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Formulation and Evaluation of *Antidesma bunius* Gel: Antibacterial Activity, Stability, and Skin Irritation

Formulasi dan Evaluasi Gel *Antidesma bunius*: Aktivitas Antibakteri, Stabilitas, dan Iritasi Kulit

Nur Khairi[1]*, Maulita Indrisari[2], Maria Ulfa[1], Fhahri Mubarak[3], Amriani Sapra[1]

[1]*Department of Pharmacy and Pharmaceutical Technology, Universitas Almarisah Madani, Makassar 90242, South Sulawesi, Indonesia*
[2]*Department of Pharmacotherapy, Faculty of Medicine, Universitas Palangka Raya, Palangka Raya 73118, Central Kalimantan, Indonesia*
[3]*Department of Pharmaceutical Analysis and Medicinal Chemistry, Universitas Almarisah Madani, Makassar 90242, South Sulawesi, Indonesia*

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ABSTRACT

Acne is associated with the proliferation of acne-related bacteria, particularly *Propionibacterium acnes* and *Staphylococcus epidermidis*. This study evaluated the antibacterial activity, physical stability, microbiological quality, and skin irritation potential of an *Antidesma bunius* extract gel. The extract was tested against both bacterial species using an agar diffusion method, and a gel containing 2.5% extract was prepared with different Carbopol concentrations. The extract produced inhibition zones of 13.31–23.64 mm, whereas the gel formulations produced inhibition zones of 17.51–19.69 mm. The formulations remained homogeneous and showed acceptable organoleptic properties, pH, viscosity, spreadability, and adhesion after stability testing. Total plate and yeast and mold counts met the applicable microbiological limits. No erythema or edema was observed in albino rabbits. These findings indicated that *A. bunius* extract gel has potential as a topical anti-acne formulation.

ABSTRAK

Jerawat berkaitan dengan proliferasi bakteri penyebab jerawat, terutama *Propionibacterium acnes* dan *Staphylococcus epidermidis*. Penelitian ini mengevaluasi aktivitas antibakteri, stabilitas fisik, mutu mikrobiologis, dan potensi iritasi kulit gel ekstrak *Antidesma bunius*. Ekstrak diuji terhadap kedua bakteri menggunakan metode difusi agar, sedangkan gel yang mengandung ekstrak 2,5% dibuat dengan variasi konsentrasi Carbopol. Ekstrak menghasilkan zona hambat sebesar 13,31–23,64 mm, sedangkan formula gel menghasilkan zona hambat sebesar 17,51–19,69 mm. Setelah pengujian stabilitas, seluruh formula tetap homogen dan menunjukkan karakteristik organoleptik, pH, viskositas, daya sebar, dan daya lekat yang dapat diterima. Angka lempeng total serta angka kapang dan khamir memenuhi batas mikrobiologis yang berlaku. Eritema dan edema tidak ditemukan pada kelinci albino. Hasil ini menunjukkan bahwa gel ekstrak *A. bunius* berpotensi dikembangkan sebagai sediaan topikal antijerawat.

**Corresponding author:*
Nur Khairi (nurkhairijalil@gmail.com)

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1. INTRODUCTION

Acne is one of the most common skin disorders, particularly among adolescents and young adults. Its pathogenesis is multifactorial and involves excessive sebum production, follicular hyperkeratinization, inflammation, and the proliferation of acne-associated bacteria, particularly *Propionibacterium acnes* and *Staphylococcus epidermidis* (Ngamlerst et al., 2019). Current treatments include topical agents, such as antibiotic creams and ointments, as well as systemic antibiotics. However, prolonged antibiotic use may promote bacterial resistance and disrupt the skin microbiota (Darkwah et al., 2022; Ferreira et al., 2017). Therefore, safer and more effective alternatives, including natural products with antibacterial and anti-inflammatory properties, are needed.

Antidesma bunius, commonly known as buni, has been used in traditional medicine and has attracted attention because of its reported antibacterial, antioxidant, and anti-inflammatory properties. The fruit contains bioactive compounds, including polyphenols, flavonoids, and anthocyanins, that may contribute to antibacterial and anti-inflammatory effects (Silalahi et al., 2022; Sonia et al., 2018). These compounds may also provide antioxidant activity and thereby help reduce oxidative stress associated with acne-related inflammation (Yasser et al., 2020).

Although previous studies have reported the antibacterial potential of *A. bunius* extracts, evidence regarding their incorporation into stable and safe topical formulations remains limited. Herbal gel formulations may be susceptible to microbial contamination, and their dermal safety must be evaluated before further development (Jung et al., 2019; Merdana et al., 2020). Therefore, this study aimed to evaluate the antibacterial activity of *A. bunius* extract against *P. acnes* and *S. epidermidis*, formulate the extract as a topical gel, assess its physical stability and microbiological quality, and evaluate its potential to cause skin irritation in an animal model.

2. METHODS

2.1. Materials

The equipment used in this study included an autoclave (IKA, Staufen, Germany), micropipettes (Eppendorf, Hamburg, Germany), a vernier caliper (Starrett, Athol, MA, United States), Petri dishes (Thermo Fisher Scientific, Waltham, MA, United States), an incubator (Memmert, Schwabach, Germany), an oven (Thermo Fisher Scientific, Waltham, MA, United States), a laminar airflow cabinet (Esco, Singapore), Erlenmeyer flasks and beakers (Pyrex, New York, NY, United States), a homogenizer (IKA, Staufen, Germany), an analytical balance (Ohaus, Parsippany, NJ, United States), a water bath (Memmert, Schwabach, Germany), and a magnetic stirrer (IKA, Staufen, Germany).

The materials used included *Antidesma bunius* fruit obtained from a local supplier in Makassar, Indonesia; Propionibacterium acnes strain 290825 and Staphylococcus epidermidis strain 25013367, obtained from the culture collection of the Microbiology Laboratory, Sekolah Tinggi Ilmu Farmasi Makassar, Indonesia; distilled water and 70% ethanol (One Med®, Sidoarjo, Indonesia); sterile blank paper discs (GE Healthcare, Chicago, IL, United States); 0.9% sodium chloride solution (Hospira, Lake Forest, IL, United States); Nutrient Agar (Oxoid®, Basingstoke, United Kingdom); dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, United States); Carbopol 940 (Lubrizol, Wickliffe, OH, United States); triethanolamine and propylene glycol (BASF, Ludwigshafen, Germany); DMDM hydantoin (Lonza, Basel, Switzerland); Potato Dextrose Agar (Merck®, Darmstadt, Germany); and adult albino rabbits used for the skin irritation test.

2.2. Extraction Procedure

The plant material used in this study consisted of *A. bunius* fruit obtained from Makassar, South Sulawesi, Indonesia. The botanical identity of the plant material was authenticated by UPT Laboratorium Herbal Materia Medica Batu, Indonesia, under voucher specimen number 000.9.3/2247/102.20/2025. The collected fruits were wet-sorted to remove foreign materials and impurities, washed under running water, and drained. The fruits were extracted by maceration. Briefly, 100 g of fruit was homogenized and transferred to a glass container. The homogenized material was immersed in 1,000 mL of 70% ethanol acidified with citric acid at a concentration of 3% w/v to improve anthocyanin stability. Maceration was conducted at room temperature in the dark for 24 h. The macerate was filtered through Whatman No. 1 filter paper, and the resulting filtrate was concentrated under reduced pressure using a rotary evaporator until a viscous extract was obtained. The extract was weighed, transferred to a tightly sealed amber container, and stored at approximately 4 °C until further use (Geronimo et al., 2020; Ngamlerst et al., 2019).

2.3. Antibacterial Activity of the Extract

The antibacterial activity of the ethanolic extract of *A. bunius* fruit was evaluated using the agar disc-diffusion method. Under aseptic conditions, 15 mL of sterile Nutrient Agar was poured into each Petri dish and allowed to solidify. Suspensions of P. *acnes* and S. *epidermidis* were prepared and adjusted to a turbidity equivalent to a 0.5 McFarland standard. The standardized bacterial suspension was evenly inoculated onto the agar surface. Extract solutions at concentrations of 2.5%, 5.0%, 7.5%, and 10.0% were prepared using dimethyl sulfoxide (DMSO) as the solvent. Subsequently, 10 µL of each extract solution was applied to a sterile paper disc measuring 6 mm in diameter. The impregnated discs were allowed to stand for approximately 15 min and were then aseptically placed on the inoculated agar using sterile forceps, with sufficient spacing to prevent overlapping inhibition zones. A tetracycline disc with a potency of 30 µg/disc was used as the positive control, whereas a disc containing 20 µL of DMSO was used as the negative control. All treatments and controls were tested in triplicate. The plates inoculated with P. acnes were incubated anaerobically at 37 °C for 24 h. The same procedure was performed for S. epidermidis, followed by incubation at 37 °C for

24 h. After incubation, the inhibition-zone diameters were measured in millimetres using a vernier caliper. The reported inhibition-zone diameters included the 6-mm paper disc diameter (Phrompanya et al., 2024; Vora et al., 2018).

2.4. Gel Formulation

The compositions of the *A. bunius* extract gel formulations are presented in **Table 1.** Carbopol 940 was dispersed in hot distilled water and allowed to hydrate until a homogeneous dispersion was obtained. Triethanolamine was added gradually while stirring to neutralize the Carbopol and obtain a gel with a target pH of 4.5–6.5. The ethanolic extract of *A. bunius* was dispersed in propylene glycol and mixed with DMDM hydantoin. The extract mixture was gradually incorporated into the gel base and stirred until homogeneous. The remaining distilled water was added while stirring to obtain a final volume of 100 mL. The resulting gel was transferred to a suitable, tightly closed container (Ansong et al., 2023).

The compositions of the *A. bunius* extract gel formulations are presented in Table 1. Carbopol 940 was gradually dispersed in distilled water heated to 40 °C while stirring and was allowed to hydrate for 24 h until a homogeneous dispersion was obtained. Triethanolamine was then added gradually while stirring to neutralize the Carbopol and adjust the final gel pH to 4.5–5.0. The ethanolic extract of *A. bunius* was dispersed in propylene glycol and mixed with DMDM hydantoin. The extract mixture was gradually incorporated into the gel base and stirred for less than 10 min until homogeneous. The remaining distilled water was added while stirring to obtain a final volume of 100 mL. The resulting gel was transferred to a suitable, tightly closed container (Ansong et al., 2023).

Table 1. Composition of the *Antidesma bunius* extract gel formulations

Ingredient	Function	F1	F2	F3	Negative control
A. bunius extract (% w/v)	Active ingredient	2.5	2.5	2.5	—
Carbopol 940 (% w/v)	Gelling agent	0.5	1.0	2.0	0.5
Triethanolamine (mL)	Neutralizing agent	0.05	0.05	0.05	0.05
Propylene glycol (% w/v)	Cosolvent and penetration enhancer	10	10	10	10
DMDM hydantoin (% w/v)	Preservative	0.5	0.5	0.5	0.5
Distilled water	Vehicle	To 100 mL	To 100 mL	To 100 mL	To 100 mL

2.5. Physical Stability Testing of the Gel

a. Cycling Test

The gel formulations were subjected to a cycling stability test consisting of six consecutive cycles. Each cycle involved storage at 4 °C for 24 h, followed by storage at 40 °C for 24 h. Thus, one complete cycle lasted 48 h, and the six-cycle test was conducted over a total period of 12 days. The organoleptic properties, homogeneity, pH, viscosity, spreadability, and adhesion of each formulation were evaluated before the cycling test and after completion of the sixth cycle.

b. Organoleptic Evaluation

The organoleptic properties of each gel formulation were evaluated by observing its physical form, color, and odor before and after stability testing. Any visible changes in these characteristics were recorded (Thote et al., 2023).

c. Homogeneity Test

Samples collected from the upper, middle, and lower portions of each gel formulation were separately spread as thin layers on transparent glass plates. A formulation was considered homogeneous when no coarse particles, aggregates, or phase separation were observed (Ermawati & Ramadhani, 2020).

d. pH Measurement

The pH of each gel formulation was measured using a calibrated Mettler Toledo pH meter. Before measurement, the instrument was calibrated using standard buffer solutions at pH 4.00 and 7.00. One gram of gel was dispersed in 10 mL of distilled water, corresponding to a gel-to-water ratio of 1:10 (w/v), and mixed until a uniform dispersion was obtained. The electrode was immersed in the dispersion, and the pH was measured at 25 °C. Measurements were performed in triplicate. The measured values were compared with the target pH range of 4.5–6.5 for topical gel formulations (Tahar et al., 2023).

e. Viscosity Measurement

The viscosity of each gel formulation was measured using a calibrated viscometer at a controlled temperature. Measurements were performed before and after stability testing and were expressed in centipoise (cP) (Putri et al., 2019).

The viscosity of each gel formulation was measured using a Brookfield viscometer equipped with spindle No. 3 at a rotational speed of 10 rpm. Measurements were conducted at 25 °C before and after the cycling stability test. Each measurement was performed in triplicate , and the viscosity values were expressed in centipoise (cP) (Putri et al., 2019).

f. Spreadability Test

For the spreadability test, 0.5 g of gel was placed at the center of a transparent glass plate positioned over graph paper. A second glass plate was placed over the sample, and a load of 50 g was applied. The gel was allowed to spread under the applied load for

1 min, after which the diameter of the spread gel was measured in centimetres (Gupta et al., 2023).

g. Adhesion Test

For the adhesion test, 0.25 g of gel was placed on a glass slide with a known surface area, and a second glass slide was positioned over the gel. A load of 50 g was applied to the upper slide for 5 min to ensure uniform contact between the gel and both glass surfaces. After 5 min, the 50-g load was removed, and the time required for the two glass slides to separate was immediately recorded in seconds. A longer separation time indicated greater adhesive capacity. The test was performed in triplicate (Thote et al., 2023).

2.6. Antibacterial Activity of the *A. bunius* Extract Gel

The antibacterial activity of the *A. bunius* extract gel formulations was evaluated using the agar-well diffusion method. A 10-mL volume of sterile Nutrient Agar was poured into each Petri dish to form a base layer and was allowed to solidify. Sterile spacers measuring 6 mm in diameter were positioned on the base layer with sufficient spacing to prevent overlapping inhibition zones. The bacterial suspensions were adjusted to a turbidity equivalent to a 0.5 McFarland standard. A seed layer was prepared by mixing 20 µL of the standardized bacterial suspension with 20 mL of molten Nutrient Agar. The inoculated agar was then poured over the solidified base layer. After the seed layer had solidified, the spacers were aseptically removed to form wells measuring 6 mm in diameter. Subsequently, 0.1 mL of each gel formulation was placed into the respective wells. A tetracycline solution at a concentration of 30 µg/mL was used as the positive control, whereas 0.1 mL of the extract-free gel base was used as the negative control. All formulations and controls were tested in triplicate (n = 3). The plates inoculated with *P. acnes* were incubated anaerobically at 37 °C for 24 h. The plates inoculated with S. epidermidis were also incubated at 37 °C for 24 h. After incubation, the inhibition-zone diameters were measured in millimetres using a vernier caliper (Papa et al., 2023).

2.7. Microbial Contamination Testing

a. Total Plate Count

A 1-mL aliquot of each sample dilution was transferred aseptically to a sterile Petri dish. Approximately 15 mL of molten Nutrient Agar was added, and the plate was gently rotated in a figure-eight motion to distribute the inoculum evenly. After solidification, the plates were incubated at 37 °C for 24 h. Colonies on countable plates were counted, and the total plate count was expressed as colony-forming units per gram of gel (CFU/g) (Darkwah et al., 2022; Jairoun et al., 2020).

b. Yeast and Mold Count

The yeast and mold count was determined for Formula 0 of the *A. bunius* extract gel. One gram of the gel was aseptically transferred into a sterile test tube containing 9 mL of sterile water for injection (Aqua Pro Injection; API) and homogenized to obtain a primary dilution of 10^{-1}. Subsequently, 1 mL of the 10^{-1} dilution was transferred into another sterile test tube containing 9 mL of API to obtain a 10^{-2} dilution. The same procedure was repeated to obtain a 10^{-3} dilution.

A 0.1 mL aliquot of each dilution (10^{-1}, 10^{-2}, and 10^{-3}) was inoculated in duplicate onto sterile Petri dishes. Approximately 15 mL of molten Potato Dextrose Agar was then added to each dish. The plates were gently rotated in a figure-eight motion to ensure even distribution of the inoculum. After the medium had solidified, the plates were inverted and incubated aerobically at 25 °C for 3 days.

The yeast and mold count was calculated using the following equation:

$$\text{CFU/g} = \frac{\text{mean number of colonies}}{\text{volume plated} \times \text{dilution tested}}$$

The maximum permissible yeast and mold count for the applicable cosmetic product category is 1×10^3 CFU/g, as stipulated in Indonesian Food and Drug Authority Regulation No. 16 of 2024 concerning microbial and contaminant limits in cosmetic products (Dabo et al., 2024).

2.8. Skin Irritation Test

The skin irritation potential of the *A. bunius* extract gel was evaluated in three healthy adult male albino rabbits using the Draize method. The animals had an average body weight of approximately 1.5 kg. Approximately 24 h before treatment, the fur on the dorsal region of each rabbit was carefully clipped, without abrading the skin, to expose separate test areas measuring 2 × 3 cm.

A 0.5-g portion of the *A. bunius* extract gel was applied uniformly to the designated test site. An equal amount of the extract-free gel base was applied to a separate site as the negative control. A 0.5-g portion of a commercially available anti-acne gel, referred to as Product X, was applied to another site as the reference treatment. Each treated site was covered with sterile gauze, secured with nonirritating adhesive tape, and maintained under an occlusive dressing for 24 h.

After the dressings were removed, any residual test material was gently removed without damaging the skin. Erythema and edema were assessed at 24, 48, and 72 h after dressing removal using the standard Draize scoring system. Erythema was scored as follows: 0, no erythema; 1, very slight erythema, barely perceptible; 2, well-defined erythema; 3, moderate-to-severe erythema; and 4, severe erythema or eschar formation that prevented erythema grading. Edema was scored as follows: 0, no edema; 1, very slight edema, barely perceptible; 2, slight edema with well-defined edges; 3, moderate edema raised approximately 1 mm; and 4, severe edema raised more than 1 mm and extending beyond the area of exposure (Kanďárová et al., 2018; Kanto et al., 2013).

The Primary Irritation Index (PII) was calculated according to the method prescribed by the Indonesian Food and Drug Authority (BPOM, 2014), using the following equation:

$$\text{PII} = \frac{A - B}{C}$$

where A is the sum of the erythema and edema scores for the test formulation at all observation times (24, 48, and 72 h), divided by the number of observation times; B is the sum of the erythema and edema scores for the negative-control sites at all observation times, divided by the number of observation times; and C is the total number of animals used in the test.

2.9. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, United States). Each antibacterial treatment was evaluated in three independent experimental replicates (n = 3), and the results were expressed as the mean ± standard deviation. Statistical analyses were performed separately for each bacterial species.

Data normality was assessed using the Shapiro–Wilk test, whereas homogeneity of variance was evaluated using Levene's test. The data met both the normality and homogeneity-of-variance assumptions and were therefore analyzed using one-way analysis of variance (ANOVA). When ANOVA indicated a statistically significant overall difference among the treatment groups, Tukey's honestly significant difference post hoc test was performed for pairwise comparisons. Differences were considered statistically significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Antibacterial Activity of *Antidesma bunius* Extract

The antibacterial activity of *A. bunius* extract against *P. acnes* and *S. epidermidis* is presented in **Figure 1**. The extract inhibited both bacterial species at all tested concentrations, and the inhibition-zone diameters generally increased as the extract concentration increased from 2.5% to 10%, indicating a concentration-dependent antibacterial response. Against *P. acnes*, extract concentrations of 2.5%, 5.0%, and 7.5% produced inhibition-zone diameters of 13.37 ± 0.32, 16.51 ± 0.24, and 19.22 ± 0.54 mm, respectively, with no significant differences among these concentrations, as indicated by the same superscript letter. By contrast, the 10% extract produced an inhibition-zone diameter of 23.64 ± 0.79 mm, which was significantly larger than those produced by the lower concentrations ($p < 0.05$). A similar pattern was observed against *S. epidermidis*, for which extract concentrations of 2.5%, 5.0%, and 7.5% produced inhibition-zone diameters of 13.31 ± 0.62, 16.32 ± 0.22, and 19.60 ± 0.29 mm, respectively, without significant differences among these concentrations, whereas the 10% extract produced a significantly larger inhibition-zone diameter of 22.49 ± 0.49 mm ($p < 0.05$). The positive control produced the largest inhibition zones, measuring 36.17 ± 1.44 mm against *P. acnes* and 34.43 ± 1.27 mm against *S. epidermidis*, and differed significantly from all extract treatments ($p < 0.05$). The solvent control produced no inhibition zone against either bacterial species and was therefore omitted from **Figure 1**, indicating that the antibacterial activity was attributable to the *A. bunius* extract rather than to the extract solvent. The observed antibacterial activity may be related to phenolic constituents, including flavonoids and other polyphenolic compounds previously reported in the fruit, which may inhibit bacterial growth by disrupting membrane integrity, interfering with essential metabolic processes, or impairing microbial proliferation. However, because the phytochemical composition of the extract was not characterized in the present study, the compounds responsible for the observed activity could not be confirmed. Previous studies have also reported the antibacterial activity of *A. bunius* extracts (Silalahi et al., 2022; Sonia et al., 2018), supporting its potential as a source of antibacterial constituents for topical anti-acne formulations. Nevertheless, differences in plant parts, extraction solvents, bacterial strains, and assay conditions should be considered when comparing inhibition-zone diameters across studies.

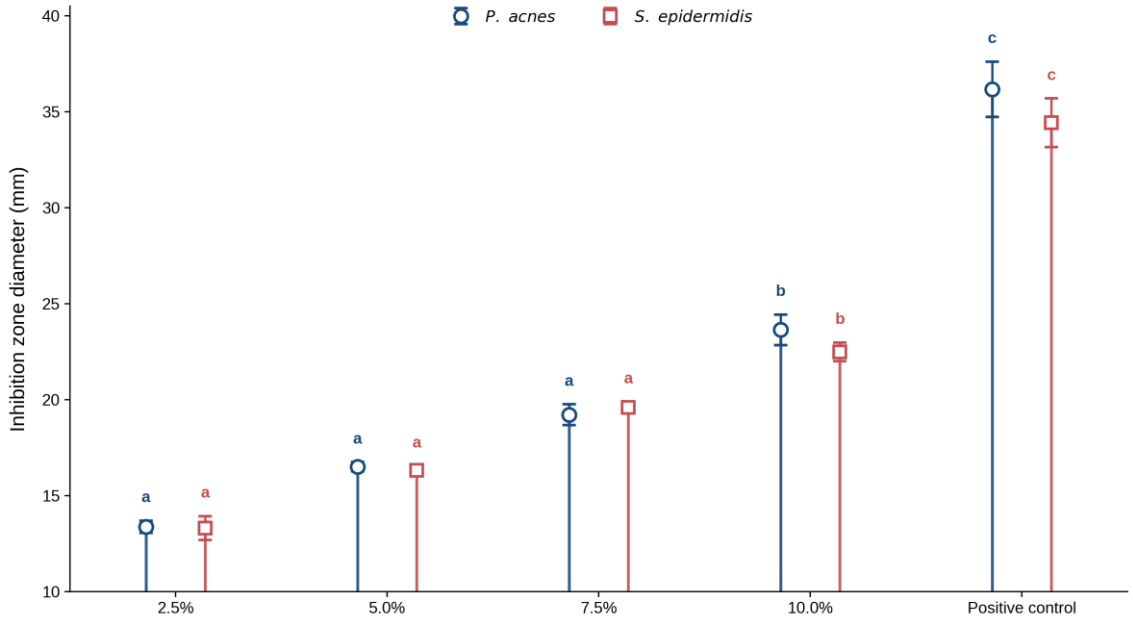

Figure 1. Inhibition-zone diameters of *A. bunius* extract against *P. acnes* and *S. epidermidis*. Data are presented as the mean ± standard deviation (n = 3). Different letters indicate significant differences within each bacterial species according to Tukey's post hoc test ($p < 0.05$). The solvent control produced no inhibition zone and is not shown.

3.2. Physical Stability of the *A. bunius* Gel Formulations

The physical appearance of formulations F1–F3 and the base gel is shown in **Figure 2**. The formulations differed primarily in Carbopol 940 concentration, whereas all extract-containing formulations contained 2.5% *A. bunius* extract.

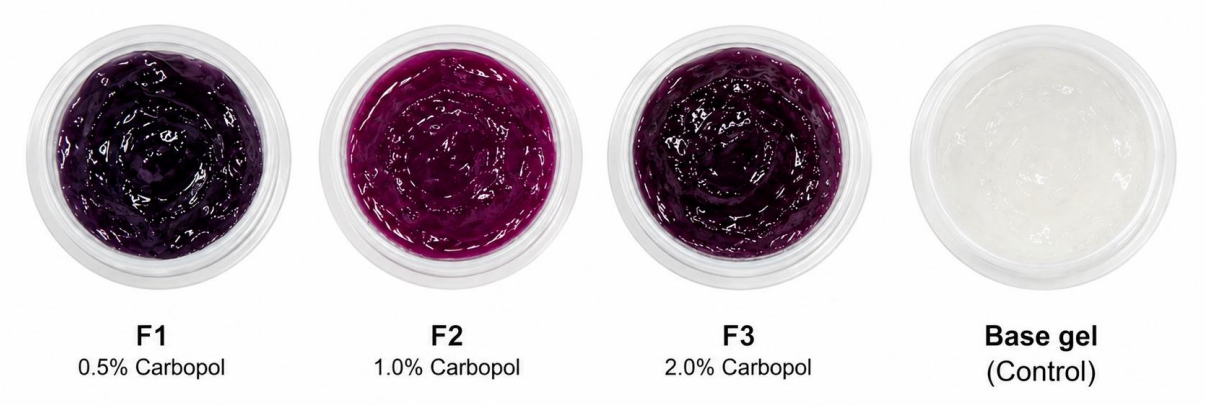

Figure 2. Physical appearance of the *A. bunius* extract gel formulations. F1, F2, and F3 contained 0.5%, 1.0%, and 2.0% Carbopol 940, respectively; the base gel contained no *A. bunius* extract.

a. Organoleptic Properties

The organoleptic properties of the gel formulations before and after the cycling test are presented in **Table 2**. No changes in physical form, color, or odor were observed after cycling. All formulations retained a semisolid consistency. F2 and F3 remained purple, whereas F1 and the base gel remained clear to white. The absence of visible organoleptic changes indicated that the formulations maintained their macroscopic characteristics under the applied cycling conditions.

Table 2. Organoleptic properties of the gel formulations before and after cycling

Formula	Observation	Physical form	Color	Odor
F1	Before	Semisolid gel	Purple	Characteristic buni odor
	After	Semisolid gel	Purple	Characteristic buni odor
F2	Before	Semisolid gel	Purple	Characteristic buni odor
	After	Semisolid gel	Purple	Characteristic buni odor
F3	Before	Semisolid gel	Purple	Characteristic buni odor
	After	Semisolid gel	Purple	Characteristic buni odor
Base gel	Before	Semisolid gel	Clear to white	Odorless
	After	Semisolid gel	Clear to white	Odorless

b. Homogeneity

The homogeneity results are presented in **Table 3**. All formulations remained macroscopically homogeneous before and after cycling, with no visible coarse particles, aggregates, or phase separation. These findings indicated that the formulation process produced physically uniform gels and that the cycling conditions did not visibly disrupt their integrity. Macroscopic homogeneity is important for the consistent distribution of formulation components; however, visual examination alone does not confirm the quantitative uniformity of the active constituents (Lubda et al., 2023).

Table 3. Homogeneity of the gel formulations before and after cycling

Formula	Observation	Homogeneity
F1	Before	Homogeneous
	After	Homogeneous
F2	Before	Homogeneous
	After	Homogeneous
F3	Before	Homogeneous
	After	Homogeneous
Base gel	Before	Homogeneous
	After	Homogeneous

c. pH

The pH values of the gel formulations before and after cycling are presented in **Table 4**. Before cycling, all formulations had a pH of 5. After cycling, the pH increased to 6 in F1, F2, and the base gel, whereas F3 reached pH 7. Because F1–F3 contained the same concentration of *A. bunius* extract, the higher pH of F3 was more likely associated with its higher Carbopol concentration, the degree of neutralization with triethanolamine, or changes occurring during cycling rather than with the extract concentration. The post-cycling pH values of F1 and F2 remained within the predetermined range of 4.5–6.5, whereas F3 exceeded this range. Therefore, F3 did not fully meet the predefined pH criterion after cycling and may require further optimization. Although no erythema or edema was observed during the irritation test, this finding does not establish that pH 7 is optimal for repeated topical use (Kaushik et al., 2021).

Table 4. pH values of the gel formulations before and after cycling

Formula	Observation	pH
F1	Before	5
	After	6
F2	Before	5
	After	6
F3	Before	5
	After	7
Base gel	Before	5
	After	6

d. Viscosity

The viscosity results are presented in **Table 5**. Viscosity increased as the Carbopol concentration increased, with F3 exhibiting the highest values both before and after cycling. This pattern was attributable to the higher concentration of the gelling agent rather than to the extract, because all extract-containing formulations contained 2.5% *A. bunius* extract. Viscosity also increased after cycling in all formulations. Higher viscosity may improve retention at the application site, but excessive viscosity may reduce spreadability and alter the release of active constituents. Consequently, viscosity should be interpreted together with the spreadability and adhesion results rather than as an independent indicator of formulation performance (Kaushik et al., 2021).

Table 5. Viscosity of the gel formulations before and after cycling

Formula	Observation	Viscosity (cP)
F1	Before	19.233
	After	20.000
F2	Before	20.060
	After	23.000
F3	Before	34.000
	After	38.230
Base gel	Before	17.633
	After	18.768

e. Spreadability

The spreadability results are presented in **Table 6**. Spreadability decreased as the Carbopol concentration increased. Before cycling, the spread diameters of F1, F2, and F3 were 6.60, 5.55, and 3.07 cm, respectively; after cycling, they decreased to 6.21, 5.21, and 2.89 cm. This inverse relationship between viscosity and spreadability was expected because the denser gel network formed at higher Carbopol concentrations provided greater resistance to flow. F3 showed the lowest spreadability both before and after cycling, which may reduce ease of application. However, therapeutic effectiveness cannot be inferred from spreadability alone and requires further release, permeation, or clinical evaluation (Lubda et al., 2023).

Table 6. Spreadability of the gel formulations before and after cycling

Formula	Observation	Spreadability (cm)
F1	Before	6.60
	After	6.21
F2	Before	5.55
	After	5.21
F3	Before	3.07
	After	2.89
Base gel	Before	6.03
	After	6.02

f. Adhesion

The adhesion results are presented in **Table 7**. Adhesion time increased with increasing Carbopol concentration, with F3 showing the longest separation time and the base gel showing the shortest. The increase in adhesion time was consistent with the higher viscosity of formulations containing greater concentrations of Carbopol. A longer adhesion time may improve retention at the application site and prolong contact between the formulation and the skin. Nevertheless, the glass-slide method used in this study did not directly measure adhesion to biological skin or demonstrate increased absorption of active constituents (Lubda et al., 2023).

Table 7. Adhesion of the gel formulations before and after cycling

Formula	Observation	Adhesion (s)
F1	Before	1.6
	After	1.7
F2	Before	1.8
	After	2.0
F3	Before	2.0
	After	2.3
Base gel	Before	1.3
	After	1.5

3.3. Antibacterial Activity of the *A. bunius* Extract Gel

The antibacterial activity of the *A. bunius* gel formulations against *P. acnes* and *S. epidermidis* is presented in **Table 8**. A concentration of 2.5% extract was selected because it was the lowest tested concentration that produced measurable antibacterial activity in the preliminary extract assay. Selecting the lowest active concentration may reduce the likelihood of unfavorable changes in the physical properties of the gel while maintaining biological activity (Putri et al., 2019; Thote et al., 2023). All formulations inhibited both bacterial species. Against *P. acnes*, F1, F2, and F3 produced inhibition-zone diameters of 19.69 ± 0.87, 18.60 ± 0.92, and 19.48 ± 0.31 mm, respectively, whereas the corresponding values against *S. epidermidis* were 17.51 ± 0.42, 17.57 ± 0.63, and 18.61 ± 0.74 mm. No significant differences were observed among F1, F2, and F3 for either bacterial species ($p > 0.05$), indicating that increasing the Carbopol 940 concentration from 0.5% to 2.0% did not significantly affect the antibacterial activity of the gel formulations under the assay conditions. Carbopol 940 primarily functions as a gelling and rheology-modifying agent; therefore, its concentration was expected to influence viscosity, spreadability, and adhesion rather than directly enhance antibacterial activity. The comparable inhibition-zone diameters among the formulations suggest that the tested Carbopol concentrations did not markedly restrict the diffusion of antibacterial constituents through the agar medium (Ansong et al., 2023; Tahar et al., 2023). The base gel produced no inhibition zone and differed significantly from the extract-containing formulations and the positive control, confirming that the excipients did not contribute to the antibacterial effect. By contrast, the positive control produced the largest inhibition zones, measuring 35.35 ± 0.32 mm against *P. acnes* and 34.16 ± 0.64 mm against *S. epidermidis*, and differed significantly from all other groups ($p < 0.05$). These findings indicate that incorporation of *A. bunius* extract into the gel matrix preserved its antibacterial activity, although the activity remained lower than that of the positive control. The observed activity may be associated with phenolic constituents previously reported in *A. bunius*, which may impair bacterial membrane integrity and essential metabolic processes (Ngamlerst et al., 2019; Phrompanya et al., 2024; Silalahi et al., 2022; Vora et al., 2018; Yasser et al., 2020). However, because the extract was not phytochemically characterized in the present study, the active compounds and their mechanisms could not be confirmed. Overall, these results support the potential of *A. bunius* gel as a topical anti-acne candidate, although further release, permeation, and clinical studies are required before its therapeutic effectiveness can be established.

Table 8. Inhibition-zone diameters of the *A. bunius* extract gel formulations

Bacterial species	F1	F2	F3	Base gel	Positive control
P. acnes	19.69 ± 0.87^{b}	18.60 ± 0.92^{b}	19.48 ± 0.31^{b}	0.00 ± 0.00^{c}	35.35 ± 0.32^{a}
S. epidermidis	17.51 ± 0.42^{b}	17.57 ± 0.63^{b}	18.61 ± 0.74^{b}	0.00 ± 0.00^{c}	34.16 ± 0.64^{a}

Data are presented as the mean ± standard deviation of three replicates. Different superscript letters within the same row indicate significant differences according to Tukey's post hoc test ($p < 0.05$). F1, F2, and F3 contained 2.5% *A. bunius* extract and 0.5%, 1.0%, and 2.0% Carbopol 940, respectively.

3.4. Microbiological Quality of the *A. bunius* Extract Gel

The microbiological quality of F1 is presented in **Table 9**. F1, containing 2.5% w/v *A. bunius* extract and 0.5% w/v Carbopol 940, was selected for microbiological testing based on the overall results of the antibacterial activity and cycling stability evaluations. The total plate count of F1 was 1.0×10^{1} CFU/g. No yeast or mold colonies were detected on the duplicate plates; therefore, the yeast and mold count was reported as not detected at the method detection limit of $<5.0 \times 10^{1}$ CFU/g. Both results were below the maximum permissible limit of 1×10^{3} CFU/g established for the applicable cosmetic-product category under Indonesian Food and Drug Authority Regulation No. 12 of 2019 concerning Contaminant Limits in Cosmetics. These findings indicate that F1 met the applicable microbiological acceptance criteria at the time of analysis. The low total plate count and the absence of detectable yeast and mold growth may reflect appropriate preparation and handling procedures, as well as the antimicrobial preservation provided by DMDM hydantoin. Nevertheless, the results represent the microbiological quality of the formulation only at the time of testing and do not independently demonstrate long-term preservative efficacy. A preservative efficacy or microbial challenge test would be required to evaluate the ability of the formulation to resist microbial contamination during storage and repeated use (Maurício et al., 2017).

Table 9. Microbial counts of the *A. bunius* extract gel

Parameter	Result (CFU/g)	BPOM limit (CFU/g)
Total plate count	1.0×10^{1}	Maximum 1×10^{3}
Yeast and mold count	2.0×10^{1}	Maximum 1×10^{3}

3.5. Skin Irritation of the *A. bunius* Extract Gel

The skin irritation response following topical application of the *A. bunius* extract gel is shown in **Figure 3**. No visible erythema or edema was observed at 24, 48, or 72 h after application of the extract-containing gel, base gel, or control treatment (A commercially available anti-acne gel, marketed as Product X), and all recorded Draize scores were zero. These findings indicated that the formulation did not produce visible primary skin irritation under the conditions of the animal test. The absence of erythema and edema was consistent with previous findings showing

favorable dermal tolerance of some natural-product formulations (Merdana et al., 2020). Nevertheless, the present results should be interpreted cautiously because a negative acute irritation response in rabbits does not establish the absence of sensitization, cumulative irritation, or adverse effects during repeated use in humans. Therefore, additional dermal safety assessments and clinical studies are required before the formulation can be considered safe for routine topical use.

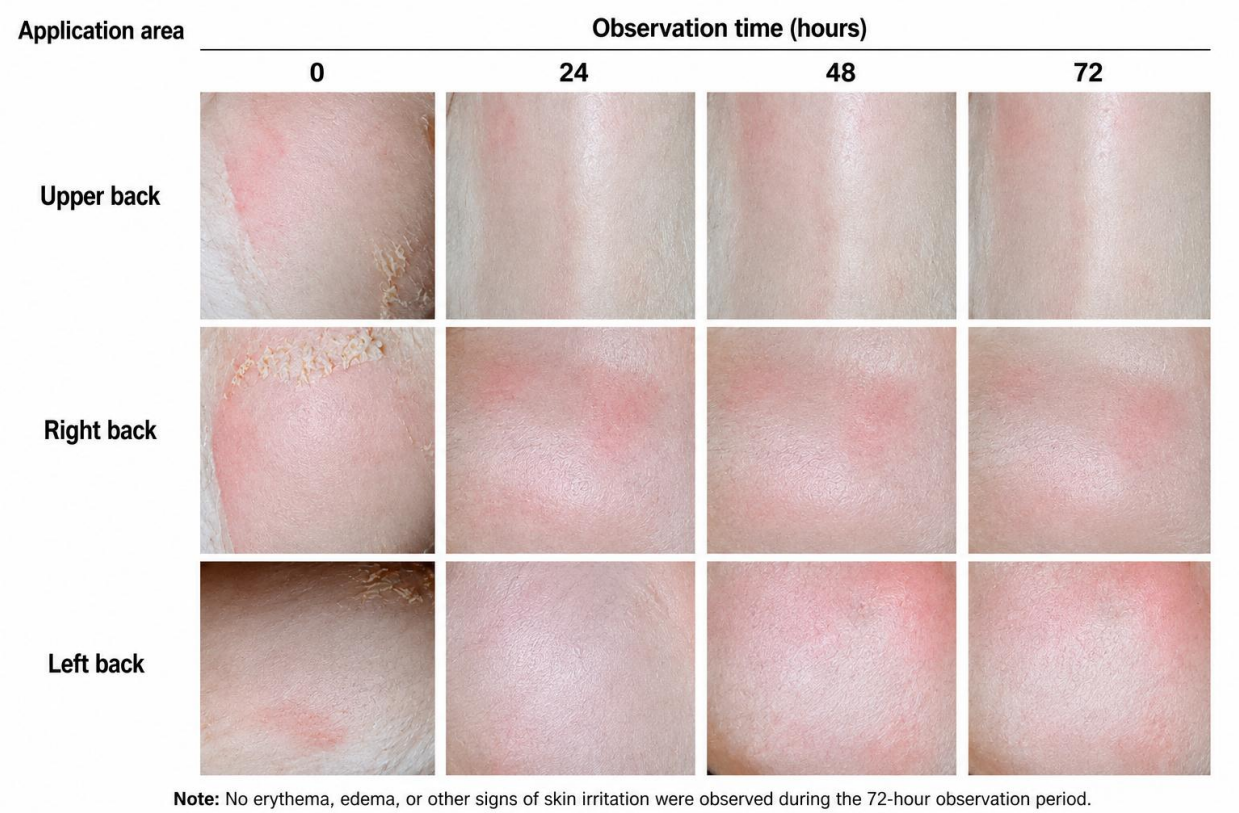

Figure 3. Representative skin observations in albino rabbits before and after topical application of the *A. bunius* extract gel, base gel, and control treatment.

4. CONCLUSION

A. bunius extract exhibited concentration-dependent antibacterial activity against *P. acnes* and *S. epidermidis*, and incorporation of 2.5% extract into Carbopol 940-based gels preserved this activity. Variations in Carbopol concentration affected the physical properties of the formulations, particularly viscosity, spreadability, and adhesion, but did not significantly alter their antibacterial performance. F1 and F2 maintained pH values within the predefined range after cycling, whereas F3 reached pH 7 and showed the lowest spreadability, indicating that further optimization of the highest-Carbopol formulation may be required. The tested gel met the stated microbiological limits and produced no visible erythema or edema in albino rabbits. Collectively, these findings support the further development of *A. bunius* extract gel as a topical anti-acne candidate. However, preservative efficacy, long-term stability, dermal permeation, repeated-dose safety, and clinical effectiveness should be evaluated before its routine topical use can be recommended.

AUTHOR CONTRIBUTIONS

Conceptualization, N.K.; methodology, M.I, M.U.; writing—original draft preparation, F.M, A.S, N.K; writing—review and editing, N.A, A.S, All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study protocol was approved by the Ethics Committee of the Faculty of Public Health, Hasanuddin University, Makassar, Indonesia (protocol code 6976/UN4.14.1/TP.01.02/2021; approval date: August 9, 2021).

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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The authors declare no conflict of interest.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, grammar, and readability of the text. After using this tool, the authors thoroughly

reviewed, edited, and verified the entire content to ensure that it accurately represented their own data, ideas, and interpretations. The authors take full responsibility for the integrity and originality of the published work.

REFERENCES

Ansong, J. A., Asante, E., Johnson, R., Boakye-Gyasi, M. E., Kuntworbe, N., Owusu, F. W. A., & Ofori-Kwakye, K. (2023). Formulation and evaluation of herbal-based antiacne gel preparations. *BioMed Research International, 2023*, Article 7838299. https://doi.org/10.1155/2023/7838299

Dabo, D. A., Zechariah, K. I., Pauline, G., Naomi, M. N., Dawal, S. P., & Vwamkat, A. S. (2024). Assessment of microbial contamination of solid herbal medicine sold in Makurdi metropolis. *World Journal of Advanced Research and Reviews, 21*(2), 496–505. https://doi.org/10.30574/wjarr.2024.21.2.0433

Darkwah, S., Agbettor, D., Codjoe, F., & Donkor, E. (2022). Microbial contamination of herbal preparations on the Ghanaian market, Accra. *Microbiology Insights, 15*. https://doi.org/10.1177/11786361221139602

Ermawati, D. E., & Ramadhani, C. I. (2020). Formulation of anti-acne gel of *Moringa oleifera* L. ethanolic extract and antibacterial test on *Staphylococcus epidermidis*. *Majalah Farmaseutik, 16*(2), 154–162. https://doi.org/10.22146/farmaseutik.v16i2.50319

Ferreira, A., Vecino, X., Ferreira, D., Cruz, J., Moldes, A., & Rodrigues, L. (2017). Novel cosmetic formulations containing a biosurfactant from *Lactobacillus paracasei*. *Colloids and Surfaces B: Biointerfaces, 155*, 522–529. https://doi.org/10.1016/j.colsurfb.2017.04.026

Geronimo, A., Bancual, M., Ko, K., Soliba, L., Ildefonso, J., Soriano, A., & Domantay, J. (2020). Free radical scavenging and in vitro cytotoxic activity of Bugnay (*Antidesma bunius*) leaves extract against A549 human lung adenocarcinoma and HCT-116 human colorectal cancer cell lines. *Indonesian Journal of Cancer Chemoprevention, 11*(3), 124–133. https://doi.org/10.14499/indonesianjcanchemoprev11iss3pp124-133

Gupta, B., Chopra, N., Dubey, S., Kohli, P., Zaidi, M. I., & Chauhan, A. (2023). Formulation of anti-acne of *Moringa oliefera* leaves powder ethanolic extract as an inhibititor of bacteria. *Current Research in Pharmaceutical Sciences, 13*(2), 102–107. https://doi.org/10.24092/CRPS.2023.130203

Jairoun, A., Al-Hemyari, S., Shahwan, M., & Zyoud, S. (2020). An investigation into incidences of microbial contamination in cosmeceuticals in the UAE: Imbalances between preservation and microbial contamination. *Cosmetics, 7*(4), Article 92. https://doi.org/10.3390/cosmetics7040092

Jung, Y., Jeong, H., Cho, Y., Lee, E., Jang, H., Kim, J., & Lim, K. (2019). Lysates of a probiotic, *Lactobacillus rhamnosus*, can improve skin barrier function in a reconstructed human epidermis model. *International Journal of Molecular Sciences, 20*(17), Article 4289. https://doi.org/10.3390/ijms20174289

Kanďárová, H., Willoughby, J., de Jong, W., Letašiová, S., Milasová, T., Bachelor, M., & Coleman, K. (2018). Pre-validation of an in vitro skin irritation test for medical devices using the reconstructed human tissue model EpiDerm™. *Toxicology in Vitro, 50*, 407–417. https://doi.org/10.1016/j.tiv.2018.02.007

Kanto, H., Washizaki, K., Itō, M., Matsunaga, K., Akamatsu, H., Kawai, K., & Mori, F. (2013). Optimal patch application time in the evaluation of skin irritation. *The Journal of Dermatology, 40*(5), 363–369. https://doi.org/10.1111/1346-8138.12004

Kaushik, V., Ganashalingam, Y., Schesny, R., Raab, C., Sengupta, S., & Keck, C. (2021). Influence of massage and skin hydration on dermal penetration efficacy of Nile red from petroleum jelly—An unexpected outcome. *Pharmaceutics, 13*(12), Article 2190. https://doi.org/10.3390/pharmaceutics13122190

Lubda, M., Pelzer, I., Salazar, A., & Hagen, J. (2023). Topically applied active ingredients establish an equilibrium dependent on their physico-chemical properties and a skin layer specific tissue capacity. *Clinical & Experimental Dermatology and Therapies, 8*(2). https://doi.org/10.29011/2575-8268.100209

Maurício, E., Rosado, C., Duarte, M., Veríssimo, J., Bom, S., & Vasconcelos, L. (2017). Efficiency of nisin as preservative in cosmetics and topical products. *Cosmetics, 4*(4), Article 41. https://doi.org/10.3390/cosmetics4040041

Merdana, I., Arjana, A., Widyastuti, S., Tetrania, T., Budiasa, K., Sudimartini, L., & Sudira, I. (2020). Assessment of the dermal acute irritation potential of natural veterinary medicine Minyak Rajas in albino rabbits. *Journal of Pharmaceutical Research International, 32*(10), 17–24. https://doi.org/10.9734/jpri/2020/v32i1030489

Ngamlerst, C., Udomkasemsab, A., Kongkachuichai, R., Kwanbunjan, K., Chupeerach, C., & Prangthip, P. (2019). The potential of antioxidant-rich Maoberry (*Antidesma bunius*) extract on fat metabolism in liver tissues of rats fed a high-fat diet. *BMC Complementary and Alternative Medicine, 19*(1). https://doi.org/10.1186/s12906-019-2716-0

Papa, C., Suciu, A., Dopcea, I., Ené, N., Singh, S., & Vamanu, E. (2023). Exploring the efficacy of extracts for cosmetic creams: In vivo and in vitro assessments. *Nutraceuticals, 3*(3), 306–314. https://doi.org/10.3390/nutraceuticals3030024

Phrompanya, P., Buncharoen, W., Tragoolpua, Y., & Saenphet, K. (2024). Antioxidant, anti-inflammatory, and antibacterial activities against acne-causing bacteria of *Miliusa velutina* (A. DC.) Hook. f. & Thomson extracts. *Journal of Pharmacy & Pharmacognosy Research, 12*(2), 243–254. https://doi.org/10.56499/jppres23.1704_12.2.243

Putri, A. N., Laila, N., & Forestryana, D. (2019). Formula optimization of *Annona muricata* folium ethanolic extract of anti acne gel formulation using factorial design method. *Borneo Journal of Pharmacy, 2*(2), 63–70. https://doi.org/10.33084/bjop.v2i2.981

Silalahi, M., Purba, E., Sawitri, I., Wahyuningtyas, R., & Sitepu, N. (2022). *Antidesma bunius* (L.) Spreng. (Foodstuffs and its

bioactivity). *Journal of Tropical Ethnobiology, 5*(1), 19–29. https://doi.org/10.46359/jte.v5i1.104

Sonia, Z., Islam, M., Koly, S., Faisal, T., & Rakib, K. (2018). Evaluation of cytotoxicity and antibacterial activities of methanolic extract of *Antidesma bunius* (Linn.) (Family Euphorbiaceae) leaf. *Journal of Advances in Medical and Pharmaceutical Sciences, 16*(2), 1–7. https://doi.org/10.9734/jamps/2018/40004

Tahar, N., Wahyuni, D., Adawiyah, R., Khaerani, & Wahyuddin, M. (2023). Formulation and antibacterial activity of porang (*Amorphophallus muelleri* Blume) extract gel against *Propionibacterium acne*. *Ad-Dawaa' Journal of Pharmaceutical Sciences, 6*(1), 83–92. https://doi.org/10.24252/djps.v6i1.38744

Thote, S., Dhoble, N., & Baheti, J. (2023). Development and evaluation of anti-acne gel containing *Luffa acutangulas*eeds extract. *International Journal of Herbal Medicine, 11*(4), 58–64. https://doi.org/10.22271/flora.2023.v11.i4a.881

Vora, J., Srivastava, A., & Modi, H. (2018). Antibacterial and antioxidant strategies for acne treatment through plant extracts. *Informatics in Medicine Unlocked, 13*, 128–132. https://doi.org/10.1016/j.imu.2017.10.005

Yasser, M., Rafi, M., Wahyuni, W., Widiyanti, S., & Asfar, A. (2020). Total phenolic content and antioxidant activities of buni fruit (*Antidesma bunius* L.) in Moncongloe Maros District extracted using ultrasound-assisted extraction. *Rasayan Journal of Chemistry, 13*(1), 684–689. https://doi.org/10.31788/rjc.2020.1315584

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