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Research Article

Formulation and Evaluation of Soursop (*Annona muricata*) Leaf Extract Nanoemulgel Against *Propionibacterium acnes*

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Abstract

Annona muricata (soursop) leaves are rich in antimicrobial compounds such as flavonoids, alkaloids, tannins, saponins, and phenols. This study aimed to develop a nanoemulgel formulation incorporating *A. muricata* leaf ethanol extract to enhance its efficacy against *Propionibacterium acnes*, a bacterium associated with acne vulgaris. Four nanoemulgel formulations containing varying concentrations of the extract (0%, 0.5%, 0.7%, and 1%) were prepared and evaluated for their physical properties (organoleptic, homogeneity, pH, spreadability, and viscosity) and stability through freeze-thaw cycles. The formulation with the highest extract concentration (Formula III) was selected for further characterization (particle size, morphology, and zeta potential) and antimicrobial testing against *P. acnes*. All formulations met the established physical property and stability criteria. Formula III exhibited a particle size of 20.5 nm and a zeta potential of 9.8 mV, indicating a stable nanoemulsion with well-dispersed particles. Antimicrobial testing revealed that Formula III demonstrated a strong inhibitory effect against *P. acnes*, with an average inhibition zone of 19.00 mm. These findings suggest that *A. muricata* leaf extract-loaded nanoemulgel has the potential to be a promising topical formulation for acne treatment. Further research is warranted to optimize the formulation and evaluate its efficacy in clinical settings.

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INTRODUCTION

Acne vulgaris is a common skin condition characterized by the inflammation of pilosebaceous units. *Propionibacterium acnes* plays a crucial role in the pathogenesis of acne by exacerbating inflammation within the hair follicles[1]. Conventional acne treatments often rely on topical formulations containing synthetic antibacterial agents, such as benzoyl peroxide, retinoids, and antibiotics. However, these agents can induce adverse side effects, including erythema, skin peeling, dryness, and burning sensations[2]. Consequently, there is a growing interest in exploring alternative therapeutic approaches utilizing natural ingredients. Natural remedies offer the potential for safer and more tolerable long-term acne management due to their generally lower risk of side effects[3].

Soursop (*Annona muricata*) has been traditionally recognized for its potential in treating acne. Previous studies have demonstrated that *A. muricata* leaves possess valuable pharmacological properties, including anti-inflammatory, antioxidant, and antibacterial activities[4]. Phytochemical analysis of *A. muricata* leaf ethanol extracts has revealed the presence of bioactive compounds such as flavonoids, saponins, tannins, and alkaloids, which are known to exhibit antibacterial properties[5-7]. Furthermore, the presence of phenols in these extracts contributes to their antioxidant activity[8]. *In vitro* studies

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have confirmed the antibacterial efficacy of *A. muricata* leaf ethanol extract against *P. acnes*, a key bacterium implicated in acne vulgaris, with significant zones of inhibition observed at 1% and 5% concentrations[9].

However, direct topical application of crude extracts can be challenging due to their often-greasy texture and potential for skin irritation. To overcome these limitations, formulating the extract into a nanoemulgel offers several advantages[10]. Nanoemulgels are characterized by their non-greasy nature, ease of spreading, water-solubility, enhanced stability, and transparent appearance, making them more cosmetically appealing and potentially more effective than conventional ointments and creams for topical delivery[11].

Nanoemulgels, colloidal systems comprising nanoemulsions incorporated within a gel matrix, offer a promising approach for transdermal drug delivery. Characterized by small droplet sizes typically ranging from 20 to 200 nm, nanoemulgels exhibit enhanced skin permeation and improved drug absorption compared to conventional emulsions[12,13]. However, a common limitation of nanoemulsions is their low viscosity, which can hinder their spreadability and stability[14]. This study aimed to address this challenge by formulating nanoemulgels incorporating *A. muricata* leaf extract. Four formulations were prepared, containing 0%, 0.5%, 0.7%, and 1% (w/w) of *A. muricata* leaf extract, respectively. These formulations were then characterized and evaluated for their antibacterial activity against *P. acnes*, a bacterium implicated in acne vulgaris.

MATERIALS AND METHODS

Materials

This study utilized *A. muricata* leaf powder obtained from CV. Lansida Group, Yogyakarta. Other materials employed included 70% ethanol (CV. C'Jaya Q-Mia, Purwokerto), isopropyl myristate (CV. C'Jaya Q-Mia, Purwokerto), surfactants such as Tween 80 and propylene glycol (both from PT. Brataco, Purwokerto), preservatives like methylparaben and propylparaben (both from PT. Brataco, Purwokerto), and a gelling agent, carbopol (CV. C'Jaya Q-Mia, Purwokerto), along with triethanolamine (PT. Brataco, Purwokerto). Paper discs (± 6 mm in diameter) were used for antimicrobial susceptibility testing. The microbiological media used were Mueller Hinton agar (MHA) (Oxoid®) and McFarland standard solution obtained from the Microbiology Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Indonesia. The study also utilized a clinical isolate of *P. acnes* obtained from the collection of the Microbiology Laboratory, Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Indonesia.

Methods

Extraction

One kilogram of dried *A. muricata* leaves was subjected to maceration with 7 L of 70% ethanol (1 : 7 w/v ratio) for 72 hours at room temperature. The mixture was filtered after each 24-hour period, and the residue was re-macerated with fresh 70% ethanol for an additional 24 hours. The combined filtrates were then concentrated using a rotary evaporator under reduced pressure at 40°C to obtain a thick ethanolic extract of *A. muricata* leaves.

Nanoemulgel formulation and preparation

The formulation of *A. muricata* ethanol extract nanoemulgel is outlined in **Table I**. The oil phase was prepared by dissolving isopropyl myristate in Tween 80. Methylparaben and propylparaben were then incorporated into the oil phase and mixed thoroughly. The aqueous phase was prepared by combining propylene glycol with distilled water. Subsequently, the oil and water phases were mixed using a magnetic stirrer at 500 rpm and 50°C for 15 minutes to form a stable nanoemulsion. *Annona muricata* ethanol extract was then added to the nanoemulsion at varying concentrations and the mixture was stirred at 500 rpm and 50°C until homogenous. The resulting nanoemulsion was allowed to stand undisturbed for 24 hours to ensure clarity and the absence of bubbles.

Separately, 25 g of Carbopol 940 was dispersed in distilled water and neutralized with triethanolamine to achieve the desired pH range (as listed in **Table I**). The Carbopol 940 solution was then allowed to swell for 24 hours. Finally, the *A. muricata* ethanol extract-loaded nanoemulsion was incorporated into the swollen Carbopol 940 gel base using a magnetic stirrer at 500 rpm. Distilled water was added to a final volume of 100 mL, and the mixture was stirred until a homogenous gel was obtained.

Table I. Nanoemulsion formula composition.

Materials	Concentration (%)			
	F0	FI	FII	FIII
Annona muricata leaf ethanol extract	0	0.5	0.75	1
Isopropyl myristate	5	5	5	5
Tween 80	22.5	22.5	22.5	22.5
Propylene glycol	22.5	22.5	22.5	22.5
Methylparaben	0.2	0.2	0.2	0.2
Propylparaben	0.01	0.01	0.01	0.01
Triethanolamine	1.5	1.5	1.5	1.5
Carbopol 940	25	25	25	25
Distilled water	ad 100	ad 100	ad 100	ad 100

Physical properties examination

Physical characterization of the nanoemulgel encompassed a comprehensive evaluation of its properties, including organoleptic assessment (color, shape, and odor), homogeneity, pH, viscosity, spreadability, and stability through freeze-thaw cycles. These physical properties were assessed at predetermined time points: day 0, 7, 14, 21, and 28, to monitor any changes over time.

Organoleptic and homogeneity tests: Organoleptic evaluation was conducted to assess the physical characteristics of the nanoemulgel, including color, odor, and shape[15]. Homogeneity testing was performed to ensure the uniform dispersion of the formulation. Homogeneity was determined by visually inspecting a small sample of the nanoemulgel applied to a glass slide. The absence of visible particles, both coarse and fine, under gentle pressure indicated a homogeneous formulation[16,17].

pH test: The pH of the nanoemulgel was determined to ensure its compatibility with the skin. Skin pH typically ranges between 4 and 6[18]. To measure the pH, 2 g of the prepared nanoemulgel were accurately weighed and dispersed in 20 mL of distilled water in a beaker glass. The pH of the resulting solution was then measured using a calibrated pH meter[17].

Viscosity test: The viscosity of the nanoemulgel was determined using a rheometer. A specific volume of nanoemulgel was placed within the measuring chamber, and the viscosity was measured at 30 rpm using spindle 63. This method provides valuable information about the flow properties of the nanoemulgel, which is crucial for its subsequent application and stability[19].

Spreadability test: The spreadability of the nanoemulgel was evaluated to assess its ease of application and distribution on the skin. A quantity of 0.5 g of nanoemulgel was placed on a clean glass slide. A second glass slide was then placed on top and a weight of 150 g was applied for 1 minute. The diameter of the spread nanoemulgel was measured after the weight was removed. This method simulates the pressure exerted during topical application of the formulation to the skin[2].

Freeze and thaw test: The freeze-thaw cycling test was conducted to evaluate the stability of the nanoemulgel during storage and transportation. The nanoemulgel was subjected to six freeze-thaw cycles, each consisting of 48 hours of refrigeration at 4°C followed by 48 hours of incubation at 40°C in an oven. Visual observations were made after each cycle to assess any changes in organoleptic properties such as phase separation, color changes, or viscosity alterations[2].

Nanoemulgel characterization

Based on the results of the physical properties tests, a single formulation of nanoemulgel was selected for further characterization. This selected formulation underwent a comprehensive characterization process to assess its physicochemical properties. Characterization studies included evaluation of morphology, particle size distribution, and zeta potential.

Morphology: Transmission electron microscopy (TEM) was employed to visualize the morphology of the nanoemulgel. This technique enabled the evaluation of crucial parameters such as the sphericity and uniformity of the nanoemulgel particles. By examining TEM images, we aimed to gain insights into the physical characteristics of the formulated nanoemulgel, which are crucial for its stability, drug delivery efficiency, and overall performance[20].

Particle size and zeta potential: Particle size and zeta potential were determined using a particle size analyzer (PSA) to assess the physical stability of the nanoemulgel. This technique provided valuable information on the average particle size and size distribution, crucial parameters for evaluating the stability and potential for *in vivo* performance of the nanoemulgel. Additionally, zeta potential measurements were conducted to assess the surface charge of the nanoemulgel, which significantly influences its colloidal stability and interactions with biological systems[21].

Antibacterial activity test

Mueller Hinton agar media was prepared according to the manufacturer's instructions and poured into Petri dishes. After solidification, the plates were inoculated with a standardized suspension of *P. acnes*. Briefly, a sterile cotton swab was dipped into the bacterial suspension and then evenly swabbed onto the surface of the MHA. The plates were incubated at 37°C for 24 hours to allow bacterial growth.

Sterile paper discs were impregnated with 50 µL of each test solution: 1% clindamycin gel (positive control), Formula 0 (negative control), selected formula, and 1% extract solution prepared in 10% DMSO. The impregnated discs were then placed onto the surface of the inoculated MHA plates. The plates were subsequently incubated at 37°C for 24 hours. The presence of antibacterial activity was determined by measuring the diameter of the zones of inhibition around the discs using vernier calipers. All experiments were conducted in triplicate, with each experiment repeated three times for reproducibility.

Data analysis

Data analysis was performed using GraphPad Prism version 8.0.1 for Windows. All data are presented as mean ± standard deviation (SD) from three independent experiments. Data normality was assessed using the Kolmogorov-Smirnov test. Statistical significance was determined using one-way ANOVA followed by appropriate post-hoc tests for multiple comparisons, such as Tukey's HSD test. For comparisons between two groups, an unpaired t-test was employed. Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Physical properties examination

Organoleptic and homogeneity tests

All *A. muricata* leaf extract nanoemulgel formulations exhibited excellent physical stability throughout the 28-day observation period, with no discernible changes in shape, odor, or color homogeneity. This observation is crucial, as it indicates that the developed formulations meet the essential physical stability criteria. A characteristic *A. muricata* leaf odor was perceptible in all formulations (**Table II**). Furthermore, visual examination revealed that all formulations were free from any coarse or fine particles, indicating a smooth and homogenous texture. These findings suggest that the developed nanoemulgel formulations possess desirable physical properties, making them suitable for potential topical application.

pH test

The pH of the *A. muricata* leaf extract nanoemulgel formulations was determined to be within a narrow range of 4.70 to 5.06. Normality of the data was assessed using the Kolmogorov-Smirnov test, which revealed a normal distribution (p-value 0.019). Subsequent analysis of variance (ANOVA) indicated no statistically significant differences in pH among the various formulations (p-value 0.566) (**Table III**).

Viscosity test

Viscosity measurements were conducted over 28 days of storage, revealing a range of 543.33 to 928.00 cP for the nanoemulgel preparations. **Table IV** summarizes these findings, indicating that Formula 0 and III exhibited greater viscosity stability compared to Formula I and II. Statistical analysis using the Kolmogorov-Smirnov test confirmed that the viscosity data were normally distributed (p-value 0.00). Furthermore, a One-way ANOVA test demonstrated significant differences in viscosity among the four formulations (p-value 0.000; **Table IV**). These findings suggest that the specific formulation and composition of the nanoemulgel significantly influence its viscosity and stability over time.

Table II. The results of nanoemulgel organoleptic observations.

Day - parameters		Formula 0	Formula I	Formula II	Formula III	Information
0	Shape	+	++	++	++	Shape :
	Color	+	++	++	+++	+ : slightly thick
	Odor	K	K	K	K	++ : thick
7	Shape	+	++	++	++	+++ : very thick
	Color	+	++	++	+++	
	Odor	K	K	K	K	Color :
14	Shape	+	++	++	++	+ : colorless
	Color	+	++	++	+++	++ : brown
	Odor	K	K	K	K	+++: dark brown
21	Shape	+	++	++	++	
	Color	+	++	++	+++	Odor :
	Odor	K	K	K	K	K: *A. muricata* leaf ethanol
28	Shape	+	++	++	++	extract
	Color	+	++	++	+++	
	Odor	K	K	K	K	

Table III. The results of nanoemulgel pH observations.

Day	pH of nanoemulgel (Average ± SD)			
	Formula 0	Formula I	Formula II	Formula III
0	5.01 ± 0.00	5.06 ± 0.06	5.00 ± 0.00	5.00 ± 0.00
7	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	4.97 ± 0.06
14	5.00 ± 0.00	4.97 ± 0.06	4.93 ± 0.06	4.90 ± 0.00
21	4.87 ± 0.06	4.80 ± 0.10	4.87 ± 0.06	4.73 ± 0.06
28	4.87 ± 0.05	4.77 ± 0.05	4.77 ± 0.06	4.70 ± 0.00

Table IV. The results of nanoemulgel viscosity observations.

Day	Viscosity of nanoemulgel (cP; average ± SD)			
	Formula 0	Formula I	Formula II	Formula III
0	543.33 ± 2.89	560.67 ± 1.15	566.67 ± 2.31	559.33 ± 1.15
7	546.67 ± 2.31	588.00 ± 0.00	894.67 ± 2.31	568.67 ± 1.15
14	550.00 ± 0.00	604.67 ± 1.15	928.00 ± 0.00	576.00 ± 0.00
21	550.67 ± 2.31	622.67 ± 2.31	928.00 ± 0.00	577.33 ± 2.31
28	546.67 ± 2.31	568.00 ± 8.00	913.33 ± 2.31	577.33 ± 2.31

Spreadability test

The spreadability of the nanoemulgel formulations was evaluated over 28 days of storage and found to range from 6.7 to 8.15 cm. Statistical analysis using the Kolmogorov-Smirnov test revealed that the data were normally distributed (p-value 0.02). Furthermore, the One-way ANOVA test indicated no significant difference in spreadability among the different formulations (p-value 0.551) during the storage period (**Table V**). This suggests that the formulations exhibited consistent spreadability characteristics throughout the storage period, indicating their potential for effective topical application.

Table V. The results of nanoemulgel spreadability observations.

Day	Spreadability of nanoemulgel (cm; average ± SD)			
	Formula 0	Formula I	Formula II	Formula III
0	8.15 ± 0.49	7.60 ± 0.57	7.33 ± 0.67	7.73 ± 0.67
7	8.00 ± 0.57	7.95 ± 0.64	6.70 ± 0.57	7.60 ± 0.57
14	7.60 ± 0.57	7.45 ± 0.64	6.98 ± 0.88	7.65 ± 0.64
21	7.50 ± 0.71	7.20 ± 1.13	7.25 ± 0.92	7.48 ± 0.95
28	7.75 ± 0.49	7.30 ± 1.27	7.30 ± 1.13	7.73 ± 0.74

Freeze and thaw test

Stability studies revealed that all formulations maintained their physical integrity throughout the testing period. No significant changes were observed in color, odor, or shape, and no phase separation occurred. These observations suggest that all materials were compatible and that the formulations were stable under various storage conditions, including low temperatures, room temperature, and elevated temperatures.

Nanoemulgel characterization

Based on a comprehensive evaluation of organoleptic properties (shape, odor, color), homogeneity, pH, viscosity, spreadability, and freeze-thaw stability, Formula III was selected for further characterization. This selection was based on its

superior overall performance across these parameters. Subsequent characterization studies included an assessment of the formulation's morphology, particle size distribution, and zeta potential.

Morphology

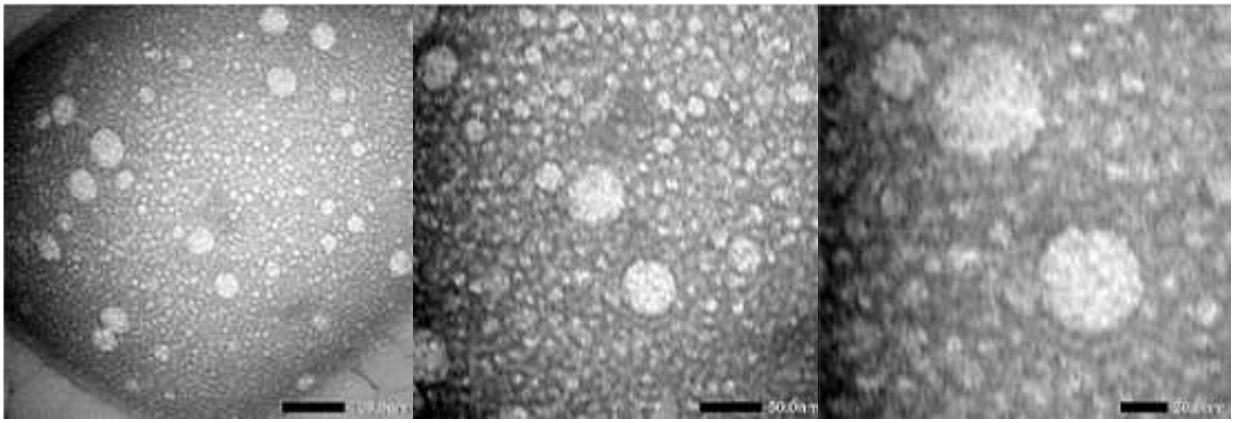
Transmission electron microscopy analysis revealed that the selected nanoemulgel exhibited a uniform dispersion of spherical globules. Notably, no signs of coalescence were observed (**Figure 1**).

Particle size and particle size distribution

The characterization of the developed nanoemulsion revealed a mean particle size of 20.5 nm with a polydispersity index (PDI) of 0.446 (**Figure 2**). This particle size falls within the desirable range for nanoemulsions, typically considered to be between 20 and 200 nm[12]. A narrow particle size distribution, as indicated by the relatively low PDI value, is crucial for achieving optimal stability and enhancing the bioavailability of developed nanoemulsion.

Zeta potential

Zeta potential measurements were conducted to assess the electrostatic stability of the developed nanoemulsion. Zeta potential reflects the surface charge of particles and plays a crucial role in determining the stability of colloidal systems. A high absolute value of zeta potential, typically above (+/-) 30 mV, is generally considered indicative of good colloidal stability, minimizing the risk of particle aggregation[22,23]. In this study, the zeta potential of the nanoemulsion was determined to be -9.8 mV, suggesting moderate electrostatic stability (**Figure 3**). While this value may not fall within the generally accepted range for high stability, the observed stability of the nanoemulsion (as evident in morphology and particle size analysis) indicates that other stabilization mechanisms, such as steric stabilization, may be contributing to the overall stability of the system.



a **b** **c**

Figure 1. Observation of nanoemulgel with TEM at (**a**) 20x, (**b**) 50x, and (**c**) 100x magnification.

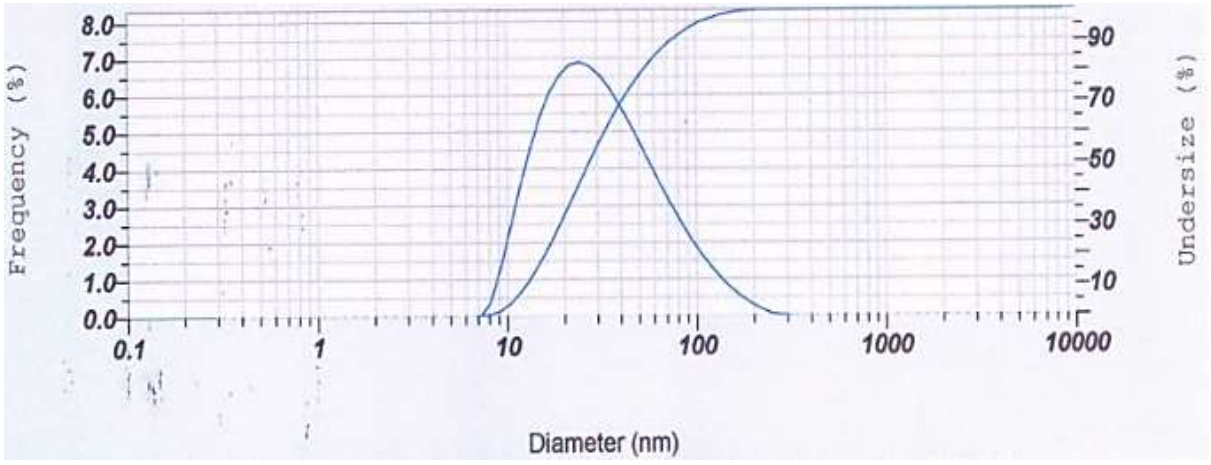


Figure 2. Nanoemulgel particle size measurement.

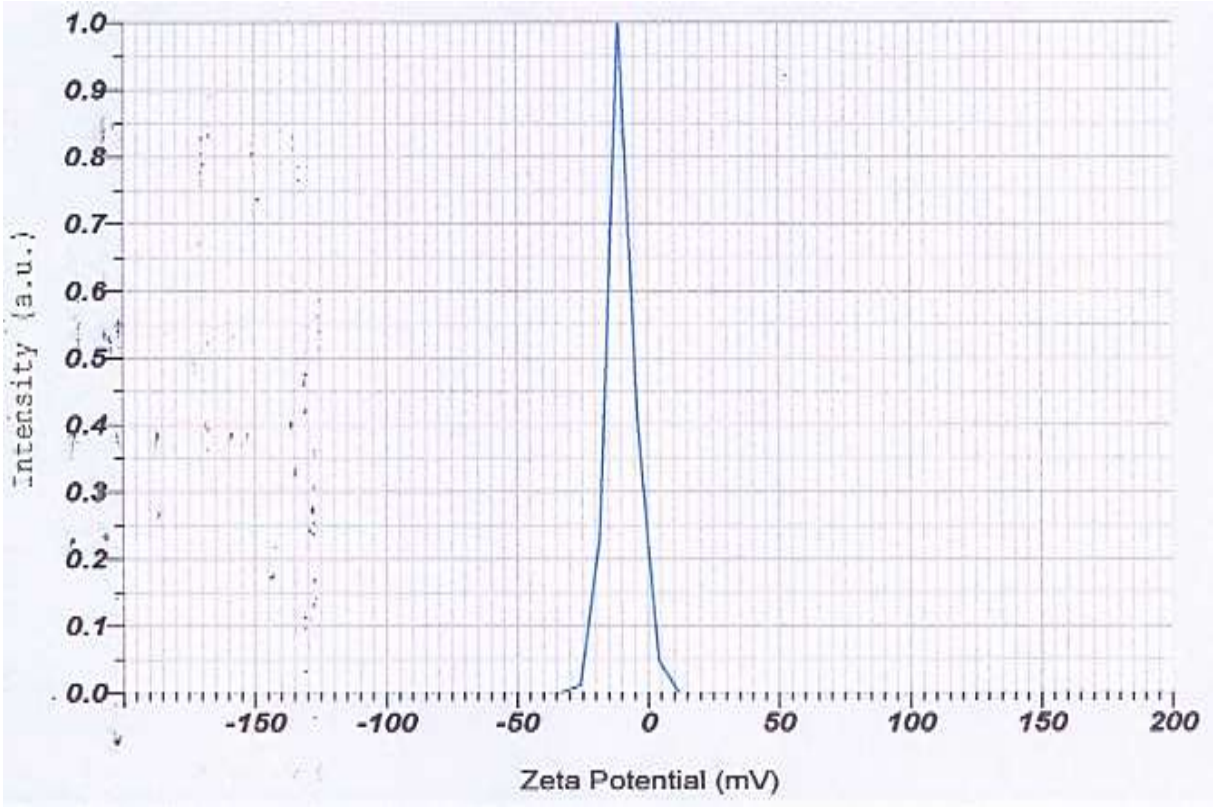
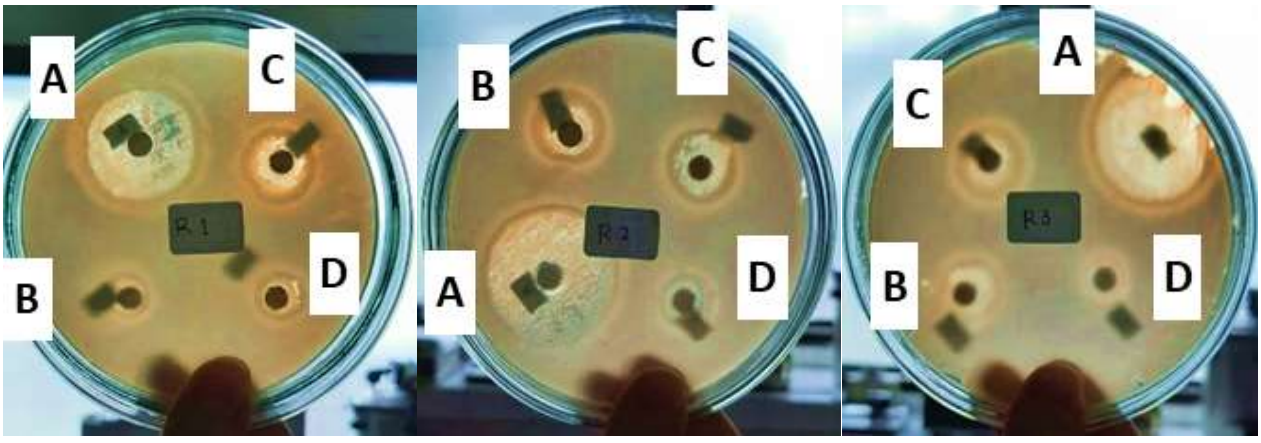


Figure 3. Nanoemulgel zeta potential.

Antibacterial activity test

The antibacterial activity of the selected formulation (Formula III) against *P. acnes* was evaluated and compared to a clindamycin gel (positive control), a negative control (base formulation without active ingredients), and a 1% *A. muricata* leaf ethanol extract solution in 10% DMSO (**Figure 4**). The results of the zone of inhibition tests, depicted in **Figure 5**, demonstrated that Formula III exhibited significant antibacterial activity against *P. acnes* with an average inhibition zone diameter of 19.00 ± 2.65 mm. This value was significantly higher ($p < 0.05$) than that observed for the negative control and the 1% extract solution. However, the antibacterial activity of Formula III was significantly lower ($p < 0.01$) than that of the clindamycin gel, which exhibited an average inhibition zone diameter of 29.33 ± 2.31 mm.



a **b** **c**

Figure 4. Antibacterial test result using disc diffusion method against *P. acnes* in triplicate (**a**: replication 1 or R1; **b**: R2; and **c**: R3). Clindamycin was used as a positive control (A) and Formula 0 as a negative control (B). Nanoemulgel formula III was selected as a sample test (C), while 1% *A. muricata* leaf ethanol extract solution in 10% DMSO was used to examine the influence of other components in the working formula (D).

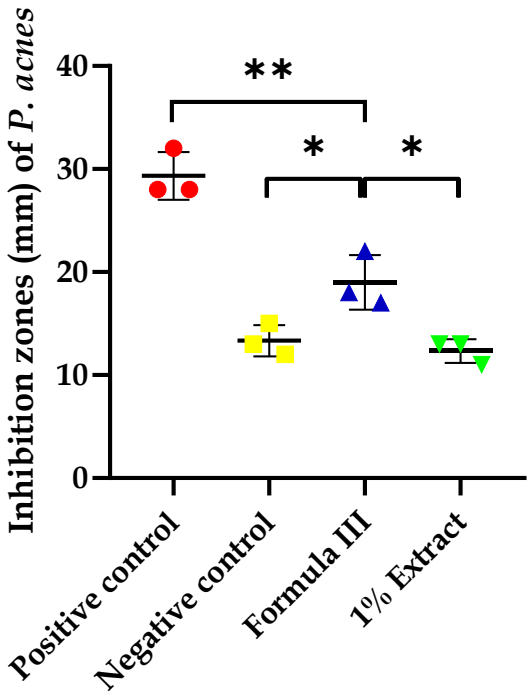


Figure 5. Inhibition zones as results of antibacterial test using disc diffusion method against *P. acnes* in triplicate. Clindamycin was used as a positive control and Formula 0 as a negative control. Data are presented as means ± SD (mm). An unpaired t-test was used for two-group comparisons. *p <0.05, **p <0.01.

Previous studies have demonstrated the potential of *A. muricata* leaf extracts in treating acne due to their inherent antibacterial and antioxidant properties[5-8]. However, direct topical application of crude leaf extracts can be challenging due to poor skin penetration and potential for irritation. To overcome these limitations, this study investigated the development of a nanoemulgel formulation to enhance the delivery and efficacy of *A. muricata* leaf extract. The combination of nanoemulsion and gel was chosen to leverage the advantages of both systems. Nanoemulsions offer enhanced skin penetration and improved drug delivery, while the gel matrix provides stability, viscosity, and improved spreadability.

All developed formulations exhibited acceptable spreadability, with no significant differences observed between them. However, significant variations in viscosity were observed among the formulations (**Tables III** and **IV**), likely influenced by the specific formulation components and their concentrations. All formulations demonstrated excellent physical stability, maintaining homogeneity and stability throughout 28 days of storage and freeze-thaw cycling. Minor color variations were observed among the formulations, which can be attributed to the varying concentrations of the plant extract (**Table I**).

The pH of all nanoemulgel formulations fell within the acceptable range for topical application (pH 4-6)[17]. This slight decrease in pH compared to the extract itself (pH 5.96-6.08)[24] is likely attributed to the use of Carbopol 940, an acidic gelling agent. To adjust the pH, the addition of TEA can be considered.

The zeta potential of the nanoemulgel was measured at -9.8 mV, indicating good stability. This negative surface charge effectively prevented particle aggregation, as confirmed by the physical stability evaluation over 28 days of storage. The successful formation of the nanoemulsion can be attributed to the high concentrations of surfactants and co-surfactants employed, which significantly reduced interfacial tension and facilitated the formation and stabilization of nanodroplets[25].

Formula III was selected for further characterization based on its superior physical stability, particularly its viscosity. As depicted in **Figures 1** and **2**, the optimized nanoemulgel exhibited a spherical morphology with an average particle size of 20.5 nm, a characteristic that is favorable for enhanced skin penetration. While a dedicated permeation study was not conducted in this investigation, the nanoemulgel demonstrated significant antibacterial activity against *P. acnes* in the effectivity testing. This activity can be attributed to the synergistic effects of several factors. Firstly, *A. muricata* leaf ethanol extract, rich in flavonoids, alkaloids, tannins, saponins, and phenols, possesses intrinsic antibacterial properties. These phytochemicals exert their antimicrobial effects through various mechanisms, including disruption of bacterial cell membranes (flavonoids), inhibition of enzyme production and cell wall synthesis (tannins), and interference with vital

cellular processes such as DNA replication (alkaloids)[5,6,8,26]. Secondly, the inclusion of methylparaben and propylparaben in the formulation further enhances its antimicrobial activity[27]. Consequently, Formula III exhibited larger inhibition zones compared to the extract solution alone (**Figure 4**). As reported by Sheskey *et al*.[28], the presence of parabens can significantly enhance the antimicrobial efficacy of formulations within the pH range of 4-8, which is relevant for topical applications. While the negative control, lacking active ingredients from the plant extract, still exhibited some antimicrobial activity due to the presence of the parabens, the positive control (clindamycin gel) demonstrated its expected inhibitory effect by interfering with bacterial protein synthesis through binding to the 50S ribosomal subunit[29].

CONCLUSION

In this study, the concentration of ethanolic extract of *A. muricata* leaves influences physical properties, including organoleptic, pH, viscosity, and spreadability. However, it did not affect freeze and thaw as well as the homogeneity of nanoemulgel. The selected nanoemulgel formula, Formula III with 1% extract content, was effective against *P. acnes* with an inhibition zone of 19.00±2.65 mm.

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AUTHORS' CONTRIBUTION

Conceptualization: Nabila Ikramına, Rehana, Dhadhang Wahyu Kurnıawan
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Funding acquisition: -
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Methodology: Nabila Ikramına, Rehana, Dhadhang Wahyu Kurnıawan
Project administration: Nabila Ikramına, Rehana
Resources: Nabila Ikramına, Rehana, Dhadhang Wahyu Kurnıawan
Software: -
Supervision: Dhadhang Wahyu Kurnıawan
Validation: Rahmad Aji Prasetya, Dhadhang Wahyu Kurnıawan
Visualization: Rahmad Aji Prasetya, Dhadhang Wahyu Kurnıawan
Writing - original draft: Nabila Ikramına, Rehana
Writing - review & editing: Rahmad Aji Prasetya, Dhadhang Wahyu Kurnıawan

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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