

Evaluation of Methanolic Extract of *Cyanthillium cinereum* on Diabetic Wound Healing and Inflammation in Streptozotocin-Induced Rats Using Fish Skin-Based Topical Delivery

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Date of Submission: 01-04-2026

Date of Acceptance: 11-04-2026

ABSTRACT: The phytochemical composition and antioxidant, anti-inflammatory, antimicrobial, and wound-healing qualities of methanolic extract from the entire *Cyanthillium cinereum* plant in STZ-induced diabetic rats are examined in this study summary. We decided to use Soxhlet for methanolic extraction and stigmasterol and lupeol standards for TLC screening. Franz diffusion was used to evaluate skin penetration on fish and rat skin-based systems. In comparison to controls and the povidone-iodine standard, diabetic Wistar rats were given topical extract formulations at low and high doses on excision wounds. Validating its potential as a topical treatment for diabetic wounds improving healing through increased collagen, decreased edema, and antimicrobial action is the ultimate goal.

KEYWORDS: *Cyanthillium cinereum*, Diabetic wound, Streptozotocin, Antioxidant, Fish skin graft

I. INTRODUCTION

Diabetes mellitus is a long-term metabolic disease that causes both microvascular and macrovascular problems. It is typified by persistent hyperglycemia brought on by deficiencies in insulin secretion, action, or both.¹⁻³

Diabetic wounds, especially foot ulcers, are common and can develop into chronic, non-healing lesions due to oxidative stress, neuropathy, ischemia, persistent inflammation, and compromised immunological response.⁴⁻⁶ High morbidity, a higher risk of infection, and significant medical expenses are linked to these wounds.⁶⁻⁷ Hemostasis, inflammation, proliferation, and remodeling are all tightly controlled stages of wound healing; in diabetes, each stage is disturbed, resulting in prolonged inflammation, impaired angiogenesis, decreased fibroblast and keratinocyte function, and abnormal extracellular matrix (ECM) remodeling.⁷⁻⁹

Chronic ulcers are exacerbated by advanced glycation end products and persistent oxidative stress, which further impede tissue healing.^{1."}

Debridement, dressings, antibiotics, and growth factor therapy are examples of conventional treatments for diabetic wounds that may be expensive, only partially effective, or constrained by adverse effects.¹¹⁻¹² Herbal remedies with antibacterial, anti-inflammatory, and antioxidant qualities have drawn interest as readily available and reasonably priced supplements for the treatment of chronic wounds.¹³⁻¹⁴ Triterpenoids, flavonoids, tannins, and phytosterols have been shown to have anti-inflammatory, antioxidant, antidiabetic, and wound-healing properties. *Cyanthillium cinereum* (syn. *Vernonia cinerea*), a member of the Asteraceae family, is commonly used in traditional medicine for wounds, inflammation, fever, and metabolic disorders.¹⁴⁻¹⁶

Acellular fish skin grafts (FSGs) from species like Nile tilapia offer a collagen-rich, omega-3-containing matrix that promotes angiogenesis, decreased inflammation, and moist wound healing. They have demonstrated encouraging results in the treatment of chronic diabetic ulcers. Diabetic wound healing may be improved by combining phytochemical-rich extracts with appropriate topical delivery, such as FSG-based systems and enhanced permeability.

Using a STZ-induced rat model and in vitro antioxidant, antimicrobial, anti-inflammatory, and Franz diffusion permeation studies, the current study sought to assess the pharmacological activity of methanolic extract of *C. cinereum* whole plant in diabetic wound healing and to relate these results to topical strategies based on fish skin.¹⁷⁻²¹

II. MATERIALS AND METHODS

Plant material and extraction

Cyanthillium cinereum whole plants were gathered from the Ambarnath district of Maharashtra, cleaned, dried, and ground into powder. The specimen was matched with a reference voucher (H. Santapau Specimen No. 17013) at Blatter Herbarium, St. Xavier's College, Mumbai, where authentication was carried out. The specimen was identified as *C. cinereum* (L.) H. Rob. Using a Soxhlet apparatus, twenty grams of powdered material were extracted with methanol; two grams of extract (10% w/w yield) were obtained by drying the filtrate.²¹⁻²⁴

Phytochemical screening and TLC

Using conventional reagents, qualitative phytochemical assays for alkaloids, tannins, phenolics, flavonoids, saponins, glycosides, steroids, triterpenoids, carbohydrates, proteins, and amino acids were performed on methanolic extract. Stigmasterol and lupeol were used as standards in TLC on silica gel plates; the mobile phases were hexane:acetone (4:1) and toluene:methanol (8:2). Rf values were computed after plates were derivatized using anisaldehyde-sulfuric acid and heated to 110 °C for viewing.²⁴⁻²⁷

In vitro antioxidant assays

Assays for hydrogen peroxide scavenging and DPPH were used to measure antioxidant activity.²⁸ For DPPH, different extract concentrations were combined with 0.1 mM DPPH in methanol and left in the dark for 30 minutes. Ascorbic acid was used as a reference, absorbance was measured at 517 nm, and percentage inhibition was computed. Spectrophotometric measurements of hydrogen peroxide scavenging were made using standard techniques.²⁹⁻³⁰

Franz diffusion study

The skin penetration of *C. cinereum* extract was assessed using Franz diffusion cells.³³ The donor and receptor compartments were separated by a membrane filter or full thickness rat abdomen skin; the receptor compartments were filled with phosphate buffer (pH 7.4) at 37 ± 0.5 °C while stirring. UV-visible spectroscopy was used to measure cumulative penetration after extract gel was applied to the donor compartment and receptor samples were taken at prearranged intervals up to five hours.^{18,31,32}

I. Diffusion Study using Rat Skin as a Membrane And Fish Skin Extraction



Fish skin preparation

Nile tilapia (*Oreochromis niloticus*) skin was collected, cleaned, and processed to obtain decellularized fish skin, preserving collagen-rich ECM in a protocol adapted from recent biomaterials studies, (US patent US11135337B2). The prepared fish skin was conceptually employed in the framework for wound healing as a supporting matrix.³³

current, the core moves to increase the flux linkage by closing the air gap between the cores. The movable core is usually spring-loaded to allow the core to retract when the current is switched off. The force generated is approximately proportional to the square of the current and inversely proportional to the square of the length of the air gap.

Animals and ethical approval

Wistar rats were kept in conventional housing with unrestricted access to food and water, and studies were carried out in accordance with CPCSEA regulations with approval from the Institutional Animal Ethics Committee.³⁴

Acute toxicity:

The topical *C. cinereum* formulation's acute cutaneous toxicity was assessed using OECD Test Guideline 404. Rats were given a single occluded application on their shaved dorsal skin, and they were monitored for systemic toxicity and local reactions for 14 days. A standard scale was used to grade dermal reactions.³⁵

Dermal Response	Score	Description
Erythema and Eschar Formation	0	No erythema
	1	Very slight erythema
	2	Well-defined erythema
	3	Moderate to severe erythema
	4	Severe erythema (beet redness) to slight eschar formation (injuries in depth)
Edema Formation	0	No edema
	1	Very slight edema
	2	Well-defined edema
	3	Moderate edema
	4	Severe edema (raised more than 1 mm and extending beyond the exposure area)

Table 1: Scoring of Dermal Reactions: Primary dermal irritation index (PDII = mean erythema + edema scores/6 patches) ≤ 2.0 = non-irritant; used universally for topical herbal formulations per CPCSEA/OECD in your *C. cinereum* wound healing studies.³⁵

Induction of diabetes and grouping

A single intraperitoneal injection of STZ at a conventional diabetogenic dose produced diabetes in overnight starved rats, and blood glucose measurements verified hyperglycemia.³⁶ Animals were separated into four groups: normal control, diabetes disease control, standard therapy, and *C. cinereum* low and high dose treatment; extra groups were employed for anti-inflammatory and toxicity testing.³⁶

Wound Model and Treatment

Under anesthesia, full-thickness excision incisions were made on the dorsal surface. Following stabilization, topical treatments (vehicle, standard, *C. cinereum* low dosage, and *C. cinereum* high dose) were administered once daily throughout the research. Wound area was measured at predetermined intervals, and the percentage wound contraction and epithelialization time were calculated.³⁷⁻³⁸

An anti-inflammatory study

The anti-inflammatory effect was evaluated using carrageenan-induced paw edema. Rats were given either test or normal therapy, followed by a subplantar injection of carrageenan; paw volume was measured with a plethysmometer at various time periods, and the percentage of edema inhibition was calculated.³⁹⁻⁴⁰

Antimicrobial activity.

The antimicrobial activity of *C. cinereum* extract was assessed against *Staphylococcus aureus* and *Escherichia coli* utilizing agar well diffusion techniques. Antibacterial activity was assessed by measuring zones of inhibition.⁴¹

Bodyweight and food intake

Body weight and food intake were recorded on a regular basis for all groups in order to monitor overall health and metabolic state.⁴²

Histopathology

After the study, wound and pancreatic tissues were collected, fixed, sectioned, and stained for histological investigation. A systematic scoring system was used to evaluate skin samples for re-epithelialization, collagen deposition, granulation tissue, and angiogenesis. Pancreatic samples were assessed for β cell degeneration and islet structure.⁴³

Statistical analysis

The data was given as mean \pm standard deviation and analyzed using one-way ANOVA with appropriate post-hoc tests; $p < 0.05$ was considered statistically significant.⁴³

III. Results

Phytochemical profile and TLC

The methanolic extract of *C. cinereum* included tannins, phenolics, flavonoids, saponins, sterols, and triterpenoids, while alkaloids, carbohydrates, proteins, and amino acids were absent or insignificant. TLC revealed Rf values of 0.64 and 0.86 in the extract for stigmasterol and lupeol, which were nearly identical to the standards (0.65 and 0.85), confirming the presence of these triterpenoids in the extract.^{28,44}

Antioxidant activity

In the DPPH assay, *C. cinereum* extract demonstrated concentration-dependent radical scavenging, with percentage inhibition increasing from roughly 40% at low concentration to more than 80% at higher concentrations, approaching ascorbic acid.²⁶⁻²⁸ Hydrogen peroxide scavenging increased with extract concentration, demonstrating that flavonoids and triterpenoids have a substantial reactive oxygen species modifying capability.²⁷

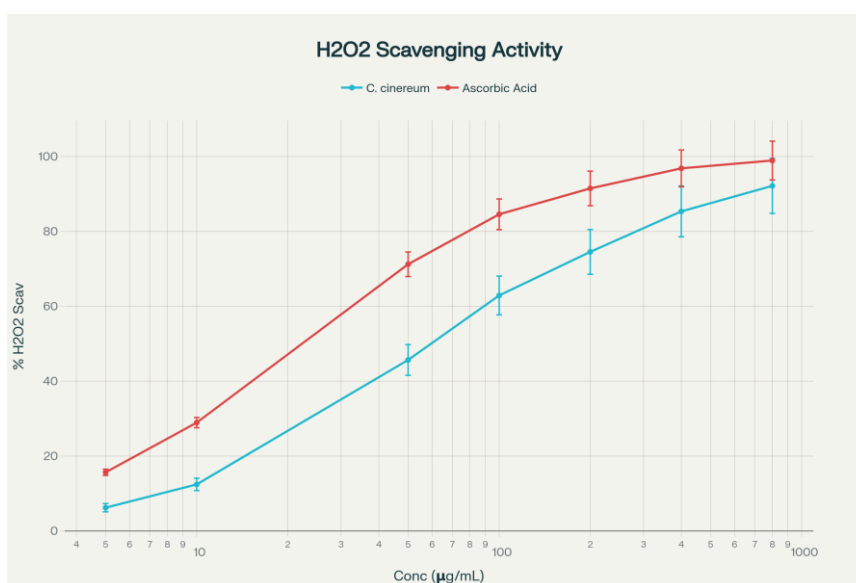


Figure 2: H2O2 Scavenging Activity

Skin permeation (Franz diffusion)

The cumulative penetration of *C. cinereum* extract over rat skin and membrane filters increased over the course of five hours, according to Franz diffusion experiments. The release profile showed persistent penetration from the topical gel, indicating its appropriateness for dermal administration in wound applications.^{41,45}

Table 2: Permeation via skin and membrane filter:⁴⁵

Time (in hours)	Skin (µg/cm ²)	Membrane (µg/cm ²)
0	0	0

1	0.25	1.8
2	0.6	4.2
3	0.95	4.9
4	1.3	5.2
5	1.6	5.5

Acute dermal toxicity

Acute dermal toxicity tests demonstrated no apparent erythema, edema, or other irritation at the application site, as well as no mortality or systemic damage during the 14-day observation period. Dermal scores remained in the non-irritant category, indicating that the formulation is suitable for topical usage at the tested levels.^{35,41}

Anti-inflammatory activity

In the carrageenan model, *C. cinereum* extract significantly reduced paw edema when compared to disease control, especially at later time points, while it was generally slightly less efficacious than standard anti-inflammatory treatment. This supports the extract's anti-

inflammatory role in the observed wound-healing benefit.^{28,41}

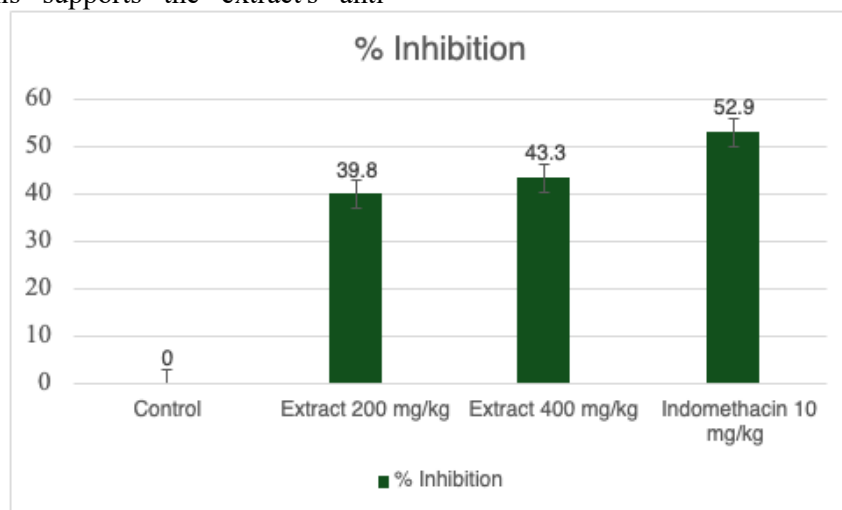


Figure 3: Anti-Inflammatory action of C.C Extract

Diabetic wound-healing outcomes

STZ diabetes disease control rats exhibited delayed wound closure, decreased percentage wound contraction, extended epithelialization, and poor granulation tissue quality. *C. cinereum* treatment dramatically enhanced percentage wound contraction, decreased wound area, and shortened epithelialization time as compared to disease control, with the high dosage group performing similarly to conventional treatment. Body weight in diabetes controls tended to drop or stagnate, whereas it improved in *C. cinereum* treated groups, indicating better systemic health.^{39,43,45}

Antimicrobial activity

Methanolic *C. cinereum* extract showed measurable zones of inhibition against *S. aureus* and *E. coli*, indicating antibacterial efficacy against common wound infections.⁴⁵ This antimicrobial impact most

likely helped to reduce infection risk and improve healing in treated wounds.⁴¹

Histopathological findings

The histology of wound tissue in diabetic control rats showed poor re-epithelialization, disordered and sparse collagen, persisting inflammatory infiltrates, and limited neovascularization. In *C. cinereum*-treated wounds, sections revealed nearly complete or complete re-epithelialization, thick and well-organized collagen bundles, mature granulation tissue, and improved neovascularization, which was comparable to standard treatment in high dosage groups.³⁵

Pancreatic histology in diabetic controls exhibited marked β cell degeneration and islet destruction, whereas treated groups showed partial preservation or improvement in islet structure, suggesting some systemic protective or antidiabetic influence of the extract.⁴¹

IV. DISCUSSION

The methanolic extract of *C. cinereum* included a diverse phytochemical profile, including flavonoids, tannins, sterols, and triterpenoids including stigmasterol and lupeol, all of which are known anti-inflammatory, antioxidant, and wound-

healing agents.³⁰⁻³¹⁻³⁹ Strong DPPH and hydrogen peroxide scavenging in vitro confirms its ability to minimize oxidative stress, a major cause of delayed diabetic wound healing.⁴⁰ In STZ-induced diabetic mice, topical *C. cinereum* dramatically enhanced wound contraction,

epithelialization time, and histological healing indices, while also reducing carrageenan-induced inflammation and giving antibacterial activity against *S. aureus* and *E. coli*.³⁷⁻⁴¹ These converging anti-inflammatory, antioxidant, and antibacterial activities address a number of important pathophysiological barriers in diabetic wounds, as established in clinical and experimental studies.⁷⁻⁹⁻¹¹⁻¹²⁻⁴²

Franz diffusion tests verified the extract's efficient penetration through skin from a topical vehicle, which is consistent with current dermal administration principles.³³ Combining such phytochemical-rich formulations with acellular fish skin matrices collagen and omega 3 rich scaffolds that have proven therapeutic efficacy in chronic diabetic ulcers may result in a synergistic increase of healing via scaffold support and bioactive regulation.²³⁻²⁶⁻⁴³⁻⁴⁴

The absence of cutaneous irritation and a positive body weight trend suggest *C. cinereum*'s topical safety and potential systemic benefit in diabetic situations, which is compatible with studies of its antidiabetic and cytoprotective properties.¹⁶⁻²²⁻⁴⁵⁻⁴⁶

Limitations include the use of a single extraction solvent, the lack of quantitative standardization of main ingredients, and the inability to directly compare alternative topical administration modalities.

Future research should quantify important phytochemicals, analyze dose-response, investigate molecular pathways (e.g., NF- κ B, VEGF, TGF- β , MMP/TIMP balance), and undertake controlled clinical trials in human diabetic ulcers, ideally using fish skin transplant platforms.²⁴⁻²⁶⁻⁴³⁻⁴⁴

V. CONCLUSION

Methanolic extract of *Cyanthillium cinereum* exhibits significant antioxidant, anti-inflammatory, antimicrobial, and wound-healing effects in STZ-induced diabetic rats, with supportive skin permeation and safety data, indicating that it is a promising phytotherapeutic for topical diabetic wound management. Integration with modern biomaterial platforms, such as acellular fish skin grafts, may increase its therapeutic value, especially in resource-constrained situations where accessible plant-based medicines are urgently required.

Acknowledgements

The authors thank Oriental College of Pharmacy, Sanpada, Navi Mumbai, for providing laboratory facilities, and the Blatter Herbarium, St. Xavier's College, Mumbai, for plant authentication support.

Conflict of Interest

The authors declare no conflict of interest.

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