



Phytochemical Screening, Cream Formulation, and Antibacterial Activity of *Cyanthillium cinereum* (L.) H. Rob. Leaf Ethanol Extract against *Staphylococcus aureus*

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ABSTRACT

Cyanthillium cinereum (L.) H. Rob. contains secondary metabolites with potential antibacterial activity. This study performed qualitative phytochemical screening of the leaf ethanol extract, formulated topical creams containing 0.75% (F1), 1.5% (F2), and 3% (F3) extract, and evaluated preliminary physical stability and antibacterial activity against *Staphylococcus aureus* ATCC 25923. Qualitative screening detected alkaloids, steroids, saponins, flavonoids, tannins, glycosides, and phenolic compounds. The creams were homogeneous; mean pH values ranged from 6.22 to 6.45 before cycling and from 6.00 to 6.05 after cycling. Antibacterial responses increased with concentration. F3 produced the greatest response among the creams (19.07 ± 0.45 mm, $n = 3$), while the blank base showed no inhibition; the corresponding unformulated 3% extract produced 19.23 ± 0.42 mm. Two-way ANOVA showed significant effects of preparation type ($p < 0.001$), concentration ($p < 0.001$), and their interaction ($p = 0.003$). The E3-F3 difference was not significant by Tukey HSD ($p = 0.996$), but this does not establish statistical equivalence. These findings indicate that an antibacterial response remains measurable after incorporation into a cream matrix and support further investigation of the 3% formulation.

Keyword: *Cyanthillium cinereum*, *Staphylococcus aureus*, Antibacterial Activity, Screening, Formulation

ABSTRAK

Sawi langit (*Cyanthillium cinereum* (L.) H. Rob.) mengandung metabolit sekunder yang berpotensi sebagai antibakteri. Penelitian ini melakukan skrining fitokimia kualitatif terhadap ekstrak etanol daun, memformulasikannya sebagai krim topikal dengan konsentrasi ekstrak 0,75% (F1), 1,5% (F2), dan 3% (F3), serta mengevaluasi stabilitas fisik awal dan aktivitas antibakteri terhadap *Staphylococcus aureus* ATCC 25923. Skrining kualitatif menunjukkan adanya alkaloid, steroid, saponin, flavonoid, tanin, glikosida, dan senyawa fenolik. Sediaan krim homogen; rerata pH berkisar 6,22-6,45 sebelum cycling dan 6,00-6,05 setelah cycling. Respons antibakteri meningkat seiring peningkatan konsentrasi. F3 menghasilkan respons terbesar di antara sediaan krim ($19,07 \pm 0,45$ mm; $n = 3$), sedangkan basis krim tidak menunjukkan hambatan; ekstrak 3% tanpa formulasi menghasilkan $19,23 \pm 0,42$ mm. ANOVA dua arah menunjukkan pengaruh bermakna dari jenis sediaan ($p < 0,001$), konsentrasi ($p < 0,001$), dan interaksinya ($p = 0,003$). Perbedaan E3-F3 tidak bermakna berdasarkan Tukey HSD ($p = 0,996$), tetapi hasil ini tidak membuktikan ekuivalensi statistik. Temuan ini menunjukkan bahwa respons antibakteri tetap terukur setelah ekstrak diformulasikan dalam matriks krim dan mendukung penelitian lanjutan terhadap formulasi 3%.

Kata Kunci: *Cyanthillium cinereum*, *Staphylococcus aureus*, Aktivitas Antibakteri, Skrining, Formulasi



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1. Introduction

Staphylococcus aureus is a clinically important Gram-positive pathogen and a major cause of skin and soft-tissue infections. The emergence and persistence of antibiotic-resistant strains, including methicillin-resistant *S. aureus* (MRSA), continue to complicate treatment and motivate the search for alternative antibacterial agents [1]. Medicinal plants are a valuable source of structurally diverse secondary metabolites, and plant-derived antibacterials remain of interest because they may act through multiple cellular targets [2]. *Cyanthillium cinereum* (L.) H. Rob. (*Asteraceae*), also widely reported under the synonym *Vernonia cinerea*, contains diverse secondary metabolites, including sesquiterpene lactones, triterpenes, flavonoids, steroids, and phenolic compounds, and extracts of this species have demonstrated antimicrobial activity [3]. A previous study specifically reported anti-staphylococcal activity of methanolic *C. cinereum* extracts, supporting further investigation of this species against *S. aureus* [4]. Recent studies further demonstrate continuing chemical and pharmacological investigation of *C. cinereum*, including essential-oil characterization, HPTLC fingerprinting, and experimental pharmacological evaluation [5-7].

Despite evidence for the phytochemical diversity and antibacterial potential of *C. cinereum*, previous work has focused mainly on crude extracts, isolated constituents, or general pharmacological properties. Less attention has been given to whether antibacterial activity is retained after incorporation into a topical semisolid formulation. This distinction is important because a cream matrix can alter the release, partitioning, availability, and diffusion of phytochemical constituents. Formulation-based studies of other medicinal plants have shown that antibacterial responses can remain measurable after incorporation into cream vehicles, while also demonstrating the importance of a blank formulation control [8]. The present study therefore performed qualitative phytochemical screening of the leaf ethanol extract of *C. cinereum*, formulated the extract into creams containing 0.75%, 1.5%, and 3% extract, evaluated preliminary physical stability, and assessed antibacterial activity against *S. aureus* ATCC 25923. The principal novelty lies in determining whether the concentration-dependent antibacterial response of the leaf ethanol extract is retained after incorporation into a topical cream matrix.

2. Materials and Methods

2.1. Plant Material and Authentication

Fresh leaves of *Cyanthillium cinereum* (L.) H. Rob. were authenticated at Herbarium Medanense (MEDA), Universitas Sumatera Utara, Medan, Indonesia. The leaves were cleaned, dried, ground, and sieved to obtain a fine powder. Approximately 521 g of powdered material was obtained and used for subsequent extraction procedures.

2.2. Chemicals, Media, and Bacterial Strain

The chemicals used for extraction and phytochemical screening included 96% ethanol, hydrochloric acid, sulfuric acid (H₂SO₄), potassium iodide (KI), sodium hydroxide, ferric chloride (FeCl₃), methanol, chloroform, and the respective reagents required for qualitative phytochemical analysis. Stearic acid, glycerin, triethanolamine, cetyl alcohol, green tea fragrance, and distilled water were used for cream preparation. Nutrient Agar (NA), Mueller–Hinton Agar (MHA), and Mueller–Hinton Broth (MHB) were used for bacterial cultivation and antibacterial testing. *Staphylococcus aureus* ATCC 25923 was used as the test microorganism. Chloramphenicol (10 mg/mL) was used as the positive antibacterial control.

2.3. Preparation of the Ethanol Extract

A total of 500 g of powdered *C. cinereum* leaves was extracted by maceration with 96% ethanol at an overall plant material-to-solvent ratio of 1:10 (w/v). The powdered material was initially macerated with 3,750 mL of 96% ethanol in a closed dark-glass container for 5 days at room temperature, with occasional stirring and protection from direct light. The mixture was filtered, and the remaining plant residue was remacerated with 1,250 mL of fresh 96% ethanol for an additional 2 days. The resulting macerates were combined and concentrated using a rotary evaporator at 45 °C, followed by further evaporation in a water bath until a thick extract was obtained. The extraction yield was calculated using the following Equation 1.

$$\text{Extraction yield (\%)} = \frac{W_e}{W_p} \times 100 \quad (1)$$

where:

W_e = weight of the concentrated extract (g);

W_p = weight of the dried plant powder used for extraction (g).

2.4. Qualitative Phytochemical Screening

The ethanol extract of *C. cinereum* leaves was subjected to qualitative phytochemical screening for alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, glycosides, and phenolic compounds. Approximately 0.5 g of extract was used for each screening test. Alkaloids were evaluated using Mayer, Dragendorff, and Wagner/Bouchardat reagents; formation of a white-cream or orange-brown precipitate was considered positive. Flavonoids were tested by adding magnesium powder followed carefully by concentrated HCl; development of an intense red, orange, pink, or yellow color indicated a positive reaction. Tannins were tested with 2–3 drops of 1% FeCl₃, with dark green, green-black, or blue-black coloration indicating a positive result. Saponins were assessed by vigorously shaking the sample with approximately 10 mL of hot distilled water and allowing it to stand for about 10 min; persistent stable froth indicated a positive reaction. Steroids/triterpenoids were evaluated using Liebermann-Burchard reagent, with green/blue coloration indicating steroids and red/purple/brown coloration suggesting triterpenoids. Glycosides were assessed using the laboratory qualitative Molisch/glycoside procedure and interpreted from the characteristic ring or color reaction. Phenolic compounds were tested with 2–3 drops of 1% FeCl₃; blue, green, or dark green-black coloration was considered positive. These tests established the presence or absence of broad phytochemical classes and did not identify or quantify individual compounds.

2.5. Preparation of Cream Formulations

Four cream formulations were prepared: a blank cream base without *C. cinereum* extract (F0) and three extract-containing formulations containing 0.75% (F1), 1.5% (F2), and 3% (F3) ethanol extract. The stepwise low-to-moderate concentration range was selected during preliminary formulation to evaluate concentration-related antibacterial response while maintaining practical incorporation and homogeneity in the cream base. This design was informed by previously reported phytochemical and antibacterial evidence for *C. cinereum* [3][4], although the exact concentrations used here were defined by the present pre-formulation design. The composition of each formulation per 100 g is presented in Table 1.

Table 1. Composition of *C. cinereum* leaf ethanol extract cream formulations (per 100 g)

Ingredient	F0	F1 (0.75%)	F2 (1.5%)	F3 (3%)
<i>C. cinereum</i> ethanol extract (g)	0	0.75	1.50	3.00
Stearic acid (g)	13	13	13	13
Glycerin (g)	12	12	12	12
Triethanolamine (g)	3	3	3	3
Cetyl alcohol (g)	3	3	3	3
Green tea fragrance	q.s.	q.s.	q.s.	q.s.
Distilled water	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g

Stearic acid and cetyl alcohol were melted in a water bath to form the oil phase. Glycerin and triethanolamine were incorporated into heated distilled water to prepare the aqueous phase. The oil and aqueous phases were combined while warm and mixed until a homogeneous cream base was obtained. The appropriate amount of *C. cinereum* ethanol extract was gradually incorporated into the cream base and mixed until homogeneous. Green tea fragrance was subsequently added, and the formulations were transferred into suitable closed containers.

2.6. Physical Evaluation and Cycling Stability Test

The cream formulations were evaluated for physical appearance, homogeneity, phase separation, and pH. The pH was measured using a calibrated digital pH meter, with three measurements performed for each formulation at each assessment point (n = 3). Preliminary accelerated stability was evaluated using a cycling test consisting of six consecutive cycles. During each cycle, the formulations were stored at 4 ± 2 °C for 24 h and subsequently at 40 ± 2 °C for 24 h. These two consecutive storage conditions constituted one complete cycle. Physical appearance, homogeneity, phase separation, and pH were evaluated before and after completion of the cycling test.

2.7. Preparation of the Bacterial Inoculum

A fresh culture of *S. aureus* ATCC 25923 was used for antibacterial testing. Bacterial colonies were aseptically suspended in the appropriate medium, and the turbidity of the bacterial suspension was adjusted to

a 0.5 McFarland standard immediately before inoculation. The standardized bacterial suspension was subsequently used to inoculate Mueller–Hinton agar plates.

2.8. Agar Well-Diffusion Assay

The antibacterial activities of the *C. cinereum* leaf ethanol extract and cream formulations were evaluated against *S. aureus* ATCC 25923 using the agar well-diffusion method. Mueller-Hinton agar plates were uniformly inoculated with a bacterial suspension standardized to 0.5 McFarland. Wells 8 mm in diameter were aseptically prepared in the inoculated agar. The ethanol extract was evaluated at 0.75% (E1), 1.5% (E2), and 3% (E3), whereas the corresponding cream formulations contained 0.75% (F1), 1.5% (F2), and 3% (F3) extract. A volume of 100 μ L of each test sample was introduced into the respective wells. Chloramphenicol (10 mg/mL) was used as the positive control, while the blank cream base without *C. cinereum* extract (F0) served as the negative formulation control. Plates were incubated at 37 °C for 24 h. The total inhibition-zone diameter, including the 8-mm well, was measured with a vernier caliper with 0.1-mm resolution in two mutually perpendicular directions, and the mean of the two diameters was recorded for each experimental replicate. Three independent experimental replicates were performed on three separate agar plates under the same inoculum and sample-batch conditions ($n = 3$). F0 was recorded as no inhibition when no clear inhibitory halo was observed beyond the well.

2.9. Data Analysis

Antibacterial assays were performed as three independent experimental replicates on separate agar plates ($n = 3$), and inhibition-zone diameters are presented as mean $\pm$ sample standard deviation (SD) with 95% confidence intervals (CI). For the 2×3 factorial comparison of unformulated ethanol extract versus cream formulation at 0.75%, 1.5%, and 3% extract concentrations, data were analyzed by two-way analysis of variance (ANOVA) to evaluate the effects of preparation type, concentration, and the preparation type $\times$ concentration interaction. Partial eta squared (η^2) was calculated as an effect-size measure. Tukey's honestly significant difference (HSD) test was used for post-hoc multiple comparisons. Statistical significance was set at $p < 0.05$. The blank cream base (F0) and chloramphenicol positive control were reported descriptively as controls and were not included in the 2×3 factorial ANOVA.

3. Results and Discussion

3.1. Extraction Yield and Qualitative Phytochemical Screening

Maceration of 500 g of powdered *Cyanthillium cinereum* leaves with 96% ethanol produced approximately 4.454 mL of combined macerate. After concentration using a rotary evaporator and further evaporation, 111 g of thick extract was obtained, corresponding to an extraction yield of 22.2% (w/w). The extraction yield represents the amount of ethanol-soluble material recovered from the dried leaf powder under the applied maceration conditions. Although the relatively substantial yield indicates efficient recovery of soluble constituents, extraction yield alone cannot be considered a direct indicator of antibacterial potency because the crude extract consists of a complex mixture of compounds with different biological activities. Qualitative phytochemical screening demonstrated the presence of alkaloids, steroids, saponins, flavonoids, tannins, glycosides, and phenolic compounds.

Table 2. Qualitative phytochemical profile of *C. cinereum* leaf ethanol extract

Phytochemical constituent	Result
Alkaloids	+
Steroids	+
Saponins	+
Flavonoids	+
Tannins	+
Glycosides	+
Phenolic compounds	+

Note: (+) indicates the presence of the corresponding phytochemical group based on qualitative screening.

The detected phytochemical profile is consistent with the updated phytochemical literature on *C. cinereum/V. cinerea*, which describes approximately 92 characterized secondary metabolites, particularly sesquiterpene lactones, together with triterpenes, flavonoids, steroids, phenolics, and other constituents [3]. These classes provide a plausible chemical basis for the observed antibacterial response. Flavonoids and other polyphenols can affect bacterial membranes, proteins, nucleic acids, energy metabolism, and biofilm-related

processes [9][10]. Nevertheless, the screening performed here was qualitative; therefore, the observed activity cannot be assigned to a single constituent or phytochemical class, and quantitative profiling is required to establish compound–activity relationships. Comparable recent studies of *C. cinereum*/*V. cinerea* and other medicinal plants have likewise combined phytochemical profiling with antimicrobial or broader biological evaluation, reinforcing the value of linking chemical characterization with bioactivity data [11-20]

3.2. Physical Characteristics and Preliminary Stability of the Cream Formulations

The ethanol extract was successfully incorporated into the cream base at concentrations of 0.75% (F1), 1.5% (F2), and 3% (F3), while a blank cream base without extract was prepared as F0. The extract-containing creams were homogeneous and showed no apparent phase separation during the physical evaluation. Mean pH values before cycling were 6.45 ± 0.01 (F0), 6.39 ± 0.01 (F1), 6.31 ± 0.01 (F2), and 6.22 ± 0.01 (F3). After cycling, the corresponding values were 6.05 ± 0.01 , 6.04 ± 0.01 , 6.02 ± 0.01 , and 6.00 ± 0.01 , respectively. Human skin surface is physiologically acidic, and published literature commonly reports values around pH 4.6–5.8; this acidity contributes to barrier and antimicrobial functions [31]. Therefore, the measured formulation pH values, particularly before cycling, are somewhat higher than the typical skin-surface range and should not be interpreted as proof of dermal compatibility or safety. The modest pH decrease after cycling and absence of apparent phase separation indicate only that the formulations maintained basic physical characteristics under the applied accelerated conditions. Direct skin-compatibility assessment, irritation testing, further formulation optimization, and longer stability studies are required before topical safety or shelf-life can be concluded.

3.3. Concentration-Dependent Antibacterial Activity

The ethanol extract and extract-containing cream formulations showed a concentration-dependent antibacterial response against *Staphylococcus aureus* ATCC 25923. Based on the three independent plate replicates, mean $\pm$ SD total inhibition-zone diameters were 15.00 ± 0.36 mm (E1; 95% CI 14.10–15.90), 17.40 ± 0.50 mm (E2; 16.16–18.64), and 19.23 ± 0.42 mm (E3; 18.20–20.27) for the unformulated extracts, and 12.70 ± 0.40 mm (F1; 11.71–13.69), 15.67 ± 0.45 mm (F2; 14.55–16.79), and 19.07 ± 0.45 mm (F3; 17.95–20.19) for the corresponding creams. F0 showed no inhibition beyond the well, whereas chloramphenicol (10 mg/mL) produced 16.50 ± 0.50 mm (95% CI 15.26–17.74). Two-way ANOVA for E1-E3 and F1-F3 showed significant effects of preparation type ($F(1,12) = 47.25$, $p < 0.001$, $\eta^2 = 0.797$), concentration ($F(2,12) = 225.74$, $p < 0.001$, $\eta^2 = 0.974$), and the preparation type $\times$ concentration interaction ($F(2,12) = 9.81$, $p = 0.003$, $\eta^2 = 0.621$).

Table 3. Descriptive antibacterial response of *C. cinereum* ethanol extract and cream formulations against *S. aureus* ATCC 25923

Test sample	Concentration	Total inhibition-zone diameter (mm), mean $\pm$ SD (95% CI), n = 3
E1 – Ethanol extract	0.75%	15.00 ± 0.36 (14.10–15.90)
E2 – Ethanol extract	1.5%	17.40 ± 0.50 (16.16–18.64)
E3 – Ethanol extract	3%	19.23 ± 0.42 (18.20–20.27)
F0 – Blank cream base	0% extract	0.00 ± 0.00 (no inhibition)
F1 – Cream formulation	0.75%	12.70 ± 0.40 (11.71–13.69)
F2 – Cream formulation	1.5%	15.67 ± 0.45 (14.55–16.79)
F3 – Cream formulation	3%	19.07 ± 0.45 (17.95–20.19)
Chloramphenicol – positive control	10 mg/mL	16.50 ± 0.50 (15.26–17.74)

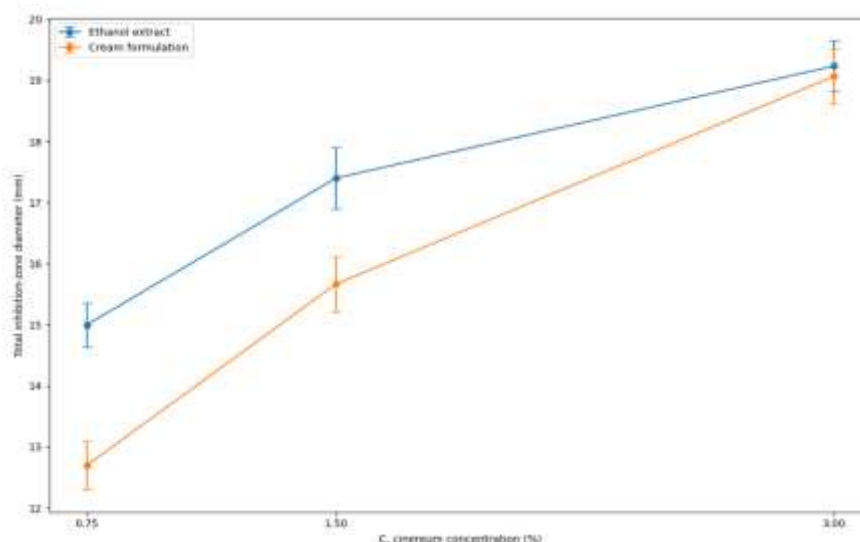


Figure 1. Concentration-dependent total inhibition-zone diameters of *C. cinereum* leaf ethanol extract and cream formulations against *S. aureus* ATCC 25923. Values are mean $\pm$ SD from three independent plate replicates ($n = 3$).

Figure 1 shows a consistent concentration-related increase for both preparations. Previous work has reported anti-staphylococcal activity of *C. cinereum*, including a methanolic leaf extract with an inhibition zone of 6.47 ± 0.17 mm at 50 mg/mL; however, that study used different extraction and diffusion conditions and therefore serves only as contextual evidence rather than a direct potency comparison [4]. More broadly, updated reviews confirm antimicrobial activity across *C. cinereum*/*V. cinerea* extracts and fractions [3].

Agar-diffusion results reflect both biological activity and the ability of constituents to diffuse through the agar matrix. Accordingly, inhibition-zone diameter should not be interpreted as a direct quantitative measure of antibacterial potency, particularly when crude extracts and formulated samples differ in physicochemical properties [2][21]. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) would provide a more rigorous characterization of antibacterial activity. In the present assay, the observed inhibition-zone diameters for E2, E3, and F3 were numerically larger than that of the chloramphenicol control (10 mg/mL). This observation should not be interpreted as evidence that the crude extract or cream is intrinsically more potent than chloramphenicol, because agar-diffusion zone size depends on sample loading, diffusion, solubility, molecular properties, and formulation matrix, and the test materials were not compared on an equimolar or standardized active-compound basis. The positive control therefore confirms assay responsiveness rather than providing a direct potency ranking between the plant preparations and chloramphenicol.

3.4. Effect of Cream Formulation on the Antibacterial Response

Comparison of the unformulated extract with the corresponding creams showed that the antibacterial response remained measurable after incorporation into the semisolid vehicle. At 0.75%, E1 and F1 produced 15.00 ± 0.36 and 12.70 ± 0.40 mm, respectively; at 1.5%, E2 and F2 produced 17.40 ± 0.50 and 15.67 ± 0.45 mm; and at 3%, E3 and F3 produced 19.23 ± 0.42 and 19.07 ± 0.45 mm. Tukey HSD showed significant extract-cream differences at 0.75% (mean difference 2.30 mm, $p < 0.001$) and 1.5% (mean difference 1.73 mm, $p = 0.004$), but not at 3% (mean difference 0.17 mm, $p = 0.996$). The absence of a significant difference between E3 and F3 is not evidence of equivalence because an equivalence margin was not prespecified and an equivalence test was not performed.

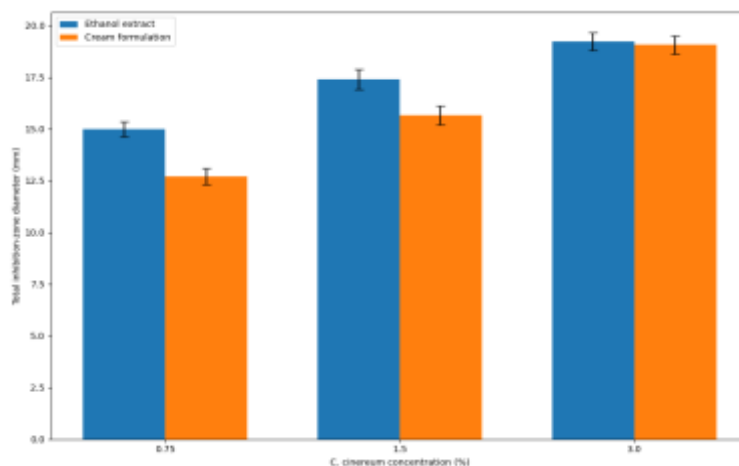


Figure 2. Comparison of total inhibition-zone diameters of *C. cinereum* ethanol extract and corresponding cream formulations against *S. aureus* ATCC 25923. Values are mean $\pm$ SD from three independent plate replicates ($n = 3$).

The smaller responses of F1 and F2 relative to E1 and E2 may reflect release and diffusion behavior from the semisolid matrix. Before producing an inhibition zone, antibacterial constituents must partition from the cream and diffuse through the agar; viscosity, phase partitioning, excipient interactions, solubility, and molecular size can therefore affect the observed zone. Importantly, F3 retained a measurable antibacterial response and was numerically close to E3, with a difference of 0.17 mm; Tukey HSD did not detect a significant difference ($p = 0.996$). This result should not be interpreted as statistical equivalence because an equivalence analysis was not performed. A formulation study using *Tephrosia vogelii* similarly found measurable activity of extract-containing creams against *S. aureus* and no antimicrobial effect from the cream base, supporting the general principle that plant-derived antibacterial constituents can remain active after incorporation into a semisolid vehicle [8]. This formulation-oriented perspective is consistent with recent studies of plant-derived ointments, gels, creams, nanoemulsions, hydrogels, and related topical systems evaluated for antimicrobial, anti-inflammatory, wound-healing, or skin-delivery applications [22-30].

3.5. Relationship Between Phytochemical Constituents and Antibacterial Activity

The concentration-dependent antibacterial response can be interpreted in relation to the phytochemical profile of the extract. Flavonoids and phenolic compounds may disrupt membrane integrity, interfere with proteins and nucleic acids, alter energy metabolism, and affect biofilm-associated processes [9][10]. Tannins, saponins, alkaloids, and terpenoid-related constituents may contribute through additional mechanisms. Because individual compounds were neither isolated nor quantified in the present study, these mechanisms are literature-supported interpretations rather than mechanisms directly demonstrated here. The observed activity may reflect additive or complementary effects among several constituents.

3.6. Significance of the 3% Cream Formulation

Among the three formulations, F3 (3%) produced the greatest measurable antibacterial response, with a total inhibition-zone diameter of 19.07 ± 0.45 mm. This value was numerically close to the corresponding unformulated extract E3 (19.23 ± 0.42 mm), and the Tukey HSD comparison was not significant ($p = 0.996$). However, lack of statistical significance does not demonstrate equivalence; therefore, the two preparations should not be described as equivalent. Previous studies have established antimicrobial activity of *C. cinereum*/*V. cinerea* extracts, including anti-staphylococcal activity, whereas the present work specifically examines the response after incorporation of the leaf ethanol extract into a topical cream matrix. Accordingly, the formulation-related contribution of this study is the preliminary demonstration that an agar-diffusion antibacterial response remains measurable after cream incorporation across the tested concentrations, with F3 showing the greatest response among the cream formulations. This finding warrants further pharmaceutical investigation using methods that can distinguish intrinsic antibacterial activity from formulation-dependent release and diffusion.

3.7. Overall Interpretation and Study Limitations

Taken together, the results demonstrate that *C. cinereum* leaf ethanol extract exhibited a concentration-dependent antibacterial response against *S. aureus* ATCC 25923 and that this response remained detectable after incorporation into a cream formulation. The significant preparation type $\times$ concentration interaction indicates that the magnitude of the formulation-associated change depended on extract concentration. F0

showed no inhibition, while F3 produced the greatest response among the creams. F3 was numerically close to E3 and the post-hoc comparison was not significant ($p = 0.996$); however, because no equivalence test was performed, these findings support a measurable antibacterial response after formulation rather than statistical equivalence or proof of unchanged antibacterial potency. Several limitations should be recognized: phytochemical screening was qualitative; agar well diffusion is influenced by diffusion as well as biological activity; MIC and MBC were not determined; the cycling test represents preliminary rather than long-term stability assessment; and the in vitro design cannot establish clinical efficacy. Future work should include quantitative phytochemical profiling, MIC/MBC determination, formulation-release and skin-permeation studies, extended stability testing, and appropriate safety assessment.

4. Conclusion

Qualitative phytochemical screening of the ethanol extract of *Cyanthillium cinereum* (L.) H. Rob. leaves indicated multiple classes of secondary metabolites, and the extract showed a concentration-dependent antibacterial response against *Staphylococcus aureus* ATCC 25923. An antibacterial response remained measurable after incorporation into a topical cream, with F3 (3%) producing the greatest inhibition-zone diameter among the tested creams (19.07 ± 0.45 mm, $n = 3$), compared with 19.23 ± 0.42 mm for the corresponding unformulated extract E3; the blank cream base showed no inhibition. Two-way ANOVA demonstrated significant effects of preparation type ($p < 0.001$), concentration ($p < 0.001$), and their interaction ($p = 0.003$). The E3-F3 Tukey HSD comparison was not significant ($p = 0.996$), but lack of significance does not establish equivalence. The principal contribution of this study is preliminary formulation-based evidence that an agar-diffusion antibacterial response remains measurable after incorporation of *C. cinereum* leaf ethanol extract into a semisolid cream matrix. These findings do not establish unchanged antibacterial potency or therapeutic efficacy. Quantitative phytochemical analysis, MIC/MBC determination, formulation-release and skin-permeation studies, long-term stability testing, and safety evaluation are required before pharmaceutical or clinical application can be considered.

5. Acknowledgements

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6. Conflict of Interest

The authors declare no conflicts of interest.

7. Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation Process

During the preparation of this work, the authors used OpenAI to assist with English-language editing and manuscript organization. After using this tool, the authors reviewed and edited the content as necessary and take full responsibility for the content of the published article.

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