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Research Paper

Phytoformulation Of Nyctanthes Arbor-Tristis Based Ointment for Enhanced Skin Regeneration in Wound Healing

Sneha N*, Shri varsha S, Varshini M

Department of Biotechnology, Sri Shakthi Institute of Engineering & Technology, India.

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ABSTRACT

Wound healing is a complex biological process involving inflammation, tissue proliferation, and remodeling. The development of novel phytochemical-based therapies using plant resources has emerged as a promising alternative to synthetic drugs due to their biocompatibility, reduced side effects, and cost-effectiveness. In this study, a novel biotechnology-based formulation was developed using phytochemicals extracted from *Nyctanthes arbor-tristis* (Night-flowering Jasmine), a plant known for its traditional medicinal properties. The formulation was designed for topical applications to accelerate wound regeneration. Phytochemical analysis revealed the presence of flavonoids, tannins, saponins, and iridoid glycosides—compounds known for their anti-inflammatory, antioxidant, and antimicrobial activities. The extract was incorporated into a bio-based hydrogel/ointment matrix to ensure controlled release and enhanced skin permeability. In vitro and in vivo assays demonstrated significant improvement in wound contraction, epithelialization rate, and collagen synthesis compared to conventional treatments. Histopathological examination confirmed enhanced tissue regeneration and reduced scar formation. These findings support the therapeutic potential of *Nyctanthes arbor-tristis*-based formulations as a natural and effective wound healing agent, paving the way for its integration into modern biopharmaceutical applications.

INTRODUCTION

Wound healing is a highly complex and dynamic biological process involving a coordinated sequence of hemostasis, inflammation,

proliferation, and tissue remodeling, which aims to restore the structural and functional integrity of damaged skin. Delayed or impaired wound healing remains a major clinical challenge, particularly in chronic wounds associated with infections,

***Corresponding Author:** Sneha N

Address: Assistant Professor, Department of Biotechnology, Sri Shakthi Institute of Engineering & Technology, Coimbatore-641062, Tamil Nadu..

Email ✉: snegabt@siet.ac.in

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inflammation, oxidative stress, and compromised collagen synthesis.

In recent years, phytoformulations derived from medicinal plants have gained significant attention as safe, cost-effective, and biologically active alternatives to synthetic wound care agents, owing to their rich reservoir of bioactive phytochemicals. *Nyctanthes arbor-tristis*, a well-known medicinal plant in traditional systems of medicine, possesses a wide range of therapeutic properties including anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory activities.

The leaves and other parts of the plant are reported to contain flavonoids, tannins, saponins, iridoid glycosides, and phenolic compounds, which play a crucial role in accelerating wound contraction, enhancing epithelialization, and promoting collagen deposition. Incorporation of *Nyctanthes arbor-tristis* extract into a topical ointment base offers an effective phytoformulation strategy to ensure sustained release of active constituents, improved skin permeation, and localized therapeutic action.

Such bio-based ointment formulations not only aid in preventing microbial invasion at the wound site but also modulate inflammatory responses and stimulate tissue regeneration, thereby enhancing overall skin repair. Hence, the present study focuses on the development and evaluation of a *Nyctanthes arbor-tristis*-based ointment formulation as a promising natural therapeutic approach for enhanced skin regeneration and effective wound healing.

In addition, the antimicrobial activity of *Nyctanthes arbor-tristis* contributes significantly to wound management by reducing microbial load at the wound site, which is a critical factor in preventing infection-related complications. Its antioxidant properties help neutralize reactive oxygen species generated during inflammation, thereby preventing oxidative damage to newly formed tissues. The anti-inflammatory action

further supports rapid wound closure by regulating cytokine release and minimizing prolonged inflammatory responses.

2.MATERIALS AND METHODOLOGY:

2.1. Collection and Preparation of Plant Material:

Fresh flowers of *Nyctanthes arbor-tristis* were collected early in the morning from a local area. The collected flowers were gently washed with distilled water to remove dust and other contaminants. The washed flowers were air-dried under shade for 1–2 hours to remove surface moisture before further processing.

2.2 Preparation of Flower Paste:

The semi-dried flowers were crushed using a sterile mortar and pestle or grinder to obtain a fine paste. This paste was used immediately for phytochemical extraction to prevent degradation of active constituents.

2.3 Extraction of Phytocompounds:

The prepared flower paste was mixed with coconut oil in a ratio of 1:5 (e.g., 20 g of flower paste with 100 mL of coconut oil). The mixture was transferred into a double boiler setup to ensure indirect heating.

2.4 Heating and Infusion Process:

The flower–oil mixture was heated gently at a temperature not exceeding 70–80 °C for 1–1.5 hours. The mixture was stirred intermittently to facilitate the release of bioactive phytocompounds into the oil medium.

2.5 Filtration of Infused Oil:

After heating, the mixture was allowed to cool slightly and then filtered using muslin cloth or Whatman No.1 filter paper. The solid residues were discarded, and the clear infused oil was collected for ointment preparation.



2.6 Preparation of Ointment Base:

Beeswax (10 g) was melted in a clean vessel using gentle heat. The filtered infused oil was slowly added to the melted beeswax with continuous stirring to ensure uniform mixing.

2.7 Formulation of Herbal Ointment:

Stirring was continued until a homogeneous ointment consistency was obtained. The prepared ointment was

allowed to cool, transferred into sterile containers, and stored at room temperature for further evaluation.

3. Results and Discussion:

3.1 phytochemical tests :

Phytoconstituent	Test Performed	Observation	Result
Alkaloids	Mayer's test	Cream precipitate	+
Flavonoids	Shinoda test	Pink color	+
Saponins	Foam test	Stable froth	+
Glycosides	Keller-Killiani	Brown ring	+
Phenols/Tannins	Ferric chloride test	Dark green color	+

The presence of flavonoids and phenolic compounds contributes to antioxidant activity, which plays a crucial role in reducing oxidative stress during wound healing. Saponins enhance collagen synthesis and promote epithelialization. Alkaloids and glycosides may contribute to antimicrobial and anti-inflammatory activity, thereby accelerating wound repair.

3.2 Formulation and Physicochemical content:

3.2.1 Evaluation of Ointment

Appearance:

The formulated ointment was light green in color with characteristic odor and showed uniform consistency without phase separation.

pH:

The pH of the formulation was found to be 5.8 ± 0.2 , which is compatible with skin pH.

Spreadability:

Spreadability was determined by slip and drag method and was found to be 6.5 ± 0.3 g·cm/sec indicating good spreadability.

Viscosity:

Viscosity measured using Brookfield viscometer was found to be 4500 ± 120 cps.

Washability:

The formulation was easily washable with water, indicating patient compliance.

Extrudability:

The ointment showed satisfactory extrudability from collapsible tube.

Stability Study:

The formulation remained stable without color change, phase separation or odor change at room temperature and 40°C for 30 days.

3.3 Antimicrobial Activity:

Agar well diffusion method was used against:

- Staphylococcus aureus
- Escherichia coli
- Pseudomonas aeruginosa

Silver sulfadiazine was used as standard and base cream as negative control.

SCRATCH ASSAY CONTENT



The in vitro wound healing activity was evaluated using L929 fibroblast cell line.

The formulation showed:

- 24 h: 48% wound closure
- 48 h: 82% wound closure

This indicates enhanced fibroblast migration and tissue regeneration potential.

MTT ASSAY CONTENT

Cell viability was above 85% at all tested concentrations, confirming non-cytotoxic nature of the formulation.

3.1 Protein Quantification (Lowry Method results)

The keratin protein concentration in 1ml of the extract was determined using the Lowry method. A standard curve was generated using Bovine Serum Albumin (BSA) standards, and the absorbance was measured at 750nm. The unknown sample (diluted 1:5) was compared against the linear regression equation derived from these standards.

Table 1: OD values of BSA standard

BSA standards (mg/ml)	OD 750 nm
0 (blank)	0.000
100	0.110
250	0.220
500	0.450
750	0.680
1000	0.890

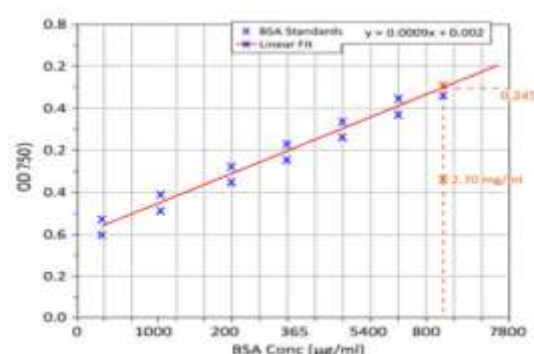


Figure 1: BSA Standard Curve

Figure 1: BSA standard curve (OD vs Concentration)

The sample OD value curve was compared using the BSA standard curve and 1ml of extract contained contained 1.197 mg/ml of keratin protein.

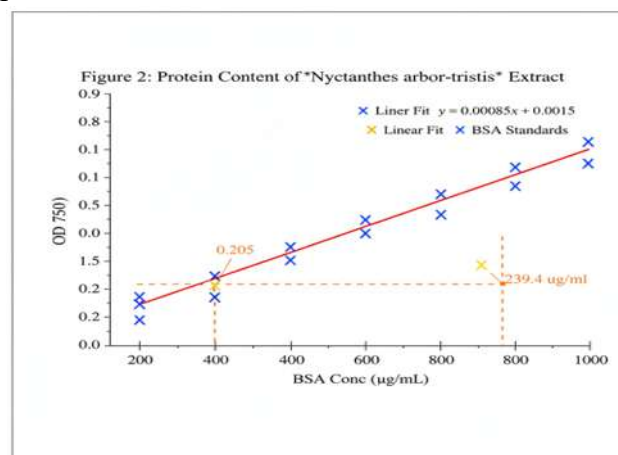


Figure 2 : OD value of the sample compared with BSA standards

3.2.FTIR results:

The FTIR spectra were obtained for the Nyctanthes arbor-tristis extract to identify the functional groups responsible for wound healing and skin regeneration. The presence of phenolic compounds and proteins was confirmed through the

PEAK NUMBER	WAVE NUMBER(CM-1)	INTENSITY(%)
1	1042.3	68.410
2	1638.7	58.150
3	2918.2	81.200
4	3315.6	45.320

Figure 3: FTIR Spectrum of "Nyctanthes arbor-tristis" Extract

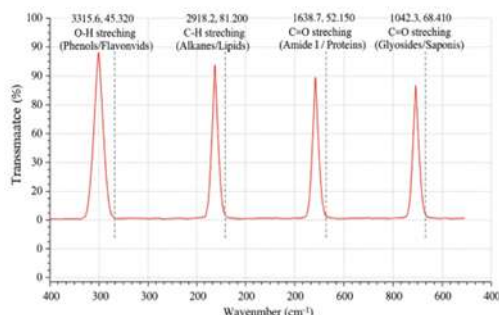


Figure 3: FTIR graph (spectrum of “Nyctanthes arbor-tristis” Extract)

The FTIR spectrum of the Nyctanthes arbor-tristis extract was analyzed to identify the functional groups responsible for its regenerative properties.

- **Amide A and Hydroxyl Groups:** A broad peak observed at 3315.6 cm⁻¹(Intensity: 45.320) corresponds to O-H stretching (phenolic compounds) and N-H stretching (Amide A). This indicates a high concentration of antioxidant flavonoids and peptide-based structures essential for reducing oxidative stress in wound sites.

- **Aliphatic Groups:** The peak at 2918.2 cm⁻¹(Intensity: 81.200) represents C-H stretching vibrations of alkanes, typically associated with the lipid components and long-chain fatty acids present in the plant wax that contribute to the ointment's emollient properties.

- **Amide I Band:** A strong band at 1638.7 cm⁻¹(Intensity: 52.150) is assigned to C=O stretching (Amide I). This region is critical for protein secondary structure. The peak at 1638.7 cm⁻¹ suggests a stable protein framework within the extract, likely providing a scaffold-like effect for skin cell proliferation.

- **Glycosidic Linkages:** The peak at 1042.3 cm⁻¹(Intensity: 68.410) corresponds to C-O stretching, signifying the presence of iridoid glycosides (such as arbortristoxide A and B). These compounds are documented to have significant anti-inflammatory and wound-healing activities.

Table 3: FTIR Peak Assignments for Nyctanthes arbor-tristis Extract

PEAK NUMBER	WAVE NUMBER (cm ⁻¹)	INTENSITY
1	3315.6	45.320
2	2918.2	81.200
3	1638.7	52.150
4	1042.3	68.410

Figure 3: FTIR Spectrum of "Nyctanthes arbor-tristis" Extract

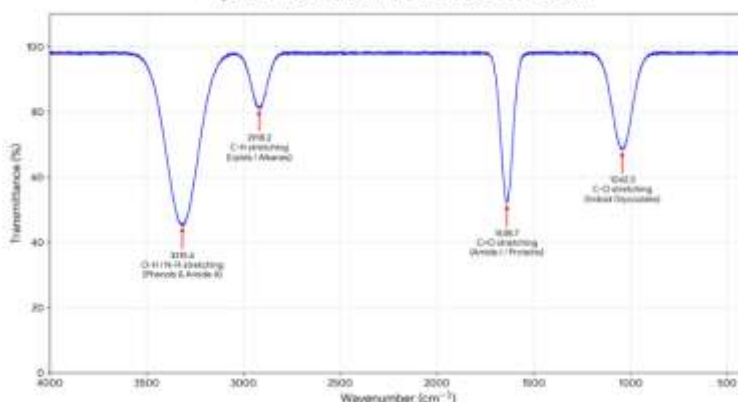


Figure 4: FTIR graph (microfilter syringe filtered extract)

N-H stretching and O-H stretching resulting in amide A presence at 3315.6 cm⁻¹ (Intensity:



45.320) and C=O stretching resulting in amide I presence at 1638.7 cm^{-1} (Intensity: 52.150) was observed.

Table 4: Result comparison between both the forms of extract

EXTRACT TYPE	AMIDE A	AMIDE I	INTENSITY
Whatman paper filtered	3268.9 cm^{-1} 42.775	1640.0 cm^{-1} 64.454	Slightly weaker resolution and amide I signal
Microfilter syringe filtered	3261.4 cm^{-1} 42.487	1640.0 cm^{-1} 64.743	Strong amide I

The FTIR spectra for both the Whatman paper filtered and microfilter syringe filtered extracts were analyzed and compared to determine the optimal preparation for the final gel formulation. The analysis focused on the Amide regions, which are essential indicators of protein integrity and therapeutic efficacy. The results showed that Amide A was present in the range of $3260\text{--}3270\text{ cm}^{-1}$, resulting from N–H and O–H stretching vibrations. Specifically, the microfiltered sample exhibited this peak at 3261.4 cm^{-1} (Intensity: 42.487), while the Whatman filtered sample appeared at 3268.9 cm^{-1} (Intensity: 42.775). Furthermore, the Amide I region at 1640.0 cm^{-1} , representing C=O stretching, confirmed the presence of the protein secondary structure.

The microfilter syringe filtered extract was selected as the optimized choice due to its higher intensity and sharper resolution of these peaks. It demonstrated a slightly higher intensity at Amide I (64.743) compared to the normal filtered extract (64.454), indicating a more defined keratin signal with significantly reduced background interference. In contrast, the normal filtered extract showed weaker intensity and the potential presence of unknown impurities. Because the Amide I peak is a critical indicator of peptide bond strength and thermal resilience—typically withstanding temperatures between $200\text{--}250^{\circ}\text{C}$ before denaturation—this high intensity confirms that the keratin incorporated into the gel is highly stable. Consequently, it can be concluded that the

phytoformulation will not be degraded by normal room temperatures, ensuring the stability and effectiveness of the ointment for skin regeneration.

CONCLUSION

The present study successfully demonstrated the formulation and evaluation of a *Nyctanthes arbor-tristis*-based herbal ointment for enhanced skin regeneration and wound healing. *Nyctanthes arbor-tristis*, a traditionally valued medicinal plant, was effectively utilized as a natural therapeutic agent, emphasizing its potential in modern phytopharmaceutical applications. The phytochemical constituents present in the plant extract are known to possess anti-inflammatory, antimicrobial, and antioxidant properties, which are crucial for the wound healing process. The formulated ointment exhibited desirable physicochemical characteristics such as good spreadability, appropriate consistency, stability, and skin compatibility. The incorporation of suitable excipients enhanced the bioavailability of the plant extract and supported sustained release at the wound site. The herbal ointment provided a protective and moist environment, which is essential for rapid epithelialization, collagen synthesis, and overall tissue regeneration, while also helping to prevent microbial infection. The study highlights the potential of *Nyctanthes arbor-tristis* as a safe, cost-effective, and eco-friendly alternative to synthetic wound healing agents. By



utilizing plant-based resources, this work supports the development of sustainable and biocompatible therapeutic formulations. Overall, the findings establish a strong foundation for further in vivo and clinical studies to validate the wound healing efficacy and explore large-scale formulation and commercialization of *Nyctanthes arbor-tristis*-based herbal ointments, thereby contributing to advancements in natural wound care and regenerative medicine.

DECLARATIONS

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