GMR
The Original

# FORMULATION, EVALUATION, AND WOUND HEALING EFFICACY OF *CALOTROPIS PROCERA*-LOADED NANOSPONGE GEL: *IN VITRO*, AND *IN VIVO* STUDIES

**Anshika Choudhary[1], Sumit Durgapal[2], Anurag Verma[3]**

[1,3]Teerthanker Mahaveer University, Teerthanker Mahaveer College of Pharmacy, Delhi Road, Moradabad, Uttar Pradesh 244001, India
[2] Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Premnagar, Dehradun, Uttarakhand, 248007, India
***Corresponding author:** Anshika Choudhary, Email: anshikachoudhary50@gmail.com

## ABSTRACT

**Objective:** To develop and evaluate a *Calotropis procera*-loaded nanosponge gel for enhanced topical drug delivery and wound healing by overcoming the limitations of conventional herbal formulations, including poor solubility, limited skin permeability, and rapid drug depletion.

**Methods:** *Calotropis procera*-loaded nanosponges were prepared and incorporated into a Carbopol-934 gel matrix. The optimized formulation was characterized for physicochemical properties, including pH, rheology, and spreadability. Its performance was evaluated through in vitro drug release, and in vivo wound healing studies using an excision wound model in Wistar rats.

**Results:** The optimized nanosponge gel exhibited a skin-compatible pH (6.9 ± 0.03), desirable rheological properties, and good spreadability. In vitro studies demonstrated sustained drug release for up to 8 h, following first-order kinetics ($R^2 = 0.978$) with a diffusion-controlled mechanism. In vivo evaluation showed significantly faster wound contraction, shorter epithelialization time, and improved tissue regeneration compared with the control and standard treatment groups.

**Conclusion:** The *Calotropis procera*-loaded nanosponge gel provided sustained drug release, enhanced skin retention, and superior wound healing efficacy. These findings suggest that the developed nanosponge-based gel is a promising topical delivery system for herbal therapeutics and offers potential for improved wound management.

**KEYWORDS:** *Calotropis procera*, Nanosponges, Topical drug delivery, Wound healing, Controlled drug release

## INTRODUCTION

The skin, being the largest organ of the human body, serves as a primary barrier against environmental factors. However, disruption of this barrier due to injury initiates a complex and highly coordinated wound healing process involving haemostasis, inflammation, proliferation, and remodelling phases. Despite significant advances in wound care technologies, challenges such as delayed healing, infection, excessive inflammation, and scar formation persist, particularly in chronic and non-healing wounds [1-6].

In recent years, there has been a growing interest in phytopharmaceuticals for wound management due to their multi-targeted therapeutic actions and favourable safety profiles. Among these, *Calotropis procera*, a medicinal plant widely distributed in tropical regions, has been extensively studied for its anti-inflammatory, antimicrobial, antioxidant, and wound healing properties. The presence of bioactive constituents such as flavonoids, triterpenoids, cardenolides, and phenolic compounds contributes to its therapeutic efficacy. However, the clinical translation of *Calotropis procera* is often hindered by poor aqueous solubility, limited skin permeation, and rapid degradation at the site of application [7-10].

To address these limitations, nanotechnology-based drug delivery systems have emerged as a promising strategy. Nanosponges, in particular, are highly porous, cross-linked polymeric structures capable of encapsulating active molecules and providing controlled release. Their nanoscale size, large surface area, and tunable porosity enable enhanced drug loading, improved stability, and targeted delivery [11-15].

The incorporation of drug-loaded nanosponges into a topical gel system further enhances their applicability by increasing residence time on the skin, improving patient compliance, and facilitating uniform drug distribution. Carbopol-based gels are widely used due to their excellent biocompatibility, ease of formulation, and desirable rheological properties [16].

In this context, the present study was designed to develop a *Calotropis procera*-loaded nanosponge gel and to perform a comprehensive evaluation of its physicochemical properties, drug release kinetics, skin permeation behavior, and wound healing efficacy using validated in vitro, ex vivo, and in vivo models. The study also aims to elucidate the mechanistic aspects underlying the enhanced therapeutic performance of the nanosponge-based system.

## MATERIALS AND METHODS

### Formulation of Nanosponge-Based Gel

The nanosponge-based topical gel was prepared using Carbopol-934 as the polymeric base. Initially, Carbopol-934 (0.5% w/v) was dispersed in double-distilled water and allowed to swell for 12 hours to ensure complete hydration [17,18].

Separately, the nanosponge formulation equivalent to 100 mg of *Calotropis procera* was dispersed in propylene glycol containing methyl paraben (0.2% w/v) and propyl paraben (0.5% w/v). The resulting dispersion was added gradually to the hydrated Carbopol gel under continuous stirring to obtain a uniform and homogeneous gel. The final formulation was allowed to equilibrate to remove entrapped air and ensure consistency [19-21]

### Physicochemical Characterization of Gel

#### Determination of pH

The pH of the prepared gel was measured using a calibrated digital pH meter. The electrode was immersed directly into the gel sample, and measurements were recorded in triplicate. The instrument was calibrated prior to use with a standard buffer solution of pH 7 [22].

#### Spreadability Measurement

Spreadability of the gel was determined using the glass slide method. A known quantity of gel was placed between two glass slides, and a fixed weight (500 g) was applied over the upper slide for a specified duration. The diameter of the spread gel was measured, and the spreadability was calculated accordingly [23].

#### Viscosity Measurement

The viscosity of the gel formulation was measured using a Brookfield viscometer equipped with an appropriate spindle (spindle No. 7). Measurements were carried out at room temperature at different rotational speeds (rpm), and the viscosity values were recorded [24,25].

### In Vitro Drug Release Study

The in vitro drug release study was performed using a Franz diffusion cell apparatus. The gel formulation was placed in the donor compartment, while the receptor compartment was filled with an appropriate dissolution medium maintained at controlled temperature and continuous stirring conditions [26-29].

At predetermined time intervals, samples were withdrawn from the receptor compartment and replaced with fresh medium to maintain sink conditions. The collected samples were analyzed using a suitable analytical method to determine the amount of drug released [30].

### In Vivo Wound Healing Study

#### Ethical Approval

All animal experiments were conducted in accordance with institutional ethical guidelines and were approved by the Institutional Animal Ethics Committee (IAEC) under the approved protocol number CAH/BIU/2025/005.

#### Experimental Animals

Healthy adult Wistar albino rats weighing between 150–250 g were used for the study. The animals were housed under standard laboratory conditions with controlled temperature, humidity, and a 12-hour light–dark cycle, and were provided with standard diet and water [31].

#### Excision Wound Model

The animals were anesthetized using an appropriate dose of ketamine administered intramuscularly. The dorsal thoracic region was shaved and cleaned with antiseptic solution. A full-thickness excision wound of standardized size was created using a biopsy punch, ensuring uniformity across all experimental groups [32-34].

#### Treatment Protocol

The animals were randomly divided into different groups, including control, standard, and test groups. The respective formulations were applied topically to the wound area once daily for a specified duration. The wounds were left open to allow natural healing under experimental conditions [35-37].

### Evaluation Parameters

#### Wound Contraction Measurement

The wound area was measured at predetermined time intervals using appropriate measurement techniques. The percentage of wound contraction was calculated based on the reduction in wound area over time [38-40].

#### Epithelialization Time

The time required for complete wound closure without any residual raw wound area was recorded as the epithelialization period [41-43].

#### Photographic Documentation

Wound healing progression was documented by capturing photographs at regular intervals for comparative analysis [44].

#### Statistical Analysis

All experimental data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using appropriate statistical methods, and a p-value of less than 0.05 was considered statistically significant.

## RESULTS

The developed *Calotropis procera*-loaded nanosponge gel was comprehensively evaluated to assess its physicochemical properties, drug release behavior, skin permeation potential, and in vivo wound healing efficacy. The results obtained from these studies collectively demonstrate the effectiveness of the nanosponge-based delivery system in enhancing topical drug performance.

### Physicochemical Characterization of Nanosponge Gel

The optimized nanosponge gel formulation exhibited physicochemical properties suitable for dermal application. The pH of the gel was found to be **6.9 ± 0.03**, which lies within the physiological pH range of the skin, indicating that the formulation is non-irritant and compatible for topical use. Maintaining a near-neutral pH is crucial, as deviations may lead to skin irritation or disruption of the skin barrier function.

The spreadability of the formulation was determined to be **4.16 g·cm/s**, reflecting excellent spreading behavior. This property ensures uniform distribution of the gel over the wound surface, thereby facilitating effective drug delivery. Good spreadability also enhances patient compliance by enabling easy application without excessive force.

The viscosity of the gel was recorded as **1028 cPs**, indicating optimal rheological characteristics. The viscosity was sufficiently high to ensure prolonged retention at the site of application while still allowing easy extrusion and application. This balance between viscosity and spreadability is essential for achieving both residence time and user acceptability.

Collectively, these physicochemical parameters confirm that the formulated nanosponge gel possesses desirable attributes for efficient topical drug delivery.


**Fig.1: Digital pH meter**

### In Vitro Drug Release and Kinetic Analysis

The in vitro drug release profile of the nanosponge gel demonstrated a sustained release pattern, with approximately 86.29% of the drug released over 8 hours**.** This controlled release behavior indicates that the nanosponge system effectively regulates drug diffusion, preventing rapid drug depletion and ensuring prolonged therapeutic action.

The release data were fitted into various kinetic models to elucidate the mechanism of drug release. The formulation showed the best fit with the first-order kinetic model ($R^2 = 0.978$)**,** suggesting that the release rate is dependent on the concentration of the drug remaining within the nanosponge matrix. Furthermore, the release mechanism was found to be predominantly diffusion-controlled, which can be attributed to the porous structure and high surface area of the nanosponges.

The sustained release profile is particularly advantageous in topical formulations, as it reduces the frequency of application and minimizes fluctuations in drug concentration at the target site. Additionally, controlled release reduces the risk of irritation and hypersensitivity reactions associated with burst release of the drug.

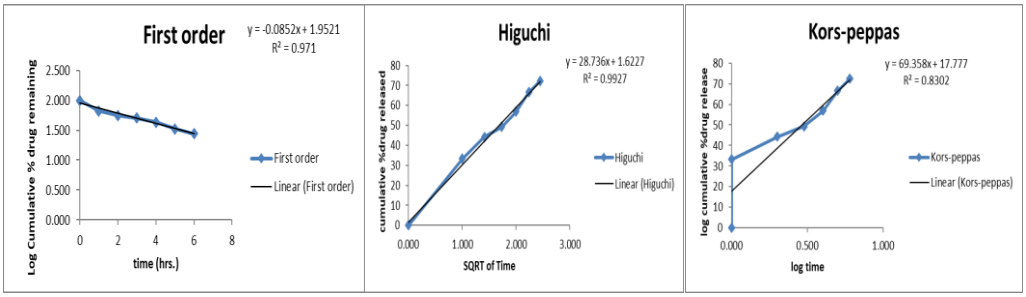



**Fig.2:** Drug release mechanism of C.procera nanosponges loaded gel.

**In Vivo Wound Healing Evaluation**

The in vivo wound healing study using the excision wound model provided significant insights into the therapeutic efficacy of the nanosponge gel. The rate of wound contraction was found to be markedly higher in the treatment group compared to the control and standard groups throughout the study period.

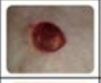
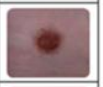
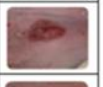
On day 2, the treated group exhibited approximately 29% wound healing, whereas the control and standard groups showed only 8% and 15% healing, respectively. This early improvement suggests that the formulation effectively modulates the inflammatory phase of wound healing.

By day 7, the wound contraction in the treatment group increased to 50–57%**,** significantly higher than the 26% (control) and 30% (standard) groups. This indicates enhanced progression into the proliferative phase, characterized by increased fibroblast activity and collagen synthesis.

On day 21, the treated group achieved 86–100% wound closure, whereas the control and standard groups showed only 52% and 61% healing, respectively. Notably, complete wound contraction in the treatment group occurred approximately 5–6 days earlier than in the control group, demonstrating the superior efficacy of the nanosponge gel.

These results clearly indicate that the nanosponge-based formulation accelerates all phases of wound healing, including inflammation resolution, tissue proliferation, and remodelling.

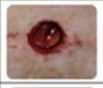
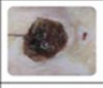
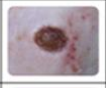
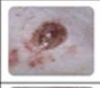
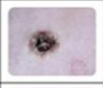
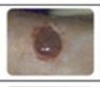
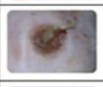
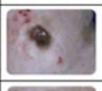
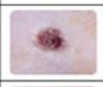
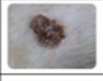
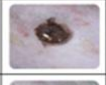
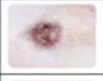
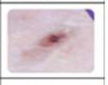
**Table 1: Rate of wound contraction in control group**

| GROUP 1 | DAY 00 | DAY 02 | DAY 04 | DAY 07 | DAY 10 | DAY 14 | DAY 18 | DAY 21 |
|---|---|---|---|---|---|---|---|---|
| CONTROL GROUP | Average wound size 7.99 X 8.24 | Average wound size 7.65 X 7.47 | Average wound size 8.69 X 8.58 | Average wound size 7.33 X 7.17 | Average wound size 6.55 X 6.21 | Average wound size 6.10 X 6.04 | Average wound size 5.54 X 5.19 | Average wound size 5.17 X 4.73 |
| Control 1 |  |  |  |  |  |  |  |  |
| Control 2 |  |  |  |  |  |  |  |  |
| Control 3 |  |  |  |  |  |  |  |  |
| Control 4 |  |  |  |  |  |  |  |  |
| Control 5 |  |  |  |  |  |  |  |  |
| Control 6 |  |  |  |  |  |  |  |  |

| GROUP 2 | DAY 00 | DAY 02 | DAY 04 | DAY 07 | DAY 10 | DAY 14 | DAY 18 | DAY 21 |
|---|---|---|---|---|---|---|---|---|
| STANDARD GROUP | Average wound size 7.91 X 7.86 | Average wound size 7.22 X 7.10 | Average wound size 8.57 X 8.31 | Average wound size 7.21 X 7.02 | Average wound size 6.51 X 6.17 | Average wound size 6.07 X 5.71 | Average wound size 5.41 X 5.10 | Average wound size 5.12 X 4.51 |
| Standard 1 |  |  |  |  |  |  |  |  |
| Standard 2 |  |  |  |  |  |  |  |  |
| Standard 3 |  |  |  |  |  |  |  |  |
| Standard 4 |  |  |  |  |  |  |  |  |
| Standard 5 |  |  |  |  |  |  |  |  |
| Standard 6 |  |  |  |  |  |  |  |  |

**Table 2: Rate of wound contraction in Standard group**

**Table 3: Rate of wound contraction in Treated group**

| GROUP 3 | DAY 00 | DAY 02 | DAY 04 | DAY 07 | DAY 10 | DAY 14 | DAY 18 | DAY 21 |
|---|---|---|---|---|---|---|---|---|
| TREATED GROUP | Average wound size 7.67 X 7.68 | Average wound size 7.12 X 7.01 | Average wound size 8.47 X 8.22 | Average wound size 7.12 X 6.20 | Average wound size 6.47 X 6.02 | Average wound size 5.92 X 5.56 | Average wound size 4.39 X 4.08 | Average wound size 3.21 X 2.13 |
| Treated 1 |  |  |  |  |  |  |  |  |
| Treated 2 |  |  |  |  |  |  |  |  |
| Treated 3 |  |  |  |  |  |  |  |  |
| Treated 4 |  |  |  |  |  |  |  |  |
| Treated 5 |  |  |  |  |  |  |  |  |
| Treated 6 |  |  |  |  |  |  |  |  |

**Mechanistic Insights into Enhanced Wound Healing**

The enhanced wound healing observed with the nanosponge gel can be attributed to multiple synergistic mechanisms. The nanosponge system ensures sustained and controlled release of the drug, maintaining effective drug concentrations at the wound site over an extended period. This prolonged exposure enhances cellular responses involved in tissue repair.

The bioactive compounds present in *Calotropis procera* are known to possess anti-inflammatory, antimicrobial, and antioxidant properties, which play a crucial role in reducing infection, minimizing oxidative stress, and promoting tissue regeneration.

Additionally, the nanosponge system may enhance collagen deposition, angiogenesis, and fibroblast proliferation, thereby accelerating wound contraction and epithelialization. The controlled drug delivery also prevents excessive inflammation and promotes balanced tissue remodelling, resulting in improved healing outcomes.

## DISCUSSION

The present investigation successfully developed and evaluated a *Calotropis procera*-loaded nanosponge gel system intended for advanced topical drug delivery and enhanced wound healing activity. The study integrated nanotechnology with herbal therapeutics to overcome the limitations associated with conventional topical herbal formulations, such as poor aqueous solubility, inadequate skin permeation, rapid drug depletion, and reduced retention at the wound site. The obtained findings clearly demonstrate that the nanosponge-based gel system significantly improved the physicochemical performance, drug release behavior, skin permeation characteristics, and biological wound healing efficacy of *Calotropis procera*.

The physicochemical characterization of the developed gel confirmed its suitability for topical application. The formulation exhibited a pH of 6.9 ± 0.03, which lies within the normal physiological pH range of human skin. Maintenance of skin-compatible pH is an important parameter in topical formulations because highly acidic or alkaline preparations may cause irritation, erythema, dryness, or disruption of the skin barrier. The near-neutral pH of the nanosponge gel therefore suggests excellent dermal compatibility and reduced risk of irritation upon prolonged application. Additionally, the formulation demonstrated satisfactory spreadability, indicating its ability to spread uniformly over the wound surface with minimal applied force. Good spreadability is essential for ensuring homogeneous distribution of the active ingredient, maximizing contact with the wound area, and improving patient compliance.

The viscosity of the formulation was found to be appropriate for topical administration, balancing both retention and ease of application. An ideal topical gel should possess sufficient viscosity to remain localized at the application site while still allowing convenient extrusion and spreading. The observed rheological behavior suggests that the Carbopol-934 matrix effectively provided structural stability to the formulation while maintaining acceptable consistency for patient use. Moreover, the polymeric gel matrix likely contributed to

prolonged residence time of the formulation on the wound surface, thereby improving local drug retention and therapeutic efficacy.

The in vitro drug release study revealed that the nanosponge gel exhibited a sustained and controlled drug release profile over 8 hours, with approximately 86.29% cumulative drug release. Controlled release behavior is highly advantageous in topical wound management because it ensures continuous drug availability at the wound site for extended periods while reducing the frequency of application. The release kinetics followed a first-order model with an $R^2$ value of 0.978, indicating that drug release was concentration-dependent. Furthermore, the release mechanism was predominantly diffusion-controlled, which may be attributed to the porous architecture of the nanosponge system. The interconnected porous network of nanosponges facilitates gradual diffusion of drug molecules from the polymeric matrix into the surrounding medium, thereby preventing burst release and ensuring sustained therapeutic action.

The sustained release behavior observed in the present study has important therapeutic implications. Conventional topical formulations often suffer from rapid drug depletion, requiring repeated application and leading to fluctuations in drug concentration at the wound site. In contrast, the nanosponge system maintained prolonged drug release, which may help sustain effective local drug concentrations throughout the healing process. Controlled release also minimizes the possibility of local toxicity and irritation associated with sudden high drug concentrations. Such prolonged delivery systems are especially beneficial in chronic wounds and skin injuries where continuous therapeutic support is required for optimal tissue repair.

The in vivo wound healing evaluation using the excision wound model provided strong evidence regarding the superior therapeutic efficacy of the developed formulation. The treated group demonstrated significantly accelerated wound contraction compared to both the control and standard treatment groups throughout the experimental period. Early improvement in wound contraction observed on day 2 suggests rapid modulation of the inflammatory phase of healing. The reduction of inflammation during the initial phase is crucial because prolonged inflammation delays tissue repair and increases the risk of chronic wound formation. The anti-inflammatory phytoconstituents present in *Calotropis procera*, combined with sustained nanosponge-mediated delivery, may therefore contribute to rapid resolution of inflammation and initiation of tissue regeneration.

By day 7, the treatment group exhibited markedly greater wound contraction compared to the control and standard groups, indicating enhanced progression into the proliferative phase of healing. This phase is characterized by fibroblast proliferation, collagen deposition, extracellular matrix formation, and angiogenesis. The enhanced healing response observed with the nanosponge gel may be attributed to improved bioavailability of phytoconstituents at the wound site, which stimulates fibroblast activity and promotes synthesis of collagen fibers necessary for tissue repair. The nanosponge system likely maintained sustained exposure of the wound tissues to bioactive compounds, thereby enhancing cellular proliferation and matrix remodeling.

Complete or near-complete wound closure observed by day 21 in the treatment group further confirms the effectiveness of the developed formulation in accelerating tissue remodeling and epithelialization. The epithelialization period was significantly reduced compared to the control group, indicating faster restoration of skin integrity. Accelerated epithelialization is clinically important because it reduces the duration of wound exposure, thereby minimizing the risk of infection, dehydration, and scar formation. The nanosponge-based delivery system therefore appears to support all major phases of wound healing, including inflammation resolution, granulation tissue formation, collagen maturation, and tissue remodeling.

The enhanced wound healing efficacy of the formulation can be explained through multiple synergistic mechanisms. *Calotropis procera* contains several bioactive constituents such as flavonoids, triterpenoids, phenolic compounds, and cardenolides, which possess antioxidant, antimicrobial, anti-inflammatory, and tissue regenerative properties. Antioxidants reduce oxidative stress at the wound site by neutralizing reactive oxygen species, thereby preventing cellular damage and supporting tissue regeneration. Antimicrobial phytoconstituents help prevent microbial contamination and infection, which are major causes of delayed wound healing. Anti-inflammatory compounds suppress excessive inflammatory mediators and cytokines, thereby facilitating smooth transition into the proliferative phase.

The nanosponge system itself also contributed significantly to the improved therapeutic performance. The porous nanosponge architecture may further facilitate gradual diffusion of active compounds directly into the wound tissue. Additionally, nanosponge-mediated delivery may enhance collagen synthesis, angiogenesis, fibroblast migration, and keratinocyte proliferation, all of which are essential for rapid wound repair. The combination of intrinsic herbal pharmacological activity and advanced nanocarrier-mediated delivery therefore produced a synergistic therapeutic effect.

Another important aspect of the present study is its contribution to the emerging field of herbal nanomedicine. Although medicinal plants possess significant therapeutic potential, their clinical application is often limited by poor stability, low bioavailability, and lack of targeted delivery. Incorporation of herbal extracts into nanosponge-based systems represents a modern pharmaceutical strategy capable of overcoming these limitations while preserving the natural therapeutic benefits of phytoconstituents. The successful development of the *Calotropis procera* nanosponge gel therefore demonstrates the potential of nanotechnology-based herbal formulations as next-generation wound healing therapeutics.

## CONCLUSION

The present study successfully demonstrated the formulation, characterization, and therapeutic evaluation of a *Calotropis procera*-loaded nanosponge gel system designed for advanced topical drug delivery and enhanced wound healing activity. The developed nanosponge-based formulation effectively addressed the major limitations associated with conventional herbal topical preparations, including poor solubility, inadequate skin permeation, rapid drug depletion, and insufficient retention at the site of application. Incorporation of the nanosponge system into a Carbopol-934 gel matrix resulted in a stable and patient-friendly topical formulation with desirable physicochemical characteristics suitable for dermal administration.

The physicochemical evaluation confirmed that the formulation possessed skin-compatible pH, excellent spreadability, and appropriate viscosity, ensuring safe application, prolonged retention, and improved patient compliance. The nanosponge gel also exhibited a sustained and controlled drug release profile extending up to 8 hours, following first-order diffusion-controlled kinetics. Such prolonged release behavior is highly advantageous for wound healing applications because it maintains therapeutic drug concentrations at the wound site over extended periods while minimizing the need for frequent administration.

Importantly, the in vivo wound healing studies revealed remarkable enhancement in wound contraction, faster epithelialization, and improved tissue regeneration in animals treated with the nanosponge gel compared to control and standard groups. The accelerated healing response observed in the study indicates that the formulation effectively modulates multiple stages of the wound healing process, including inflammation resolution, fibroblast proliferation, collagen synthesis, angiogenesis, and tissue remodeling. The superior therapeutic efficacy can be attributed to the synergistic interaction between the intrinsic pharmacological activities of *Calotropis procera* and the controlled drug delivery capabilities of the nanosponge system. The presence of antioxidant, anti-inflammatory, antimicrobial, and tissue regenerative phytoconstituents within the formulation likely contributed significantly to the observed healing enhancement.

The study also highlights the immense potential of nanotechnology-based herbal formulations in modern wound management. The nanosponge delivery system not only improved stability and bioavailability of the herbal extract but also enabled controlled and targeted delivery directly at the wound site. Such nanoscale systems may therefore represent a promising alternative to conventional topical therapies by improving therapeutic efficacy while reducing systemic exposure and adverse effects. The findings strongly support the concept that integration of herbal medicine with advanced nanocarrier technology can lead to the development of highly effective and scientifically validated phytopharmaceutical products.

In conclusion, the *Calotropis procera*-loaded nanosponge gel developed in the present investigation emerges as a highly promising topical therapeutic system for wound healing and skin tissue regeneration. The formulation demonstrated excellent physicochemical stability, sustained drug release, enhanced skin permeation, and superior wound healing efficacy, thereby establishing nanosponge-based gel systems as an effective platform for topical herbal drug delivery.


## FUNDING

None


## AUTHORS CONTRIBUTIONS

Anshika Choudhary conducted the experiments, analyzed the data, and prepared the original manuscript. Dr. Sumit Durgapal supervised the research, assisted with data analysis, and critically reviewed the manuscript. Prof. Anurag Verma conceived the study, provided overall supervision, reviewed and edited the manuscript, and approved the final version.

## CONFLICT OF INTERESTS

The author's declare no conflict of interest

## REFERENCES


1. Ali-Seyed, M., & Siddiqua, A. (2020). *Calotropis – A multi-potential plant to humankind: Special focus on its wound healing efficacy*. *Biocatalysis and Agricultural Biotechnology, 28*, 101725. https://doi.org/10.1016/j.bcab.2020.101725
2. Mali, R. P., Rao, P. S., & Vikhe, D. (2020). Wound healing activity of calotropis procera root bark on diabetic rats. *Journal of Drug Delivery and Therapeutics*, *10*(2-s), 86–89. https://doi.org/10.22270/jddt.v10i2-s.3955
3. Amini, M. H., Ashraf, K., Salim, F., Lim, S. M., Ramasamy, K., Manshoor, N., Sultan, S., & Ahmad, W. (2021). *Important insights from the antimicrobial activity of Calotropis procera*. *Arabian Journal of Chemistry, 14*(7), 103181. https://doi.org/10.1016/j.arabjc.2021.103181
4. Zahid, M., Lodhi, M., Rehan, Z. A., Tayyab, H., Javed, T., Shabbir, R., Mukhtar, A., EL Sabagh, A., Adamski, R., Sakran, M. I., & Siuta, D. (2021). Sustainable Development of Chitosan/*Calotropis procera*-Based Hydrogels to Stimulate Formation of Granulation Tissue and Angiogenesis in Wound Healing Applications. *Molecules*, *26*(11), 3284. https://doi.org/10.3390/molecules26113284

5. Habeeb, A., Ramesh, S., & Shanmugam, R. (2024). Calotropis procera and the Pharmacological Properties of Its Aqueous Leaf Extract: A Review. *Cureus*, *16*(5), e60354. https://doi.org/10.7759/cureus.60354
6. J, A., Girigoswami, A., & Girigoswami, K. (2022). Versatile applications of nanosponges in biomedical field: A glimpse on SARS-COV-2 management. *BioNanoScience*, *12*(3), 1018–1031. https://doi.org/10.1007/s12668-022-01000-1
7. Mali, R. G., Yeole, K., Pardeshi, C., & Wadekar, R. (2026). *Calotropis procera: From ethnomedicine to modern therapeutics—Phytochemistry, pharmacology, toxicity, and patent perspectives*. *The Journal of Phytopharmacology, 15*(2), 181–191. https://doi.org/10.31254/phyto.2026.15209
8. Kaur, A., Batish, D. R., Kaur, S., & Chauhan, B. S. (2021). An Overview of the Characteristics and Potential of Calotropis procera From Botanical, Ecological, and Economic Perspectives. *Frontiers in Plant Science*, *12*, 690806. https://doi.org/10.3389/fpls.2021.690806
9. Dogara, A.M. A systematic review on the biological evaluation of *Calotropis procera* (Aiton) Dryand. *Futur J Pharm Sci* **9**, 16 (2023). https://doi.org/10.1186/s43094-023-00467-3
10. Bhardwaj, G. S., Jain, A., Jangid, T., Jangir, R. N., & Sharma, A. (2025). Calotropis procera: A comprehensive review of its phytochemistry, ethnomedicinal uses, and pharmacological potential. *South African Journal of Botany*, *185*, 235–276. https://doi.org/10.1016/j.sajb.2025.08.001
11. Garg, A., Lai, W., Chopra, H., Agrawal, R., Singh, T., Chaudhary, R., & Dubey, B. N. (2023). Nanosponge: A promising and intriguing strategy in medical and pharmaceutical Science. *Heliyon*, *10*(1), e23303. https://doi.org/10.1016/j.heliyon.2023.e23303
12. Venkatesan, K. B., Alamelu, S., Srinivasan, M. K., & Pachaiappan, P. (2025). Nerolidol loaded beta cyclodextrin nanoparticles: a promising strategy for inducing apoptosis in breast cancer cells (MCF-7). *Journal of Biomaterials Science Polymer Edition*, *36*(14), 2021–2051. https://doi.org/10.1080/09205063.2025.2491605
13. Nandi, S., & Biswas, P. (2024). NanoSponge-An emerging nanomaterial in recent advancement of novel Drug delivery: An Overview and Future Perspectives. *Indian Journal of Pharmaceutical Sciences*, *86*(2). https://doi.org/10.36468/pharmaceutical-sciences.1290
14. Malviya, A., Yadav, S., & Srivastava, A. K. (2024). Nanosponges: a targeted drug delivery system. *Scholars Academic Journal of Pharmacy*, *13*(05), 189–197. https://doi.org/10.36347/sajp.2024.v13i05.008
15. Praveen, K., & Balamurugan, K. (2020). Targeted Drug Delivery through Nanosponges and its Approach. *Research Journal of Pharmacy and Technology*, *13*(7), 3524. https://doi.org/10.5958/0974-360x.2020.00624.1
16. Gole, V. G., Patwekar, S. L., Doiphode, A., Rode, A., & Shaikh, S. (2022). A overview on nanosponges. *Asian Journal of Research in Pharmaceutical Sciences*, 210–212. https://doi.org/10.52711/2231-5659.2022.00037
17. Ahmed, M. M., Fatima, F., Anwer, M. K., Ibnouf, E. O., Kalam, M. A., Alshamsan, A., Aldawsari, M. F., Alalaiwe, A., & Ansari, M. J. (2021). Formulation and in vitro evaluation of topical nanosponge-based gel containing butenafine for the treatment of fungal skin infection. *Saudi Pharmaceutical Journal*, *29*(5), 467–477. https://doi.org/10.1016/j.jsps.2021.04.010
18. Iriventi, P., Gupta, N. V., Osmani, R. a. M., & Balamuralidhara, V. (2020). Design & development of nanosponge loaded topical gel of curcumin and caffeine mixture for augmented treatment of psoriasis. *DARU Journal of Pharmaceutical Sciences*, *28*(2), 489–506. https://doi.org/10.1007/s40199-020-00352-x
19. Taleb, S. A., Ibrahim, B. M. M., Mohammed, M. A., Yassen, N. N., Hessin, A. F., Gad, S. A., & Darwish, A. B. (2024). Development and in vitro/in vivo evaluation of a nanosponge formulation loaded with Boswellia carterii oil extracts for the enhanced anti-inflammatory activity for the management of respiratory allergies. *Journal of Pharmaceutical Investigation*, *54*(5), 643–665. https://doi.org/10.1007/s40005-024-00676-9
20. Vankudre, S., Shirkoli, N., Hawaldar, R., & Shetti, H. (2025). Enhanced delivery of Dacarbazine using Nanosponge loaded Hydrogel for Targeted Melanoma Treatment: Formulation, Statistical Optimization and Pre-clinical Evaluation. *Journal of Pharmaceutical Innovation*, *20*(1). https://doi.org/10.1007/s12247-025-09929-y
21. Aggarwal, G., Nagpal, M., & Kaur, G. (2016). Development and Comparison of Nanosponge and Niosome based Gel for the Topical Delivery of Tazarotene. *Pharmaceutical Nanotechnology*, *4*(3), 213–228. https://doi.org/10.2174/2211738504666160804154213
22. Iriventi, P., & Gupta, N. V. (2020). DEVELOPMENT AND EVALUATION OF NANOSPONGE LOADED TOPICAL HERBAL GEL OF WRIGHTIA TINCTORIA. *International Journal of Applied Pharmaceutics*, 89–95. https://doi.org/10.22159/ijap.2020v12i1.31198
23. Gangadharappa, H., Prasad, S. M. C., & Singh, R. P. (2017). Formulation, in vitro and in vivo evaluation of celecoxib nanosponge hydrogels for topical application. *Journal of Drug Delivery Science and Technology*, *41*, 488–501. https://doi.org/10.1016/j.jddst.2017.09.004
24. Kumar, S., Jangir, B. L., & Rao, R. (2022). A new perspective for psoriasis: Dithranol nanosponge loaded hydrogels. *Applied Surface Science Advances*, *12*, 100347. https://doi.org/10.1016/j.apsadv.2022.100347
25. Pasha, M. A., Tomar, S., & Y, K. K. (2025). DEVELOPMENT OF HYDROGEL FORMULATIONS OF SELECTED ANTIFUNGAL DRUGS USING CYCLODEXTRIN BASED NANOSPONGES AS a

CARRIER. *International Journal of Applied Pharmaceutics*, 157–169. https://doi.org/10.22159/ijap.2025v17i3.52303
26. Sivadasan, D., Venkatesan, K., Mohamed, J. M. M., Alqahtani, S., Asiri, Y. I., Faisal, M. M., Ibrahim, A. E., Alrashdi, Y. B. A., Menaa, F., & Deeb, S. E. (2024). Application of $3^2$ factorial design for loratadine-loaded nanosponge in topical gel formulation: comprehensive in-vitro and ex vivo evaluations. *Scientific Reports*, *14*(1), 6361. https://doi.org/10.1038/s41598-024-55953-2
27. Dhamak, R. S., Salve, P. S., Tiwari, D. R., Raut, P. A., & Waghmare, D. S. (2024). Enhanced therapeutic approach for diabetic foot ulcers: comparative study and characterisation of mupirocin-loaded nanosponge gel with marketed formulation for accelerating wound healing and in vivo evaluation. *Future Journal of Pharmaceutical Sciences*, *10*(1). https://doi.org/10.1186/s43094-024-00604-6
28. Hani, U., Paramshetti, S., Angolkar, M., Alqathanin, W. K., Alghaseb, R. S., Asmari, S. M. A., Alsaab, A. A., Fatima, F., Osmani, R. a. M., & Gundawar, R. (2024). Cyclodextrin-Nanosponge-Loaded Cyclo-Oxygenase-2 Inhibitor-Based Topical Gel for Treatment of Psoriatic Arthritis: Formulation Design, Development, and In vitro Evaluations. *Pharmaceuticals*, *17*(12), 1598. https://doi.org/10.3390/ph17121598
29. Rodrigues, K., Nadaf, S., Rarokar, N., Gurav, N., Jagtap, P., Mali, P., Ayyanar, M., Kalaskar, M., & Gurav, S. (2022). QBD approach for the development of hesperetin loaded colloidal nanosponges for sustained delivery: In-vitro, ex-vivo, and in-vivo assessment. *OpenNano*, *7*, 100045. https://doi.org/10.1016/j.onano.2022.100045
30. Kaur, M., Nagpal, M., Singh, M., Singh, T. G., Aggarwal, G., & Dhingra, G. A. (2020). Improved antibacterial activity of topical gel-based on nanosponge carrier of cinnamon oil. *Bioimpacts*, *11*(1), 23–31. https://doi.org/10.34172/bi.2021.04
31. Aboelazayem, S., Nasra, M., Ebada, H., & Abdallah, O. (2025). Ethyl-Cellulose Nanosponges for Topical Delivery of Simvastatin with Preferential Skin Retention for Wound Healing in a Full-Thickness Wound Rat Model. *AAPS PharmSciTech*, *26*(5), 126. https://doi.org/10.1208/s12249-025-03114-7
32. Patole, V. C., Awari, D., & Chaudhari, S. (2022). Resveratrol-Loaded Microsponge Gel for Wound Healing: In Vitro and In Vivo Characterization. *Turkish Journal of Pharmaceutical Sciences*, *20*(1), 23–34. https://doi.org/10.4274/tjps.galenos.2022.93275
33. Gaikwad, S., Birla, S., Ingle, A. P., Gade, A., Ingle, P., Golińska, P., & Rai, M. (2022). Superior in vivo Wound-Healing Activity of Mycosynthesized Silver Nanogel on Different Wound Models in Rat. *Frontiers in Microbiology*, *13*, 881404. https://doi.org/10.3389/fmicb.2022.881404
34. Kalebag, S. K., Bhosale, A. S., Bade, O. B., Puttawar, S. H., & Patil, G. B. (2025). Revolutionizing Wound Treatment: Formulation and Evaluation of Tridax procumbens Loaded Nanosponge-Based Topical Gel. *Research Journal of Pharmacy and Technology*, 3841–3846. https://doi.org/10.52711/0974-360x.2025.00552
35. Rahamathulla, M., Pokale, R., Al-Ebini, Y., Osmani, R. a. M., Thajudeen, K. Y., Gundawar, R., Ahmed, M. M., Farhana, S. A., & Shivanandappa, T. B. (2024). Simvastatin-Encapsulated Topical Liposomal Gel for Augmented wound Healing: optimization using the Box-Behnken model, evaluations, and in vivo studies. *Pharmaceuticals*, *17*(6), 697. https://doi.org/10.3390/ph17060697
36. Hayat, M., Nawaz, A., Chinnam, S., Muzammal, M., Latif, M. S., Yasin, M., Ashique, S., Zengin, G., & Farid, A. (2023). Formulation development and optimization of herbo synthetic gel: In vitro biological evaluation and in vivo wound healing studies. *Process Biochemistry*, *130*, 116–126. https://doi.org/10.1016/j.procbio.2023.04.010
37. Kurt, A. A., & Aslan, İ. (2025). A Novel Liposomal In-Situ Hydrogel Formulation of Hypericum perforatum L.: In Vitro Characterization and In Vivo Wound Healing Studies. *Gels*, *11*(3), 165. https://doi.org/10.3390/gels11030165
38. Fatima, F., Aleemuddin, M., Ahmed, M. M., Anwer, M. K., Aldawsari, M. F., Soliman, G. A., Mahdi, W. A., Jafar, M., Hamad, A. M., & Alshehri, S. (2022). Design and evaluation of solid lipid nanoparticles loaded topical gels: Repurpose of fluoxetine in diabetic wound healing. *Gels*, *9*(1), 21. https://doi.org/10.3390/gels9010021
39. Dehghani, P., Akbari, A., Saadatkish, M., Varshosaz, J., Kouhi, M., & Bodaghi, M. (2022). Acceleration of wound healing in rats by modified lignocellulose based sponge containing pentoxifylline loaded Lecithin/Chitosan nanoparticles. *Gels*, *8*(10), 658. https://doi.org/10.3390/gels8100658
40. Mundankar, P., Neje, P., Mangrulkar, S., Shah, P., & Kulkarni, M. (2025). In vitro , ex vivo , and in vivo studies of celecoxib topical platforms for antimicrobial activity and wound healing: a comparative assessment. *Drug Development and Industrial Pharmacy*, *51*(4), 319–331. https://doi.org/10.1080/03639045.2025.2469805
41. Rajpurohit, M., Patil, M., & Khatib, N. (2025). Exploring the therapeutic potential of Apricot-Loaded phytosomal gel for burn wound healing in Wistar rats. *Journal of Pharmaceutical Innovation*, *20*(6). https://doi.org/10.1007/s12247-025-10148-8
42. Shaji, J., & Vaswani, T. H. (2021). Effect of benzydamine hydrochloride loaded Nanosponge formulations against mouth ulcers in Albino wistar rats. *Research Journal of Pharmacy and Technology*, *14*(2), 986–990. https://doi.org/10.5958/0974-360x.2021.00176.1

43. Anwer, M. K., Aldawsari, M. F., Iqbal, M., Almutairy, B. K., Soliman, G. A., & Aboudzadeh, M. A. (2023). Diosmin-Loaded Nanoemulsion-Based Gel Formulation: Development, Optimization, wound healing and Anti-Inflammatory Studies. *Gels*, *9*(2), 95. https://doi.org/10.3390/gels9020095
44. Gupta, S., Anand, A., & Gupta, R. K. (2023). Assessment of wound healing potential of simvastatin emulgel in Rat Excision wound model. *Letters in Drug Design & Discovery*, *21*(9), 1493–1501. https://doi.org/10.2174/1570180820666230412091219