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Research Paper

Development of a Natural Antifungal Shampoo Containing Clove Bud Oil (*Syzygium aromaticum*): Formulation and in Vitro Evaluation

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Abstract
Dandruff affects approximately 50% of the global population during their lifetime. Clove bud oil (*Syzygium aromaticum*) (CBO), which is currently utilized mainly in the cigarette industry, has potential as an anti-dandruff agent because of its antifungal activity. This study aimed to optimize cocamide DEA and sodium lauryl sulfate (SLS) concentrations in CBO shampoo formulations and to evaluate the preliminary antifungal activity of the optimum formulation against *Candida albicans* as a model fungal organism, compared with a commercial ketoconazole shampoo (Ketomed®). CBO quality was characterized through organoleptic evaluation, specific gravity determination, ethanol solubility testing, Thin Layer Chromatography (TLC), and GC–MS analysis. GC–MS confirmed eugenol (76.88%) and β-caryophyllene (16.18%) as the major constituents of the oil. The shampoo formulations were prepared using the emulsification method followed by a two-stage surfactant optimization process. The first optimization identified 8% cocamide DEA as the optimum concentration, producing a viscosity of 3915 ± 59 cPs and foam height of 10.43 ± 0.20 cm. The second optimization demonstrated that 8% SLS provided the most favorable physicochemical characteristics and washing performance. The optimum formulation exhibited antifungal activity against *C. albicans* with an inhibition zone of 24.06 ± 3.01 mm, compared with 32.50 ± 1.44 mm for Ketomed®, which corresponded to approximately 74% relative antifungal efficacy. These findings suggest that CBO shampoo possesses promising potential as an antifungal anti-dandruff formulation while also providing an alternative value-added utilization of clove oil. This study was limited to *in vitro* evaluation against a single microorganism, *C. albicans*, and further *in vivo* and clinical studies remain necessary to confirm the efficacy and safety of the developed formulation.

Keywords
Cocamide DEA, Sodium Lauryl Sulfate, *C. albicans*, Optimization

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1. INTRODUCTION

Dandruff affects approximately half of the global population during their lifetime (Grimshaw et al., 2019). In tropical and humid countries such as Indonesia, dandruff is highly prevalent and affects a large proportion of the population. The condition is associated with excessive sebaceous gland activity, which promotes fungal colonization, particularly by *Malassezia* sp., the primary microorganisms involved in dandruff pathogenesis (Galizia et al., 2024; Kumari et al., 2022; Locker et al., 2025; Poojary et al., 2024; Trüeb and Gavazzoni Dias, 2023). Dandruff may cause itching, scaling, hair loss leading to alopecia, and reduced self-confidence, thereby affecting daily comfort and quality of life (Pagaran et al., 2023).

In recent years, the trend toward natural-based cosmetic and health products has increased significantly (Lourenço-Lopes et al., 2020). Various medicinal plants have been developed into evidence-based herbal preparations. One plant with potential antifungal activity for hair care applications is clove (*Syzygium aromaticum*). Eugenol, the major constituent of CBO, accounts for approximately 30–95% of its composition (Biernasiuk et al., 2022). Previous studies have demonstrated that eugenol exhibits strong activity against *Candida albicans*, with an inhibition zone diameter of approximately 35.2 mm (Saracino et al., 2022). Eugenol exhibits antifungal activity by disrupting the membrane and interfering with ergosterol synthesis, leading to membrane damage and inhibition of cell growth (Ahaik et al., 2026). In addition, eugenol induces excessive reactive oxygen species (ROS) production, leading to oxidative stress, cellular leakage, and fungal cell death (Shahina and Dahms, 2024).

Clove is one of Indonesia's major export commodities and contributes substantially to the national economy through the

agricultural and cigarette industries. However, the majority of clove production is still utilized for tobacco products (Pratama et al., 2020). Although the tobacco industry generates considerable economic value, tobacco consumption is widely recognized as a major risk factor for premature morbidity and mortality (Abozahra et al., 2024; Benowitz and Liakoni, 2022; Coleman-Cowger et al., 2018; Holipah et al., 2020). Therefore, diversification of clove utilization into alternative value-added products is important to support economic sustainability while promoting public health (Iryono, 2017).

CBO has considerable potential as a natural antifungal agent and may be developed into pharmaceutical or cosmetic preparations, including anti-dandruff shampoos. Essential oils are commonly formulated into emulsion systems to improve their stability and efficacy (Sari et al., 2026). In addition to antifungal activity, shampoo formulations should provide desirable cosmetic properties such as softness, glossiness, and ease of hair management (Cornwell, 2018). Studies on the development and optimization of CBO shampoo formulations remain limited. Existing antifungal shampoos are predominantly formulated using synthetic active ingredients such as ketoconazole, climbazole, and piroctone olamine (Ergin et al., 2024; Mayser et al., 2026; Poojary et al., 2024). Previous studies on herbal anti-dandruff shampoos have mainly focused on antimicrobial activity and formulation stability (Al-Rimawi et al., 2025; Filatov et al., 2023). Investigations regarding the optimization of surfactant composition to achieve desirable physicochemical characteristics, washing performance, foam properties, and antifungal activity in clove oil–based shampoos are still scarce (Mawani et al., 2023).

Therefore, this study aimed to optimize the concentrations of cocamide DEA and SLS in CBO shampoo formulations. The optimum formulation was evaluated based on its physicochemical characteristics, washing performance, and antifungal activity. Although *Malassezia* spp. are recognized as the primary fungi associated with dandruff pathogenesis, *Candida albicans* was selected as a preliminary fungal model because it is widely used in antifungal susceptibility testing and has also been reported in scalp disorders and dandruff-related conditions (Jain et al., 2022; Kumari et al., 2022; Poojary et al., 2024; Sopyan et al., 2025; Umar et al., 2022). This study may support the diversification of clove-derived products while contributing to the development of plant-based antifungal shampoo formulations.

2. EXPERIMENTAL SECTION

2.1 Materials

CBO derived from *Syzygium aromaticum* was purchased from PT Syailendra Bumi Investama (Karanganyar-Gondangrejo, Central Java, Indonesia). Other materials included SLS, cocamide DEA, HPMC, methyl paraben, SDA, NaCl, 96% ethanol, and 70% ethanol of pharmaceutical grade (Merck). Candida albicans was obtained from the Bandar Lampung City Health Laboratory.

2.2 Methods

2.2.1 Clove Bud Quality Control Test

Prior to formulation, CBO underwent quality control evaluation, including organoleptic assessment, specific gravity determination, and ethanol solubility testing. Organoleptic evaluation of CBO was performed through visual and sensory observation, including color, appearance, and odor assessment (Syahadat and Diningsih, 2022).

For specific gravity analysis, an empty pycnometer was weighed before being filled with the oil sample. The pycnometer was maintained at 27.5°C for 15 min using a thermostat and then reweighed. Specific gravity was calculated according to Equation (1) (Syahadat and Diningsih, 2022).

$$\text{Specific gravity } (\rho) = \frac{m_2 - m}{m_1 - m} \times \rho_{\text{water}} \quad (1)$$

where:

m mass of the empty pycnometer
m_1 mass of pycnometer + distilled water
m_2 mass of pycnometer + sample

CBO solubility in ethanol was evaluated by measuring one milliliter of CBO and gradually mixing it with 70% ethanol until a clear solution was obtained. Additional ethanol was added to confirm the absence of precipitation. If turbidity occurred, it was compared with a reference turbidity solution prepared by adding 0.5 mL of 0.1 N silver nitrate solution to 50 mL of 0.0002 N sodium chloride solution followed by one drop of 25% nitric acid. The solution was observed after 5 min (Syahadat and Diningsih, 2022).

2.2.2 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

GC–MS analysis was performed to identify the chemical constituents of CBO according to a previous study with slight modifications (Hameed et al., 2021). The analysis was conducted using an Agilent Technologies 7890B Gas Chromatography system coupled with an Agilent 5977B Mass Selective Detector (MSD). Separation was carried out using a capillary column with a sample injection volume of 0.2 mL in splitless mode. Helium was used as the carrier gas under constant flow conditions. The oven temperature was initially maintained at 40°C for 1 min and then increased at a rate of 5°C/min to 290°C without a holding period, resulting in a total run time of 51 min. The mass spectrometer operated in electron impact ionization mode at 70 eV. The temperatures of the MSD transfer line, ion source, and quadrupole were maintained at 300°C, 230°C, and 150°C, respectively. Mass spectra were recorded in full-scan mode over an m/z range of 20–600 with a solvent delay of 5 min.

Peak integration was performed using the ChemStation Integrator (autoint1.e) system. Compound identification was conducted by comparing retention times and mass fragmentation patterns with the National Institute of Standards and

Technology (NIST20.L) library database. Only compounds with a match quality above 80% were considered positively identified.

2.2.3 Shampoo Formulation

All ingredients were weighed according to the formulation presented in Table 1 using an analytical balance. Shampoo preparation was carried out using the emulsification method consisting of aqueous and oil phases.

The aqueous phase consisted of SLS, HPMC, cocamide DEA, and methyl paraben, whereas the oil phase consisted of CBO. SLS was dissolved in 30 mL distilled water at 40°C under magnetic stirring at 300 rpm. Separately, HPMC was dispersed in 30 mL hot water and stirred at 400 rpm. Methyl paraben was dissolved in a small amount of ethanol before being added to the aqueous phase. Subsequently, the HPMC solution, methyl paraben, cocamide DEA, and CBO were combined using a hot plate magnetic stirrer at 60–70°C and 500 rpm. Distilled water was then added to volume, and the mixture was stirred until homogeneous.

2.2.4 Surfactant Optimization

The first optimization stage evaluated different concentrations of cocamide DEA (Table 1). The selected concentration range was based on previous studies demonstrating acceptable shampoo characteristics following cocamide DEA incorporation (Eryaputri et al., 2023). Optimization parameters included viscosity and foam height. The optimum formulation obtained from the first stage was used in the second optimization stage.

The second optimization evaluated different concentrations of SLS (Table 1). The optimum formulation was determined based on organoleptic properties, pH, homogeneity, viscosity, wetting power, detergency, and specific gravity. Selected formulations were subsequently evaluated for antifungal activity.

2.2.5 Physicochemical Evaluation

Organoleptic evaluation included assessment of color, appearance, and odor of the shampoo formulations. Homogeneity was examined by spreading 1 g of sample on a glass slide and visually observing the presence of coarse particles (Novaryatiin et al., 2024). For pH measurement, 1 g of shampoo was dispersed in 10 mL distilled and then analyzed by pH-meter (Paredes-Sulca et al., 2026).

Viscosity was measured using a Brookfield viscometer equip ped with spindle number 8 at 50 rpm (AlQuadeib et al., 2018). The specific gravity of the shampoo, in comparison to distilled water, was measured using a pycnometer at room temperature. The specific gravity test was measured using the Equation (1) (Syahadat and Diningsih, 2022).

2.2.6 Foam Height Test

A 0.1 g shampoo sample was dispersed in 10 mL distilled water and transferred into a test tube. The tube was shaken consistently for 20 s and allowed to stand for 5 min before foam height was measured (AlQuadeib et al., 2018).

2.2.7 Wetting Power and Washing Power Test

In the wetting power test, canvas cloth was cut into discs with a diameter of 3 cm and an approximate weight of 0.44 g. One gram of shampoo was dissolved in 100 mL of distilled water, after which the cloth disc was immersed in the solution. The time required for the cloth to sink completely was recorded using a stopwatch (AlQuadeib et al., 2018).

Washing power was evaluated using clean hair strands approximately 7 cm in length with a total weight of 5 g. The hair samples were exposed outdoors for four days until visibly dirty and subsequently immersed in 200 mL of shampoo solution containing 1 g of shampoo while being stirred for 4 min. After rinsing with water and drying using a hair dryer, the hair samples were reweighed, and the weight difference was used to determine the washing performance of the formulation.

2.2.8 TLC Test

TLC analysis was performed using silica gel F254 as the stationary phase and ethyl acetate:n-hexane (15:18) as the mobile phase (Hemalatha et al., 2016). The TLC chamber was saturated with the mobile phase prior to analysis. Samples were spotted 1 cm above the lower edge of the plate and developed until the solvent front reached the predetermined limit. The plate was dried, sprayed with reagent, and observed under UV light at 254 nm.

2.2.9 Antifungal Activity Assay

All equipment used for antifungal testing was sterilized prior to use. Glassware was sterilized in an oven at 170°C for 1 h, while media were sterilized using an autoclave at 121°C for 15 min (Jain et al., 2020).

Candida albicans was cultured on Sabouraud Dextrose Agar (SDA) slants and incubated at 37°C for 48 h. SDA medium was prepared by dissolving 9.75 g SDA powder in 250 mL distilled water, followed by sterilization at 121°C for 15 min (Gholampour-Azizi et al., 2015).

Fungal suspension preparation was performed by suspending *C. albicans* colonies in sterile NaCl solution. Turbidity was adjusted to match the McFarland standard corresponding to approximately 1×10^8 CFU/mL using UV–Vis spectrophotometry at 625 nm with an absorbance range of 0.08–0.13 (Kurniawansyah et al., 2021).

Antifungal activity was evaluated using the well diffusion method. Wells with a diameter of 5 mm were prepared in SDA plates inoculated with *C. albicans*. Each well received 0.2 g sample. Tested samples included distilled water, DMSO, Ketomed® shampoo, F8 and F9 formulations, F8 and F9 base formulations, and 4% CBO. Plates were incubated at 37°C for 48 h before inhibition zone diameters were measured using a caliper (Gholampour-Azizi et al., 2015).

2.2.10 Short-Term Stability and Centrifugation Test

Emulsion stability was evaluated using short-term storage and centrifugation tests. The formulations were stored at room

Table 1. The Optimization of Shampoo Preparations with Varying Concentrations of Surfactants

Optimization	Formula	Concentration (%)					
		CBO	SLS	Cocamide DEA	Methyl paraben	HPMC	Distilled Water
1	1	4	10	4	0.1	0.5	ad 100
	2	4	10	6	0.1	0.5	ad 100
	3	4	10	8	0.1	0.5	ad 100
	4	4	10	10	0.1	0.5	ad 100
	5	4	10	12	0.1	0.5	ad 100
2	6	4	2	*	0.1	0.5	ad 100
	7	4	4	*	0.1	0.5	ad 100
	8	4	6	*	0.1	0.5	ad 100
	9	4	8	*	0.1	0.5	ad 100
	10	4	10	*	0.1	0.5	ad 100

(*): Concentration based on optimization 1

temperature for 30 days (Zhou et al., 2025). For centrifugation testing, samples were centrifuged at 5000 rpm for 5 min (Špaglová et al., 2025). Organoleptic properties including color, odor, texture, and phase separation were evaluated before and after testing. Stability was determined based on the absence of visible changes in these parameters.

2.2.11 Ethical Consideration

This study was conducted entirely through *in vitro* experimental procedures and did not involve human participants, human biological samples, or experimental animals. Therefore, ethical approval and informed consent were not required. Nevertheless, further safety evaluation and clinical studies are necessary prior to potential human application of the developed shampoo formulation.

2.2.12 Data Analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA followed by Tukey's post-hoc test to determine significant differences among groups, with $p < 0.05$ considered statistically significant. Different letters in the figures indicate significant differences between groups (Rosdianto et al., 2023).

3. RESULTS AND DISCUSSION

3.1 CBO Quality Control

The results of CBO quality control are presented in Table 1. The oil showed a yellow color with a characteristic clove odor. The specific gravity of the oil was 1.04 ± 0.002 g/mL, which was within the acceptable range of 1.04–1.07 g/mL, and the oil exhibited clear solubility in ethanol (1:2), indicating suitable quality for formulation purposes (Syahadat and Diningsih, 2022).

3.2 Chemical Composition and Purity of CBO

The chemical composition of CBO used in the shampoo formulation was characterized by GC–MS analysis (Table 3). The total ion chromatogram (TIC) revealed a chemical profile dominated by several major phytoconstituents, particularly eugenol and β-caryophyllene. Based on the integration of the peak areas, eugenol was identified as the primary constituent, accounting for 76.88% of the total volatile profile. The second major compound was identified as beta-caryophyllene with an area percentage of 16.18%. A total of 33 different components were identified using GC-MS analysis. Monoterpenoids, sesquiterpenoids, and phenylpropanoids make up the majority of clove essential oils, with small amounts of alcohols, aldehydes, and ketones (Hameed et al., 2021).

The prominent percentage of eugenol indicates that the chemical composition of the CBO is consistent with previous reports describing high-quality clove bud oil intended for pharmaceutical and cosmetic applications (Campelo et al., 2021; Hameed et al., 2021; Santos et al., 2020). The high abundance of this bioactive compound suggests that the oil possesses considerable potential for incorporation into topical antifungal formulations. Structural identification of the major constituents was validated using the NIST20.L library match program, and no major unexpected components were detected.

3.3 Shampoo Formulation

Following characterization of the active ingredient, the shampoo formulation was prepared using the emulsification method to ensure proper dispersion of CBO within the aqueous phase. HPMC functioned as a viscosity enhancer and stabilizing agent, while SLS and cocamide DEA acted as surfactants contributing to cleansing ability and foam formation (Kalinowska-Lis and Mucha, 2026; Sachdev et al., 2023; Zhou et al., 2020). Heating during emulsification facilitated ingredient mixing and reduced interfacial tension between the oil and water phases (Perazzo et al., 2015). However, CBO contains volatile com-

Table 2. CBO Quality Control Results

Control Test	Results	Requirements	Remarks
Organoleptic	Yellow, typical smell of CBO	Dark yellow-brown, typical of CBO	meet the requirement
Specific Gravity	1.04 ± 0.002 g/mL	1.04–1.07 g/mL	
Solubility in ethanol	1:2 clear	1:2 clear	
RF by TLC	0.48	0.48	

Table 3. Main Volatile Constituents of Clove Essential Oil by GC-MS

Constituents	Retention Time (min)	% Area
Eugenol	20.387	76.88
Caryophyllene	22.064	16.18
Humulene	22.853	1.84
Caryophyllene oxide	25.818	0.61

pounds that may undergo evaporation or partial degradation during heating. Previous studies reported that clove oil exhibits relatively high volatility and lower thermal stability because of the presence of eugenol and related compounds (Gamayel et al., 2022). Nevertheless, controlled heating conditions are commonly applied during clove oil extraction and topical formulation processes, including hydrodistillation and emulgel preparation at temperatures of 60–70°C, while still maintaining physicochemical characteristics and biological activity (Bennabi et al., 2025; Krishnamoorthy et al., 2022). Accordingly, the emulsification process in this study was conducted under controlled temperature and limited heating duration to minimize excessive volatilization during shampoo preparation.

To obtain an optimum shampoo formulation, surfactant composition was optimized in two stages involving cocamide DEA and SLS concentrations. Regarding formulation characterization, critical micelle concentration (CMC) analysis of the surfactant system was beyond the scope of this study. Nevertheless, the surfactants used, namely SLS and cocamide DEA, are conventional surfactants with well-established micellization behavior reported in the literature (Bejczi and Nagy, 2025; Nivón-Ramírez et al., 2022; Perinelli et al., 2020), and the concentrations used in the formulation were substantially higher than their reported CMC values.

3.4 Shampoo Optimization Using Different Cocamide DEA Concentration

The physicochemical evaluation results of formulations F1–F5 are presented in Figures 1 and 2A, and the corresponding numerical data are summarized in Table 4. The foam height of formulations F1–F5 ranged from 7.06 to 14.73 cm and fulfilled the required foam height range for shampoo preparations (Atmanto and Ambarwati, 2023). Statistical analysis showed that increasing cocamide DEA concentration significantly affected both foam height and viscosity ($p < 0.05$). As shown in Figure 1A, higher concentrations of cocamide DEA progressively increased foam formation due to its well-established function as a foam booster and viscosity enhancer (Rieger, 2017). This effect was particularly evident in formulations F4 and F5, which exhibited the highest foam heights among the tested formulations (Figure 1A).

Table 4. Results Foam Height and Viscosity Test of Shampoo Formulations with Different Cocamide DEA Concentration

Formula	Average ± SD	
	Foam Height (cm)	Viscosity (cPs)
F1	7.16 ± 0.15	914 ± 74.22
F2	8.53 ± 0.35	2486 ± 4.73
F3	10.43 ± 0.21	3915 ± 4.04
F4	12.60 ± 0.20	5387 ± 45.62
F5	14.73 ± 0.25	6425 ± 1.53

All formulations exhibited a clear yellow appearance, semi-solid consistency, and characteristic clove odor (Figure 2A). The transparent appearance of formulations containing higher surfactant concentrations (Figure 2A, F5) may be attributed to reduced interfacial tension and improved dispersion of oil droplets within the emulsion system (Ravera et al., 2021). Similar findings have been reported in previous clove oil emulsion studies involving oil-in-water systems (Gul et al., 2022; Singh et al., 2023). Appropriate mixing temperature and stirring conditions also contributed to formulation homogeneity by preventing early solidification and improving ingredient distribution (Abed et al., 2019; Alharbi and Abdulhamid, 2023; Tadros, 2018).

Foam generation is an important quality attribute in shampoo formulations because consumers often associate abundant and stable foam with effective cleansing and overall product quality (Mehta and Paul Choudhury, 2025; Zhou et al., 2020), although foam volume does not necessarily correlate with actual cleansing performance. In addition to enhancing foam formation, increasing cocamide DEA concentration also increased formulation viscosity. While formulations F1–F3 remained within the acceptable viscosity range for shampoo preparations, F4 and F5 exceeded the recommended limit. Excessive viscosity may negatively affect product handling, spreading, and pourability during use. Therefore, formulation F3 containing 8% cocamide DEA was selected as the optimum formulation because it provided a favorable balance between foam performance and viscosity while remaining within the acceptable quality specifications.

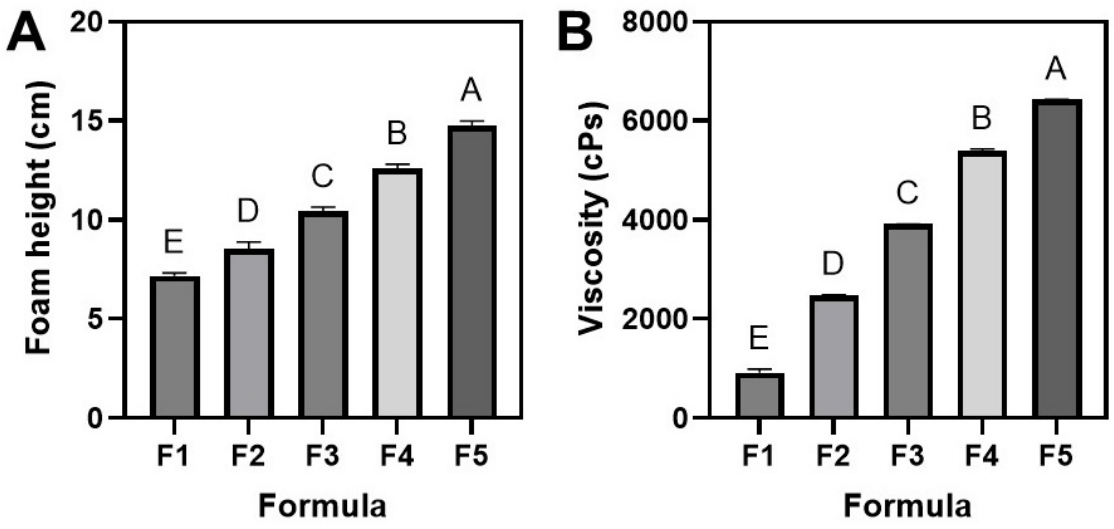

Figure 1. Results of the Foam Height Test (A) and Viscosity Test (B) of CBO Shampoo Formulations with Varying Cocamide DEA Concentrations, Analyzed Using One-Way ANOVA Followed by Tukey's Post-Hoc Test. Significance is Shown in Different Letters (p <0.05)

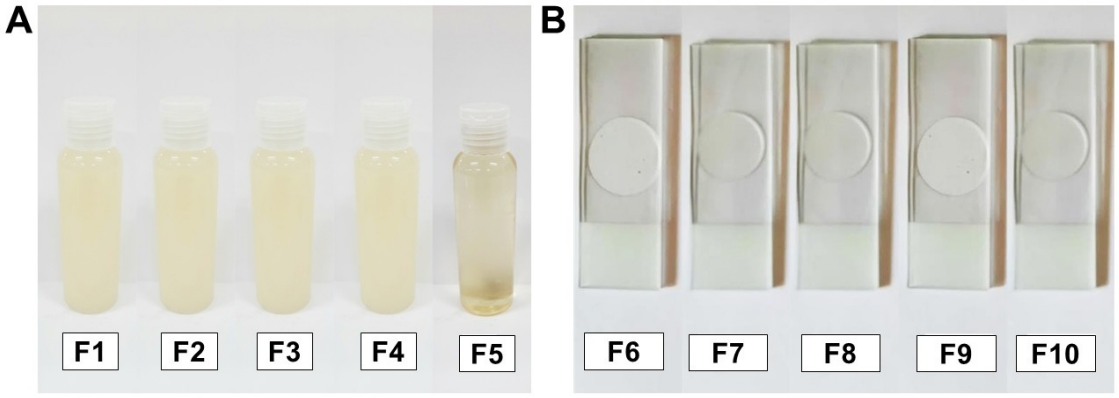

Figure 2. Results of the Foam Height Test (A) and Viscosity Test (B) of CBO Shampoo Formulations with Varying Cocamide DEA Concentrations, Analyzed Using One-Way ANOVA Followed by Tukey's Post-Hoc Test. Significance is Shown in Different Letters (p <0.05)

3.5 Shampoo Optimization Using Different SLS Concentration

After determining the optimum cocamide DEA concentration, a second optimization was performed by varying the SLS concentration. The physicochemical evaluation results of formulations F6–F10 are presented in Figures 2B and 3, while the complete numerical data are summarized in Table 5. Homogeneity testing confirmed that all formulations were homogeneous and free from coarse particles or phase separation (Figure 2B).

The pH values of all formulations ranged from 5.72 to 7.08, which fell within the acceptable range for shampoo preparations (Paredes-Sulca et al., 2026). Increasing SLS concentration significantly increased pH, viscosity, and specific gravity values ($p < 0.05$) (Figures 3A–C). Similarly, wetting and washing power evaluations demonstrated that higher SLS concentrations improved cleansing performance, as evidenced by shorter wetting times and greater washing power percentages (Figures 3D and 3E). Among the tested formulations, F10 exhibited the fastest wetting time and the highest washing power.

In addition to organoleptic properties, pH, viscosity, and specific gravity are important parameters affecting shampoo quality and user acceptability. The pH values obtained in all formulations indicate suitability for scalp application and are expected to minimize irritation while maintaining scalp physiological balance (George and Potlapati, 2021). The increase in pH observed with higher SLS concentrations may be related to the alkaline nature of the surfactant. Likewise, the significant increases in viscosity and specific gravity may be attributed to changes in micelle structure and intermolecular interactions

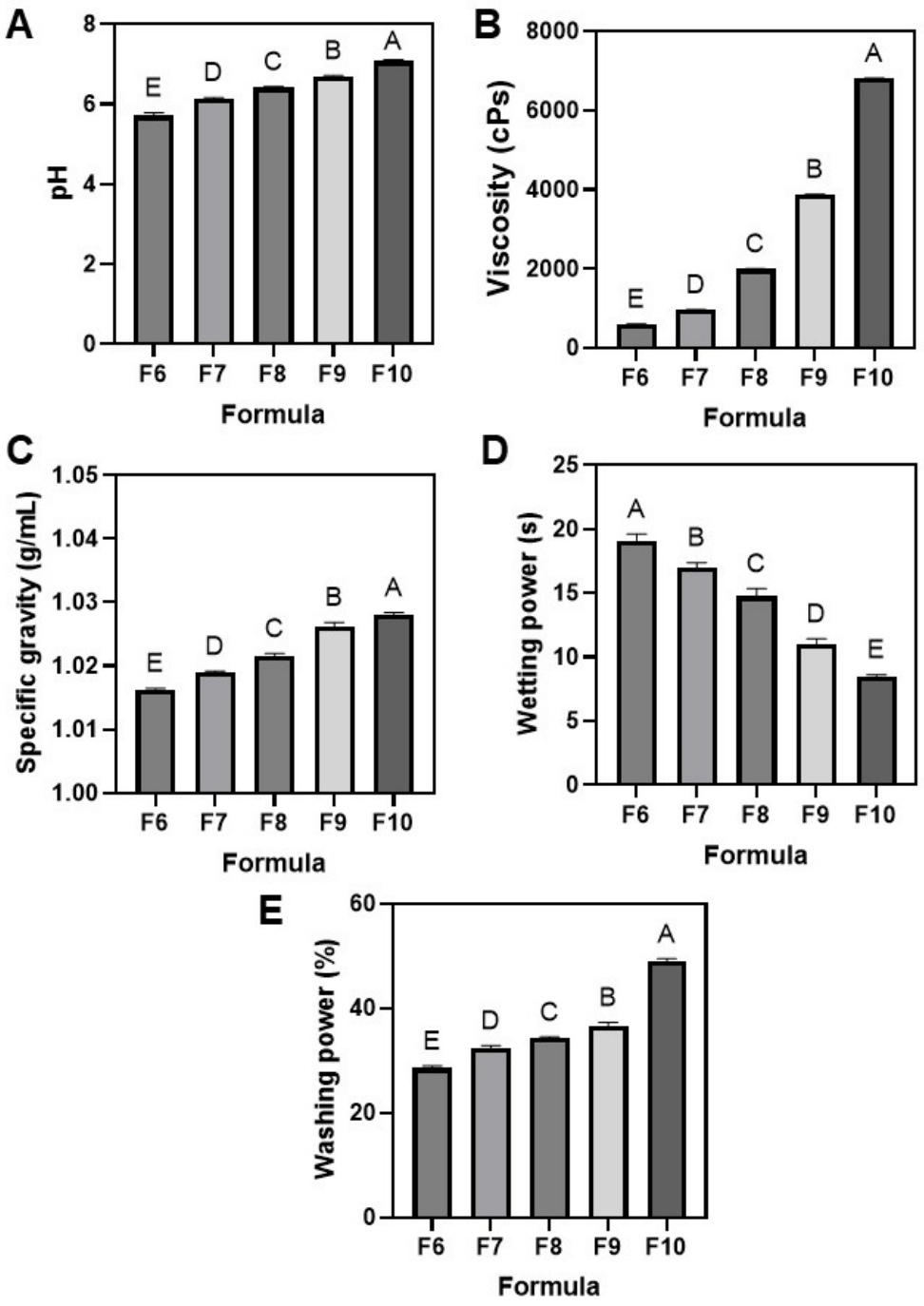

Figure 3. The Results of pH (A), Viscosity (B), Specific Gravity (C), Wetting Power (D), and Washing Power (E), of CBO Shampoo Formulation with the Variation of Cocamide DEA Analyzed with ANOVA Statistical Analysis and Post-Hoc Tukey (p <0.05). Significance is Shown in Different Letters (p <0.05). SLS = Sodium Lauryl Sulfate

among surfactant molecules, resulting in greater resistance to flow and increased formulation density (Tadros, 2018).

The improvements in wetting and washing performance can be explained by the role of SLS as an anionic surfactant with strong detergency and foam-forming properties (Zhou et al., 2020). Higher surfactant concentrations promote micelle formation, thereby enhancing the removal of dirt and oil from the hair surface and improving cleansing efficiency (Tadros, 2018). However, increasing SLS concentration also resulted in higher viscosity and pH values, which may reduce formulation

Table 5. Results of Physicochemical Characterization and Performance Testing of Shampoo Formulations with Different SLS Concentrations

	Average ± SD				
Formula	pH	Viscosity (cPs)	Specific Gravity (g/mL)	Wetting Power (s)	Washing Power (%)
F6	5.72 ± 0.07	606 ± 2.00	1.0162 ± 0.00	19.11 ± 0.51	28.57 ± 0.46
F7	6.13 ± 0.04	966 ± 2.00	1.0190 ± 0.00	16.98 ± 0.41	32.46 ± 0.42
F8	6.23 ± 0.25	1995 ± 6.43	1.0214 ± 0.00	14.76 ± 0.58	34.28 ± 0.24
F9	6.70 ± 0.02	3885 ± 4.93	1.0262 ± 0.00	10.98 ± 0.45	36.70 ± 0.61
F10	7.08 ± 0.03	6816 ± 8.00	1.0281 ± 0.00	8.48 ± 0.13	48.95 ± 0.51

acceptability and potentially increase the risk of scalp irritation. Consequently, although F10 demonstrated the highest cleansing performance, F9 provided a more favorable balance between physicochemical characteristics, washing performance, and formulation suitability. Therefore, F9 containing 8% SLS was selected as the optimum formulation because it provided the most favorable balance between physicochemical characteristics, cleansing performance, and formulation suitability while avoiding the excessive viscosity and higher pH observed in F10.

3.6 TLC Profile

The TLC chromatographic profiles of CBO, F8, and F9 are presented in Figure 4A. The RF values of CBO, F8, and F9 were 0.477, 0.482, and 0.485, respectively, which are consistent with previous reports for compounds commonly found in CBO, including eugenol (Hemalatha et al., 2016). The similarity of the chromatographic profiles between CBO and the shampoo formulations suggests that major constituents of the oil remained detectable after formulation.

However, qualitative TLC analysis alone is insufficient for definitive identification and quantification of CBO constituents. Therefore, the major compounds detected in the oil were further confirmed by GC–MS analysis. Future studies may further strengthen analytical characterization through densitometric or HPTLC analysis, particularly for quantitative determination of eugenol and more comprehensive evaluation of CBO stability after formulation.

3.7 Antifungal Activity

Following physicochemical characterization of the optimized formulations, antifungal activity was evaluated against *C. albicans*. The antifungal activity results are presented in Figures 4B and 4C. Distilled water and DMSO did not produce inhibition zones against *C. albicans* (Figures 4C-1 and 4C-2), confirming the absence of antifungal activity from the solvents used. Base formulations F8 and F9 produced inhibition zones of 9.25 ± 0.90 mm and 11.42 ± 3.82 mm, respectively, whereas CBO alone exhibited a substantially larger inhibition zone of 29.79 ± 2.23 mm (Figure 4B; Figures 4C-4 to 6).

The optimized shampoo formulations F8 and F9 produced inhibition zones of 15.78 ± 3.48 mm and 24.06 ± 3.01 mm, respectively. Statistical analysis demonstrated that F9 exhibited significantly greater antifungal activity than F8 ($p < 0.05$) (Figure 4B). This finding was visually supported by the larger clear zone observed for F9 (Figure 4C-8) compared with F8 (Figure 4C-7). Nevertheless, both formulations produced lower inhibition zones than the positive control ketoconazole shampoo (32.50 ± 1.44 mm; Figure 4C-3). Based on the overall evaluation, F9 was selected as the optimum formulation because it demonstrated significantly greater antifungal activity than F8 while maintaining favorable physicochemical characteristics and washing performance. Thus, formulation selection was based on a balanced assessment of physicochemical quality, cleansing efficacy, and biological activity rather than on a single parameter.

The substantial antifungal activity observed for CBO may be associated with its chemical composition. GC–MS analysis confirmed eugenol (76.88%) and β-caryophyllene (16.18%) as the major constituents of the oil. Eugenol is widely recognized as the principal bioactive compound responsible for the antifungal activity of clove oil (Ahaik et al., 2026; Saracino et al., 2022; Shahina and Dahms, 2024), while β-caryophyllene has also been reported to possess antimicrobial and antifungal properties. Previous studies have demonstrated that eugenol exerts antifungal effects by disrupting fungal cell membrane integrity, interfering with ergosterol biosynthesis, and inducing oxidative stress, ultimately leading to fungal cell death (Ahaik et al., 2026; Shahina and Dahms, 2024). Therefore, the antifungal activity observed in the present study may be attributed to the combined contribution of these major constituents, particularly the high eugenol content.

The greater antifungal activity of F9 compared with F8 suggests that increasing SLS concentration may have enhanced antifungal efficacy. This effect may be attributed to the ability of surfactants to alter fungal membrane permeability, facilitating leakage of intracellular components and promoting fungal cell death (Sinko, 2023). However, despite the improved activity of F9, both formulations remained less effective than the commercial ketoconazole shampoo under the tested conditions.

The lower inhibition zones observed in the shampoo formulations compared with pure CBO may also be influenced by the formulation matrix. Entrapment of active compounds within the shampoo base can reduce diffusion of the active constituents into the agar medium, resulting in smaller inhibition zones (Cunha et al., 2022). In contrast, pure CBO can

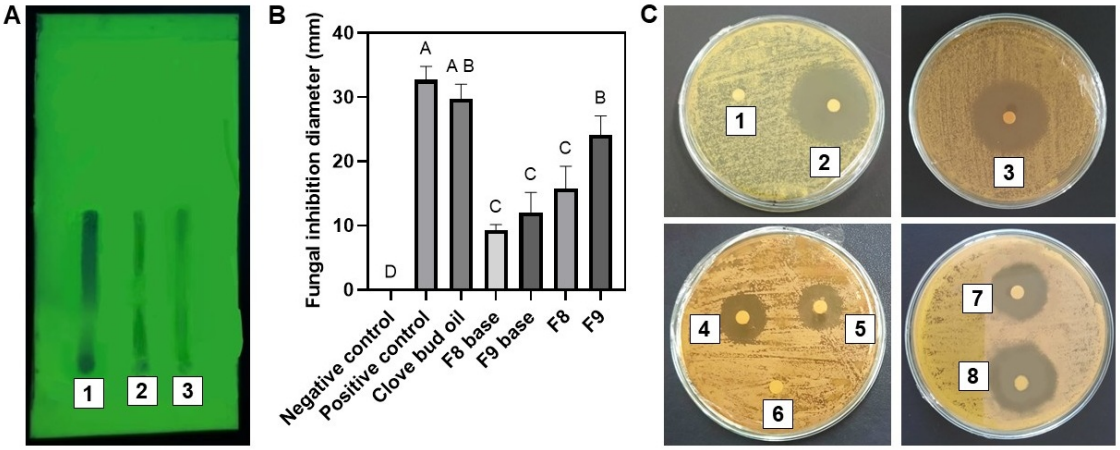

Figure 4. (A) TLC Chromatographic Profile of (1) CBO (2) Formula F8, and (3) Formula F9. (B) Inhibition Zone Diameter Against Candida albicans. (C) Representative Images of Antifungal Activity: (1) DMSO, (2) CBO, (3) Positive Control, (4) F8 Base (Blank Shampoo Formulation without CBO), (5) F9 Base (Blank Shampoo Formulation without CBO), (6) Distilled Water, (7) F8, and (8) F9. All Petri Dishes Had a Diameter of 90 mm and All Wells Had a Diameter of 5 mm. Data are Presented as Mean ± SD (n = 3). Different Letters Above the Bars Indicate Statistically Significant Differences Between Groups According to Tukey's Post-Hoc Test (p <0.05)

diffuse more freely through the medium and therefore produce stronger antifungal activity. Furthermore, methyl paraben present in the shampoo base may have contributed to the antifungal activity observed in the base formulations because parabens are known to possess antimicrobial and antifungal properties (Poddębniak and Kalinowska-Lis, 2024).

Although *Malassezia* spp. are widely recognized as the primary fungi associated with dandruff pathogenesis (Galizia et al., 2024; Locker et al., 2025), *C. albicans* was selected as a preliminary fungal model because of its reproducible growth characteristics and widespread use in antifungal susceptibility testing. Recent advances in scalp microbiome research suggest that dandruff development involves complex alterations in microbial communities and interactions among scalp microorganisms (?Tao et al., 2021). Several studies have reported an increased relative abundance of *Candida* species in patients with seborrheic dermatitis and dandruff-related conditions (Soares et al., 2016), whereas other investigations identified *Malassezia* spp. as the predominant fungi with minimal involvement of *Candida* (Tao et al., 2022; Yu et al., 2025). These inconsistent findings indicate that dandruff pathogenesis is multifactorial and may vary according to individual scalp microbiota composition, geographical factors, sampling sites, and analytical methods. Therefore, the present findings should be interpreted as preliminary evidence of antifungal potential, and further studies involving *Malassezia* species are necessary to provide a more clinically relevant assessment of the anti-dandruff efficacy of the developed formulation.

Compared with previous studies, anti-dandruff shampoo research has predominantly focused on synthetic antifungal agents such as ketoconazole, climbazole, and piroctone olamine (Ergin et al., 2024; Poojary et al., 2024). Studies involving natural-based anti-dandruff formulations have mainly emphasized antimicrobial activity and stability evaluation (Al-Rimawi et al., 2025; Filatov et al., 2023), whereas investigations concerning surfactant optimization in CBO-based shampoo formulations remain limited. In addition, previous optimization studies have primarily evaluated biosurfactants or combinations of synthetic antifungal agents rather than CBO as the principal active ingredient (Mawani et al., 2023). Therefore, the present study provides novel information regarding the development and surfactant optimization of CBO-based anti-dandruff shampoo formulations and their influence on physicochemical characteristics, washing performance, and antifungal activity.

Future studies should include *Malassezia* species, incorporate MIC and MFC determinations, and perform *in vivo* safety and efficacy evaluations to provide a more clinically relevant assessment of the developed shampoo formulation.

3.8 Short-Term Stability and Centrifugation Test

Observations showed that all formulations maintained consistent color, aroma, and texture without evidence of phase separation after storage and centrifugation tests. Physical instability in emulsions is generally characterized by creaming, flocculation, coalescence, caking, viscosity changes, or phase separation (Yang et al., 2026). However, none of these phenomena were observed, indicating good physical stability of the formulations. The absence of phase separation after centrifugation may be attributed to strong interfacial interactions between the oil and water phases, which contributed to maintaining emulsion stability during accelerated stress conditions (Zhou et al., 2025). Although the formulations demonstrated acceptable physical stability during short-term storage and centrifugation testing,

future studies incorporating droplet size distribution analysis may provide additional insight into emulsion homogeneity and long-term stability.

4. CONCLUSION

In conclusion, the present study successfully optimized a clove bud oil shampoo formulation containing SLS and cocamide DEA as the surfactant system. Among the tested formulations, F9, consisting of 8% SLS and 8% cocamide DEA, demonstrated the most favorable physicochemical characteristics, including acceptable pH, viscosity, homogeneity, foamability, washing performance, and physical stability. The developed shampoo formulation also exhibited promising antifungal activity against *C. albicans*, indicating its potential application as a natural-based anti-dandruff shampoo. Overall, the findings support the potential utilization of CBO as a value-added natural ingredient in topical cosmetic formulations. Further studies involving long-term stability evaluation, quantitative phytochemical standardization, DOE-based formulation optimization, MIC/MFC determination, broader antifungal spectrum analysis, and *in vivo* assessments could provide deeper insight into the scientific basis, safety profile, and therapeutic potential of the developed CBO shampoo formulation for future topical applications.

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