

# Formulation and antibacterial activity of black mulberry fruit (*Morus nigra* L.) extract cream against cutibacterium acnes and staphylococcus epidermidis

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## ABSTRACT

Acne vulgaris is an inflammatory disorder of the pilosebaceous unit characterized by sebum overproduction, abnormal keratinization, microbial proliferation, and inflammation, with *Propionibacterium acnes* and *Staphylococcus epidermidis* as key contributing bacteria. To reduce the side effects associated with conventional acne therapies, natural ingredients have been explored as alternative approaches. Black mulberry (*Morus nigra*) fruit is one such natural source known to contain bioactive compounds, including flavonoids, tannins, and terpenoids, which possess antibacterial properties. This study aimed to develop and evaluate a topical cream formulation containing ethanolic extract of black mulberry fruit and to assess its antibacterial activity and physicochemical stability. The extract was obtained through 96% ethanol maceration and formulated into creams at concentrations of 1.25%, 2.5%, and 5%. Antibacterial activity was evaluated using the agar well diffusion method, while cream quality was assessed through organoleptic properties, homogeneity, pH, viscosity, spreadability, and centrifugation stability. The results showed that the extract exhibited strong antibacterial activity against both tested bacteria. All concentrations demonstrated inhibition zones categorized as strong, with a concentration-dependent increase in activity. At 1.25% inhibition zones against *P. acnes* were  $11.7 \pm 0.25$  m, while against *S. epidermidis* were  $11.9 \pm 0.20$  mm at day 28. All formulations showed acceptable physicochemical characteristics and remained stable during 28 days of storage. The novelty of this study lies in the development of a stable topical cream formulation of *M. nigra* extract with confirmed antibacterial activity, providing a potential plant-based alternative for anti-acne therapy.

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## INTRODUCTION

Acne represents one of the most prevalent dermatological conditions among Indonesian adolescents. This disorder can lead to significant psychological effects, including depression, anxiety, and reduced self-esteem. Epidemiological studies reveal a peak prevalence of 80–85% in females aged 15–18 years, mainly attributed to heightened sebaceous gland activity resulting in excessive sebum production. The accumulation of sebum combined with corneocytes causes obstruction of the pilosebaceous unit, leading to the development of various lesion types. This process is often worsened by bacterial infections, particularly from *Propionibacterium acnes* and *Staphylococcus epidermidis*, which amplify the skin's inflammatory response. Potential complications encompass scarring and post-inflammatory hyperpigmentation, frequently necessitating extended therapeutic management to improve skin health (Lynn et al., 2016; Vasam et al., 2023).

Standard regimens for acne treatment frequently include antibiotics (e.g., clindamycin, tetracycline, erythromycin) aimed at suppressing bacterial proliferation. Nevertheless, indiscriminate antibiotic administration poses a substantial risk of inducing antimicrobial resistance. Furthermore, topical therapeutics incorporating benzoyl peroxide or salicylic acid are prone to eliciting cutaneous irritation as an adverse effect (Vasam et al., 2023). Due to these limitations, the development of plant-based extracts with antibacterial activity has emerged as an alternative approach. Black mulberry (*Morus nigra* L.) fruit has potential as a natural anti-acne agent due to its high content of bioactive compounds. Its phenolic content is reported to be higher than that of other *Morus* species, making it more effective (Ferlinhayati et al., 2012.; Wutsqo & Budiman, 2018).

Previous research by Budiman et al., (2017) reported that the 96% ethanolic extract of black mulberry fruit exhibited significant minimum inhibitory concentration (MIC) values against acne-causing bacteria, with a value of 2.5% against *P. acnes* and *S. epidermidis*. Based on these findings, a topical cream formulation containing black mulberry (*Morus nigra*) fruit extract was developed to evaluate its antibacterial activity. Although *Morus nigra* extract has demonstrated antibacterial activity against *P. acnes* and *S. epidermidis*, studies focusing on its development into a stable and pharmaceutically applicable anti-acne cream formulation remain limited. (Budiman et al., 2017).. Most previous research has been restricted to in vitro antibacterial testing without further exploration into topical dosage forms suitable for clinical or cosmetic use. In addition, there is still limited research evaluating the relationship between the adhesive characteristics of cream formulations and the enhanced antibacterial effectiveness of black mulberry extract against acne-causing bacteria.

The selection of a cream dosage form was based on its advantages for topical application, particularly its strong adhesion to the skin surface. This high adhesiveness helps ensure that the extract is not easily removed by friction from clothing or physical activity, thereby prolonging its residence time on the skin and providing sufficient contact time for penetration into the skin tissue. In addition, cream exhibits higher adhesive properties than gel, allowing it to remain longer on the skin, resist mechanical removal, and enhance the interaction between the active compound and the target site. This extended contact time may improve drug penetration and antibacterial effectiveness. Furthermore, cream has more emollient properties that help maintain skin moisture, which is often disrupted in acne conditions (Febriyanti et al., 2023; Nurjanah & Rahmawati, 2019; Sirajul Firdaus et al., 2024). Therefore, this study aims to develop a stable topical cream formulation containing *Morus nigra* L. fruit extract and to evaluate its antibacterial activity against acne-causing bacteria, as a potential alternative for topical anti-acne therapy.

## RESEARCH METHOD

### Tool

The instruments used included a water bath, autoclave (My Life®), 5 mm perforator, incubator (Mettler), Laminar Air Flow (ERSA Scientific), rotary evaporator (IKA®), viscometer (Brookfield), pH meter (Mettler Toledo), homogenizer, maceration apparatus, and various laboratory glassware required for screening and formulation processes.

### Material

The materials used in this study included black mulberry fruit obtained from the Cibodas Mulberry Plantation, Lembang; reagents for phytochemical screening; stearic acid, liquid paraffin, cetyl alcohol, propyl paraben, methyl paraben, glycerin, triethanolamine (TEA), 96% ethanol, and distilled water (PT. Dwilab). Nutrient agar (PT. Dwilab), clindamycin, and test bacteria *P. acnes* and *S. epidermidis* were obtained from Universitas Padjadjaran, Jatinangor, Bandung.

### Characterization of Simplicia

Characterization of the simplicia involved comprehensive evaluations, including determination of moisture content, total ash value, water-soluble extractive value, and ethanol-soluble extractive value, in accordance with established pharmacopoeial standards to ensure quality and standardization of the raw material (Dzulfadhli Utomo et al., 2022).

### Extraction of Black Mulberry Fruit

Maceration was utilized in this investigation for 3 × 24 hours to isolate bioactive compounds. Specifically, 500 g of simplicia was introduced into an appropriate vessel and immersed in 96% ethanol, ensuring the solvent level remained approximately 2.5 cm above the material surface during extraction. The resultant mixture was filtered through Whatman filter paper to yield the filtrate, which was then concentrated via rotary evaporation at 45–50°C to obtain a viscous extract. Finally, the extraction yield was quantified to ascertain the percentage of extract recovered (Yanti et al., 2024).

### Phytochemical Screening

Phytochemical screening was conducted on both simplicia and extract to identify the presence of secondary metabolites, including saponins, quinones, steroids and triterpenoids, monoterpenes and sesquiterpenes, polyphenols, tannins, flavonoids, and alkaloids (Dzulfadhli Utomo et al., 2022).

### Antibacterial Activity Test of Black Mulberry (*Morus nigra*) Fruit Extract

The study employed an experimental laboratory design using the agar well diffusion method to evaluate the antibacterial activity of black mulberry (*Morus nigra* L.) fruit extract in cream formulation. Bacterial isolates were initially revitalized on nutrient agar slants and incubated at 37°C for 18 hours. Subsequently, the cultures were resuspended in 0.9% NaCl solution, with turbidity standardized to 25% transmittance at 580 nm via spectrophotometry. A 50 µL aliquot of the standardized suspension was uniformly plated onto agar surfaces, overlaid with 20 mL sterile nutrient agar, and allowed to solidify. Five 5 mm-diameter wells were then created using a sterile borer. Wells were filled with 50 µL of extract solutions at concentrations of 1.25%, 2.5%, and 5%. Clindamycin functioned as the positive control, while sterile distilled water served as the negative control. Plates were incubated at 37°C for 18 hours, after which zones of inhibition were measured (Devin Suwandi et al., 2023; Febriyanti et al., 2023). The experiment was performed in triplicate (n=3).

### Preparation of Black Mulberry Cream

Cream formulations were developed incorporating ethanolic extract of black mulberry fruit at three varying concentrations, with a base cream lacking extract serving as the control. The oil phase comprising stearic acid, cetyl alcohol, liquid paraffin, and propyl paraben was

liquefied in a water bath at 70–75°C. Concurrently, the aqueous phase (triethanolamine [TEA], glycerin, methyl paraben, and distilled water) was heated to the identical temperature

**Table 1.** Formulation of black mulberry (*morus nigra* l.) fruit extract cream

Ingredients	Formula (% b/v)			
	F0	F1	F2	F3
Black Mulberry Fruit Extract	0	1,25	2,5	5
Stearic Acid	8	8	8	8
Cetyl Alcohol	2	2	2	2
Liquid Paraffin	6	6	6	6
Triethanolamine	1	1	1	1
Glycerin	15	15	15	15
Methyl Paraben	0,1	0,1	0,1	0,1
Propyl Paraben	0,05	0,05	0,05	0,05
Distilled Water	ad 100	ad 100	ad 100	ad 100

The aqueous phase was progressively integrated into the oil phase under continuous agitation to yield a homogeneous emulsion. The extract was then methodically incorporated and blended to uniformity. The final creams were packaged suitably and evaluated for physicochemical properties. (Dzulfadhli Utomo et al., 2022; Pratasik et al., 2019; Zam Zam & Musdalifah, 2022).

#### Evaluation of Physical Properties of the Cream

All physical evaluations were repeated on days 0, 7, 14, 21, and 28 during storage. Organoleptic evaluation of the cream formulations entailed sensory assessment of color, odor, and texture. Homogeneity was determined by dispersing 1 g of cream between glass slides, spreading uniformly, and inspecting visually for consistency. pH values were quantified by diluting 1 g cream in distilled water to 10 mL, followed by measurement with a calibrated pH meter. Viscosity was assessed employing a Brookfield viscometer with spindle number 64 at a speed of 6 rpm. (Nurleni et al., 2022). Spreadability was evaluated by applying 0.5 g of cream onto a glass plate and subjecting it to incremental loads of 50 g, 100 g, and 200 g for 1 minute each, while measuring the resultant spread diameter (Devin Suwandi et al., 2022). Physical stability was further assessed via centrifugation test, wherein 5 g of cream was placed in a tube and spun at 5000-10000 rpm for 30 minutes (Pratasik et al., 2019).

#### Antibacterial Activity Test of Black Mulberry Cream

Antibacterial efficacy of cream formulations (F0, F1, F2, F3) was assessed using the well diffusion assay. A 50 µL standardized bacterial suspension was evenly plated on Petri dishes, overlaid with 20 mL nutrient agar, and allowed to solidify. Five wells of 5 mm diameter were created using a sterile borer. Each formulation (50 µL) was introduced into the wells, with F0 as negative control and clindamycin as positive control. Plates were incubated at 37°C for 18 hours, followed by measurement of inhibition zones (Asthyanda & Bakri, 2024; Devin Suwandi et al., 2023; Febriyanti et al., 2023). The experiment was performed in triplicate (n=3).

## RESULTS AND DISCUSSIONS

The preparation of simplisia was carried out systematically. The initial weight of raw material after wet sorting was 7.17 kg. After drying, 708.15 g of dried simplisia was obtained. From 500 g of processed simplisia, 152.93 g of thick ethanolic extract was produced. The characterization results in table 1 showed a drying shrinkage <10%, indicating low moisture and volatile compound content, which supports storage stability. The moisture content <10% reflects optimal drying and resistance to microbial contamination (Rusmawati et al., 2021). The ash content of 5.4% indicates moderate mineral content and good purity of the simplisia. Extractive value determination reflects the amount of bioactive compounds present (Veninda et al., 2023). The results showed water-

soluble extractive value of 40.3% and ethanol-soluble extractive value of 40%. The relatively comparable values, with a slightly higher tendency in the aqueous solvent, indicate the predominance of polar compounds in the simplisia. Overall, the characterization results indicate that the simplisia is of good quality and suitable for use. The extraction yield of ethanolic extract was 30.586% (152.93 g from 500 g simplisia), indicating the effectiveness of the maceration process. Ethanol was an appropriate solvent due to its compatibility with phenolic compounds, allowing optimal extraction of bioactive constituents (Budiman et al., 2017; Ferlinhayati et al., 2012).

**Table 2.** Characterization of black mulberry fruit simplisia

Characterization	Result (%)
Loss on drying	0.5
Moisture content	4
Total ash content	5.4
Water-soluble extractive	40.3
Ethanol-soluble extractive	40

**Table 3.** Phytochemical screening results

Sample	Alkaloids	Flavanoids	Tannins	Phenols	Quinones	Monoterpens and sesquiterpens	Steroids and Triterpenoids	Saponins
Simplisia	-	+	+	+	-	+	-	-
Ekstrak	-	+	+	+	-	+	-	-

**Note:**

Detected = (+)

Not detected = (-)

Post-phytochemical screening, which revealed the presence of bioactive compounds with potential antibacterial properties, the black mulberry (*Morus nigra*) fruit extract was further evaluated for its antibacterial activity. Sterile distilled water was used as the negative control, while clindamycin served as the positive control. As a broad-spectrum lincosamide antibiotic, clindamycin is effective against anaerobic and Gram-positive aerobic bacteria, exhibiting both bacteriostatic and bactericidal mechanisms. This is particularly relevant, as both *Propionibacterium acnes* and *Staphylococcus epidermidis* are Gram-positive bacteria associated with acne infection (Rana et al., 2024).

**Table 4.** Antibacterial activity results of black mulberry fruit extract against *p. acnes* and *s. epidermidis*

Sample	Concentration (%)	Inhibition Zone (mm)	
		<i>Propionibacterium acnes</i>	<i>Staphylococcus epidermidis</i>
Black mulberry extract	7.5	23.4 ± 0.26	29.5 ± 2.00
	5	21.4 ± 0.35	25.9 ± 3.37
	2.5	17.9 ± 0.71	19.8 ± 1.55
	1.25	12.0 ± 0.90	12.8 ± 0.59
Positive control	10	27.8	19.4
Negative control	-	-	-

**Note:**

Positive control = Clindamycin

Negative control = Sterile distilled water

(+) = Inhibition zone observed

(-) = No inhibition zone observed

Table 4 shows the antibacterial activity of the extract at 1.25% against *P. acnes* (inhibition zone: 12.0 ± 0.90 mm) and *S. epidermidis* (12.8 ± 0.59 mm), which serves as a reference for determining the optimal extract concentration in cream formulations. Based on inhibition zone criteria, black mulberry fruit extract exhibited very strong antibacterial activity at concentrations of 7.5% and 5%, and strong activity at 2.5% and 1.25%. The positive control (clindamycin) exhibited superior inhibition against both pathogens, underscoring its greater potency versus *P. acnes*,

whereas the extract proved more effective against *S. epidermidis*. The negative control (sterile distilled water) yielded no zones, verifying that antibacterial effects were exclusively attributable to the extract (Sarah Tumundo et al., 2024).

The ethanolic extract of black mulberry fruit effectively suppresses acne-associated bacteria, including *S. epidermidis* and *P. acnes*, primarily through bioactive constituents such as flavonoids, polyphenols, and tannins. Flavonoids disrupt bacterial proliferation by hydrogen bonding with cell wall proteins, inducing structural destabilization. Phenolics exert effects by denaturing cellular proteins, whereas tannins compromise cell wall integrity, culminating in cellular damage and lysis (Budiman et al., 2017; Wutsqo & Budiman, 2018). Subsequent to confirming the extract's antibacterial efficacy, cream formulations were developed incorporating extract at 1.25%, 2.5%, and 5%, alongside a control base. These were rigorously assessed for physicochemical attributes encompassing organoleptic properties, homogeneity, viscosity, pH, spreadability, and centrifugation stability over 28 days of storage.

Based on Budiman et al., (2017) antibacterial testing of black mulberry fruit extract was conducted starting from a 10% concentration. The results showed that at 10%, the extract produced an inhibition zone of  $6.50 \pm 2.19$  mm against *S. epidermidis* and showed no activity against *P. acnes*. In contrast, in the present study, concentrations below 10% still demonstrated strong antibacterial activity, with larger inhibition zones compared to those reported by Budiman et al., (2017). This indicates that the black mulberry extract in this study exhibits more effective antibacterial potential at lower concentrations.

**Table 5.** Evaluation results of black mulberry (*Morus nigra* L.) extract cream

Parameters	Formula							
	Day 1 Evaluation				Day 28 Evaluation			
	F0	F1	F2	F3	F0	F1	F2	F3
Organoleptic	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid	semi-solid
Consistency	White	Cream	Brown	White	Cream	Brown	White	Cream
Color	White	Cream	Brown	White	Cream	Brown	White	Cream
Odor	Base	Typical mulberry	Typical mulberry	Typical mulberry	Typical mulberry	Typical mulberry	Typical mulberry	Typical mulberry
Homogeneity	Crean	mulberry	mulberry	mulberry	mulberry	mulberry	mulberry	mulberry
pH	H	H	H	H	H	H	H	H
Viscosity (cP)	6.24	6.11	5.84	5.71	6.24	6.07	5.81	5.64
Spreadability (cm)	46000	39000	31000	25000	44000	34000	28000	20000
Centrifugation	4,63	5,12	5,31	5,52	4,71	5,19	5,39	5,62
Centrifugation	-	-	-	-	-	-	-	-

Note:

- H = Homogeneous
- (+) = Phase separation observed
- (-) = No phase separation observed

Based on Table 5, no changes were observed in all formulations in terms of color, form, and odor during storage up to day 28. This indicates that all cream formulations met the required quality standards, as a good cream should maintain stable color and odor without significant changes. The color of the cream became darker brown with increasing extract concentration, which is consistent with the natural reddish-brown color of black mulberry extract. Homogeneity testing showed that all formulations were uniform, with no coarse particles observed throughout the storage period. This confirms that the formulations met quality standards, as a good cream should exhibit high homogeneity with uniform mixing of oil and water phases and no visible lumps (Nurleni et al., 2022).

pH assessment aimed to verify compatibility with skin physiological pH, promoting user safety and comfort. Findings indicated a progressive pH decline over storage in Table 5, attributable to the lack of buffering agents. Nonetheless, all formulations sustained values within

the optimal dermatological range of 4.5–6.5 (Lukić et al., 2021; Pratasik et al., 2019). Higher extract concentrations resulted in lower pH values, which may influence antibacterial activity. Additionally, skin pH below 6.0 is known to help suppress inflammation and support skin barrier recovery (Aspadih et al., 2024).

Viscosity profiling across all formulations revealed a consistent decline throughout the storage duration. Notwithstanding this reduction, values persisted within the pharmacologically acceptable limits of 2,000–50,000 cP (Dominica et al., 2023). Notably, elevated extract concentrations correlated with diminished viscosity, thereby enhancing spreadability and facilitating improved dermal application.

Spreadability testing showed that all formulations exhibited increased spreadability over time, likely due to the reduction in viscosity. Adequate spreadability facilitates ease of application and enhances drug diffusion across the skin. The spread diameter values were within acceptable limits, indicating good application properties (Datak et al., 2016; Dzulfadhli Utomo et al., 2022).

Centrifugation stability assays confirmed physical integrity across all formulations, exhibiting no phase separation post-centrifugation at 5000 rpm for 30 minutes. These results underscore the robust physical stability of the creams, demonstrating resilience to gravitational forces under accelerated storage conditions (Kurniawati et al., 2025). Overall, cream formulations containing black mulberry extract exhibited good physical stability and met the required quality parameters, making them suitable for topical application.

**Table 6.** Antibacterial activity test of black mulberry fruit extract cream

Sample	Inhibition Zone (mm) <i>Propionibacterium acnes</i>		Inhibition Zone (mm) <i>Staphylococcus epidermidis</i>	
	Day 1 Evaluation	Day 28 Evaluation	Day 1 Evaluation	Day 28 Evaluation
F0	-	-	-	-
F1	12.2 ± 0.20	11.7 ± 0.25	12.5 ± 0.10	11.9 ± 0.20
F2	13.5 ± 0.12	12.8 ± 0.20	14.7 ± 0.12	14.3 ± 0.15
F3	15.4 ± 0.10	15.1 ± 0.36	16.6 ± 0.20	15.8 ± 0.20
Positive control	24.5	23.9	22.7	22.3
Negative control	-	-	-	-

Note:

Positive control = Clindamycin

Negative control = Sterile distilled water

(+) = Inhibition zone observed

(-) = No inhibition zone observed

F0 = Cream Without Black Mulberry Fruit Extract

Subsequently, antibacterial activity testing was conducted on the cream formulations to determine whether the black mulberry fruit extract retained its antibacterial activity after being formulated into a topical cream, as well as to evaluate the formulation's ability to maintain its antibacterial effectiveness over 28 days of storage. The well diffusion method was used because it is suitable for evaluating the antibacterial activity of semisolid formulations such as creams, where samples cannot be evenly absorbed using the disc diffusion method. This method allows the active compounds from the extract to diffuse from the well into the agar medium, resulting in a measurable inhibition zone. In addition, the well diffusion method is more representative for assessing the release and diffusion of active compounds from topical formulations (Nurhayati et al., 2020).

The results presented in Table 6 show that the cream formulations containing black mulberry fruit extract retained their antibacterial activity against *P. acnes* and *S. epidermidis* after the formulation process. The concentration selection was based on previous findings by Budiman et al., (2017) where the MIC of black mulberry extract against *S. epidermidis* was reported in the range of 1.25–2.5%, while against *P. acnes* it ranged from 2.5–5%. In this study, the tested concentrations were 1.25%, 2.5% and 5%, representing the lower effective limit and higher levels to evaluate the

dose-dependent antibacterial response and the extent of activity increase with rising extract concentration.

This is evidenced by the presence of inhibition zones on both day 1 and day 28 in formulations F1, F2, and F3, which fall into the category of strong antibacterial activity with inhibition diameters of 10–20 mm (Sarah Tumundo et al., 2024). Conversely, F0 (base formulation and negative control) exhibited no antibacterial activity, confirming that the preservatives methyl paraben and propyl paraben exerted no inhibitory effects on the test bacteria. Thus, the demonstrated antibacterial efficacy is exclusively attributable to the black mulberry fruit extract.

## CONCLUSION

This study demonstrated that the ethanolic extract of black mulberry (*Morus nigra* L.) possesses strong antibacterial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis*, with notable inhibition zones. In formulation studies, Formula F1 (1.25%) exhibited antibacterial activity, with inhibition zones of  $11.7 \pm 0.25$  mm for *P. acnes* and  $11.9 \pm 0.20$  mm for *S. epidermidis*. All formulated creams (F1, F2, and F3) remained physically stable over a 28-day storage period, meeting standard requirements for organoleptic properties, homogeneity, pH, viscosity, spreadability, and centrifugation stability. Furthermore, the results showed a concentration-dependent effect, where increasing extract concentration led to larger inhibition zones, indicating stronger antibacterial activity. These findings indicate that black mulberry extract has potential as an active ingredient in topical anti-acne formulations. However, further studies are recommended, particularly skin irritation tests and in vivo evaluations, to ensure the safety and effectiveness of the formulation for topical use.

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