resistance under all storage conditions for three months without phase separation or significant size change. The MBC against *Streptococcus mutans* was 0.4% w/v essential oil following 30 seconds of exposure. A corresponding mouthwash with 0.4% w/v essential oil demonstrated equivalent bactericidal activity.

Conclusion: Nanodispersion offers a promising strategy for white basil essential oil in mouthwash formulations. The developed formulation shows favorable stability and rapid bactericidal action, supporting further evaluation for clinical and commercial development.

Keywords: Oral hygiene; Nanomaterials; Essential oil; *Ocimum gratissimum*

*Corresponding author: Nguyen Phuoc Vinh, Ph.D, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Health Sciences, Vietnam National University Ho Chi Minh City, Ho Chi Minh City, Vietnam; Department of Pharmaceutics, Research Center Of Discovery and Development Of Healthcare Products, Vietnam National University Ho Chi Minh City, Vietnam. Tel: +84795757572 Fax: +842871021212 Email: npvinh@uhsvnu.edu.vn

INTRODUCTION

Vietnam is among the countries with the highest prevalence of oral diseases, reaching over 90% in some studies of specific age groups or locations. While not directly life-threatening, oral diseases exert significant impacts on systemic health, particularly the digestive system, as well as on psychological well-being and quality of life. Consequently, the prevention and management of dental diseases represent an important public health priority and a growing concern within society (1). To effectively prevent the progression of dental diseases, it is essential to implement preventive strategies that are simple, cost-effective, and feasible within the socioeconomic context of Vietnam. Such measures not only reduce the risk of disease onset but also play a critical role in minimizing recurrence, thereby contributing to improved long-term oral health outcomes (2).

Several antimicrobial agents, including triclosan and chlorhexidine, have been widely employed in oral hygiene products. Although effective in reducing plaque accumulation and preventing gingivitis, their use is often associated with undesirable side effects, such as tooth discoloration and alterations in taste perception. In addition, the emergence of antimicrobial resistance among oral microorganisms has become a growing concern within the dental and pharmaceutical fields. In this context, herbal-derived compounds, particularly essential oils, have attracted increasing attention as potential alternatives. Numerous studies have demonstrated their antibacterial and anti-inflammatory activities, highlighting their promise as safe and effective agents for oral health management (3-7).

Essential oils are secondary metabolites of plants that contain a complex mixture of terpenic hydrocarbons, especially monoterpenes, sesquiterpenes, and their oxygenated derivatives (8) Some of common herbs with essential oils can be used in dental care like Lavender (*Lav andula angustifolia* Lamiaceae) (9); Cinnamon (*Cinnamomum cassia* Lauraceae) (10), Clove (*Syzygium aromaticum* Myrtaceae) (11), Tea tree (*Melaleuca alternifolia* Myrtaceae) (12), Eucalyptus (*Eucalyptus globulus* Myrtaceae) (12, 13), Peppermint (*Mentha piperita* Lamiaceae) (14), Lemon (*Citrus limon* Rutaceae) (15).

Among the essential oils with potential applications in oral health, white basil (*Ocimum gratissimum*, Lamiaceae), a species widely utilized in traditional medicine and culinary practices, has emerged as a particularly promising candidate. Its essential oil is rich in bioactive constituents, with eugenol identified as the major component. Eugenol has been extensively reported to possess strong antibacterial and antioxidant properties, supporting its therapeutic use in the management of various conditions, including coughs, fever, and conjunctivitis. (16-18). White basil essential oil has demonstrated notable antibacterial activity against a range of oral pathogens. Specifically, it has been shown to inhibit *Streptococcus mutans*, a primary initiator of dental caries; *Enterococcus faecalis*, commonly associated with persistent endodontic infections; *Lactobacillus paracasei*, implicated in the progression of advanced carious lesions; and *Porphyromonas gingivalis*, a key etiological agent in periodontitis. These pharmacological activities underscore the potential of *O. gratissimum* essential oil as a natural agent for oral hygiene formulations because of its broad-spectrum antimicrobial potential across different stages of oral disease (19). Although the therapeutic potential of white basil essential oil in oral hygiene has been increasingly recognized within the scientific community (20), However, a substantial gap persists between the documented therapeutic potential of white basil essential oil and its practical application, primarily due to its poor aqueous solubility, high volatility, and physicochemical instability. For oral hygiene, various dosage forms have been explored, including toothpastes, mouthwashes, gels, and eugenol-containing dental materials. Additionally, eugenol has been incorporated into restorative applications such as zinc oxide–eugenol (ZOE) cements and dental implants (21). Eugenol can be immobilized onto or in proximity to material surfaces, enabling covalent interactions while preserving its intrinsic antibacterial and antioxidant properties (22). Nanocarriers designed for eugenol delivery encompass a wide range of systems, including both organic-based and inorganic-based platforms. Nanotechnology also provides an effective strategy to overcome the weak point of the essential oil by enhancing solubility, protecting volatile components, and improving bioavailability at the site of action (23, 24).

This study aimed to develop a nanosystem for the delivery of essential oils in oral hygiene applications, accompanied by comprehensive physicochemical characterization and supporting scientific evidence for its suitability in oral use.

MATERIALS AND METHODS

White basil essential oil was purchased from a domestic supplier (Thien Nhien Xanh Co. Ltd.), which met the standards for essential oils as indicated in the Vietnamese Pharmacopoeia V (Batch number PTEO-035; CoA number HNHU 1121/2). Reference standard of eugenol was purchased from Sigma-Aldrich (Batch number BCCC7425, CoA number 35995). Methanol and water were HPLC grade. All other solvents and reagents used in the analyses were of analytical grade.

Quantification of eugenol in essential oil. Eugenol, the principal constituent of white basil essential oil, was selected as the marker compound for quality control of the material. According to the Vietnamese Pharmacopoeia V, white basil essential oil must contain not less than 60% v/v eugenol. Based on the density values provided in the certificate of analysis (CoA) for eugenol (1.067 g/mL) and for the essential oil (1.015 g/mL), this specification corresponds to approximately 63% w/w.

Quantification of eugenol. The eugenol content in the essential oil was determined by high-performance liquid chromatography (HPLC) using a SHIMADZU LC-2050C system equipped with a SUPERCOSIL® LC-18-S column (250 × 4.6 mm). The mobile phase consisted of methanol and water (60:40, v/v) in isocratic mode, with a flow rate of 1.0 mL/min. The column oven was maintained at 35°C, and the injection volume was set at 10 µL. Detection was performed at a wavelength of 281 nm by PDA detector. For calibration, a reference solution of eugenol (0.10 mg/mL in methanol) was prepared.

To prepare the test solution, 250 mg of white basil essential oil was diluted with methanol to obtain a final concentration of 0.10 mg/mL. The percentage of eugenol in the essential oil (P%, w/w) was calculated using the following equation:

$$P(\%) = \frac{A_T}{A_C} \times C \times P \times \frac{F}{m \times 1000} \times 100\%$$

Wherein:

- A_T: Area of eugenol's peak in the test sample's chromatogram.

- A_C: Area of eugenol's peak in the reference sample's chromatogram.

-C: Concentration of eugenol in the reference solution (mg/mL).

-P: The purity of eugenol in the reference standard.

-F: The dilution factor of the test sample.

-m: The weight of the essential oil used to prepare the test sample (g).

Formulation of nanodispersion containing white basil essential oil. The nanodispersion formulation was developed using a combination of surfactant and co-surfactant. Specifically, the effects of surfactant/co-surfactant type and the surfactant-to-co-surfactant ratio were investigated as follows:

- Selection of surfactant and co-surfactant: To evaluate compatibility with essential oil, 0.10 g of essential oil was mixed with 1.0 g of each candidate surfactant or co-surfactant in Eppendorf tubes, followed by vigorous vortexing for 30 seconds. Only those agents capable of producing a clear, transparent solution were selected for subsequent studies. A total of four surfactants (Labrasol, Tween 20, Tween 80, and Cremophor RH40) and four co-surfactants (ethanol, propylene glycol, glycerol, and isopropanol) were screened in this process.

- Selection of surfactant-to-co-surfactant ratio: Various ratios of the selected surfactant and co-surfactant (1:1, 2:1, 3:1, 4:1, and 5:1, w/w) were prepared to obtain S_{mix}. Subsequently, 0.10 g of essential oil was incorporated into S_{mix}, with the essential oil-to-S_{mix} ratio (w/w) ranging from 1:10 to 1:50. Water was then titrated into the mixture using a burette in 0.10 mL (≈ 0.10 g) increments, under continuous magnetic stirring at ambient temperature, until a final volume of 10 mL was reached. Only S_{mix} combinations that yielded clear and transparent dispersions upon aqueous dilution were selected for further optimization.

Characterization of the obtained nanodispersion. - Physico-chemical characterization: The particle size, polydispersity index (PDI), and zeta potential of the nanodispersion were measured using dynamic light scattering (DLS) with a Zetasizer Nano ZS90 (Malvern, UK) and further analyzed by the nanoparticle tracking analysis (NTA) method. (ZetaView® X30, Particle Metrix).

- pH: The pH of the nanodispersion was measured using a calibrated digital pH meter under ambient conditions (S220-Kit, Mettler Toledo).

- Centrifugation stability: The phase stability of the nanodispersion was evaluated by centrifugation at 4,000 rpm for 30 minutes and 12,000 rpm for 15

minutes. Formulations were considered stable only if they remained clear and exhibited no evidence of phase separation following centrifugation.

- Thermal stability: The thermal stability of the nanoformulation was assessed under three storage conditions: 2-8°C, 50°C, and ambient temperature, each for 24 hours. This cycle was repeated six times consecutively. The formulation was considered thermally stable if it remained clear and free of phase separation after all cycles.

- Encapsulation efficiency (EE%): The EE of the nanocarriers was determined using an indirect method by quantifying the amount of non-encapsulated eugenol. Briefly, 1.5 mL of the nanodispersion was centrifuged at 12,000 rpm for 10 minutes. An aliquot of 0.5 mL of the supernatant was transferred into a 10-mL volumetric flask, diluted to volume with methanol, and filtered prior to HPLC analysis, as described above. The concentration of eugenol (X, mg/mL) in the supernatant was calculated according to Equation (2.2), and the encapsulation efficiency (EE%) was determined using Equation (2.3). All measurements were performed in triplicate.

$$X\ (mg/mL) = \frac{A_T}{A_C} \times C \times P \times F$$

Wherein:

- A_T: Area of eugenol's peak in the test sample's chromatogram.
- A_C: Area of eugenol's peak in the reference sample's chromatogram.
- C: Concentration of eugenol in the reference solution (mg/mL).
- P: The purity of eugenol in the reference standard.
- F: The dilution factor of the test sample.

$$EE\ (\%) = (1 - \frac{X}{a \times P}) \times 100\%$$

Wherein:

- X: Concentration (mg/mL) of eugenol in the free-eugenol sample
- a: Concentration (mg/mL) of essential oil in the sample
- P: The percentage (P%) of eugenol within the essential oil.

- Long-term stability: Centrifugation and thermal stability tests were re-evaluated at 1, 2, and 3 months post-production to assess the long-term stability of the formulation under accelerated conditions (40°C, 75% RH) and stressed conditions (60°C, 75% RH). In addition, some physicochemical parameters, including appearance, particle size, polydispersity index (PDI), zeta potential, and pH, were monitored throughout the study period.

Formulation of nano-based mouthwash. The concentration of white basil essential oil incorporated into the mouthwash formulation was determined based on the MBC. Additional excipients, including sweeteners (aspartame, sodium saccharin, acesulfame K, and mannitol) and flavoring agents (menthol), were evaluated to enhance the palatability of the final product. The mouthwash was further characterized with respect to its physicochemical properties, following the same parameters applied to the nanodispersion.

Antimicrobial assay on *Streptococcus mutans* ATCC 25175. The minimum bactericidal concentration (MBC) of white basil essential oil, the nanodispersion, and the nano-based mouthwash was evaluated against *Streptococcus mutans* ATCC 25175. To simulate oral rinsing conditions, bacterial suspensions at 10^6 CFU/mL were exposed to each test sample, with essential oil concentrations ranging from 0.1% to 0.4% (w/v), for 30 seconds. Following exposure, 1 mL of the bacterial suspension was centrifuged at 13,000 rpm for 3 minutes, and the pellet was resuspended in sterile saline to restore the original volume, thereby minimizing essential oil carryover.

Serial tenfold dilutions were prepared in BHI broth, and 100 µL aliquots were spread on nutrient agar plates. Plates were incubated at 37°C for 48 hours, after which colony-forming units (CFU) were enumerated. The microbial load of the original sample was calculated based on the dilution factor. The limit of detection (LOD) was 1.3 $\log_{10}$ CFU/mL. The MBC was defined as the lowest tested concentration of essential oil at which no bacterial growth was observed.

RESULTS

Eugenol content in the essential oil. As shown in Figs. 1 and 2, the eugenol peak was identified in the sample chromatogram at a retention time of 5.772 min, closely matching that of the reference standard (5.786 min). Quantitative analysis indicated an euge-

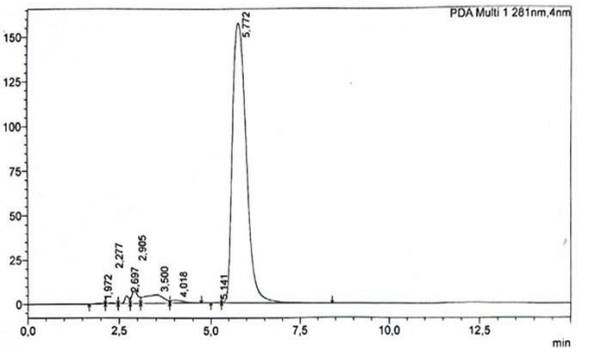

Fig. 1. Chromatogram of the reference solution

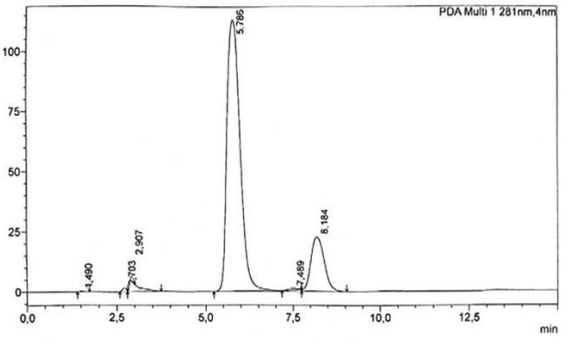

Fig. 2. Chromatogram of the sample solution

nol content of 63.93 ± 1.55% w/w. This value complies with the specification for white basil essential oil established in the Vietnamese Pharmacopoeia V, thereby confirming that the material met the quality requirements for pharmaceutical use. On this basis, the essential oil was deemed suitable for subsequent experimental investigations.

Validation of the quantification procedure. The eugenol assay method was validated for system suitability, linearity, precision (repeatability and intermediate precision), and accuracy in accordance with the International Council for Harmonisation (ICH) guideline Q2 (R2) on analytical procedure validation.

For the system suitability, the results showed that the RSD of retention time, area of peak, number of theoretical plates, and symmetry factor of peak were 0.44%, 0.33%, 0.23%, and 0.37%, respectively.

In terms of linearity (Fig. 3), the regression equation was $\hat{y}$ = 37,403x – 108,845 with R^2 = 0.9991 (α = 0.05). The results of repeatability and intermediate precision also proved that validation of this analytical method with an RSD = 0.17% and 0.16%, respectively. Finally, the accuracy test showed that this analytical procedure achieves accuracy, with the recovery limit within each concentration: Level 80% is 98.6% (RSD = 0.14%) ; Level 100% is 99.4% (RSD = 0.36%) ; Level 120% is 99.8% (RSD = 0.05%). The procedure also achieved the selectivity, as per the requirements in the ICH guideline.

According to these results, this analytical method was validated and eligible for further tests in the current study. This result is also in line with other research about the chemical constituents of the white basil, which ranges from over 45% v/v (or 47% w/w) (25) and can be up to 60% v/v (or 63% w/w) (26).

Selection of surfactant and co-surfactant. The solubility of the essential oil in 04 surfactants and 04 co-surfactants was assessed, and the results are shown in Table 1. The results showed that the essential oil exhibited good solubility in Labrasol, Tween 80, ethanol, and isopropanol. Therefore, these solvents were chosen for the next step of this study.

Selection of surfactant-to-co-surfactant ratio. The surfactants (S01 to S05) and co-surfactants (CS01 to CS03) were combined to create 75 formulations of Smix as shown in Tables 2-4.

Each S_{mix} was combined with essential oil at nine different ratios (essential oil: S_{mix} = 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, and 1:50, w/w), followed

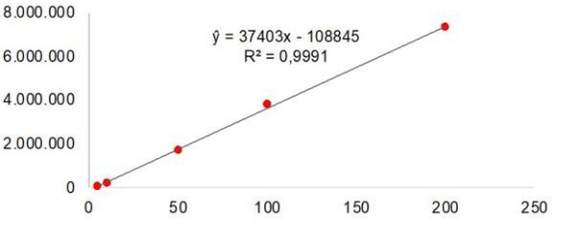

Fig. 3. Linearity of the assay method

Table 1. Screening for the solubility of essential oils in surfactant and co-surfactant

No.	Name	Results
1	Labrasol	Clear
2	Tween 80	Clear
3	Tween 20	Opaque
4	Cremophor RH40	Opaque
5	Ethanol	Clear
6	Isopropanol	Clear
7	Propylen glycol	Opaque
8	Glycerol	Opaque

Table 2. The ratio between Labrasol & Tween 80 in mixtures of surfactant

Mixture	S01	S02	S03	S04	S05
% Labrasol	0	25	50	75	100
% Tween 80	100	75	50	25	0

Table 3. The ratio between ethanol or isopropanol in the of co-surfactant

Mixture	CS01	CS02	CS03
% Ethanol	0	50	100
% Isopropanol	100	50	0

Table 4. The percentage of surfactant and co-surfactant in the S_{mix}

S_{mix}	% Labrasol	% Tween 80	% Ethanol	% Isopropanol
S01	0%	50%	0%	50%
S02	0%	67%	0%	33%
S03	0%	75%	0%	25%
S04	0%	80%	0%	20%
S05	0%	83%	0%	17%
S06	0%	50%	25%	25%
S07	0%	67%	17%	17%
S08	0%	75%	13%	13%
S09	0%	80%	10%	10%
S10	0%	83%	8%	8%
S11	0%	50%	50%	0%
S12	0%	67%	33%	0%
S13	0%	75%	25%	0%
S14	0%	80%	20%	0%
S15	0%	83%	17%	0%
S16	13%	38%	0%	50%
S17	17%	50%	0%	33%
S18	19%	56%	0%	25%
S19	20%	60%	0%	20%
S20	21%	63%	0%	17%
S21	13%	38%	25%	25%
S22	17%	50%	17%	17%
S23	19%	56%	13%	13%
S24	20%	60%	10%	10%
S25	21%	63%	8%	8%
S26	13%	38%	50%	0%
S27	17%	50%	33%	0%
S28	19%	56%	25%	0%
S29	20%	60%	20%	0%
S30	21%	63%	17%	0%
S31	25%	25%	0%	50%
S32	33%	33%	0%	33%
S33	38%	38%	0%	25%
S34	40%	40%	0%	20%
S35	42%	42%	0%	17%
S36	25%	25%	25%	25%
S37	33%	33%	17%	17%
S38	38%	38%	13%	13%
S39	40%	40%	10%	10%
S40	42%	42%	8%	8%
S41	25%	25%	50%	0%
S42	33%	33%	33%	0%
S43	38%	38%	25%	0%
S44	40%	40%	20%	0%
S45	42%	42%	17%	0%
S46	38%	13%	0%	50%
S47	50%	17%	0%	33%
S48	56%	19%	0%	25%
S49	60%	20%	0%	20%
S50	63%	21%	0%	17%
S51	38%	13%	25%	25%
S52	50%	17%	17%	17%
S53	56%	19%	13%	13%
S54	60%	20%	10%	10%
S55	63%	21%	8%	8%
S56	38%	13%	50%	0%
S57	50%	17%	33%	0%
S58	56%	19%	25%	0%
S59	60%	20%	20%	0%
S60	63%	21%	17%	0%
S61	50%	0%	0%	50%
S62	67%	0%	0%	33%
S63	75%	0%	0%	25%
S64	80%	0%	0%	20%
S65	83%	0%	0%	17%
S66	50%	0%	25%	25%
S67	67%	0%	17%	17%
S68	75%	0%	13%	13%
S69	80%	0%	10%	10%
S70	83%	0%	8%	8%
S71	50%	0%	50%	0%
S72	67%	0%	33%	0%
S73	75%	0%	25%	0%
S74	80%	0%	20%	0%
S75	83%	0%	17%	0%

by the addition of water to obtain a final dispersion volume of 10 mL. Only formulations exhibiting a clear and transparent appearance were considered suitable for further evaluation. As summarized in Table 5, four formulations containing more than 50% water and meeting the clarity criteria were selected for subsequent characterization.

Characterization of nanodispersion. The selected formulations were further evaluated for pH, stability (by centrifugation and thermal cycling), and physicochemical properties, including droplet size, size distribution, and surface charge. As presented in Table 6, all formulations exhibited a pH of approximately 3.5, which is within the acceptable range for oral products. In addition, they demonstrated high stability under centrifugation and were characterized by nearly neutral surface charge values.

Among the tested nanodispersion, only the formulations containing isopropanol as a co-surfactant (M3 and M4) achieved an appropriate droplet size for nanodispersion (less than 200 nm). Both formulations also demonstrated favorable thermal stability. However, M3 exhibited superior size homogeneity compared to M4, as reflected by its lower polydispersity index (0.23 vs. 0.81, respectively). Consequently, formulation M3, consisting of essential oil, Labrasol, Tween 80, and isopropanol at a ratio of 1:27:9:9 (w/w), was selected as the optimized system in this study. The morphology of the M3 nanodispersion, as observed by ZetaView® X30 analysis, is presented in Fig. 4.

Characterization of the optimal nanodispersion formulation. The optimized formulation was evaluated for encapsulation efficiency and stability over three months under ambient, accelerated (40°C, 75% RH), and stressed (60°C, 75% RH) conditions. As summarized in Table 7, the physicochemical parameters, including pH, particle size, polydispersity index (PDI), and zeta potential, remained stable across all storage conditions. The nanodispersion maintained a mean particle size of approximately 130 nm, with near-neutral surface charge and a PDI below 0.30, confirming its high colloidal stability. Thermal- and centrifugation-based assessments further supported the robustness of the formulation after three months of storage. A slight, non-significant decline in encapsulation efficiency was observed from the second month onward, likely due to structural reorganization

Table 5. The formulation of the nanodispersion contains the white basil essential oil

Ingredients	M1	M2	M3	M4
White basil essential oil	1%	1%	1%	1%
Labrasol	9%	11%	30%	24%
Tween 80	25%	11%	7%	8%
Ethanol	11%	23%	-	4%
Isopropanol	-	-	7%	4%
Water	54%	54%	55%	59%

Fig. 4. The morphology of M3 nanodispersion under ZetaView® X30, Particle Metrix

Table 6. The properties of the nanodispersion.

Nanodispersion	M1	M2	M3	M4
pH	3.55 ± 0.10	3.60 ± 0.10	3.42 ± 0.10	3.55 ± 0.10
Centrifugation stability	Stable	Stable	Stable	Stable
Thermal-cycle stability	Non-stable	Non-stable	Stable	Stable
Droplet size (nm)	65.34 ± 28.49	72.10 ± 35.86	128 ± 2.1	21 ± 17
	182.50 ± 64.85	105.10 ± 42.58		
PDI	0.51 ± 0.24	0.49 ± 0.40	0.23 ± 0.12	0.81 ± 0.21
Zeta potential (mV)	0.44 ± 1.20	0.63 ± 6.40	-0.21 ± 2.92	0.71 ± 2.57

Table 7. Properties of the M3 nanodispersion

Characterization parameters	Temparature	Time-line		
		1st month	02nd month	03rd month
pH	Ambient	3.42 ± 0.10	3.67 ± 0.15	3.29 ± 0.20
	40°C, RH 75%	3.21 ± 0.10	3.23 ± 0.05	3.19 ± 0.10
	60°C, RH 75%	3.22 ± 0.12	3.22 ± 0.10	3.15 ± 0.15
Droplet size (nm)	Ambient	128 ± 2.1	129 ± 1.1	128 ± 1.6
	40°C, RH 75%	127 ± 2.1	130 ± 0.2	128 ± 1.8
	60°C, RH 75%	129 ± 1.9	130 ± 2.8	133 ± 0.8
PDI	Ambient	0.23 ± 0.12	0.17 ± 0.11	0.24 ± 0.17
	40°C, RH 75%	0.25 ± 0.21	0.27 ± 0.18	0.22 ± 0.17
	60°C, RH 75%	0.26 ± 0.18	0.27 ± 0.13	0.27 ± 0.19
Zeta potential (mV)	Ambient	-0.21 ± 2.92	-0.12 ± 2.24	-0.17 ± 1.47
	40°C, RH 75%	-0.18 ± 2.80	-0.12 ± 1.14	-0.19 ± 1.49
	60°C, RH 75%	-0.21 ± 2.16	-0.20 ± 1.19	-0.21 ± 2.77
Centrifugation stability	Ambient	Stable	Stable	Stable
	40°C, RH 75%	Stable	Stable	Stable
	60°C, RH 75%	Stable	Stable	Stable
Thermal-cycle stability	Ambient	Stable	Stable	Stable
	40°C, RH 75%	Stable	Stable	Stable
	60°C, RH 75%	Stable	Stable	Stable
Encapsulation efficiency	Ambient	98.37 ± 0.09%	95.52 ± 0.12%	94.98 ± 0.33%
	40°C, RH 75%	98.45 ± 0.15%	95.11 ± 0.05%	94.66 ± 0.21%
	60°C, RH 75%	97.88 ± 0.21%	95.15 ± 0.24%	94.32 ± 0.16%

of the surfactant/co-surfactant matrix. Collectively, these results demonstrate the long-term stability of the selected nanodispersion, supporting its feasibility for practical application.

Formulation of nano-based mouthwash. The finalized formulation of the nano-based mouthwash is presented in Table 8.

Characterization and stability study of the finalized nano-based mouthwash. The concentration of white basil essential oil in the nano-based mouthwash was fixed at 0.4% (w/v), based on the MBC results. To enhance palatability, several sweetening agents were evaluated, including aspartame, sodium saccharin, acesulfame potassium, and mannitol. Both acesulfame potassium and aspartame at 0.5% (w/v) produced a pleasant sweetness; however, acesulfame potassium imparted a bitter aftertaste. Therefore, aspartame at 0.5% (w/v) was selected as the sweetener. For flavoring, menthol was tested at 0.25% and 0.50% (w/v), with the higher concentration providing a more desirable cooling sensation. The nano-based mouthwash was subsequently evaluated for pH, particle size, polydispersity index, zeta potential, and stability over a three-month storage period under ambient, accelerated, and stressed conditions.

Table 8. Formulation of the finalized nano-based mouthwash.

Component	Role	Percentage (%) w/v
White basil essential oil	Active ingredient	0.40
Labrasol	Surfactant	12.0
Tween 80	Surfactant	2.80
Isopropanol	Co-surfactant	2.80
Aspartame	Sweetener	0.50
Menthol	Palatibility enhancer	0.50
Water		81.0

As presented in Table 9, the droplet size, PDI, and zeta potential of the nanodispersion incorporated into the mouthwash formulation remained comparable to those of the standalone system (≈130 nm, PDI < 0.20, and near-neutral surface charge). This suggests that the additional excipients did not adversely affect

Table 9. Properties of the M3 nano-based mouthwash

Characterization parameters	Temparature	Time-line		
		1st month	02nd month	03rd month
pH	Ambient	4.82 ± 0.1	4.75 ± 0.1	4.77 ± 0.1
	40°C, RH 75%	4.64 ± 0.1	4.93 ± 0.1	4.98 ± 0.1
	60°C, RH 75%	4.99 ± 0.1	4.96 ± 0.1	4.88 ± 0.1
Droplet size (nm)	Ambient	131.3 ± 0.07	130.0 ± 0.05	128.4 ± 0.05
	40°C, RH 75%	135.9 ± 0.14	137.1 ± 0.12	135.4 ± 0.14
	60°C, RH 75%	127.9 ± 0.57	132.1 ± 0.46	129.4 ± 0.53
PDI	Ambient	0.10 ± 0.06	0.17 ± 0.15	0.13 ± 0.07
	40°C, RH 75%	0.13 ± 0.06	0.15 ± 0.15	0.11 ± 0.07
	60°C, RH 75%	0.16 ± 0.05	0.16 ± 0.07	0.17 ± 0.13
Zeta potential (mV)	Ambient	-1.01 ± 0.55	-1.13 ± 0.59	-1.26 ± 1.14
	40°C, RH 75%	-1.21 ± 0.41	-1.25 ± 0.46	-1.23 ± 0.44
	60°C, RH 75%	-1.24 ± 0.44	-1.21 ± 0.34	-1.26 ± 0.21
Centrifugation stability	Ambient	Stable	Stable	Stable
	40°C, RH 75%	Stable	Stable	Stable
	60°C, RH 75%	Stable	Stable	Stable
Thermal-cycle stability	Ambient	Stable	Stable	Stable
	40°C, RH 75%	Stable	Stable	Stable
	60°C, RH 75%	Stable	Stable	Stable

the nanodispersion structure. Notably, the PDI of the mouthwash (0.10) was lower than that of the nanodispersion alone (0.26), reflecting improved size homogeneity, potentially due to stabilizing effects of the added ingredients.

After three months of storage under ambient, accelerated, and stressed conditions, the mouthwash maintained stable physicochemical and thermodynamic properties. Collectively, these findings confirm the robustness of the finalized nano-based mouthwash formulation and support its suitability for scale-up studies.

Antibacterial assay of nanodispersion and nano-based mouthwash. As illustrated in Fig. 5, the MBC of the essential oil after 30 seconds of exposure was slightly higher for the nanodispersion compared with the non-encapsulated essential oil (0.4% vs. 0.3%, respectively). This minor increase may be attributed to the time required for nano-encapsulated oil to be released into the aqueous phase before exerting antibacterial activity. However, the difference was not statistically significant, and the 0.4% nanodispersion was selected for the mouthwash formulation. The finalized nano-based mouthwash demonstrated efficient antibacterial activity against *Streptococcus mutans* within 30 seconds, supporting its potential as a practical alternative to conventional oral care products.

DISCUSSION

Gas chromatography is the standard technique for essential oil analysis due to their volatile nature; however, it is relatively sophisticated and costly compared to high-performance liquid chromatography (HPLC). In this study, an HPLC method was adapted and validated as a more accessible and economical alternative for routine analysis. The procedure complied with ICH Q2 (R2) guidelines for analytical validation. Eugenol was selected as the marker compound for quantification, in accordance with the specifications of the Vietnam Pharmacopoeia V for white basil essential oil.

Given the intrinsic water insolubility of white basil essential oil, the selection of suitable surfactant and co-surfactant types and their ratios is critical for the development of an efficient nanoformulation. Non-ionic surfactants and co-surfactants, such as Labrasol, Tween, ethanol, and isopropanol, are commonly employed owing to their high compatibility with neutral active compounds. In addition, these

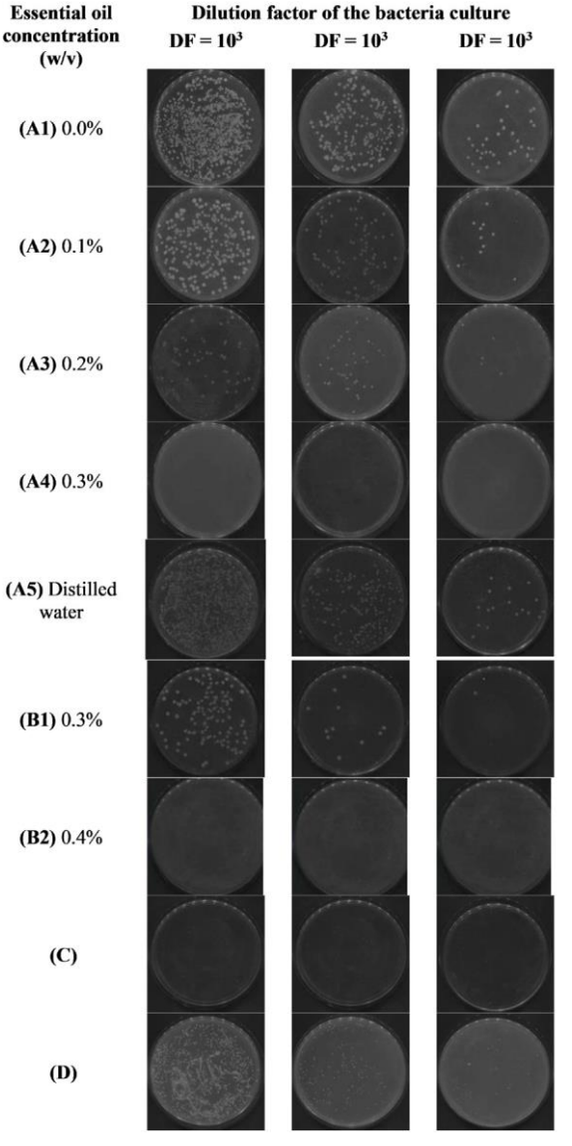

Fig. 5. MBC determination on S. mutans after 30 seconds of contact (DF: dilution factor). (A1-A5) Non-encapsulated essential oil, (B1 B2) Essential oil- encapsulated nanodispersion, (C) Nano-based mouthwash, (D) Saline 0.85%.

excipients are recognized for their biocompatibility, biodegradability, and favorable safety profile, being non-irritating and non-carcinogenic, which further supports their suitability for oral pharmaceutical applications (27). The ratio of surfactant to co-surfactant is a key determinant of the physicochemical properties of nanodispersions. In this study, the optimized composition yielded a formulation with a mean droplet size of approximately 130 nm, near-neutral surface charge, narrow size distribution, and favorable thermodynamic stability.

Another concern in the nanodispersion formulation is the high concentration of the surfactant and co-surfactant. Nevertheless, it was significantly decreased in the finalized nano-based mouthwash to 12% m/v. In addition, the intended use of the product further minimizes any potential toxicity risk: the exposure is short (about 30 seconds), using 20 mL twice daily, without ingestion, and users may rinse the mouth with water immediately afterward. Taken together, these considerations suggest that the applied concentration of Labrasol does not raise safety concerns in the proposed formulation.

In terms of herbal-based mouthwash formulation, one of the biggest concerns is its high content of monoaromatics and fats. As a result, it is important to ensure the thermodynamic stability of the dispersion to avoid phase separation, especially in hot and humid countries, including Vietnam (28). In our study, a high thermodynamic stability was proven for our finalized formulation after three months of storage under different conditions, reflecting its high potential for real application (29, 30). Although the MBC value was slightly increased in nanodispersion compared to non-encapsulated essential oil, this increase was not significant, and the finalized mouthwash exhibited an efficient antibacterial activity on the tested strain. To explain this slight increase, it may be because the essential oil has been encapsulated in the nanocarriers, so the liberation process must take place before its antimicrobial effect. Moreover, as the active component is highly volatile, the manufacturing process critically influences product quality. To minimize degradation and loss, a heat-free fabrication approach was employed (28, 31). This process is cost-effective, environmentally friendly, and easy to implement and scale up.

Nanoparticle Tracking Analysis (NTA) and Dynamic Light Scattering (DLS) are both techniques used to measure the size distribution of nanoparticles in liquid nanodispersions. While they share similarities, they differ in methodology, data interpretation, and specific applications. NTA visualizes individual particles using a microscope, tracking their Brownian motion to calculate hydrodynamic diameters, while DLS analyzes the intensity fluctuations of scattered light from an ensemble of particles. DLS is more influenced by larger particles in heterogeneous samples and is commonly used for quality control in nanoparticle production (32-34).

Furthermore, NTA is capable of analyzing samples

at lower concentrations, detecting particles that are up to 1000 times more dilute than those detectable by DLS, which requires higher particle concentrations for accurate measurements and may face challenges with samples containing a broad size distribution. NTA also allows for real-time observation of particle behavior, facilitating the detection of aggregation or other dynamic changes. In this research, we have diluted the sample 10.000 times before using the NTA to characterize the nanodispersion. The NTA report showed a size distribution but did not include the polydispersity index, so we needed to perform the evaluation again by the DLS method to confirm the results shown by NTA.

Streptococcus mutans plays a central role in the development of dental caries and other oral diseases. This bacterium is highly cariogenic due to its strong ability to adhere to tooth surfaces and form biofilms, commonly known as dental plaque. Within these biofilms, *Streptococcus mutans* metabolizes dietary sugars through fermentation, producing organic acids such as lactic acid. These acids lower the local pH in the oral cavity, leading to the demineralization of tooth enamel and initiating cavity formation. In addition, *Streptococcus mutans* exhibits aciduricity, meaning it can survive and thrive in acidic environments that inhibit the growth of many other oral microbes, thereby reinforcing its dominance in cariogenic niches. The accumulation of this bacterium and its metabolic byproducts not only contributes to dental decay but also disrupts the balance of the oral microbiota, further aggravating oral health problems. For this reason, we tested the effectiveness of the mouthwash against *Streptococcus mutans* to evaluate its potential in preventing dental caries.

Recently, nanotechnology has been widely recognized as a promising strategy to deliver herbal extracts with antimicrobial effects, especially in improving oral health and combating *Streptococcus mutans* infections (35). In particular, the use of nanotechnologies for loading essential oils by encapsulating them into nanoparticles or nanocapsules can enhance stability, improving solubility and prolonged release, which makes them ideal candidates for oral care products like mouthwash (36). However, nanocarriers themselves are not qualified to be marketed for oral hygiene due to their poor palatability and/or unsuitability for administration pathways. To overcome this problem, an appropriate dosage form based on these nanodispersions is required. For oral hygiene, several dosage forms can be used, such as toothpastes, mouthwashes, gels, or dental implants with a particle size in the range of 20 to 200 nm (37).

In this study, a micellar nanosystem was selected over alternative nanocarriers (e.g., lipid-based or polymeric nanoparticles) for several reasons. First, micelles formed by non-ionic surfactants (Labrasol, Tween 80, and isopropanol) efficiently solubilize lipophilic compounds such as eugenol, while maintaining a clear and transparent dispersion suitable for mouthwash formulations. Second, surfactant-based micelles are thermodynamically stable, simple to prepare without heat or organic solvents, and thus cost-effective, environmentally sustainable, and scalable for industrial production. In contrast, lipidic nanocarriers (e.g., liposomes, solid lipid nanoparticles) and polymeric nanoparticles, while effective, require more complex processes (high-pressure homogenization, solvent evaporation, or polymer synthesis), higher production costs, and may present regulatory challenges for oral care applications (36). Therefore, micellar nanodispersion was identified as the most appropriate delivery platform, balancing stability, efficacy, safety, and feasibility for large-scale production of a nano-based mouthwash.

Our finalized mouthwash formulation containing nano-encapsulated essential oil exhibited appropriate characteristics for oral hygiene, including appropriate physico-chemical properties (size around 130 nm, PDI inferior to 0.3, neutral charge, pH around 4.5, high thermodynamic stability, high stability after 3 months of storage under different conditions, and promising antibacterial activity. This study demonstrates, for the first time, the successful incorporation of white basil essential oil into a stable nanodispersion system suitable for mouthwash applications. Importantly, the nano-based mouthwash retained strong antibacterial activity against *Streptococcus mutans* within clinically relevant exposure times, underscoring its potential as an effective alternative to synthetic agents such as chlorhexidine. The use of a heat-free, scalable, and cost-efficient fabrication process further enhances the potential of this product for industrial production. Another advantage of the selected nanodispersion in this study is alcohol-free, which may help to popularize the finalized product. These findings highlight both the novelty and practical relevance of our work to the advancement of oral healthcare formulations by offering a promising pathway toward natural, friendly alternatives herb-

al-based oral care products in dental hygiene.

While the present study provides valuable insights, it is important to acknowledge certain limitations. Since biofilm formation is central to the pathogenicity of *Streptococcus mutans*, biofilm inhibition assays should have been performed. In this research, our primary focus was on evaluating the direct antimicrobial effect of the mouthwash against S*treptococcus mutans* in planktonic form as a first step. However, we fully recognize that biofilm formation represents the natural growth condition of *Streptococcus mutans* in the oral cavity and plays a critical role in its cariogenic potential. Therefore, we plan to conduct further experiments on *Streptococcus mutans* biofilms in future work to provide a more comprehensive evaluation of the mouthwash's effectiveness under conditions that closely mimic the in vivo oral environment.

Future research will be directed toward expanding the study population, prolonging the observation period, and employing more advanced analytical techniques to enhance the generalizability of our findings. Particular attention will be given to evaluating the cytotoxicity of the formulation through both in vitro and in vivo studies to confirm its biosafety. Furthermore, as palatability is a key determinant of patient compliance, sensory evaluation in human volunteers will be undertaken, subject to ethical approval and informed consent. By addressing these aspects, we aim to generate more comprehensive evidence and thereby support the translation of this approach into practical application.

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