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Original Research Article

Development of Anti-Acne Gel Preparation of Binahong Leaf Extract (Anredera Cordifolia (Ten.) Steenis) Against Staphylococcus Epidermidis Bacteria

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Abstract: ***Introduction*:** Acne vulgaris is a common skin disorder frequently associated with *Staphylococcus epidermidis*, an aerobic bacterium. The increasing incidence of antibiotic resistance in *S. epidermidis* has encouraged the exploration of alternative therapies, including herbal medicines. Binahong leaves (*Anredera cordifolia* (Ten.) Steenis) are widely used in traditional medicine and have demonstrated potential antibacterial properties. ***Aims*:** To evaluate and analyze the gel formulation of binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis) to identify a formulation with optimal physical characteristics and effective antibacterial activity against *Staphylococcus epidermidis*. ***Method*:** The study employed a true experimental design with a post-test–only control group. Treatment groups received binahong leaf extract gel at concentrations of 40%, 60%, 80%, and 100%. Clindamycin gel served as the positive control, while a gel base without extract was the negative control. *Staphylococcus epidermidis* was used as the test organism. Antibacterial activity was evaluated using the disc diffusion method, and data were analyzed using the Kruskal–Wallis test. ***Results*:** All gel formulations demonstrated acceptable physical characteristics. The inhibition zone diameters of binahong leaf extract gel against *Staphylococcus epidermidis* at concentrations of 40% (7.6 mm) and 60% (8.3 mm) were classified as moderate, whereas concentrations of 80% (10.86 mm) and 100% (12.80 mm) exhibited strong antibacterial activity. Statistical analysis revealed significant differences among treatment groups, with a p-value of < 0.05. ***Conclusion*:** Binahong leaf extract gel formulations at concentrations of 80% and 100% were identified as optimal, as they combined satisfactory physical properties with strong antibacterial activity against *Staphylococcus epidermidis*.

Keywords: Acne Vulgaris, Staphylococcus Epidermidis, Binahong Leaf Extract, Antibacterial Activity, Gel Formulation, Herbal medicine.

INTRODUCTION

The skin is an organ that covers the entire human body and serves as a protective barrier against external influences. As one of the most visible parts of the body, skin disorders can significantly affect personal appearance; therefore, the skin requires proper care, maintenance, and health preservation. (Rahmalia, 2020) One of the most prevalent skin problems globally, which often causes concern particularly among adolescents and young adults, is acne vulgaris. (Putri *et al*., 2021; Basri *et al.,* 2021) Acne is an inflammatory condition of the pilosebaceous unit characterized by excessive sebum production, as well as the presence of comedones, papules, pustules, and cysts. (Fitriana *et al*., 2018)

According to data from the Global Burden of Disease in 2019, acne ranked eighth among the most common diseases worldwide, with a prevalence rate of 9.4%. (Wang *et al*., 2022) Surveys conducted in Southeast Asia reported that 40–80% of cases involved acne vulgaris. (Sifatullah & Zulkarnain, 2021) In addition, a survey by MarkPlus, Inc. and Zap Clinic (2018) involving 17,889 women in Indonesia revealed that 58.7% experienced acne-related problems, indicating a high prevalence of acne among women. (Rahmalia, 2020)

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The etiology of acne is multifactorial, one of which includes excessive sebaceous gland activity that may be exacerbated by bacterial infection. (Meilina & Hadi, 2018) Research conducted by Lovena *et al*., (2020) at Adam Malik Hospital identified *Staphylococcus epidermidis* as one of the aerobic bacteria frequently associated with acne. (Sari *et al*., 2020) More recent research in 2022 by Lili Legawati *et al*., from the Department of Dermatology and Venereology, Faculty of Medicine, Indonesia, also reported that *Staphylococcus epidermidis* is the second most common acne-causing bacterium in Indonesia after *Propionibacterium acnes*. (Legiawati *et al*., 2023)

Two main treatment approaches are commonly used in acne management: topical therapy, which is applied directly to the affected area to produce local effects, and oral therapy, which works systemically. Both topical and oral antibiotics are routinely used in acne treatment. However, in recent years, antibiotic therapy for acne has faced significant challenges. One of the primary concerns associated with improper and prolonged antibiotic use is bacterial resistance, where bacteria become insensitive to certain antibiotic classes. Furthermore, long-term antibiotic use may result in organ damage and immunohypersensitivity reactions. (Damayanti *et al*., 2022; Kindangen *et al*., 2018)

According to the latest World Health Organization (WHO) report on Antimicrobial Resistance Global Surveillance, Southeast Asia has the highest rate of antibiotic resistance worldwide. (World Health Organization, 2022) A study conducted in 2019 at Cipto Mangunkusumo Hospital, Indonesia, reported that *Staphylococcus epidermidis* exhibited resistance to clindamycin in 52.2% of cases, erythromycin in 65.2%, doxycycline in 89.1%, and tetracycline in 32.6% of cases. Multidrug resistance (MDR) was also identified in isolates of *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, with similar resistance patterns observed among isolates from the same patients. (Sitohang *et al*., 2019; Pariury *et al*., 2021)

Given that antibiotic resistance has become a major public health concern, numerous studies have focused on identifying safer alternative antibacterial agents, particularly those derived from medicinal plants. The use of natural products has increased due to their relatively lower side effects, reduced risk of resistance, accessibility, and safety compared to conventional drugs. (Damayanti *et al*., 2022) One such plant is binahong (*Anredera cordifolia* (Ten.) Steenis), with its leaves commonly utilized. Binahong leaves contain various secondary metabolites, including flavonoids, alkaloids, saponins, tannins, and steroids/triterpenoids, which contribute to their antibacterial activity. (Hanum *et al*., 2022)

Previous studies have demonstrated the antibacterial potential of binahong leaf extract. Sri Puan (2022) reported that binahong leaf extract effectively inhibited the growth of Gram-negative bacteria such as *Salmonella typhi*, *Escherichia coli*, and *Shigella flexneri*, with the largest inhibition zone observed at a concentration of 100%. (Hanum *et al*., 2022) Additionally, Indarto *et al*., (2019) showed that ethyl acetate extract of binahong leaves exhibited antibacterial activity against *Propionibacterium acnes*, with higher concentrations resulting in greater inhibitory effects. (Indarto *et al*., 2019)

To optimize the topical antibacterial benefits of binahong leaf extract, it is necessary to formulate it into a suitable pharmaceutical dosage form to ensure practicality and efficiency in use. (Yati *et al*., 2018) Gel formulations are commonly used in acne treatment due to their good spreadability, ease of removal, non-sticky nature, and efficient drug release. (Dermawan, 2021) A previous study by Sony Dermawan (2022) reported that binahong leaf extract gel formulations at three different concentrations demonstrated weak antibacterial activity. Moreover, the gel base used in that study did not meet acceptable spreadability standards. (Dermawan, 2021)

Based on these considerations, this study aims to optimize the concentration and gel base formulation of binahong (*Anredera cordifolia*) leaf extract to enhance antibacterial activity against *Staphylococcus epidermidis* and improve physical characteristics, thereby overcoming limitations of previous studies and providing a more effective and clinically applicable herbal gel for acne management.

METHODS

The type of research employed in this study was a true experimental design using a posttest-only control group design. The bivariate analysis applied was the Kruskal–Wallis test, followed by post hoc analysis using the Dunnett T3 test. This research was conducted at the Integrated Laboratory of the Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana. The study was carried out from June to August 2023. The antibacterial activity was evaluated using the disc diffusion method. This study did not involve human participants or experimental animals; therefore, ethical committee approval was not required.

Bacterial Strain and Test Materials

The binahong leaves (Anredera cordifolia (Ten.) Steenis) used in this study were collected by the researchers in the Kupang City area, East Nusa Tenggara (NTT). The binahong leaves selected were fresh and green in color. Samples of Staphylococcus epidermidis were obtained from the Center for Health Laboratory (BBLK) Surabaya. The sample size in this study consisted of six groups, including four treatment groups of binahong leaf extract gel formulations at concentrations of 40%, 60%, 80%, and 100%, a negative control group consisting of gel preparations without extract, and a positive control group consisting of

clindamycin gel. Each group underwent four repetitions or replications.

Experimental Procedure

The extract preparation process began with the provision of 10 kg of binahong leaves. The leaves were washed thoroughly and air-dried at room temperature for 6 hours, followed by oven-drying at a temperature of 50°C for 24 hours. The dried leaves were then ground into powder. The powder was subsequently macerated by soaking it in 70% ethanol solvent for three days with daily stirring. After maceration, the mixture was filtered to obtain the liquid binahong leaf extract. The liquid extract was then evaporated using a rotary evaporator to obtain a thick extract.

The binahong leaf extract was subjected to an ethanol-free test by reacting potassium dichromate ($K_2Cr_2O_7$) with ethanol in an acidic environment. If the solution did not contain ethanol, a mixed color formed from the extract solution and potassium dichromate solution with the addition of sulfuric acid (H_2SO_4). Conversely, if ethanol was present, a blue color would appear. In addition, phytochemical screening was performed on the extract to identify the presence of active antibacterial compounds. The phytochemical screening of the binahong leaf extract included tests for alkaloids, flavonoids, tannins, saponins, and triterpenoids.

The formulation of the anti-acne gel preparation was carried out by placing 2 grams of carbopol 940 into a mortar containing 10 mL of distilled water and stirring rapidly. Subsequently, 1 mL of triethanolamine (TEA) was added gradually while stirring slowly until a clear gel was formed, followed by the addition of 5 mL of glycerin. Binahong leaf extract at the desired concentration was mixed with 0.2 grams of methyl paraben and then incorporated into the gel base. Distilled water was added to a final volume of 50 mL, and the mixture was stirred until homogeneous.

Prior to antibacterial testing, all equipment used was sterilized. Glassware and media were wrapped in paper and aluminum foil and sterilized in an autoclave at 121°C for 15–20 minutes, while inoculating needles and forceps were sterilized by direct flaming using spirit. Plastic equipment was sterilized using 70% alcohol.

Staphylococcus epidermidis underwent bacterial confirmation testing using Gram staining and catalase tests, followed by preparation of rejuvenation media. Nutrient agar media were prepared, sterilized, poured into Petri dishes, and allowed to solidify. The media were then inoculated with Staphylococcus epidermidis and incubated at 37°C for 24 hours. Subsequently, a bacterial suspension was prepared using 0.9% NaCl solution and 1–2 inoculating loops of bacteria until a turbidity equivalent to the 0.5 McFarland standard was achieved.

The antibacterial testing procedure involved using sterile cotton swabs dipped into the bacterial suspension and evenly spreading it over the surface of nutrient agar media. One paper disc with a diameter of 6 mm was placed on each Petri dish using sterile forceps. The paper discs had previously been immersed in each binahong leaf extract gel formulation for 30 minutes. All media were then incubated at 37°C for 24 hours. The same procedure was performed for the negative control (gel base without extract) and the positive control (clindamycin gel). The final step involved measuring the diameter of the inhibition zones using a caliper. The inhibition zone measurements were recorded as the research results.

RESULTS

Binahong Leaf Extraction

A total of 10 kg of binahong leaves (Anredera cordifolia (Ten.) Steenis) were cleaned by washing under running water and then air-dried at room temperature for 6 hours. The drying process was continued using an oven at 50°C for 24 hours. The dried leaves were subsequently ground using a blender to obtain 0.8 kg of binahong leaf powder. The powdered leaves were then subjected to maceration using 70% ethanol at a ratio of 1:10, consisting of 0.8 kg of binahong leaf powder and 8 liters of 70% ethanol, and soaked for 3 days with occasional daily stirring. The macerated mixture was then filtered using filter paper, yielding 6.5 liters of binahong leaf extract. This extract was subsequently concentrated using a vacuum rotary evaporator to obtain 183 grams of thick binahong leaf extract.

Ethanol Free Testing

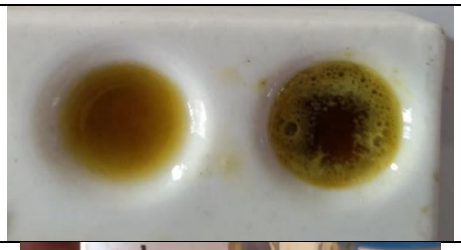
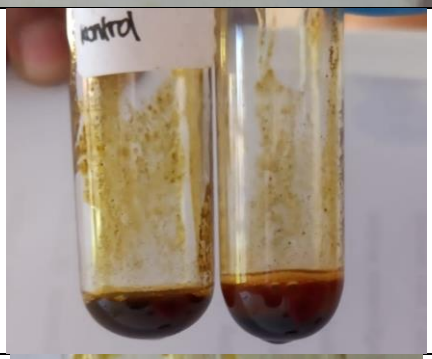
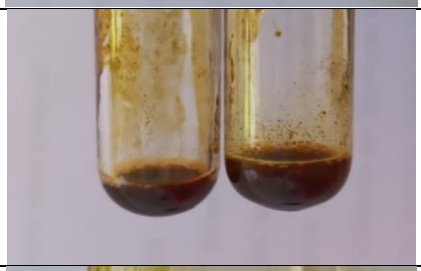
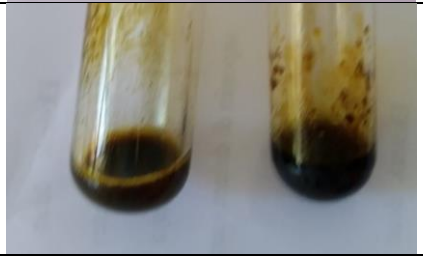
Table 1: Ethanol-Free Test

Identification	Results	Interpretation
Ethanol-Free Test		The extract was confirmed to be ethanol-free, as indicated by the formation of an orange coloration or a mixed color resulting from the reaction between the extract, potassium dichromate ($K_2Cr_2O_7$), and sulfuric acid (H_2SO_4).

Phytochemical Screening

The results of the phytochemical screening indicated that the binahong leaf extract contained active compounds including alkaloids, flavonoids, saponins, and tannins, while triterpenoids were not detected.

Table 2 : Phytochemical Screening

Compound Group	Results	Interpretation
Flavonoid		The extract tested positive for flavonoids, indicated by the formation of an orange–reddish color reaction
Alkaloid		The extract tested positive for alkaloids, as evidenced by the formation of a brown precipitate following the addition of Wagner's reagent.
Saponin		The extract tested positive for saponins, indicated by the formation of stable foam persisting for 10 minutes with a height of approximately 1 cm.
Tanin		The extract tested positive for tannins, as shown by the appearance of a dark-colored reaction (reddish-black).
Triterpenoid		The extract tested negative for triterpenoids, as no brown or violet ring was observed at the interface between the two solvent layers.

Evaluation of Physical Characteristics of the Anti-Acne Gel

Table 3 : Formulations of the Binahong Leaf Extract Gel Preparations

Ingredients Name	Formulations (%)					Function
	F0	F1	F2	F3	F4	
Binahong leaf ethanol extract	0	20 mL	30 mL	40 mL	50 mL	Active ingredient
Carbopol 940	2 gr	2 gr	2 gr	2 gr	2 gr	Gel base
Triethanolamine (TEA)	1 mL	1 mL	1 mL	1 mL	1 mL	pH neutralizer
Glycerin	5 mL	5 mL	5 mL	5 mL	5 mL	Humectant
Methyl Paraben	0,2 gr	0,2 gr	0,2 gr	0,2 gr	0,2 gr	Preservative
Aquadest ad.	50 ml	50 ml	50 ml	50 ml	50 ml	Solvent

Table 4 : Evaluation of Physical Characteristics of the Anti-Acne Gel

Evaluation	F0 (0%)	F1 (40%)	F2 (60%)	F3 (80%)	F4 (100%)	Standard value
Organoleptic:						
Colour	Clear	Light brown	Brown	Dark brown	Dark brown	-
Odor	Characteristic of gel base	Characteristic of binahong extract	Characteristic extract odor	Characteristic extract odor	Characteristic extract odor	-
Konsistensi	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid
Homogenity	Homogen	Homogen	Homogen	Homogen	Homogen	Homogen
pH	5,40	4,95	5,59	5,63	5,56	4,5-6,5
Spradability	5,6 cm	6,5 cm	5,7 cm	6,5 cm	6,8 cm	5-7 cm

Bacterial Confirmation Test

The Gram staining results showed that the test bacteria appeared purple with a cocci morphology, indicating that the bacteria were Gram-positive. The catalase test yielded a positive result, as evidenced by the formation of oxygen gas bubbles (O_2), which is characteristic of bacteria belonging to the genus *Staphylococcus*.

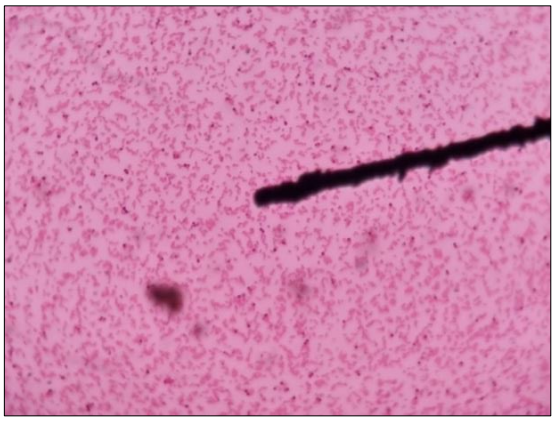


Figure 1 : Gram Staining Test Indicated Staphylococcus epidermidis

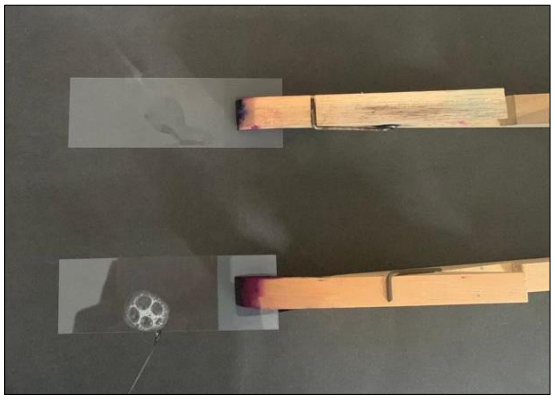


Figure 2 : Katalase Test Indicated Staphylococcus epidermidis

Antibacterial Test

The results of the antibacterial activity testing of binahong leaf extract gel formulations at concentrations of 40%, 60%, 80%, and 100%, along with the negative control (gel base without extract) and the positive control (clindamycin gel), against the growth of *Staphylococcus epidermidis* are presented. Antibacterial activity was indicated by the presence of inhibition zones, as evidenced by the diameter of the clear area formed, or by the absence of bacterial growth surrounding the paper discs. The results of the antibacterial activity testing of the binahong leaf extract presented below.

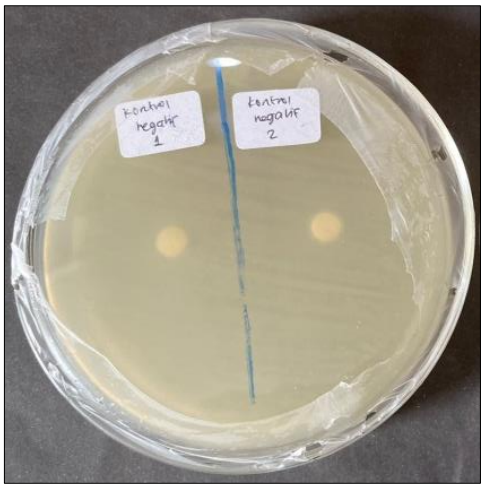


Figure 3 : Negative Control

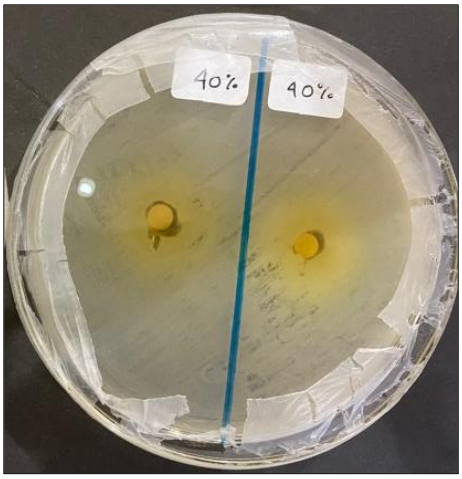


Figure 4 : 40% Gel Extract

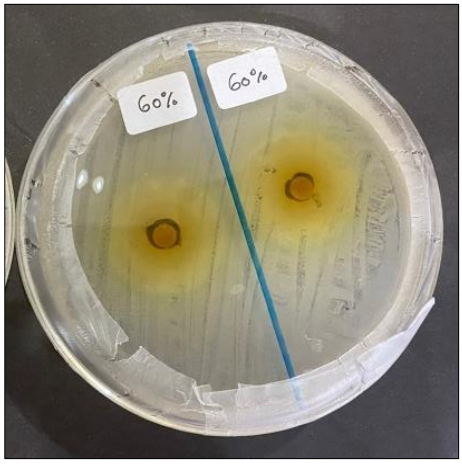


Figure 5 : 60% Gel Extract

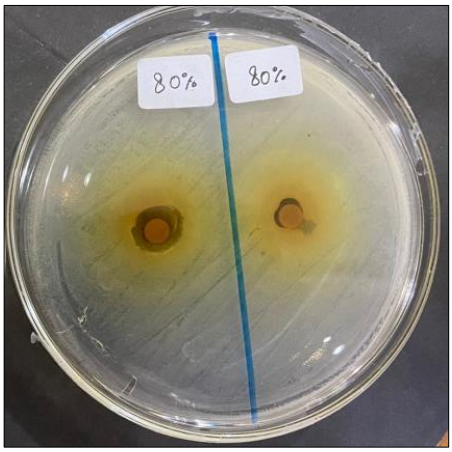


Figure 6 : 80% Gel Extract

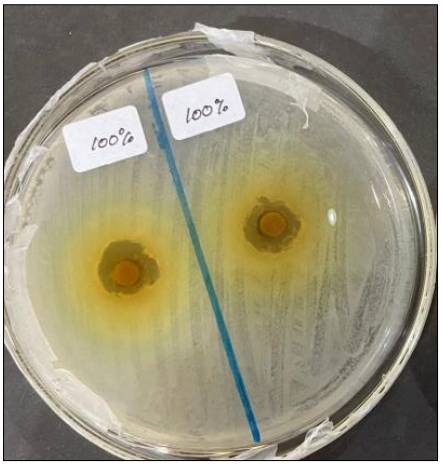


Figure 7 : 100% Gel Extract

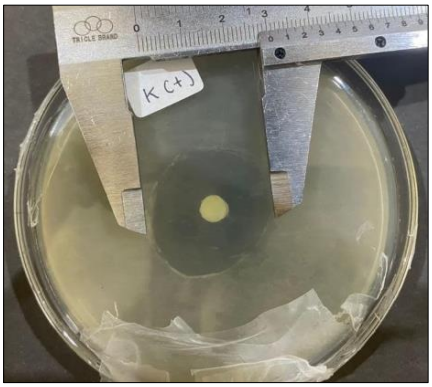


Figure 8: Positive Control

Table 5: Measurement Results of Inhibition Zone Diameters of Binahong Leaf Extract Anti-Acne Gel Against Staphylococcus epidermidis

Treatment Groups	Inhibition Zone Diameter (mm)					Potency
	1	2	3	4	Mean	
F0	0	0	0	0	0	Weak
F1	7,33	7,47	8,25	7,4	7,6	Moderate
F2	8,33	9,28	8,23	7,5	8,33	Moderate
F3	8,95	10,56	11,33	12,61	10,86	Strong
F4	11,78	14,13	12,11	13,21	12,80	Strong

(+) Control	28,8	29,5	28,1	27,9	28,5	Very Strong

Data Analysis

Table 6: Results of the Normality Test

Treatment Group	P Value*	Data Distribution
F0	*omitted*	-
F1	0,040	Abnormal
F2	0,780	Normal
F3	0,982	Normal
F4	0,631	Normal
Positive Control	0,603	Normal

***Notes**: Normality test using Shapiro-Wilk test

Based on the results of the normality test presented in the table 6, formulations F2 (60% concentration), F3 (80% concentration), F4 (100% concentration), and the positive control demonstrated normally distributed data, whereas formulation F1 (40% concentration) showed a non-normal data distribution. The negative control yielded a value of zero, indicating no inhibition zone formation and therefore was excluded from the normality assessment. Consequently, it can be concluded that the overall normality test results for the inhibition zone diameters of the binahong leaf extract anti-acne gel against *Staphylococcus epidermidis* were not normally distributed.

Table 7: Results of the Homogeneity Test

	*Levene statistic**	Notes
Inhibition Zone Diameter	0,039	Not homogeneous

Based on the results of the homogeneity test for the inhibition zone diameters presented in the table above, a p-value of 0.039 was obtained with a significance level (α) of 0.05. Since 0.039 < 0.05, it can be concluded that the data in this study are not homogeneous.

Table 8: Results of the Kruskal–Wallis Test

	*Asym. Sig**	Notes
Inhibition Zone Diameter	0,002	There was a statistically significant difference in mean values.

Based on the results of the normality test of the inhibition zone diameters of the binahong leaf extract anti-acne gel against the growth of *Staphylococcus epidermidis*, the data were found to be not normally distributed; therefore, the Kruskal–Wallis test was used for data analysis.

The Kruskal–Wallis analysis yielded a p-value of 0.002, which is less than 0.05, indicating that the null hypothesis (H_0) was rejected and the alternative hypothesis (H_1) was accepted. This result demonstrates that the binahong leaf extract anti-acne gel exhibited antibacterial activity, as evidenced by a significant difference in mean inhibition zone diameters among the treatment groups.

Table 9: Results of Post Hoc Analysis Using the Dunnett T3 Test

Treatment Group	Treatment Group					
	F0	F1	F2	F3	F4	K(+)
F0		0,000*	0,001*	0,005*	0,001*	0,000*
F1	0,000*		0,726	0,128	0,007*	0,000*
F2	0,001*	0,726		0,250	0,008*	0,000*
F3	0,005*	0,128	0,250		0,535	0,000*
F4	0,001*	0,007*	0,008*	0,535		0,000*
(+) Control	0,000*	0,000*	0,000*	0,000*	0,000*	

Based on the results of the Dunnett T3 post hoc test presented in Table 9, several significant differences were identified among the study groups. Formulation 0 showed statistically significant differences compared with Formulations 1, 2, and 3, as well as with the positive and negative control groups ($p < 0.05$). Formulation 1 differed significantly from Formulation 0, Formulation 4, and the positive control ($p < 0.05$), but showed no significant differences when compared with Formulations 2 and 3 ($p > 0.05$). Similarly, Formulation 2 demonstrated significant differences relative to Formulation 0, Formulation 4, and the positive control (p

< 0.05), while no significant differences were observed in comparison with Formulations 1 and 3 (p > 0.05).

Formulation 3 exhibited significant differences compared with Formulation 0 and the positive control (p < 0.05), but did not differ significantly from Formulations 1, 2, and 4 (p > 0.05). Formulation 4 showed significant differences when compared with Formulations 0, 1, and 2, as well as with the positive control (p < 0.05), whereas no significant difference was found between Formulation 4 and Formulation 3 (p > 0.05). The positive control group demonstrated statistically significant differences when compared with all formulation groups (Formulations 0–4), with p-values less than 0.05.

DISCUSSION

The formulation and antibacterial activity testing of binahong leaf extract anti-acne gel against *Staphylococcus epidermidis* were conducted by observing the presence of bacterial inhibition zones, defined as areas around the disc where no bacterial growth occurred. The initial stage of this study involved an extraction process aimed at obtaining secondary metabolite compounds required for antibacterial activity. (Teroreh *et al*., 2015) The extraction was performed using the maceration technique with 70% ethanol as the solvent. The selection of 70% ethanol was based on its ability to extract a greater amount of active compounds compared with other organic solvents. (Suhendra *et al*., 2019)

Maceration was chosen because it is one of the most commonly used extraction methods and can reduce the risk of degradation of thermolabile compounds present in plant materials. (Badaring *et al*., 2020) The dried and powdered binahong leaves were soaked in the solvent in a closed container for three days. During this period, daily stirring was carried out to facilitate the dissolution of the required secondary metabolites into the solvent. The maceration product was subsequently filtered, and solvent evaporation was performed to obtain a concentrated and purified extract with a high concentration of active compounds. (Munazar, 2019)

The concentrated binahong leaf extract was subjected to an ethanol-free test, and the results indicated that the extract did not contain ethanol. This test was conducted to ensure that the extract was free from ethanol contamination, as ethanol itself possesses antibacterial and antifungal properties that could lead to false-positive results during antibacterial testing. (Kurniawati, 2015) In addition, phytochemical screening was performed to identify the secondary metabolite compounds present in the extract. (Kumalasari & Andiarna, 2020) The screening results revealed the presence of flavonoids, alkaloids, tannins, and saponins, which is consistent with the findings reported by Cut Bidara and Walfa Syafidya (2022), who also demonstrated that binahong leaf extract contains these compounds. (Sariwating, 2022) In contrast, triterpenoids were not detected, which may be attributed to the solvent used.

Triterpenoids are nonpolar compounds, whereas 70% ethanol is a polar solvent. This observation aligns with the “like dissolves like” principle, whereby polar compounds dissolve in polar solvents and nonpolar compounds dissolve in nonpolar solvents. (Hanifah & Anjani, 2022; Karim, 2017) Flavonoids exert antibacterial activity by inhibiting nucleic acid synthesis, disrupting cytoplasmic membrane function, and interfering with bacterial energy metabolism. (Cushnie & Lamb, 2005) Alkaloids act by inhibiting bacterial cell wall synthesis, altering membrane permeability, suppressing bacterial metabolism, and inhibiting nucleic acid and protein synthesis. (Othman *et al*., 2019) Tannins exhibit antibacterial effects by inhibiting bacterial adhesion to surfaces, inducing bacterial cell death, and limiting bacterial growth through inhibition of sugar and amino acid absorption. (Kaczmarek, 2020) Saponins exert antibacterial activity by disrupting cell wall permeability, leading to bacterial cell lysis and death. (Tagousop *et al*., 2018)

The binahong leaf extract containing these secondary metabolites was formulated into a gel preparation using a gel base and gelling agent. Five gel formulations were prepared with varying concentrations of binahong leaf extract: F0 (negative control), F1 (40%), F2 (60%), F3 (80%), and F4 (100%). The gel preparations were evaluated for physical characteristics, including organoleptic properties, homogeneity, pH, and spreadability. Based on Table 4, all formulations exhibited stable semi-solid consistency. Formulations F1, F2, F3, and F4 possessed the characteristic odor of the extract, whereas F0 (gel base) used as the negative control exhibited the characteristic odor of the gel base.

Differences in odor and color were influenced by the concentration of extract present in each formulation. Homogeneity testing showed that none of the formulations contained coarse particles, indicating that all formulations were homogeneous. The pH values obtained were 4.95 for F1, 5.59 for F2, 5.63 for F3, 5.56 for F4, and 5.40 for F0. The acceptable pH range for topical preparations that are compatible with skin and non-irritating is 4.5–6.5. Therefore, all binahong leaf extract gel formulations met the pH requirements, indicating that they are safe for topical application and unlikely to cause skin irritation. (Thomas *et al*., 2023)

The final evaluation involved antibacterial activity testing of the binahong leaf extract gel formulations. The test was conducted using the diffusion method, in which the inhibition zone or area devoid of bacterial growth surrounding the disc was observed and measured, indicated by a clear zone around the disc. Based on the criteria for antibacterial inhibition activity proposed by Davis and Stout (1971), the mean inhibition zone diameters of the binahong leaf extract gel against *S.*

epidermidis for Formulation 4 (100% concentration; 12.80 mm) and Formulation 3 (80% concentration; 10.86 mm) were categorized as strong. Formulation 2 (60% concentration; 8.33 mm) and Formulation 1 (40% concentration; 7.6 mm) were categorized as moderate. Formulation 0 (negative control) was categorized as weak, as no inhibition zone was observed (0 mm).

The positive control using clindamycin gel was categorized as very strong, with an inhibition zone diameter of 28.5 mm. These results indicate that the binahong leaf extract anti-acne gel exhibits antibacterial activity, leading to the rejection of H0 and acceptance of H1. Furthermore, the data demonstrate a direct relationship between extract concentration and inhibition zone diameter, indicating that higher extract concentrations result in greater antibacterial activity. This finding is consistent with the study conducted by Cut Bidara *et al*., (2022) on the antibacterial activity of binahong leaf extract against *Staphylococcus epidermidis*. (Bidara *et al*., 2022)

When compared with previous similar research on the formulation of ethanol extract gel of binahong leaves against *Staphylococcus epidermidis* by Sony Dermawan (2021), the results of this study showed discrepancies. In that study, the mean inhibition zone diameters at extract concentrations of 25%, 30%, and 35% were 9.4 mm, 10.36 mm, and 11.56 mm, respectively. (Dermawan, 2021) In contrast, the present study demonstrated smaller inhibition zones at concentrations of 40%, 60%, and 80%, namely 7.6 mm, 8.33 mm, and 10.86 mm, respectively. One factor influencing the diameter of the bacterial inhibition zone is the concentration of the gel base, specifically carbopol 940. Higher concentrations of carbopol 940 increase gel viscosity, which in turn reduces the diffusion rate of antibacterial compounds into the agar medium, resulting in smaller inhibition zones. (Nailufar, 2013; Masyithah *et al*., 2015)

This study employed clindamycin gel as the positive control and a gel base without extract (F0) as the negative control. Clindamycin is a lincosamide antibiotic used to treat serious bacterial infections by inhibiting bacterial growth. It exhibits high activity against various facultative anaerobic bacteria and is particularly effective against Gram-positive bacteria. Clindamycin acts by inhibiting protein synthesis at the bacterial ribosome, thereby disrupting peptide chain formation. It can suppress the production of bacterial proteins, toxins, enzymes, and cytokines within tissues. (Athaillah & Sugesti, 2020).

In this study, the clindamycin gel as a positive control produced a clear inhibition zone, confirming its antibacterial activity. Conversely, the negative control (gel base without extract) showed no inhibition zone, indicating that the antibacterial activity observed in this study was attributable to the binahong leaf extract. (Rasyid & Amody, 2020)

Data analysis began with univariate analysis using the Shapiro–Wilk normality test, as the sample size was fewer than 50. The normality test results indicated that the data were not normally distributed, as Formulation 1 showed a p-value < 0.05. Given the non-normal distribution of the data, the Kruskal–Wallis test was employed for bivariate analysis. The results of this test yielded a p-value < 0.05, leading to the rejection of H0 and acceptance of H1, indicating a significant difference in the mean inhibition zone diameters among the treatment groups.

Subsequent analysis involved a homogeneity test using Levene's statistic, which indicated that the data were not homogeneous ($p > 0.05$). Consequently, the Dunnett T3 post hoc test was used to identify which treatment groups exhibited significant differences in mean inhibition zone diameters. The Dunnett T3 test results showed significant differences ($p < 0.05$) between Formulation 1 and the negative control, Formulation 4, and the positive control; Formulation 2 and the negative control, Formulation 5, and the positive control; Formulation 3 and the negative control and positive control; Formulation 4 and the negative control and positive control; the positive control and all formulations and the negative control; as well as the negative control and Formulation 5 and the positive control.

CONCLUSION

There is antibacterial activity exhibited by the anti-acne gel formulation of binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis) against *Staphylococcus epidermidis*.

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