

The potential microemulsion of black cumin oil (*Nigella sativa* L.) as an antiacne agent in facial soap

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Abstract

Background: Acne vulgaris is a skin disorder caused by *Propionibacterium acnes* requiring effective natural-based topical treatment.

Method: Black cumin oil (*Nigella sativa* L.) was formulated into a microemulsion-based facial wash soap in three concentrations (F1: 0.4 %, F2: 0.5 %, F3: 0.6 %) and evaluated for physical characteristics, stability, irritation, and antibacterial activity using the agar well diffusion method.

Results: Formula F3 (0.6 %) was the best formula with particle size 668 nm, transmittance 97.2%, and inhibition zone 13 mm. The facial wash soap showed pH 5 ± 0 , foam height 17.67 ± 0.57 mm, stable over 21 days, non-irritating, and antibacterial inhibition zones of 13.1–14.0 mm (strong category).

Conclusion: Black cumin oil microemulsion facial wash soap is stable, safe, and effective against acne-causing bacteria.

Keywords: Black cumin oil; microemulsion; facial wash soap; antiacne; *Propionibacterium acnes*.

INTRODUCTION

Acne, also known as acne vulgaris, is a common skin condition that typically occurs during adolescence. It can be caused by the production of excessive amounts of sebum, which leads to blocked skin pores and fosters the growth of bacteria, particularly *Propionibacterium acnes* (1). One significant contributing factor is the increase in androgen hormones during puberty, which can result in heightened stress levels. This stress, prevalent among adolescents, may further exacerbate acne development on the face. According to the Indonesian Health Survey data from 2023, 6% of adolescents aged over 15, or approximately 14 million individuals, experience stress due to anxiety (2).

Acne can be effectively managed by consistently caring for and maintaining facial cleanliness using appropriate skincare products, such as facial soap. Many people often resort to excessive use of chemical products or topical antibiotics as a quick solution for acne. However, these methods can lead to skin irritation and may even result in antibiotic resistance. Facial soaps that are

considered safe typically contain natural ingredients, like black cumin oil (*Nigella sativa* L.), which is known for its antiacne properties due to the presence of *Thymoquinone*. This compound can inhibit or kill bacteria such as *Propionibacterium acnes* (3).

In the community, high-value facial soaps are often available in liquid form (4). However, these liquid facial soaps may have limitations in delivering active ingredients that are crucial for treating acne (5). Therefore, there is a need for new delivery systems that can enhance oil solubility, improve the stability of the formulation, increase skin penetration, and offer more effective antiacne benefits through controlled and thorough release of substances on the facial surface (6).

Previous research has indicated that black cumin oil possesses a strong resistance, with a resistance value of 21.55 mm (3). The purpose of this journal is to identify challenges and provide recommendations for further research. It aims to review the potential of black cumin oil microemulsion as an antiacne agent in facial

soap formulations and to overview existing studies on the effectiveness of black cummin oil (*Nigella sativa* L.) in various preparations and delivery systems for treating acne.

METHOD

This study is a laboratory experimental research that aims to create and evaluate facial soap preparations based on black cummin oil microemulsions (*Nigella sativa* L.) and testing its activity against the bacteria *Propionibacterium acnes*. The approach used was quantitative and comparative, by comparing three formulations (F1, F2, and F3) based on differences in the concentration of black cummin oil, namely 0.4%, 0.5%, and 0.6%, against physical parameters, stability, and antimicrobial effectiveness. The subject of the study is a liquid facial soap preparation containing black cummin oil microemulsion obtained from Kisbiokim Medilab with a Certificate of Analysis (CoA) as a guarantee of quality and authenticity of raw materials.

The tools used in this study include rotary vacuum evaporator (Buchi®), ultrasonicator (Kudos®), water bath (Stuart), particle size analyzer (Beckman Coulter Delsa Nano C®), zeta sizer (Beckman Coulter Delsa Nano C®), UV-Vis spectrophotometer, analytical scale, magnetic stirrer, hotplate, pH paper, stopwatch, cork borer, caliper, ose needle, laminar air flow (LAF), autoclave, bunsen fire, as well as glass and metal equipment such as beakers (Pyrex), erlenmeyer flask (Pyrex), measuring flask (Pyrex), test tube, petri dish (Pyrex), watch glass, glass jar, funnel, volume pipette (Pyrex), drip pipette, measuring cup, porcelain cup, spatula, and stirring rod. The ingredients used include black cummin oil, VCO (virgin coconut oil), Tween 80, PEG 400, olive oil, stearic acid, potassium hydroxide (KOH) 10%, Sodium Carboxymethyl Cellulose (Na-CMC), Sodium Lauryl Sulfate (SLS), BHT, peppermint oil, ethanol 96%, aquadest, alcohol 70%, physiological NaCl solution 0.9%, Mueller Hinton Agar (MHA) media, and *Propionibacterium acnes* test culture.

The first step is the characterization of black cummin oil through organoleptic tests, which included visual observation of color,

aroma, and form (7). Subsequently, relative density was determined using a pycnometer (8), optical rotation was measured using a polarimeter zeroed with n-hexane as a blank, refractive index was measured using a refractometer at room temperature (9), and acid number was determined by titrating 2 grams of the sample dissolved in 50 mL of 95% ethanol using 0.1 N NaOH with a phenolphthalein indicator until a persistent light pink color formed for at least 15 seconds after which a microemulsion formulation is made with reference to the modified formula from Mariska *et al.* (2025), consisting of black cummin oil as the oil phase and active substance, VCO as the supporting oil phase, Tween 80 as surfactant, PEG 400 as cosurfactant, and aquadest as the water phase, with variations in the concentration of black cummin oil at F1 (0.4%), F2 (0.5%), and F3 (0.6%) (10).

The manufacture of liquid facial soap preparations using black cummin oil microemulsions is based on a modified formula from Damayanti & Ermawati (2023). The process begins by weighing all the ingredients according to the formula. Olive oil and stearic acid are then melted in a beaker over heat. Once melted, a potassium hydroxide (KOH) solution is added slowly at a temperature of 70–80°C while stirring continuously until a soap mass (referred to as mass I) is formed. Next, sodium carboxymethyl cellulose (Na-CMC) is dissolved in hot water and allowed to expand. This mixture is then incorporated into mass I, creating a viscous solution (mass II). Following this, sodium lauryl sulfate, butylated hydroxytoluene (BHT), peppermint oil, and black cummin oil microemulsions are added sequentially to mass II. The mixture is ground until all the ingredients are evenly combined, and then diluted with distilled water (aquadest) to achieve a total volume of 100 mL. For comparison, a commercial product, Himalaya Purifying Neem Face Wash, is used as a positive control, while a soap base without active substances serves as the negative control (11).

Data collection was conducted using a series of evaluation tests, which included

tests for microemulsion characteristics, physical properties, stability, irritation, and antiacne activity. The microemulsion characteristic tests comprised an organoleptic test, particle size analysis (PSA), polydispersity index measurement, zeta potential assessment, and transmission percent measurement (7). The physical evaluation of the preparation included organoleptic assessments (such as color, smell, and shape) (12), homogeneity tests by applying a sample to a object glass (13), and pH measurement using pH indicators, targeting a required pH range of 4.5–6.5 (11).

The stability test was performed by storing samples under three different temperature conditions: 4°C, 25°C, and 40°C. Observations were made on days 1, 3, 5, 7, 14, and 21 to evaluate organoleptic properties, pH, and foam height (14). The irritation test utilized the patch test method, where the preparation was applied to the inner arm of 9 participants using Whatman paper coated with a 2 cm diameter polyethylene film for 24 hours. Reactions such as redness, heat, itching, or stinging were assessed 25 to 30 minutes after the patch was removed (15).

The antiacne activity test was performed in vitro using the well diffusion method to target the bacteria *Propionibacterium acnes* on Mueller Hinton Agar (MHA). We utilized a cork borer to create five wells of uniform diameter on the surface of the media, previously inoculated with the test bacteria. Each well was carefully filled with samples: F1 (replication 1), F2 (replication 2), F3 (replication 3), alongside a positive control and a negative control, using a micropipette to deliver a precise volume of 10–100 µL. Aseptic techniques were strictly adhered to throughout the inoculation process in a laminar air flow (LAF) cabinet to ensure accuracy. After the incubation period, we measured the inhibition zones using calipers, providing quantitative data for analysis. This comprehensive data set was evaluated descriptively to highlight the impact of varying concentrations of black cumin oil on the physical properties, stability, and antiacne efficacy of the formulated facial soap

preparations. By emphasizing these results, we can demonstrate the potential benefits of our formulations in combating acne effectively (16).

RESULTS

Table 1. Result of Characterization Black Cumin Oil

Parameters	Result		Methods
Relative density	0,922 ± 0,034	-	Pycnometer
Optical rotation	0,106 ± 0,028	(°)	Polarimeter
Refractive index	1,4729 ± 0,0006	(nD20 °)	Refractometer
Acid number	6,3 ± 0,116	-	Volumetric

Table 2. Organoleptic Test Results of Black Cumin Oil Microemulsion

Formula	Results		
	Shape	Color	Smell
F1	Thick	Clear yellowish	Specialty Oil
F2	Thick	Clear yellowish	Specialty Oil
F3	Thick	Clear yellowish	Specialty Oil

Table 3. Emulsion Type Test Results

Formula	Emulsion Type
F1	o/w
F2	o/w
F3	o/w

Table 4. Transmitter Percent Test Results

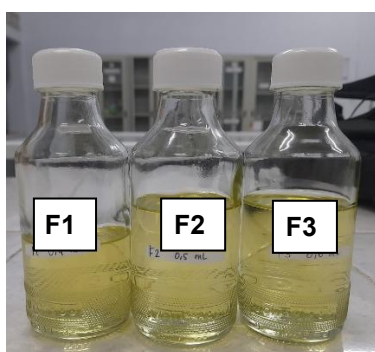
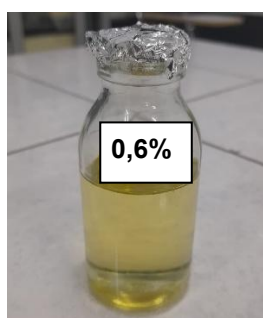
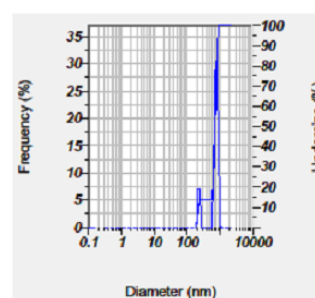
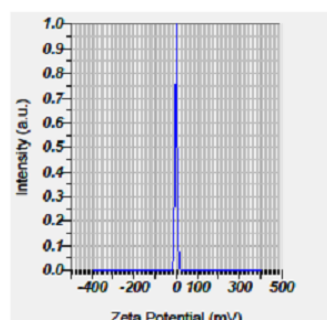
Formula	T %
MJH Microemulsion 0.6%	97,2%

Table 5. Facial Soap Test Results

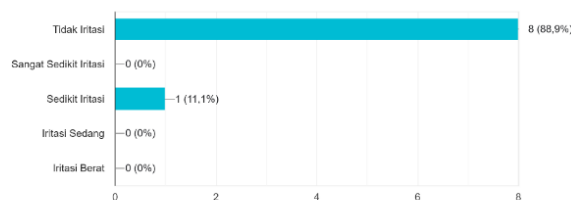
Parameters	Results		
	R1	R2	R3
Organoleptis	Liquid Milky White Pappermint	Liquid Milky White Pappermint	Liquid Milky White Pappermint
Homogeneity	Homogeneous	Homogeneous	Homogeneous
pH	5	5	5
Foam height	18 mm	17 mm	18 mm

Table 6. 21 Day Stability Test Results of Facial Soap

0,6 %	Parameters	Results	
		Before	After
R1	Organoleptis	Liquid Milky White Pappermint	Liquid Milky White Pappermint
	pH	5.10 ± 0.02	5.20 ± 0.02
	Foam Height	18 mm	17.3 ± 0.02 mm
R2	Organoleptis	Liquid Milky White Pappermint	Liquid Milky White Pappermint
	pH	5.13 ± 0.02	5.24 ± 0.03
	Foam Height	17 mm	16.7 ± 0.02 mm
R3	Organoleptis	Liquid Milky White Pappermint	Liquid Milky White Pappermint
	pH	5.12 ± 0.02	5.26 ± 0.04
	Foam Height	18 mm	16.9 ± 0.02 mm

**Figure 1. Results of MJH Microemulsion Formulation****Figure 2. Best Formulation 0.6%****Figure 3. Formulation Results of MJH Microemulsion Facial Soap 0.6%****Figure 4. PSA and PDI Test Results****Figure 5. Potential Zeta Test Results**

Bagaimana reaksi kulit lengan anda ketika dilakukan pengujian selama 24 jam?
9 jawaban

**Figure 6. Irritation Test Results**

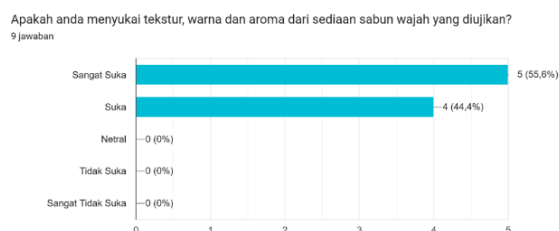


Figure 7. Hedonic Test Results

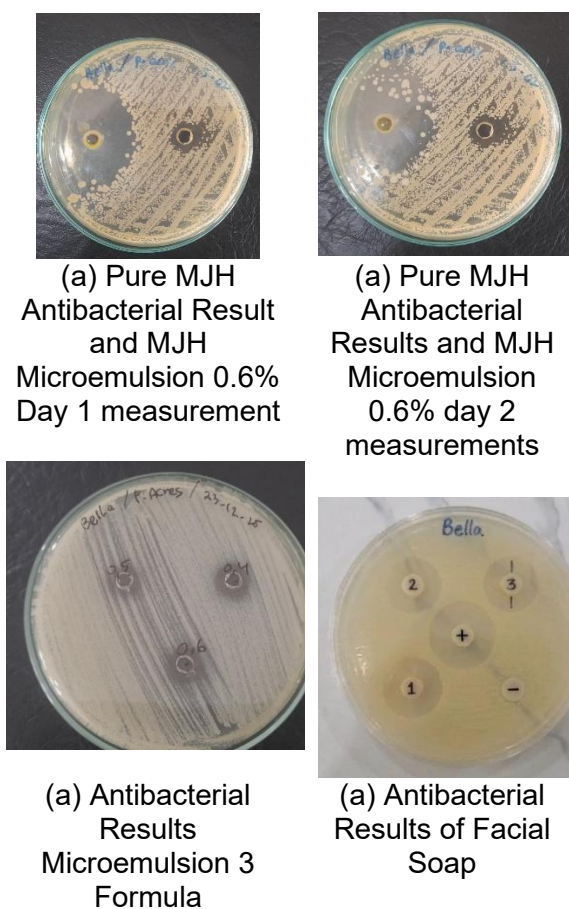


Figure 8. *Propionibacterium acnes* Antibacterial Test Results

DISCUSSION.

Characterization Results of Black Cumin Oil

Characterization of black cumin oil was conducted on specific parameters to validate the quality of the raw material in accordance with applicable standards. Organoleptically, black cumin oil is yellowish-brown in color, oily in texture, and has a distinctive volatile aroma (17).

The non-specific parameter test results are presented in Table 1. The relative density value of 0.922 ± 0.034 g/mL remains within the density range of volatile oils ($0.8589\text{--}0.9748$ g/mL) (18). The optical rotation value of $0.106 \pm 0.028^\circ$ reflects the composition of the oil's constituent compounds. The refractive index is 1.4729 ± 0.0006 nD 20° . The acid number of 6.3 ± 0.116 mg KOH/g indicates a value slightly higher than the ideal range (1–2 mg KOH/g), suggesting relatively low oxidative stability in the oil (19).

This is consistent with previous research by Lestari *et al.* (2025), which stated that black cumin oil organoleptically exhibits a slightly bitter taste, with a slightly dark brown color and a sharp, spicy aroma. Furthermore, it was also noted that black cumin oil has a specific gravity of $0.900\text{--}0.930$ g/cm ^{3} , a refractive index of $1.465\text{--}1.480$, and an acid number that is slightly higher in value (1).

MJH Microemulsion Antibacterial Test Results

Testing for antiacne activity was conducted using the radiant diffusion method against *Propionibacterium acnes*. The results showed the following inhibition zones for the three formulas tested: F1 (0.4 %) had an inhibition zone of 10.5 mm, F2 (0.5 %) had 7 mm, and F3 (0.6 %) had 13 mm. Consequently, F3 was selected as the best formula, exhibiting moderate to strong inhibition. In comparing pure black cumin oil to the F3 microemulsion, the inhibition zones measured 32 mm and 22 mm, respectively, with both being classified as very strong (18).

This aligns with research conducted by Rahayu *et al.* (2026), which indicates that variations in results can be attributed to differences in the concentrations of the formulas used. These differences affect the release process of the active material, influencing diffusion in the media (20).

Additionally, Lestari *et al.* (2025) reported an inhibition zone of 21.55 mm for black cumin oil, also categorized as very strong (3). Although pure black cumin oil demonstrated a larger inhibition zone, its preparation is generally less stable compared to the microemulsion form. Therefore, the

microemulsion system is regarded as superior in terms of stability (21).

Characteristics Test Results of MJH Microemulsion

Organoleptic tests on the three microemulsion formulas (F1, F2, F3) showed uniform results, namely viscous in shape, clear yellowish in color, and with a typical oil odor. The emulsion type test by the dilution method shows that the entire formula can be perfectly dispersed in the water phase without separation, so it is categorized as an oil-in-water (o/w) type emulsion, which is influenced by the content of hydrophilic components such as Tween 80 and aquades (7).

MJH Microemulsion Test Results 0.6%

Particle size testing on the optimal formula (F3) using the Particle Size Analyzer (PSA) yielded an average size of 668.0 ± 196.6 nm ($0.668 \mu\text{m}$), which is still within the required range of 0.5–10 μm (22). Research conducted by Aini *et al.* (2022) explains that the size of submicron particles is influenced by and commonly found in formulations containing active ingredients derived from plants (23).

The polydispersity index (PDI) value of 0.576 indicates a less uniform distribution of globule sizes, known as polydispersion. This variability may be attributed to factors such as storage time and temperature fluctuations (24). According to research by Jamaludin *et al.* (2025), PDI values ranging from 0.277 to 0.827 are influenced by the choice of co-surfactants, which play a crucial role in determining particle homogeneity (25).

The measured zeta potential of -0.2 mV is very low and significantly below the required electrostatic stability threshold of $>\pm 30$ mV, indicating that further optimization of surfactant types and concentrations is necessary (26). Meanwhile, the transmittance percentage of 97.2% is close to 100%, demonstrating that the microemulsion preparation is good and transparent (27). This result is consistent with findings from a previous study by Rahmadevi (2020), which reported a transmittance score of 97% using a combination of Tween 20 and Span 60, despite the particle size being at the micrometer scale (28).

Results of Physical Evaluation and Stability Test of Facial Soap

Physical evaluation of facial soap preparations was carried out against three replication formulas (R1, R2, R3) along with negative controls. The results of the organoleptic test showed that all four formulas were liquid, milky white, and had a distinctive peppermint flavor. The homogeneity test on all formulas shows an even mixture without any coarse particles or sediments, thus meeting the SNI requirements (29). The pH value of the three replicates was obtained at 5 ± 0 , which is within the required pH range of facial soap (4.5–6.5), indicating that the preparation is safe to apply to the skin without the risk of irritation (12).

The average foam height obtained is 17.67 ± 0.57 mm, still within the SNI standard range (13–220 mm) (4). The addition of SLS by 1% provides adequate lather ability without overdoing it, so it does not have the potential to dry out the skin (30). Stability tests over 21 days at three temperature variations (4°C, 25°C, and 40°C) showed that all three replicates did not undergo organoleptic changes. The pH value after the test ranged from 5.20–5.26, slightly increased but still within the required limits. The foam height after 21 days ranges from 16.7–17.3 mm, still meeting SNI standards. These results indicate that the facial soap preparation is stable during storage (15).

Based on Figure 6, the results of the patch test for skin irritation were obtained from 9 respondents over a 24-hour period. The findings showed that 8 out of 9 respondents did not experience any irritant reactions, such as redness (erythema), heat, itching, or pain. Consequently, the calculated Irritation Index score (IIP) is 0.22. This indicates that the facial soap formulation is safe for use on the skin, which is further supported by its pH value, which falls within the physiological range of the skin (1). This aligns with the research conducted by Fidayanti *et al.* (2025), which states that a score of 0.0–0.4 is classified as non-irritating (31).

In the Hedonic Test shown in Figure 7, 55.6% of respondents (5 participants) reported that they really liked the texture,

aroma, and color of the preparation, while 44.4% (4 participants) indicated that they liked it. This results in a combined total of 91.11% of respondents expressing positive feedback, categorizing the preparation in the "very much liked" range. According to Lestari *et al.* (2025), preparations that receive favorable ratings are considered to have strong potential for further development and research in the industrial sector (1).

Antibacterial Test Results of Facial Soap

We tested the antibacterial activity of facial soap preparations against *Propionibacterium acnes* using the suction diffusion method. All three trials indicated inhibition zones ranging from 13.1 to 14.0 mm, which are classified as strong. In comparison, the positive control exhibited an inhibition zone of 24.3 mm, classified as very strong, while the negative control did not show any inhibition zone (32). These results confirm that black cumin oil microemulsions in facial soap preparations have significant antibacterial activity against *P. acnes* (33).

CONCLUSIONS

This study demonstrates that black cumin oil can be effectively formulated into a safe facial soap microemulsion preparation that acts as an antiacne agent. The optimal formula, with a concentration of 0.6 %, produces an oil-in-water microemulsion system with a particle size and clarity that meet established standards. It also exhibits strong inhibitory activity against *Propionibacterium acnes*. The resulting facial soap preparation fulfills all physical and stability requirements, does not irritate the skin, and maintains organoleptic stability, pH, and lathering ability for 21 days of storage. Therefore, black cumin oil microemulsions are shown to enhance the stability and effectiveness of active substances compared to pure oils. This indicates their great potential for further development as a natural skincare product that is safe, stable, and exhibits significant antibacterial activity.

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