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RESEARCH ARTICLE

Formulation and Characterization of Microemulgel Containing *Boesenbergia rotunda* (L.) Mansf. Essential Oil as an Anti-Acne Agent

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Formulation and Characterization of Microemulgel Containing Boesenbergia rotunda (L.) Mansf. Essential Oil as an Anti-Acne Agent

SUMMARY

This study aimed to formulate and evaluate the physicochemical and antibacterial effects of a microemulgel of Boesenbergia rotunda essential oil (BREO) developed as an anti-acne agent. The BREO microemulgel was prepared using Tween 80 as a surfactant, PEG 400 as a co-surfactant, and Viscolam as a gelling agent. The microemulgel was evaluated for homogeneity, pH value, viscosity, spreadability, adhesion test, and stability. The droplet size and zeta potential were measured using a Particle Size Analyzer (PSA). The antibacterial effects were tested using the diffusion method against Propionibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis. BREO microemulsion using Tween 80-PEG 400 (25%:25%) obtained a clear and transparent microemulsion with a transmittance percentage of 98.65 ± 0.40%. BREO microemulgels showed a homogenous system, pH value of 5.95-6.43, viscosity of 6411-36569 cps, spreadability of 5.2-5.5 cm, adhesion 3.62-4.48 seconds, and no visible separation in the stability test. Each formulation exerted an antibacterial effect against bacteria-related acne, with 22.6-24.6 mm inhibition zone diameters. The formulation of F1B had a droplet size of 22 nm, and zeta potential -31.58 ± 0.30 mV. The BREO microemulgel formulation (F1B) met the physical properties, the stability, and the antibacterial activity to be developed as an anti-acne.

Keywords: *Acne vulgaris, fingerroot, microemulsion, Propionibacterium acnes, surfactant.*

Akne Karşıtı Bir Ajan Olarak Boesenbergia rotunda (L.) Mansf. Uçucu Yağı İçeren Mikroemüljelin Formülasyonu ve Karakterizasyonu

ÖZ

Bu çalışmanın amacı, akne karşıtı bir ajan olarak geliştirilen Boesenbergia rotunda esansiyel yağı (BREO) mikroemüljelinin fizikokimyasal ve antibakteriyel etkilerini formüle etmek ve değerlendirmektir. BREO mikroemüljeli, yüzey aktif madde olarak Tween 80, yardımcı yüzey aktif madde olarak PEG 400 ve jelleştirici olarak Viscolam kullanılarak hazırlanmıştır. Mikroemüljel homojenlik, pH değeri, viskozite, yayılabilirlik, yapışma testi ve stabilite açısından değerlendirilmiştir. Damlacık boyutu ve zeta potansiyeli bir Parçacık Boyutu Analizörü (PSA) kullanılarak ölçülmüştür. Antibakteriyel etkiler Propionibacterium acnes, Staphylococcus aureus ve Staphylococcus epidermidis'e karşı difüzyon yöntemi kullanılarak test edilmiştir. Tween 80-PEG 400 (%25:25) kullanılan BREO mikroemülsiyonu, %98,65 ± 0,40 geçirgenlik yüzdesi ile berrak ve şeffaf bir mikroemülsiyon elde etmiştir. BREO mikroemüljelleri homojen bir sistem, 5,95-6,43 pH değeri, 6411-36569 cps viskozite, 5,2-5,5 cm yayılabilirlik, 3,62-4,48 saniye yapışma ve stabilite testinde gözle görülür bir ayrılma göstermemiştir. Her bir formülasyon, 22,6-24,6 mm inhibisyon zon çapları ile bakterilerle ilişkili aknelere karşı antibakteriyel etki göstermiştir. F1B formülasyonu 22 nm damlacık boyutuna ve -31,58 ± 0,30 mV zeta potansiyeline sahipti. BREO mikroemüljel formülasyonu (F1B), akne karşıtı olarak geliştirilecek fiziksel özellikleri, stabiliteyi ve antibakteriyel aktiviteyi karşılamıştır.

Anahtar Kelimeler: *Akne vulgaris, mikroemülsiyon, parmak kökü, Propionibacterium acnes, yüzey aktif madde.*

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INTRODUCTION

Acne vulgaris is a prevalent skin disorder among young adults and teenagers. Acne can be caused by various factors, including androgens that activate the sebaceous glands, microbiota imbalances in the pilosebaceous follicle, and innate and cellular immunological responses (O'Neill & Gallo, 2018). Inflammatory papules and pustules are derived from the skin's commensal bacteria, such as *Propionibacterium acnes*, *Staphylococcus epidermidis,* and *Staphylococcus aureus*, stimulating the innate and adaptive immune systems (Dréno, 2017). Follicle rupture causes keratin, pro-inflammatory lipids, and germs to be discharged into the dermis, which exacerbates the inflammation (Thiboutot & Zaenglein, 2024).

Antibiotics have long been used to treat acne, leading to a clinical improvement in the condition, but this can lead to bacterial resistance to antibiotics (Dessinioti & Katsambas, 2017). A study in China reported a resistance rate of *P. acnes* to clindamycin, erythromycin, and azithromycin (Zhu et al., 2019). Another study in France showed that *P. acnes* were shown to be resistant to erythromycin (75%) and doxycycline (100%) (Dumont-Wallon et al., 2010). The widespread occurrence of resistance strains in acne patients is a result of the prolonged treatment courses for acne, the usage of topical and/or systemic antibiotics, and the accessibility of over-the-counter antibiotic medications (Dessinioti & Katsambas, 2022). According to this study, choosing non-antibiotic treatments for acne wherever feasible may have major benefits given the rising risk of antibiotic resistance.

Boesenbergia rotunda (L.) Mansf. Essential oil (BREO), also known as the fingerroot essential oil, has the potential to be developed into an anti-acne preparation. It mainly contains camphor, cis-ocimene, geraniol, and methyl cinnamate, which are known for their antibacterial activity. The previous study reported that BREO had antibacterial activity against acne-causing bacteria (Mulyaningsih et al., 2024). Otherwise, this oil has the potential to be developed into anti-acne preparations. However, this essential oil provides volatile properties and is easily decomposed by heat, light, and oxygen. In addition, it can cause skin irritation when it is used directly. The development of a convenient formulation is necessary to address this instability and to optimize its activity and efficacy (Kartika, 2018).

Microemulsion gels or also known as microemulgels are used to deliver a variety of medications, including analgesics, anti-inflammatories, antifungals, and anti-acne (Talat et al., 2021). In addition, microemulgel has some benefits, including a long shelf life, being translucent, emollient, thixotropic, greaseless, easily removable, and emollient. Currently, microemulgel is a promising drug delivery system that is widely used to administer hydrophobic compounds (Jain et al., 2019). Microemulgel containing essential oils can increase its antibacterial efficacy because the smaller the particle size of the dispersed phase, the better its bioavailability of antibacterial substances (Karimah & Aryani, 2021). To date, no studies have been conducted on the formulation of BREO in the form of a microemulgel for treating acne. Therefore, this study aimed to investigate the BREO microemulgel formulations with various gelling agents in relation to their physical and antibacterial properties as an anti-acne agent.

MATERIALS AND METHODS

Materials

BREO was distilled from *B. rotunda* rhizomes, and its chemical composition has been verified by gas chromatography-mass spectrometry (GC-MS), *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, and *P. acnes* ATCC 6919.

Optimization of surfactant and co-surfactant of the BREO microemulsion base

Surfactant and co-surfactant were optimized using various concentrations of Tween 80 as surfactant and PEG 400 as co-surfactant (Table 1). Tween 80, PEG 400, and BREO were mixed in a glass beaker and then added with distilled water to 100 mL. The mixture was stirred with a magnetic stirrer at 300 rpm for 30 minutes. Emulsification of 3% BREO in Tween 80 and PEG 400 was checked by measuring the percentage of transmittance using a spectrophotometer (Shimadzu, Japan) at 650 nm (Islam & Uddin, 2022). The formulation with the highest percentage transmittance was followed by its microemulsion globule, polydispersity index (PI), and zeta potential determination using a Particle Size Analyzer (PSA) (Malvern Panalytical Ltd.) (Zainol et al., 2015).

Table 1. Formulation of microemulsion with various percentages of Tween 80 and PEG 400

Formulation	Tween 80 (%)	PEG 400 (%)	BREO (%)
F1	25	25	3
F2	20	30	3
F3	30	20	3

Formulation of BREO microemulgel

The optimum surfactant and co-surfactant combination in BREO microemulsion was used as a microemulgel formulation base with various concentrations of Viscolam as a gelling agent (Table 2). Microemulgel was prepared based on a previous study (Priani et al., 2019). First, Viscolam 8% was prepared by dispersing 8 g of Viscolam in distilled water to a volume of 100 mL. Then, it was weighed according to formulas F1A (15 g), F1B (20 g), and F1C (25 g). Tween 80, PEG 400, BREO, and water were mixed and homogenized using a magnetic stirrer (Jieo Tech Japan Inc) at 300 rpm for 30 minutes. Each formulation was mixed with Viscolam, followed by homogenizing using a magnetic stirrer at 300 rpm for 15 minutes. Subsequently, propyl paraben, methyl paraben, and α-tocopherol were added.

Table 2. Formulation of BREO microemulgels

Composition	Concentration (%)		
	F1A	F1B	F1C
BREO	3	3	3
Tween 80	25	25	25
PEG 400	25	25	25
8% Viscolam	**15**	**20**	**25**
Methyl paraben	0.36	0.36	0.36
Propyl Paraben	0.04	0.04	0.04
Tocopherol	0.01	0.01	0.01
Glycerine	0.126	0.126	0.126
Distilled water	until 100 mL	until 100 mL	until 100 mL

Characterization of BREO microemulgel formulations

Organoleptic observations

Organoleptic observations were carried out visually with human senses, including observations of clarity, color, odor, and texture of the microemulgel that was made.

Homogeneity testing

About 1 gram of the microemulgel was uniformly and thinly applied to the transparent glass. If there was no coarseness, the microemulgel was considered homogenous (Dewi et al., 2023).

pH measurement

The pH values were determined by a pH meter (Ohaus Starter ST300). The pH meter was calibrated using a standard solution with a pH value of 4, pH 7, and pH 10 before use. One gram of BREO microemulgel was dispersed in 10 ml of distilled water. Afterwards, the electrode of the pH meter was immersed in the diluted solution until a stable pH measurement was obtained (Rahmania et al., 2020).

Spreadability test

For this test, 0.5 grams of microemulgel were placed in the center of a glass plate. Then, a pre-weighed cover glass was placed on top of the gel. Next, 150-gram weights were added and left for one minute. Spreadability was determined by measuring the average diameter of the spread on several sides (Nurman et al., 2019). This test was replicated three times.

Adhesion test

This test involved placing 0.5 grams of gel on an object glass, covering it with another object glass, and leaving it for five minutes. Then, a 1 kg weight was placed on top for five minutes. Next, both slides were placed on a testing device with an 80-gram load. The time it took for the slides to separate after removing the 80-gram load was recorded (Rahmawati & Setiawan, 2019). This process was repeated three times.

Viscosity measurement

The viscosity of the BREO microemulgel was assessed using a Rheosys Merlin VR rheometer with a 2.0/30 mm cone-plate spindle. The gel was placed on the plate and sealed with the parallel plate. The rheometer was connected to a computer with the Rheosys Micra application installed. The measurement parameters were set as follows: temperature: 25 °C; start speed: 10; end speed: 100; direction: up/down; steps: 12; delay time: 30 seconds; and an integration time of 10 seconds. The viscosity measurement was initiated by pressing "Start," which lasted for a period of time. The viscosity of the three microemulgels was measured using the same treatment and parameters (Sugihartini et al., 2021).

Globule characterization of BREO microemulsion and microemulgel

The produced microemulgel was analyzed for droplet size and polydispersity index (PI), using dynamic light scattering. Zetasizer (Nano ZS, Malvern Panalytical, U.K.) was used to measure the BREO microemulgel. The prepared sample was put into cuvettes, and scattering intensity was observed at a temperature of 25 °C.

Morphology of Microemulgel

Transmission electron microscopy (TEM) imaging was used to examine the microemulgel's morphology. In short, a droplet of the water-diluted microemulgel suspension was put on a 200-mesh formvar copper grid, allowed to adsorb, and then the excess was wiped off using filter paper. After adding a drop of 2% (w/v) uranyl acetate solution, the sample was allowed to come into contact with it for five minutes. Before the vesicles were photographed using an HR-TEM (Talos F200C G2), the material was dried at room temperature (Sulastri et al., 2025).

Stability studies of BREO microemulgel

a) Centrifugation assay

Five milliliters of each BREO microemulgel formulation were centrifuged at 3500 rpm for 30 minutes. All formulations were observed for the presence of separation, creaming, or cracking (Kiromah et al., 2023).

b) Heating-cooling cycle

The study was run for three cycles. A cycle involved storage of the BREO microemulgel at 4 °C for 24 hours and then storage at 40 °C for the following 24 hours (Priani et al., 2019). The separation, creaming, or cracking was evaluated for each formulation.

Antibacterial assay of BREO microemulgel

The antibacterial assay used the well diffusion method against bacteria-related acne, including *S. epidermidis, S. aureus*, and *P. acnes*. Bacterial suspensions with a concentration of 10^8 CFU/mL were taken with a sterile cotton swab and then spread onto Mueller-Hinton Agar (MHA) media (HiMedia). After that, wells were made on MHA media using a perforator. A total of 100 mg of each formulation, F1A, F1B, and F1C, was placed in the wells and incubated at 37 °C for 24 hours. The same procedure was applied using a microemulgel base as a negative control and 1% clindamycin gel as a positive control. After incubation at 37 °C for 24 hours, the diameter of the inhibition zone was measured.

Data analysis

The data on the physical properties of the microemulgel and the inhibition zone diameter were analyzed using GraphPad Prism 8. A data normality test was performed using the Shapiro-Wilk test ($P > 0.05$). The data were analyzed using one-way ANOVA followed by a Tukey post hoc test at a 95% confidence level.

RESULTS AND DISCUSSION

Formulation of BREO microemulgel

The correct ratio of oil phase, water phase, and surfactant plays an important role in the stable microemulsion formation. The optimal surfactant and co-surfactant were selected based on the transmittance percentage. Transmittance values close to 100% are characteristic of high-quality microemulsions that form a clear and transparent microemulsion (Pathan et al., 2012). This indicates that they can disperse light due to their small droplet size. F1 formulation had the highest transmittance percentage compared to F2 and F3 (Table 3). The transmittance percentage of F1 microemulsion was found to be 98.65 ± 0.40 %, confirming the good transparent nature of the formulations. The optimum ratio was achieved in F1 with a 25%:25% ratio of Tween 80 and PEG 400 to make a clear and transparent BREO microemulsion. Previous studies have shown that surfactant and co-surfactant solutions with a 1:1 ratio of Tween 80: PEG 400 were thermodynamically stable and transparent. PEG 400, as a cosolvent, is promising for microemulgel formulation stability (Taher et al., 2022).

Table 3. Transmittance percentage of the BREO microemulsion formulation with different percentages of Tween 80 and PEG 400

Formulation	Tween 80 (%)	PEG 400 (%)	BREO (%)	Transmittance (mean ± SD) (%)
F1	25	25	3	98.65 ± 0.40
F2	20	30	3	12.07 ± 1.11
F3	30	20	3	50.11 ± 10.9

One of the factors responsible for the variation of the transmittance value of the microemulsions is droplet size. Smaller droplets increase transparency (higher transmittance) because they scatter less light (Naganuma & Kagawa, 2002). Measurement of the droplet size of formulation F1 was performed to confirm the microemulsion formation (Table 4). The average droplet size of F1 was 12 nm, which meets the ideal range of 10-200 nm for a microemulsion system. The PI value of formulation F1 was 0.24, between 0.01 and 0.7, and is classified as a monodispersed system (Kiromah et al., 2023). The zeta potential of F1 was -8.235 mV. It is not too negative for skin application due to the possible repulsion. Therefore, it is suitable for topical application to deliver the active ingredient. The Formulation 1 indicated clear or transparent, with a very small droplet size. Production of the microemulsions using a magnetic stirrer yielded small droplet sizes with a relatively low PI. This result is consistent with those of other studies that prepared microemulsions with a magnetic stirrer, producing droplet sizes of less than 200 nm (Nunes de Godoi et al., 2025; Syapitri et al., 2022).

Table 4. Particle characterization of BREO microemulsion (F1 formulation)

Measurement	Microemulsion (F1)
Droplet size (nm)	12.00 ± 0.07
Polydispersity index (PI)	0.24 ± 0.02
Zeta potential (mV)	-8.235 ± 0.13

Numerous investigations have looked into the physicochemical characteristics of different Tweens. For microemulsion formulations, these references take into account every characteristic and observation that proves Tween to be a convenient cosmetic excipient (Kaur & Mehta, 2017). However, existing guidelines should be followed when using Tween 80 as a surfactant to avoid adverse effects.

Characterization of BREO microemulgel

All formulations of BREO microemulgels were yellowish-white in color and exhibited the characteristic odor of fingerroot, as shown in Table 5. The difference observed among the formulations was in their consistency: F1C indicated the thickest compared to the other formulations. This difference is mainly affected by the amount of the gelling agent Viscolam. The higher the concentration of the gelling agent, the thicker the gel (Forestryana et al., 2022). During gel formation, the gelling agent absorbs water and remains in the gel, forming a thick liquid. Therefore, the higher the concentration of the gelling agent, the more liquid will be bound and retained, which can lead to an increase in viscosity. Table 5 shows that each microemulgel is homogeneous, with no particles or coarse grains, thus reducing the potential for skin irritation.

Table 5. The organoleptic and homogeneity of BREO microemulgel

Test		F1A	F1B	F1C
Organoleptic	**Clarity**	Clear	Clear	Clear
	Color	Yellowish white	Yellowish white	Yellowish white
	Odor	Typical aromatic	Typical aromatic	Typical aromatic
	Consistency	Slightly thick	Thick	Thicker
Homogeneity		Homogenous	Homogenous	Homogenous

Ideally, the pH value of the microemulgel is neither too acidic nor too alkaline and matches the natural pH of human skin. The normal skin pH is between 4 and 6 (Lukić et al., 2021; Prakash et al., 2017). Another study reported that the skin preparation for acne should be at a pH between 5.4 and 6 (Kulthanan et al., 2015). If the resulting pH is too acidic, it may irritate, while if the pH obtained is too alkaline, it may cause the skin to dry out and become sensitive (Hwang et al., 2022). Figure 1 presents the pH value of three formulations, which were in the range of 5.95 to 6.43. Thus, the BREO microemulgel can be applied to the facial skin without irritation. Statistical analysis shows that the pH values of the three formulations do not have significant differences ($p>0.05$).

A spreadability test was conducted to evaluate how well the microemulgel spreads on the skin during application. Good spreadability ensures that active ingredients are evenly distributed on the skin. However, if the microemulgel spreads too easily, it can cause discomfort during application. An ideal spreadability test result is between 5 and 7 cm (Nurman et al., 2019). Viscosity is inversely proportional to spreadability. This occurs because higher viscosity results in lower spreadability (Szulc-Musioł et al., 2017). The three BREO microemulgel formulations have a spreadability in the range of 5 to 5.5 cm and no statistically significant difference, meeting the requirements for good spreadability ($p>0.05$).

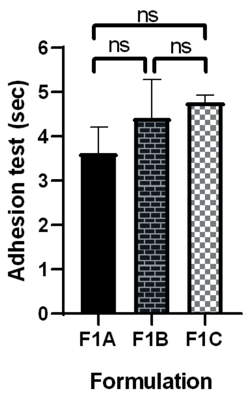
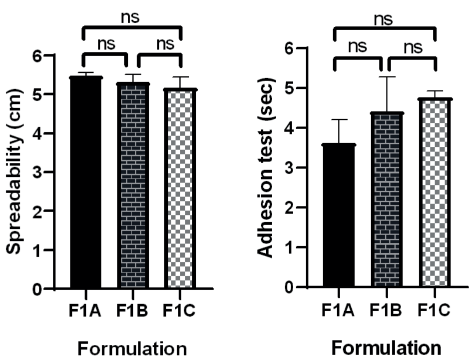


Figure 1. pH value of BREO microemulgel formulations. The asterisk denotes a significant difference (p<0.05), and the letter “ns” denotes not significant (p>0.05).



A B

Figure 2. Spreadability (A) and adhesion tests (B) of the BREO microemulgel formulations. The letter “ns” denotes not significant (p>0.05).

Adhesion testing determines a preparation’s ability to adhere to the skin. A good adhesion study requires a contact time of more than 4 seconds on the skin surface (Tania et al., 2022). BREO microemulgel formulations that meet this requirement are F1b and F1c, whereas F1a demonstrates an adhesion time of less than four seconds (Figure 2). The adhesion value of F1A is associated with its lower consistency and viscosity, which prevents it from adhering for an extended duration. For topical preparations, such as microemulgels, it is necessary to determine their adhesion time. The amount of gelling agent could affect the adhesivity as well as the viscosity. The higher the adhesion value, the stronger the contact between the microemulgel and the skin. Good adhesion is associated with better efficacy and absorption of active ingredients (Kurniawan & Aryani, 2024). Thus, good adhesion relates not only to the convenience of use but also to the therapeutic effectiveness of an active ingredient. Nonetheless, the adhesion value and spreadability value are inversely correlated; the higher the spreadability, the lower the adhesion value.

Figure 3 shows that the viscosity of F1A, F1B, and F1C was 6,411.78, 23,361.22, and 36,569.51 cps, respectively. The difference in the results is due to variations in the amount of the gelling agent Viscolam. F1C indicated the highest viscosity due to the most Viscolam added, resulting in a thicker consistency. One-way ANOVA statistical analysis showed a significant difference in the viscosity of the three formulations (p<0.05). This demonstrates that the addition of Viscolam significantly affects the viscosity of BREO microemulgel.

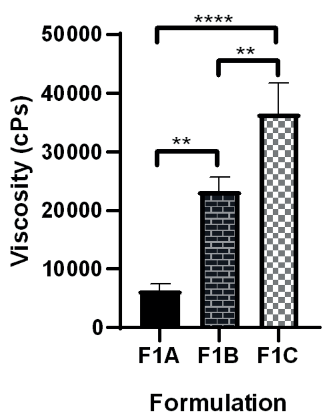


Figure 3. The viscosity of BREO microemulgel formulations. The asterisk denotes a significant difference (p<0.05).

Viscosity influences the distribution of particle sizes, the physical state, and the interactions between drug molecules and excipients, all of which have an impact on drug stability. It is important to avoid phase separation or deterioration while being stored. Viscosity affects the rheological characteristics, influencing the administration route, flow characteristics, and residence time at the intended location, particularly in gel dosage devices. Finally, viscosity influences the therapeutic outcomes (Kottke, 2023).

The BREO microemulgels flow type was determined by examining the rheogram (Figure 4.) and the results of the linear regression equations between shear stress and shear rate, as well as log shear stress and log shear rate. The log shear stress vs. log shear rate equation for all microemulgels (F1A, F1B, and F1C) produced an r-value close to one and a slope value greater than one, indicating that they have a pseudoplastic flow type. The viscosity of all microemulgels decreases with increasing shear stress. This is beneficial when the gel is in a package (e.g., a tube) because squeezing the package will cause the gel to flow out. This is also convenient during the filling process because the gel flows easily when given frictional pressure (Calienni et al., 2023).

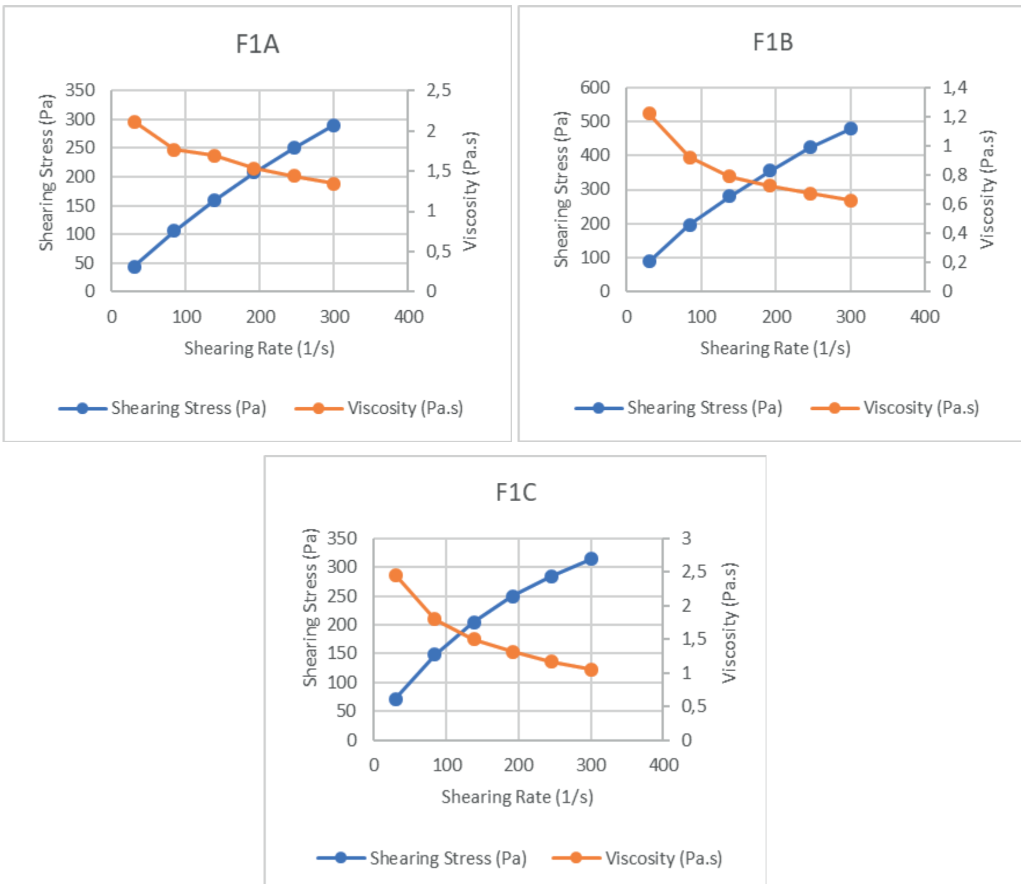


Figure 4. The rheogram of BREO microemulgel formulations

Accelerated stability studies of BREO microemulgel formulations

The accelerated stability studies were performed to determine the physical stability of the microemulgels. Two methods were used: centrifugation and exposure to extreme temperatures. The purpose of the centrifugation study was to ascertain whether or not there was a phase separation brought on by gravity. The heating-cooling cycle study was conducted to show the microemulgels resistance to extreme temperatures. This stability is important to prevent damage to the preparation due to thermal expansion and contraction, maintain optimal system performance in the long term, and reduce the risk of failure due to repeated thermal stress. All microemulgel formulations produced stable results, characterized by an absence of separation, creaming, or cracking (Table 6).

Table 6. The result of the stability studies of BREO microemulgel formulations

Formulation	Centrifugation method	Heating-cooling cycle
F1A	No separation, no creaming, no cracking	No separation, no creaming, no cracking
F1B	No separation, no creaming, no cracking	No separation, no creaming, no cracking
F1C	No separation, no creaming, no cracking	No separation, no creaming, no cracking

Measurements of microemulgels globule size and zeta potential

The particle size measurement indicated that the average globule size of F1A, F1B, and F1C was about 22-210 nm, placing F1B within the microemulsion size range of 10-200 nm (Seng & Loong, 2019). Based on the PI value obtained as 0.37, the droplet size uniformity level in the F1B microemulgel is close to monodispersion (Mahbubul, 2019). Interestingly, the droplet size of F1A was larger than that of F1B. F1A had a zeta potential value of -19.61 ± 0.29, which is closer to 0, while F1B had a zeta potential value of -30. The closer the value is to zero, the greater the chance of coagulation between droplets. Additionally, lower viscosity increases the likelihood of aggregation in gel preparations (Namburu et al., 2007).

F1B appears clear or transparent due to its small microemulsion droplet size, stability, homogeneity, and negligible light scattering. The PI measurement results in this study demonstrated that all microemulsion base formulations are monodisperse, resulting in a more homogenous microemulsion that can avoid creaming or cracking. Microemulsion base systems have a consistent particle size distribution and are typically more stable (Kamaria et al., 2015). This implies that Tween 80, functioning as a surfactant, has created micelles by adhering to the oily phase droplets' surface. By lowering interfacial tension, these micelles can produce a microemulsion with tiny particles (Ashara et al., 2016). Furthermore, as a co-surfactant, PEG 400 has a role in preventing the recombined phase from separating.

Table 7. Particle characterization of BREO microemulgel

Measurement	F1 A	F1B	F1C
Droplet size (nm)	86.2 ± 1.63	21.7 ± 0.19	210.2 ± 2.9
Polydispersity index (PI)	0.67 ± 0.13	0.37 ±0.06	0.38 ± 0.03
Zeta potential (mV)	-19.61 ± 0.29	-31.58 ± 0.30	-31.38 ± 0.50

Zeta potential is one of the parameters used to evaluate the physical stability of materials in a colloidal environment. It is the surface charge of the globules that maintains an optimal distance to prevent coalescence. Due to electrostatic repulsion, zeta potential affects the stability of microemulsion systems. Emulsions with larger zeta potential values are more stable than emulsions with smaller values. The stability of a system can be categorized based on its zeta potential value: good stability (ζ 40-60), somewhat good stability (ζ 30-40), incipient stability (ζ 10-30), or easy coagulation or flocculation (ζ 0-5) (Prakash et al., 2014) The F1B and F1C microemulgels had zeta potential values of -31.58 mV and -31.38 mV, respectively (Table 7). This value indicates that the F1B microemulgel is stable. The zeta potential of the three BREO microemulgels is approximately two to four times more negative than that of the microemulsions. This difference is probably due to Viscolam®›s components, Sodium Polyacryloyldimethyl Taurate, which releases sodium cations and amide protons when dispersed in water. This results in the accumulation of anions on the surface of the microemulgel droplets, causing them to become negatively charged (negative zeta potential).

Figure 5 shows the TEM image of the spherical microemulgel droplets had smooth surfaces, and were fairly uniform in size. TEM analysis (F1A) revealed droplet sizes ranging from 50 to 100 nm. This result was consistent with the particle measurement results obtained using PSA, which showed droplet sizes of 80 nm.

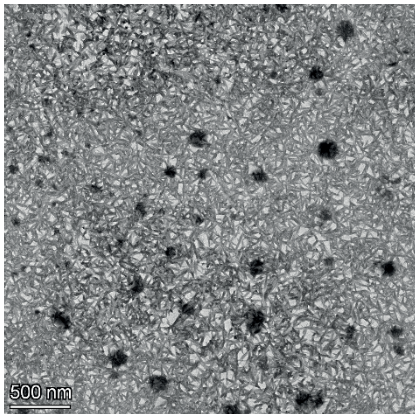


Figure 5. TEM analysis of BREO microemulgel morphology

Antibacterial activity of BREO microemulgel formulations

Figure 6 shows that BREO microemulgel can inhibit the growth of *S. epidermidis*, *S. aureus*, and *P. acnes* bacteria. The negative control, which contained only the gel base and no BREO, did not inhibit all three bacteria. Based on the diameter of the inhibition zone, F1B exhibited the greatest antibacterial activity against all three bacteria, especially against *P. acnes*. The three formulations exhibited similar antibacterial activity against *S. aureus* and *S. epidermidis*. All three formulations contain BREO at the same concentration; the difference lies in the amount of Viscolam added. The amount of Viscolam 8% added does not affect the antibacterial activity of the BREO microemulgels. The significant variation of antibacterial activity is likely due to differences in particle size among the three microemulgels. F1B had the smallest particle size, so it more easily penetrated the cytoplasmic membranes of microorganisms, increasing antimicrobial activity (Monteiro et al., 2023).

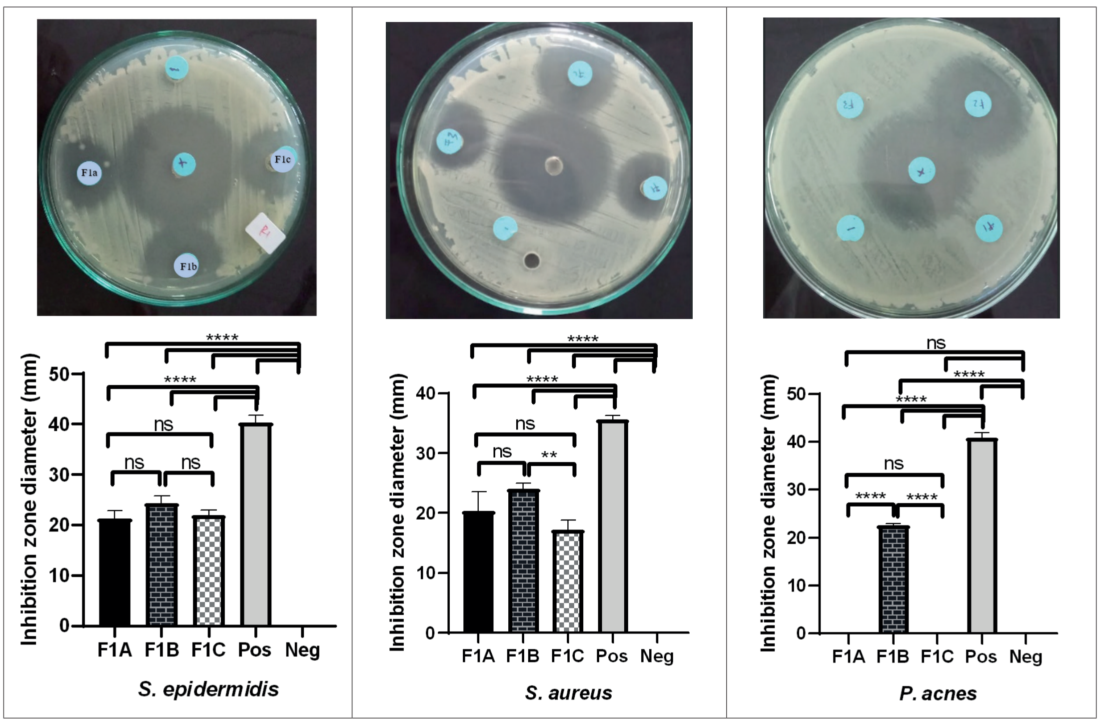


Figure 6. Antibacterial activity of BREO microemulgel against bacteria-related acne (*means significantly different. The asterisk denotes a significant difference (p<0.05), the letter "ns" denotes not significant (p>0.05).

CONCLUSION

The BREO microemulgel can be prepared with 3% BREO, 8% Viscolam, and Tween 80-PEG 400 (25%:25%). Organoleptic characteristics, pH, spreadability, adhesion test, viscosity, physical stability, globule size, polydispersity index, and zeta potential were all found to be best suited for Formula F1B. It was also effective against *P. acnes*, *S. aureus*, and *S. epidermidis*. BREO microemulgel has the potential to be developed as an anti-acne product.

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AUTHOR CONTRIBUTION STATEMENT

SM: Study concept and design, supervision, manuscript drafting and review. ANWS: Data collection, statistical analysis and manuscript review. ED: Technical support and manuscript review. NAE: Literature collection and manuscript review.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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