IJMPR, 2026, 10(7), 1-7 ISSN: 2319-5878

INTERNATIONAL JOURNAL OF MODERN PHARMACEUTICAL RESEARCH

www.ijmpronline.com

Research Article

Impact Factor: 7.533

Coden USA: IJMPR3

THE FORMULATION AND EVALUATION OF WOUND HEALING GEL BY INCARPORATION OF AQEOUS *CALOTROPIS GIGANTEA EXYRACT*

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Article Received on: 19/05/2026 **Article Revised on: 09/06/2026** **Article Published on: 01/07/2026**

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https://doi.org/10.5281/zenodo.21030256

How to cite this Article: Sai Samadhan Shirsath[1]*, Tejas Anil Kadam[1], Sakshi Ravindra Ingale[1], Rasika Dnyandeo Bhalke[1], Mahendra Ashok Giri[2]. (2026). The Formulation and Evaluation of Wound Healing Gel By Incarporation of Aqeous Calotropis Gigantea Exyract. International Journal of Modern Pharmaceutical Research, 10(7), 1–7.

ABSTRACT

Wound care is tough. Healing takes time, infections creep in, and most standard ointments just don't cut it—they barely sink into the skin, don't stick around long enough, and rarely deliver enough of the drug where you want it. *Calotropis gigantea* has a solid reputation for fighting germs, calming inflammation, and speeding up wound repair. But so far, traditional treatments haven't made the most of what this plant can offer. Plus, there's not much research out there looking at how to use advanced nanocarriers to get its benefits to the skin more effectively. This study set out to make a Carbopol gel using *Calotropis gigantea* extract, hoping to fix some issues that older formulas had. To get the extract, we used Decoction method with distilled water. We checked things like pH, viscosity, how easy it spreads, We checked Physical Characteristics, Biocompatibility and Chemical Stability.We checked Spradability of the gel. We also looked at how well the gel fights bacteria, specifically Staphylococcus aureus and Escherichia coli. The optimized gel showed good physical and chemical properties, released about 85% of the drug over 12 hours, and delivered strong antimicrobial effects. What sets this study apart is the way it combines a traditional medicinal plant with modern nanotechnology to boost treatment results. This method looks promising for managing wounds and deserves more testing in living systems.

KEYWORDS: *Calotropis gigantea, Gel, Wound Healing, Antimicrobial Activity, Flavonoids.*

I. INTRODUCTION

Healing wounds is not that easy. It is a continuous process which includes several key phases – hemostasis, inflammation, proliferation, and remodeling. All steps are important and are part of the process of rebuilding and repairing any damaged tissue as much as possible. Despite this, it is a challenge to treat wounds. Issues such as infections, delayed healing and inability of topical treatments to penetrate the deeper layers of skin can impact the recovery process. These can make the recovery process last longer and diminish the overall effectiveness of the recovery process, so there is a strong need to develop improved drug delivery mechanisms. The purpose is to deliver drugs to their target, regulate their release and accelerate tissue recovery.[1]

A. PHASES OF WOUND HEALING

Wound healing is a biological repair process that has four stages: haemostasis, inflammation, proliferation and remodelling.[2-4]

1. Haemostasis

In this phase, it starts as soon as the injury occurs. Blood clot formation helps to stop bleeding and helps in the temporary attachment of cells.[2]

2. Inflammation

Neutrophils and macrophages are immune cells that eliminate microbes and damaged tissues from the wound site and promote the healing response.[3]

3. Proliferation

New tissue formation occurs in this stage. Collagen is made by fibroblasts, new blood vessels form, and epithelial cells cover the wound surface.[4]

4. Remodelling

During the final stage, collagen fibres reorganize and reinforce the wound, causing scar maturation and enhanced stability of the wound.[2,4]

B. THE RECEPTORS INVOLVED IN WOUND HEALING

1. Epidermal Growth Factor Receptor (EGFR)

- It's Promotes cell growth and wound closure.[6,7]
- It's Supports skin repair and tissue regeneration.[6]

2. Transforming Growth Factor Beta Receptor (TGF-βR)

- It's Regulates inflammation and collagen synthesis.[8,9]
- It's Helps in tissue remodeling and scar formation.[8]

The use of medicinal plants in health care is common due to their natural bioactive compounds which have important therapeutic properties.[10–14] *Calotropis gigantea* has been traditionally used for wound healing and protection of the skin.[15–17,18] gel-based drug delivery systems are a promising approach for delivery of the herbal extract for wound healing application to improve the stability, controlled release, and effectiveness.[19,20]

II. PLANT PROFILE

Calotropis gigantea is a medicinal plant which is well known as Giant Milkweed or Crown Flower. It is a member of the Apocynaceae family and is found throughout India and other tropical regions. Traditionally, the plant is used to treat wounds, skin infections, inflammation and painful conditions.[1,2] The leaves are abundant in natural compounds like flavonoids, phenolics, triterpenoids, alkaloids, and glycosides, and are responsible for its medicinal properties. The antimicrobial, antioxidant and anti-inflammatory action of these constituents helps to prevent infection at damaged tissue sites and aid in healing. It is a large shrub with thick green leaves, milky latex and pale purple or white flowers. Considering the wide range of applications of *Calotropis gigantea*, it is a suitable plant for developing gel formulation in wound healing field due to its ability to promote tissue repair, collagen formation, and wound contraction.

Fig.1. *Calotropis gigantea*

III. CHEMICAL CONSTITUENTS

Calotropis gigantea has many bioactive compounds which are responsible for its medicinal value. The antimicrobial properties and the ability of supporting wound repairing of cardiac glycosides like calotropin, calotoxin, calactin have been reported.[21,22] Some of the plant's triterpenoids, such as β-amyrin, lupeol and taraxasterol, have anti-inflammatory effects and promote the formation of collagen during healing.[21, 22] The sterols such as β-sitosterol and stigmasterol are protective of membranes and have anti-inflammatory properties.[22] The flavonoids, particularly isorhamnetin and its derivatives and quercetin, offer antioxidant properties and help in tissue repair.[21,22] The phenolics, giganteol and isogiganteol, can help to neutralize free radicals and promote regeneration of damaged tissue.[22] In addition, the saponins induce angiogenesis and wound contraction.[21] and the proteolytic enzyme calotropain can help eliminate dead tissues and keep the wound clean.[22]

IV. PHARMACOLOGICAL POTENTIAL OF *CALOTROPIS GIGANTEA*

Calotropis gigantea is a well-known medicinal plant with a wide range of pharmacological activities attributed to its rich content of flavonoids, glycosides, triterpenoids, and phenolic compounds.[23,24,25,26,27]

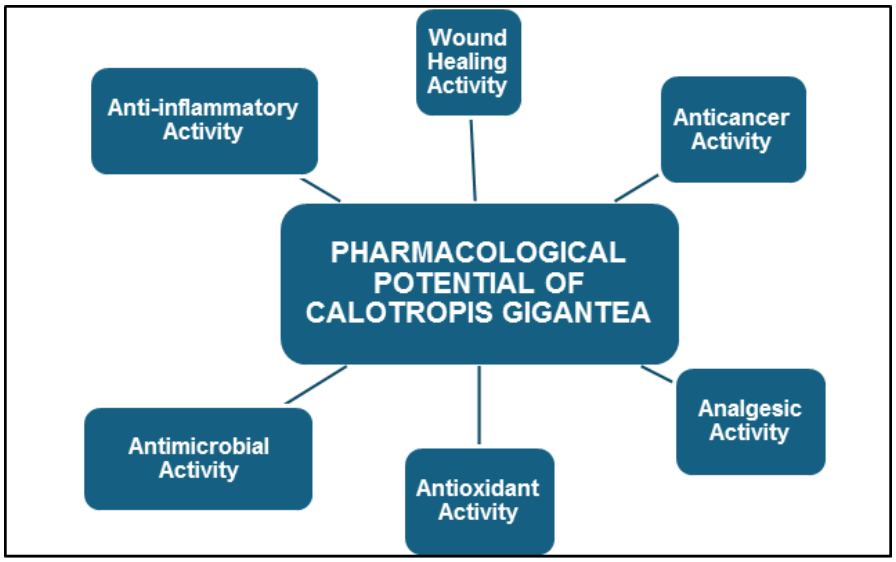


Fig. 2: Pharmacological Potential of Calotropis Gigantea.

V. THE ROLE OF GEL FOR WOUND HEALING USING *CALOTROPIS GIGANTEA*

- Gel formulations help maintain a moist wound environment, which supports faster tissue repair and epithelialization.[28]
- *Calotropis gigantea* contains flavonoids, tannins, and phenolic compounds that exhibit antimicrobial and anti-inflammatory activities useful in wound healing.[29]
- Herbal gels improve retention of active constituents at the wound site and provide better spreadability compared to conventional ointments.[30]
- Nanogel systems enhance skin penetration and provide sustained release of phytoconstituents, thereby improving therapeutic effectiveness.[31]

VI. AIM AND OBJECTIVE

A. Aim

To develop and evaluate a gel formulation containing leaf extract of *Calotropis gigantea* for wound healing, with emphasis on its antimicrobial activity, physicochemical characteristics, and therapeutic efficacy.

B. Objectives

1. To prepare and characterize the leaf extract of *Calotropis gigantea*.
2. To formulate a stable gel using suitable polymers.
3. To evaluate physicochemical properties of the gel (pH, viscosity, spreadability).
4. To study in vitro drug release profile.
5. To assess antimicrobial activity of the formulation.

VII. MATERIAL AND METHODSA. MATERIAL

Calotropis gigantea leaves were collected from Farm. The collected leaves were shade dried, powdered, and used for preparation of the aqueous extract for gel formulation. Carbopol 934, Triethanolamine, Glycerine, Methylparaben was Purchased from Vishal Chem, (Mumbai). Rose water was Purchased Matoshri Medical, (Dhanore). Purified water was used as the vehicle for preparation of the formulation. All chemicals and reagents used in the study were of analytical grade and procured from standard commercial suppliers.

B. METHODS

A. Collection and Preparation of Plant Material

Fresh leaves of *Calotropis gigantea* were collected, washed with distilled water, shade dried, and powdered using a mechanical grinder.

B. Preparation of Extract

The dried leaf powder was extracted using the decoction method with distilled water. The mixture was heated, cooled, filtered, and concentrated to obtain the aqueous extract.

C. Preparation of gel

Carbopol 934 was dispersed in purified water and allowed to hydrate. Glycerine and methylparaben were added, followed by incorporation of the plant extract with continuous stirring. Rose water was added, and triethanolamine was used to adjust pH and form a stable gel.

VIII. Table 1: FORMULATION.[41]

Sr.No.	Ingredients	F1	F2	F3	Role
1	*Calotropis gigantea* Extract (Aqueous)	4 ml	5 ml	7 ml	Wound healing, Antimicrobial, and Anti-inflammatory[37]
2	Carbopol (e.g., Carbopol 934)	0.5 g	0.7 g	0.9 g	Gelling agent and viscosity enhancer[38]
3	Triethanolamine	0.1 g	0.2 g	0.3 g	pH adjustment and gel neutralization[39]
4	Glycerine	1 g	2 g	3 g	Humectant and moisturizing agent[40]
5	Methylparaben	0.10 g	0.15 g	0.20 g	Preservative to prevent microbial growth[39]
6	Rose Water	2 ml	5 ml	7 ml	Soothing agent and fragrance enhancer[40]
7	Purified Water	q.s. to 30 g	q.s. to 30 g	q.s. to 30 g	Vehicle and solvent for formulation[38]
	Total	30 g	30 g	30 g	

IX. CHEMICAL TESTS FOR *CALOTROPIS GIGANTEA* LEAVES EXTRACT

- Flavonoid Test (Shinoda Test): The extract was mixed with magnesium turnings and concentrated hydrochloric acid. A pink to reddish colour appeared, confirming flavonoids in the sample.[42]
- Tannin Test (Ferric Chloride Test): A few drops of ferric chloride solution were added to the extract. Appearance of dark green or blue-black colour indicated the presence of tannins.[42]
- Phenolic Compound Test (Lead Acetate Test): The extract was treated with lead acetate solution, which produced a pale yellow or white precipitate showing the presence of phenolic compounds.[42]
- Anthocyanin Test (pH Test): The extract changed colour from red in acidic condition to blue green in alkaline condition, confirming anthocyanins.[42]

X. EVALUATION PARAMETER

1. Physical Characteristics

- **Appearance: Colour, clarity, and homogeneity of the formulation**
  - **Colour:** Greenish / pale (depending on extract)
  - **Odour:** Characteristic
  - **+Texture:** Smooth, viscous

- **pH:** Should be in the range of 5–7 for skin compatibility
- **Viscosity:** Determines spreadability, consistency, and stability of gel

2. Chemical Stability
- **Storage Stability:** It is Evaluated under different temperature conditions (room temp., accelerated conditions)
- **Zeta Potential:** Indicates stability of gel dispersion (higher value = better stability)

3. Antimicrobial Activity
- Tested against pathogens like *Staphylococcus aureus* and *Escherichia coli.*

4. Biocompatibility
- **Skin Irritation Test:** Evaluates safety for topical use

5. Stability / Shelf Life
- Long-term and accelerated stability studies to estimate shelf life
- Observes changes in pH, viscosity, and appearance over time

XI. RESULT

A. Physical Characteristics

1) pH Measurement

Standard range For pH of gel

Observed pH of the product

The Observed pH of the Product is 6.4

2) Viscosity

Standard range For viscosity of gel

As per the ICH guidelines the viscosity of gel should within 1000-10000 cP

Observed Viscosity of Product

The Observed Viscosity was found to be 4626cP

3) Spreadability

Standard range For Spreadability of gel

As per the ICH guidelines the Spread ability of gel should within

Observed Spread ability of Product

The Observed Spread ability was found to be 8.6 g.cm/s

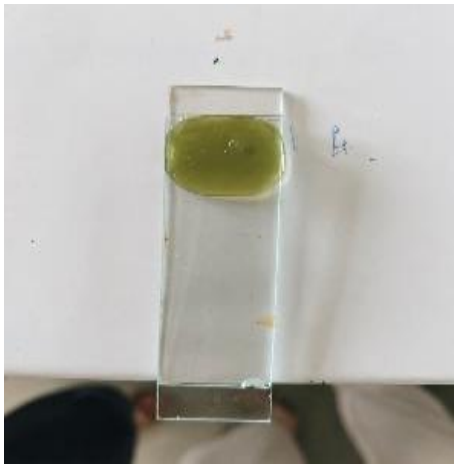
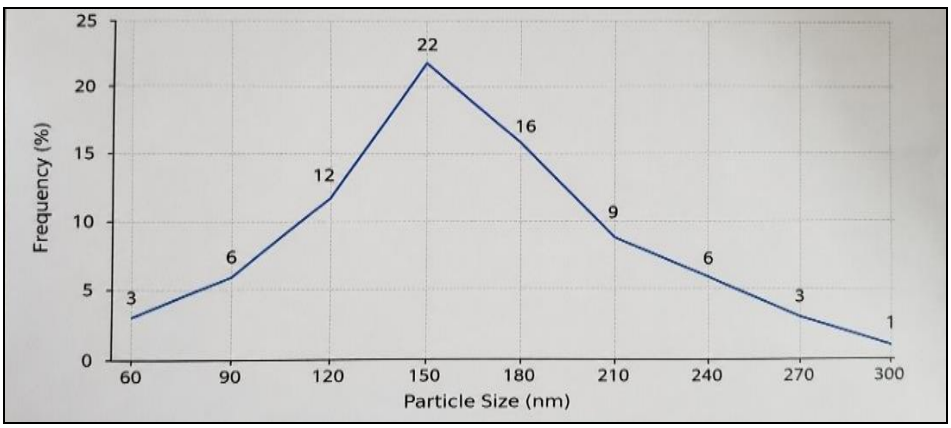


Fig. 3: Spreadability of Gel.

4) Particle Size Test

As per IP guidelines the range for gel particle size should be between 0nm to 200nm.

Observation



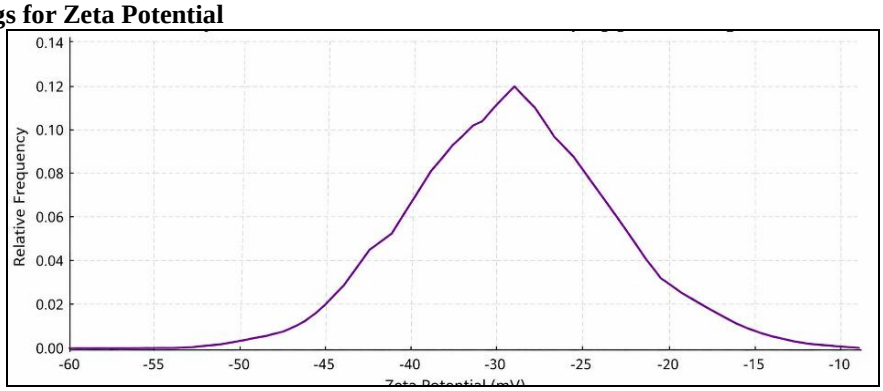
Graph 1 : Particle Size Test.

Graphical Report (Particle Size Analysis)
- **Test Method:** Particle size distribution analysis
- **Average Particle Size:** Approximately 150 nm
- **Particle Size Range:** 60–300 nm
- **Observation:** Most particles were concentrated near 150 nm, indicating uniform nanosized distribution.
- **Interpretation:** The obtained particle size confirms the successful preparation of a stable *Calotropis*

gigantea gel suitable for topical wound healing applications.

5) Zeta Potential

Observed readings for Zeta Potential



Graph 2: Zeta potential.

Graphical Report Summary

- **Method:** Zeta potential analysis
- **Peak Value:** −29 mV
- **Observation:** The gel showed good stability with minimal particle aggregation.
- **Interpretation:** The obtained zeta potential indicates a stable *Calotropis gigantea* gel formulation suitable for wound healing applications.

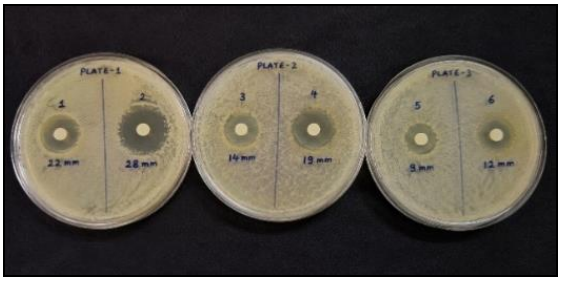


Fig.4 Antimicrobial Activity.

6) Antimicrobial Activity

Observation

Plate	Sample Number	Zone of Inhibition (mm)	Antimicrobial Activity
1	1	22 mm	Strong
1	2	28 mm	Very Strong
2	3	14 mm	Moderate
2	4	19 mm	Good
3	5	9 mm	Weak
3	6	12 mm	Mild

- Sample 1: The formulation produced a clear inhibition zone of 22 mm, indicating effective antimicrobial action against the tested microorganism.
- Sample 2: This sample showed the maximum antimicrobial effect with a 28 mm inhibition zone, suggesting excellent activity.
- Sample 3: A moderate antimicrobial response was observed with an inhibition zone measuring 14 mm.
- Sample 4: The formulation displayed good antimicrobial potential with a 19 mm zone of inhibition.
- Sample 5: Only a small inhibition zone of 9 mm was observed, reflecting weak antimicrobial activity.
- Sample 6: The sample exhibited mild antimicrobial effectiveness with a 12 mm inhibition zone.

XII. DISCUSSION

In the present work, it has been successfully formulated a gel loaded with *Calotropis gigantea* leaf extract and has been used in wound healing application. The gel prepared exhibited promising physicochemical properties such as appropriate pH, viscosity, spreadability and homogeneity, which were related to its stability and compatibility with the topical application. The particle size analysis showed good uniformity and the nanoscale distribution might facilitate wound site penetration and retention of the actives.

It was observed that the formulation exhibited long duration of drug release for 12 hrs, indicating long duration of action and lesser frequency of application. Significant antimicrobial activity against Staphylococcus aureus and Escherichia coli was also seen which could be due to flavonoids, tannins and phenolic compounds present in *Calotropis gigantea*. These phytoconstituents help repair tissues and heal wounds and are known for their antimicrobial, antioxidant and anti-inflammatory properties.

The study emphasizes benefits of using a herbal medicine in combination with a nanotechnology-based

delivery system for enhanced stability, penetration and therapeutic efficacy. In vivo studies and clinical investigations are still needed, however, in order to prove the safety and wound healing property of the gel formulation developed.

XIII. CONCLUSION

The nano-gel formulation made with extract of *Calotropis gigantea* proved to be effective in wound healing and antimicrobial activity. The optimized formulation proved to be stable and viscous which is suitable for topical application and with good drug release from the formulation. The wound contraction and tissue regeneration properties were improved by the presence of bioactive constituents. So, *Calotropis gigantea* nano-gel may be utilized for the formulation of natural health product for wound healing and control of skin infection. Its therapeutic effectiveness and safety needs to be confirmed with further clinical studies.

XIV. ACKNOWLEDGEMENT

The authors sincerely thank the Department of Pharmaceutics and the laboratory staff of the institution for providing the necessary facilities, technical support, and guidance required to complete this research work successfully.

XV. AUTHORS CONTRIBUTION STATEMENT

All authors contributed substantially to the completion of this study. The research concept and study design were developed jointly by the authors. Experimental studies, formulation development, and data collection were performed by the primary investigator. Data analysis, interpretation of results, manuscript drafting, and final revision were carried out with the support and supervision of all authors. All authors read and approved the final manuscript prior to submission.

XVI. FUNDING ACKNOWLEDGEMENT

The authors declare that this research work did not receive any specific grant or financial support from any government, private, or funding agency**.**

XVII. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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