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Formulation of facial liquid soap wıth 4-(Dimethylamino) chalcone and virgin coconut oil as antibacterial agents against acne-causing bacteria

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ABSTRACT: Acne vulgaris is a multifactorial skin disorder commonly associated with infections caused by *Staphylococcus epidermidis* and *Cutibacterium acnes*. This study aimed to develop and evaluate an antibacterial facial liquid soap containing 4-(Dimethylamino) chalcone and virgin coconut oil (VCO). The synthesized chalcone, a flavonoid derivative obtained through the Claisen–Schmidt condensation reaction, was characterized using TLC, melting point analysis, UV-Vis spectrophotometry, and LC-MS/MS. Formulations containing 1.25% and 2.5% chalcone combined with 40% VCO were prepared and tested. Antibacterial activity was evaluated using the agar diffusion method, while physical properties were assessed through organoleptic observation, homogeneity, foaming ability, pH measurement, stability testing, and skin irritation tests. The 1.25% chalcone formulation demonstrated strong antibacterial activity, producing inhibition zones of 19.40±0.39 mm against *C. acnes* and 18.97±0.45 mm against *S. epidermidis*. All formulations were stable, homogeneous, and non-irritating. These findings indicate a synergistic antibacterial effect between chalcone and VCO, supporting their potential use as natural active ingredients in anti-acne facial soap formulations.

KEYWORDS: 4-(Dimethylamino) chalcone; acne; antibacterial; facial liquid soap; virgin coconut oil.

INTRODUCTION

Cosmetic products play a vital role in daily life for both women and men, serving not only to maintain skin health but also to enhance physical appearance[1]. The growing availability of cosmetic products has provided consumers with more options to improve personal hygiene and aesthetic appeal. Among these products, soap is one of the most widely used for skin cleansing and care, helping to remove impurities, excess oil, and microorganisms that may cause dermatological problems [1].

The skin, as the body's outermost layer, serves as a flexible yet protective barrier against environmental aggressors, including bacteria, viruses, and other pathogens. Acne vulgaris is a common inflammatory skin condition resulting from clogged pores, leading to pustule formation and inflammation. It affects more than 80% of individuals aged 12–44 years and is particularly prevalent during puberty due to increased androgen production, which stimulates excessive sebum secretion [2]. Chronic inflammation of the pilosebaceous unit and bacterial colonization, particularly by *Staphylococcus epidermidis* and *Cutibacterium acnes*, are key factors in acne development [3]. This condition can occur across all age groups and significantly impacts self-confidence and psychological well-being.

Facial cleansers, including soaps, are essential in acne management as they help remove dirt, sebum, and bacteria from the skin surface. Soaps are produced through saponification reactions involving triglycerides and alkaline agents [4]. Virgin Coconut Oil (VCO) is a natural triglyceride commonly used in soap manufacturing due to its superior properties compared to regular coconut oil, including lower free fatty acid and water content, longer shelf life, and additional cosmetic benefits. VCO is recognized for its antimicrobial, antioxidant, anti-inflammatory, and moisturizing properties, making it a valuable ingredient in pharmaceutical and cosmetic formulations, particularly in acne treatment [5].

Chalcones, secondary metabolites belonging to the flavonoid family, have garnered significant attention due to their wide range of biological activities, including antibacterial, anti-inflammatory, and antioxidant

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effects. *4-(Dimethylamino)* chalcone can be synthesized via Claisen–Schmidt condensation, an aldol cross-condensation reaction between benzaldehydes and ketones under basic or acidic catalysis [6]. Previous studies have demonstrated that chalcone derivatives exhibit inhibitory effects against acne-related bacteria such as *S. aureus*, *S. epidermidis*, and *Bacillus* spp., with varying inhibition zones and antibacterial efficacy [7].

Liquid facial cleansers have become a preferred formulation compared to solid soaps due to their ease of use, faster reaction on the skin surface, and superior cleansing efficiency. However, many commercial facial soap products still contain synthetic chemicals, such as surfactants and antibacterial agents, which may cause irritation, dryness, or disrupt the natural balance of the skin microbiota. This condition indicates the need for developing facial soap formulations that are not only effective in inhibiting acne-causing bacteria but also safe and capable of maintaining the skin's natural moisture. Anti-acne facial cleansers formulated with natural bioactive compounds offer a promising solution, as these compounds can inhibit bacterial growth while maintaining the skin's protective and moisturizing functions. Therefore, this study was conducted to address this research gap by developing a liquid facial soap formulation that combines 4-(Dimethylamino) chalcone, synthesized through the Claisen–Schmidt condensation reaction, with Virgin Coconut Oil (VCO) as a natural moisturizing and antibacterial ingredient [8].

Commercial liquid facial cleansers often contain synthetic surfactants and antimicrobial agents that may cause irritation, dryness, and disruption of the skin's natural microbiota. This limitation underscores the need for safer formulations that maintain skin moisture while effectively targeting acne-causing bacteria. 4-(Dimethylamino) chalcone, synthesized via the Claisen–Schmidt condensation reaction, has demonstrated promising antibacterial activity. Meanwhile, Virgin Coconut Oil (VCO) is recognized for its natural moisturizing and antimicrobial properties. Combining these two ingredients offers a potential strategy to develop a liquid facial cleanser that provides effective antibacterial protection while preserving skin hydration.

MATERIALS AND METHODS

Materials

4-Dimethylaminobenzaldehyde, acetophenone, sodium hydroxide (NaOH), 96% ethanol, distilled water (aquadest), virgin coconut oil, chalcone compound, potassium hydroxide (KOH), glycerin, propylene glycol, cocamide DEA, butylated hydroxytoluene (BHT), and citric acid.

The equipment used in this study included an analytical balance, glassware (Iwaki, Japan), micropipette (Fraser, UK), digital balance, parchment paper, spatula, dropper pipette, chromatography chamber, chromatography column, silica gel GF254 plate (Merck, Germany), ultrasound processor, rotary vacuum evaporator, magnetic stirrer, pycnometer, test tubes, Petri dishes, incubator (36 ± 1 °C), vernier caliper, oven, crucible, ground-glass Erlenmeyer flask, and digital thermometer.

Synthesis of 4-(dimethylamino) chalcone

The synthesis of 4-(dimethylamino) chalcone was carried out using 50% sodium hydroxide (NaOH) as a base catalyst. A separate beaker was prepared containing 1169 µL of acetophenone and 6 mL of 96% ethanol, which was then subjected to ultrasonic bath treatment at 40 kHz for 10, 20, and 30 minutes at 40 °C [9]. The reaction mixture was cooled in an ice bath for 2 hours, followed by the addition of hydrochloric acid (HCl) and allowed to stand at room temperature for 1 hour. The resulting crystalline precipitate was washed with distilled water until neutral pH was achieved, filtered, and dried in an oven at 40°C for 3 hours. The dried powder was used for subsequent analysis.

Characterization of synthesized chalcone

Organoleptic and melting point analysis

Physical properties (color, odor, texture) were observed, and the melting point was determined using a melting point apparatus [10].

Thin-layer chromatography (TLC)

Approximately 5 mg of the synthesized compound 4-(dimethylamino)chalcone was accurately weighed and dissolved in 2 mL of 98% ethanol until homogeneous. The solution was then analyzed using Thin-Layer Chromatography (TLC) by spotting 5 µL onto a silica gel $_{GF254}$ plate, at a distance of 0.7 cm from the bottom

edge, with a migration distance of 10 cm. The plate was then placed in a chamber and eluted using a mobile phase of ethyl acetate and n-hexane (1:5) until the solvent front reached the limit. The spots were observed and marked under UV light at 254 nm and 366 nm [10].

UV-Vis Spectroscopy

The compound was dissolved in methanol, diluted to 10 mL, and analyzed in the 200–800 nm wavelength range to determine λmax [10].

LC-MS

The synthesized compound was dissolved in methanol and 5 µL of the solution was injected into the sample port of the LC-MS/MS instrument for separation and analysis. The analysis was performed using liquid chromatography–mass spectrometry (LC-MS/MS) with a mobile phase consisting of water containing 5 mM ammonium formate (A) and acetonitrile containing 0.05% formic acid (B) [11].

Formulation of liquid facial soap

Facial soap was prepared using a controlled heating and stirring method. VCO was saponified with KOH at 75 °C to form the soap base. Chalcone was dissolved in glycerin, mixed with propylene glycol and water, and incorporated into the base along with cocamide-DEA and preservatives. The mixture was homogenized using a magnetic stirrer at 200 rpm and adjusted to neutral pH with citric acid. Details of the formulations can be found in Table 1. The prepared liquid soap formulations were evaluated for their physicochemical properties to ensure product quality and stability [12].

Table 1. Liquid facial soap formulation.

No.	Ingredients	Function	Ingredient composition (%)		
			F0	F1	F2
1	4-(Dimethylamino) chalcone	Active compound	-	1.25	2.5
2	Virgin coconut oil (VCO)	Active compound	40	40	40
3	Potassium hydroxide 10%	Saponifying agent	4.5	4.5	4.5
4	Glycerin	Humectant	3.41	3.41	3.41
5	Propylene glycol	Solvent	7.5	7.5	7.5
6	Cocamide-DEA	Surfactant & Emulsifier	1.82	1.82	1.82
7	Butylated hydroxytoluene	Antioxidant	0.02	0.02	0.02
8	Citric acid	pH Neutralizer	0.15	0.15	0.15
9	Phenoxyethanol	Presservative	0.5	0.5	0.5
10	Purified water	Solvent	Ad 100	Ad 100	Ad 100

Evaluation of soap formulation

Organoleptic test

The formulations were visually inspected for changes in appearance, color, and odor. Observations were recorded weekly over a period of 8 weeks to monitor potential physical changes [13].

Homogeneity tests

A small amount of the soap was spread onto a clean glass plate and gently rubbed with a finger. The formulation was considered homogeneous if no coarse particles or solid residues were detected. This evaluation was performed throughout the 8-week study period [13].

Foam height

Foam height was measured by preparing a 1% soap solution in 10 mL of distilled water, which was then transferred into a 100 mL graduated cylinder. The solution was shaken, and the foam height was measured immediately. After 5 minutes, the foam height was measured again [13].

Viscosity measurement

Viscosity was determined using a Brookfield viscometer operated at 30 rpm. Samples were placed into a designated container, and readings were recorded in centipoise (cP) [3].

Density test

A clean, dried pycnometer was weighed, filled with distilled water at 25°C, equilibrated for 10 minutes, and weighed again. The procedure was repeated with the liquid soap formulation. Specific gravity was calculated by comparing the weights of the soap and water [14].

Surface tension measurement

Surface tension was evaluated using a tensiometer equipped with a platinum ring. The ring was immersed into the sample and gradually lifted until detached from the liquid surface. The force required for detachment was measured and reported in dyne/cm [15].

pH measurement

The pH meter was calibrated using standard buffer solutions of pH 4 and 7. One gram of the soap sample was diluted in 10 mL of distilled water, and the electrode was immersed in the solution to record the pH value [3].

Stability testing

The stability of the liquid soap formulations was evaluated using a cycling test method. Samples were alternately stored at refrigeration temperature (4±2 °C) for 24 hours and in a hot oven (40±2 °C) for 24 hours to simulate temperature fluctuations during storage and transportation. A total of six cycles were performed over 12 days. After each cycle, the physical appearance of the formulations including color, odor, phase separation, and consistency was visually inspected and recorded to determine their stability under varying temperature conditions [16].

Skin irritation test

Conducted on rabbits according to BPOM No. 10/2022 guidelines, observing erythema and edema over 5 days. Primary irritation index was calculated [17].

Preparation of antibacterial assay

Glassware was sterilized in an autoclave (121 °C, 15 min), while metallic tools were sterilized by flaming after ethanol immersion. Nutrient agar medium was prepared by suspending 5 g of nutrient agar in 250 mL of distilled water, heated, stirred, and autoclaved under the same conditions. Agar plates were poured and allowed to solidify. Bacterial strains (*C. acnes* and *S. epidermidis*) were cultured on agar plates and incubated at 37 °C for 24 hours. A 0.5 McFarland standard (prepared from $BaCl_2$ and H_2SO_4 solutions) was used to adjust bacterial turbidity [5].

Antibacterial activity assay

The antibacterial activity of the formulated liquid soaps was evaluated using the agar well diffusion method against *Cutibacterium acnes* and *Staphylococcus epidermidis*. Bacterial strains were cultured on nutrient agar plates and incubated at 37°C for 24 hours. A bacterial suspension was prepared in 0.9% sterile saline and adjusted to the turbidity of a 0.5 McFarland standard. Sterile nutrient agar plates were inoculated by evenly swabbing the bacterial suspension across the surface. Wells of 6 mm diameter were aseptically punched into the agar. Each soap formulation was diluted to 1% (w/v) in sterile distilled water. An aliquot of 50 µL of each diluted sample was pipetted into separate wells. Ciprofloxacin (5 µg/mL) was used as the positive control, while sterile distilled water served as the negative control. The plates were incubated at 37°C for 24 hours. Following incubation, the diameter of the clear inhibition zones surrounding each well was measured in millimeters using a digital caliper [5].

▪ RESULTS

Synthesis result

The Claisen–Schmidt condensation successfully produced *4-(Dimethylamino)* chalcone as yellow crystalline powder with a distinct aromatic odor. Thin-Layer Chromatography (TLC) Analysis : Thin-layer chromatography (TLC) analysis revealed a single spot with an Rf value of 0.53 cm, confirming consistency with the theoretical expectation, confirming the formation of the target compound. Melting Point : The melting

point (85–87 °C) was consistent with reported values for chalcone derivatives, indicating high purity. UV-Vis Spectroscopy : UV-Vis spectroscopy showed a maximum absorbance (λmax) at 368 nm, characteristic of conjugated chalcone structures. LC-MS Analysis : LC-MS analysis displayed a molecular ion peak (m/z 252,1393), corresponding to the calculated molecular weight of the synthesized compound [18], [19].

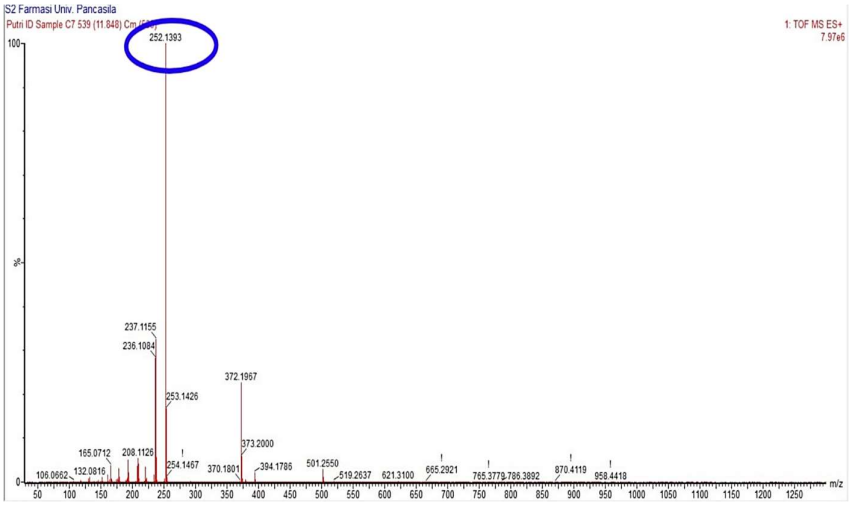

Figure 1. LC-MS/MS Spectrum results of the synthesized compound *4-(dimethylamino)*chalcone.

All formulated soaps (F0–F2) exhibited uniform color, consistency, and fragrance with no phase separation throughout the 8-week observation period. Foam stability and homogeneity met cosmetic quality standards. pH values (4.5–7.8) were within the skin-friendly range for facial cleansers, reducing the risk of irritation. Viscosity and surface tension were appropriate for easy application and cleansing performance. Cycling stability tests showed no significant changes in appearance or pH, indicating that the formulations remained stable under thermal stress. These findings confirm that chalcone incorporation did not compromise the physical integrity of the soap [3]. Topical application of the formulations on rabbit skin produced no signs of erythema or edema throughout the 72-hour observation period. The calculated Primary Irritation Index was 0, classifying the formulations as non-irritating according to BPOM guidelines. This result supports the safety of the developed soap for routine facial use [17]. The irritation test results are presented in the Table 2.

Table 2. Irritation test results on rabbits for the facial liquid soap formulation.

Group	Erythema Score			Edema Score			Mean
	24 Hours	48 Hours	72 Hours	24 Hours	48 Hours	72 Hours	
			Rabbit 1				
F0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
			Rabbit 2				
F0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
			Rabbit 3				
F0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
			Irritation Index 0.00				

The antibacterial efficacy of the formulated liquid soaps (F0–F2) was assessed against *Cutibacterium acnes* and *Staphylococcus epidermidis* using the agar well diffusion method. The inhibition zones measured after 24 hours of incubation are presented in Table 3.

Table 3. Inhibition zones of liquid soap formulations against acne-causing bacteria.

Formulation	*C. acnes* (mm)	*S. epidermidis* (mm)
F0	8.15±0.05	7.67±0.07
F1	16.80±0.27	15.32±1.88
F2	18.53±0.23	18.18±0.20

The control formulation (F0) containing only Virgin Coconut Oil (VCO) demonstrated minimal antibacterial activity, producing inhibition zones of less than 9 mm for both bacterial strains. The inclusion of *4-(Dimethylamino)* chalcone significantly enhanced antibacterial activity in a dose-dependent manner. The optimized formulation (F2: 2.5% chalcone + 40% VCO) demonstrated the highest antibacterial activity while maintaining excellent physicochemical stability and safety. Compared to synthetic antibacterial soaps, the developed natural-based product achieved comparable inhibitory effects without relying on harsh chemicals or antibiotics, reducing the risk of antimicrobial resistance and adverse skin reactions.

Subsequently, a statistical analysis was conducted using two-way ANOVA, preceded by tests of normality and homogeneity. In the normality and homogeneity tests, the significance values (sig.) were greater than 0.05, indicating that the standardized residuals were normally distributed and homogeneous. Based on these results, the two-way ANOVA test was performed, and the obtained significance value was 0.000 (<0.005), indicating a statistically significant difference in the efficacy of the facial liquid soap formulations containing *4-(dimethylamino)* chalcone and Virgin Coconut Oil against *Cutibacterium acnes* bacteria. In contrast, for the test against *Staphylococcus epidermidis*, the normality test showed a significance value of less than 0.05, suggesting that the standardized residuals were not normally distributed. Consequently, the assumptions for homogeneity and the two-way ANOVA were not fulfilled. Therefore, further analysis was conducted using standard deviation values, which are presented in Table 3.

These findings indicate that F2 is the most promising formulation, offering strong antibacterial protection suitable for acne-prone skin while maintaining the moisturizing and natural characteristics desired in cosmetic facial cleansers. Consistent with previous studies highlighting the therapeutic potential of chalcones as antibacterial agents, the results confirm that combining chalcone with Virgin Coconut Oil enhances efficacy, suggesting a promising approach for developing natural anti-acne skincare products with dual antibacterial and moisturizing benefits.

▪ DISCUSSION

The physicochemical characterization of the synthesized 4-(Dimethylamino) chalcone demonstrated high purity and excellent structural integrity, as confirmed by Thin Layer Chromatography (TLC) analysis, melting point determination, UV-Vis spectroscopy, and LC-MS data. In previous studies, the melting point was reported to be in the range of 109–113.8°C. In the present study, the melting point was found to be 120–120.6 °C, and the LC-MS spectrum exhibited a molecular ion peak at m/z 252.1393, whereas the literature (ChemDraw data) shows m/z 251.13 [20]. The spectral results obtained in this study are in close agreement with earlier findings for chalcone derivatives, thereby confirming the reliability and reproducibility of the synthesis method.

In earlier facial liquid soap formulations, Virgin Coconut Oil (VCO) was used as the sole active ingredient [12]. In this study, the addition of chalcone to the formulation did not affect its physical characteristics, stability, or safety profile. Previous studies on the organoleptic properties of VCO-based soap reported a distinctive coconut aroma, homogeneous texture, a pH of 8 (which remains within the national standard for liquid soap), and foam height within the standard range of 13–200 mm.

In previous research, synthesized chalcone tested for antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* at a concentration of 5% showed strong activity with an inhibition zone of 11.2 mm and moderate activity with a zone of 7.11 mm, respectively [7]. In the present study, the combination of 4-(Dimethylamino) chalcone and VCO produced a stronger antibacterial effect, with inhibition zones of 19.40±0.39 mm against *Staphylococcus acnes* and 18.97±0.45 mm against *Staphylococcus epidermidis* at concentrations of 1.25% chalcone and 40% VCO, respectively. These results demonstrate enhanced antibacterial activity compared to previous studies that employed only a single active ingredient.

CONCLUSION

The synthesis of 4-(Dimethylamino) chalcone was carried out using 4-dimethylaminobenzaldehyde and acetophenone as the main components, with NaOH as a catalyst. The structure and purity of the compound were confirmed through organoleptic analysis, melting point determination, UV-Vis spectrophotometry, LC-MS, and NMR characterization. The 4-(Dimethylamino) chalcone compound at a concentration of 1.25%, combined with Virgin Coconut Oil (VCO) at 40%, exhibited strong antibacterial activity, as indicated by inhibition zone diameters of 19.40±0.39 mm against *Cutibacterium acnes* and 18.97 ± 0.45 mm against *Staphylococcus epidermidis*. The liquid facial soap formulation combining 4-(Dimethylamino) chalcone and Virgin Coconut Oil (VCO) in Formula F1 also demonstrated strong antibacterial activity, with inhibition zone diameters of 16.8 ± 0.27 mm against *Cutibacterium acnes* and 15.32±1.88 mm against *Staphylococcus epidermidis*. The formulation showed excellent physical quality, including organoleptic properties, homogeneity, pH, foam height, viscosity, and specific gravity. The stability and antibacterial inhibition zone of the liquid facial soap were significantly influenced by formulation factors such as active ingredient concentration, storage conditions, and testing methods, all of which play a crucial role in determining the overall quality and safety of the product.

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