

# ***Calotropis gigantea* Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections**

**V.G. Akilandeswari<sup>1</sup>, Dr.G. Geetha<sup>2</sup>, Dr N. Selvasudha<sup>3</sup>, A.M. Renosa sarun<sup>4</sup>, J. Deepthi<sup>4</sup>, N.Dhivya sri<sup>4</sup>**

1. Associate professor, Department of Pharmaceutics, Kamarajar college of Pharmacy, Cuddalore, Tamilnadu
2. Principal, Kamarajar college of Pharmacy, Cuddalore, Tamilnadu
3. HOD, Department of Pharmaceutics, Kamarajar college of Pharmacy, Cuddalore, Tamilnadu
4. Students, Kamarajar college of Pharmacy, Cuddalore, Tamilnadu

**Corresponding author: V.G. Akilandeswari**, Department of Pharmaceutics, Kamarajar college of Pharmacy, The Tamil Nadu Dr. M.G.R. Medical University, Chidambaram, Cuddalore 608602, Tamilnadu, India  
E-mail address: [santhasabarivg@gmail.com](mailto:santhasabarivg@gmail.com) Tel: +917904404057

## **Abstract:**

Chronic wounds, especially amongst human beings with diabetes, pose a challenging assignment in healthcare due to their sluggish healing, higher chance of infection, and awful effectiveness of elegant treatments. Lately, redress the usage of plant-based treatment have received a true deal interest due to the fact of a range of restoration benefits. This look up desires to create and decide a new kind of transdermal patch made from latex extracted from *Calotropis gigantea*, a medicinal plant identified for its healing, anti-inflammatory, and antimicrobial effects, which need to be beneficial in treating diabetic wounds. The latex used to be meticulously extracted and blended into a biocompatible patch made from polyvinylpyrrolidone (PVP) and hydroxypropyl methylcellulose (HPMC). The patches have been definitely examined for a quantity of bodily and chemical properties, such as thickness, strength, durability, moisture levels, and pH. Studies on drug launch validated that the patches allowed for a regular and managed launch over time. To think about antibacterial properties, checks have been carried out in opposition to *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are frequent germs placed in diabetic wounds. The patches created essential inhibition zones of 18 mm and 21 mm, respectively. Further assessments the use of Franz diffusion cells validated first-class drug shipping by way of the skin, while compatibility checks on HaCaT human keratinocyte cells demonstrated that the latex used to be secure at therapeutic levels. These penalties spotlight the achievable of the usage of *Calotropis gigantea* latex-based transdermal patches for superb diabetic wound treatment. This formulation gives targeted drug transport alongside with sturdy antimicrobial houses and a proper protection profile. The findings encourage similarly research in living organisms and scientific trials to completely discover the therapeutic workable of this plant-based treatment.

**Keywords:** *Calotropis gigantea*, Transdermal patch, Diabetic wound, Antimicrobial, Wound healing

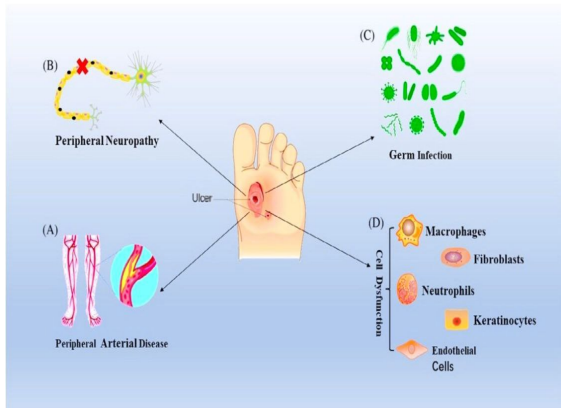
**How to cite this article:** Akilandeswari VG, Geetha G, Selvasudha N, Renosa sarun AM, Deepthi J, Dhivya sri N. *Calotropis gigantea* Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections. *Int J Drug Deliv Technol.* 2026;16(11s): 274-286; DOI: 10.25258/ijddt.16.11s.26

## **Introduction:**

Diabetes mellitus is a world health concern, affecting over 537 million adults in 2021, with projections estimating this range will upward shove to more than 783 million by using way of 2045 [1]. Among its several complications, persistent wounds including diabetic foot ulcers pose massive challenges due to delayed healing, infection, and accelerated hazard of amputation, cumulatively contributing to massive morbidity, mortality, and healthcare costs [1,2]. The elaborate pathophysiology underlying diabetic wounds entails peripheral neuropathy, microvascular impairment, multiplied oxidative stress, and immune desktop deficiencies, impairing tissue fix mechanisms

and predisposing the wounds to continual infection [2,3].

# Calotropis gigantea Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections



**Figure 1:** Pathophysiology of *Calotropis gigantea*. Bacterial colonization, ordinarily by way of way of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and antibiotic-resistant *Escherichia coli* strains, in a similar fashion aggravates wound chronicity and complicates scientific outcomes [4,1]. In this context, transdermal drug transport structures (TDDS) have emerged as promising alternatives, providing localized, sustained launch of bioactive marketers that decrease systemic toxicity and optimize therapeutic outcomes [5]. Additionally, TDDS can keep a moist wound environment, vital for mobile migration and tissue regeneration [6]. Phytotherapeutics, with their multifactorial mechanisms including anti-inflammatory, antimicrobial, and antioxidant activities have garnered renewed pastime in wound management [7]. *Calotropis gigantea*, a medicinal plant historically employed in Ayurveda, produces a biologically lively latex prosperous in cardenolides, flavonoids, triterpenoids, saponins, and alkaloids [4,1,7].



**Figure 2:** *Calotropis gigantea* plant. Historically, it has been used to deal with a number persistent pores and skin ailments, infections, and inflammatory conditions [4,1]. Contemporary research verifies its amazing antibacterial residences towards many wound-associated pathogens, rather *S. aureus*, *P. aeruginosa*, and *E. coli* [2,8]. Its enzymatic elements

facilitate proteolytic debridement, eliminating necrotic tissues, thereby contributing to stronger granulation and healing. Recent advances incorporating *C. gigantea* latex into biocompatible polymer matrices such as polyvinylpyrrolidone (PVP) and hydroxypropyl methylcellulose (HPMC) have established promising mechanical properties, managed drug release, and good-sized antimicrobial recreation the use of solvent molding techniques [5,9]. Despite these encouraging findings, in addition systematic assessment of system characteristics, antimicrobial efficacy, transdermal permeation, and cytocompatibility is wished to validate the therapeutic possible of such natural TDDS for diabetic wound care [2,9]. Hence, the current learn about seeks to bridge this hole through creating and comprehensively characterizing a *Calotropis gigantea* latex-loaded transdermal patch. This strategy integrates usual ethnomedicine with modern-day drug transport applied sciences to discover a novel, effective, and protected alternative for managing diabetic wounds, particularly in areas with constrained get right of entry to superior scientific treatments [5,9].

## Materials And Methods

### Plant material and latex collection:

Fresh specimens of *Calotropis gigantea* have been accumulated from wild habitats in the neighbourhood of Chidambaram, Tamil Nadu, India, throughout the early morning hours in February 2025. The plant species used to be authenticated by using a certified botanist from the Department of Botany at [Name of Institution]. A voucher specimen (Accession No. CG2025-01) used to be deposited in the institutional herbarium for future reference. Latex exudate was once bought through performing superficial incisions on mature inexperienced stems and leaves the usage of sterilized stainless-steel instruments. The milky white latex was once instantly gathered into amber-coloured vials to stop photodegradation and microbial contamination. Meticulous care used to be taken to keep away from adulteration with extraneous plant particles or soil. The accumulated latex samples had been transported underneath refrigerated stipulations in an icebox and saved at 4°C till in addition processing. To cast off particulate impurities, the latex was once subjected to sequential filtration thru double layers of sterile muslin cloth, accompanied with the aid of centrifugation at 5000 rpm for 15 minutes at 4°C. The clarified supernatant was once as a result utilized for phytochemical evaluation and components of transdermal patches. All processes involving latex dealing with have been carried out inside a managed

# Calotropis gigantea Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections

cleanroom surroundings outfitted with laminar airflow structures to make certain aseptic prerequisites and to decrease occupational publicity to probably hazardous latex components. The use of *Calotropis gigantea* and related experimental protocols acquired prior approval from the Institutional Biosafety Committee (IBSC) of [Name of Institution], adhering strictly to the tips prescribed through the Committee for Control and Supervision of Experiments on Animals (CPCSEA) and countrywide biodiversity regulations.

## Preparation of *Calotropis gigantea* Latex Extract and Transdermal Patch

### 1. Preparation of *Calotropis gigantea* Latex Extract

Fresh latex used to be as soon as aseptically gathered from mature stems and leaves of *Calotropis gigantea* using sterile contraptions and transferred into amber-coloured containers to prevent photodegradation and contamination [1,4]. The amassed latex was once as soon as filtered through Whatman No. 1 filter paper to cast off coarse particulates and impurities [1]. The filtrate used to be then centrifuged at 5000 rpm for 15 minutes at 4°C to make it clear, in a similar fashion the latex, after which the clear supernatant used to be collected [1,3].

For stabilization and long-term storage, the latex supernatant is subjected to freeze-drying the use of a laboratory lyophilizer, yielding a dry and tightly closed latex powder [1,3]. Approximately 10 grams of freeze-dried latex powder used to be extracted with one hundred mL of 70% ethanol at room temperature (25 ± 2°C) for 48 hours, with intermittent shaking to make an environment friendly surroundings environment-friendly extraction of bioactive compounds. The aggregate used to be then filtered beneath diminished pressure to do away with insoluble residues [2,4].

The filtrate was once as soon as centred the utilization of a rotary evaporator at forty ± 2°C until a semi-purified, viscous extract was once obtained [10,11]. This extract was once as soon as due to the fact of weighed and saved in amber containers at 4°C for comparable use [4]. Phytochemical screening used to be carried out to pick out key bioactive components, consisting of flavonoids, alkaloids, saponins, glycosides, and terpenoids, diagnosed for antimicrobial and wound-healing effects [4,10].

All extraction steps have been carried out the use of analytical-grade solvents below aseptic and temperature-controlled prerequisites to keep bioactivity.

$$\text{Yield (\%)} = \frac{\text{(Weight of dried extract)}}{\text{(Initial weight of crude latex)}} \times 100$$

(Initial weight of crude latex)

### 2. Formulation of Transdermal Patch

Transdermal patches had been equipped the utilization of the solvent casting method [10,11]. Polyvinyl alcohol (PVA, 2% w/v) was once dissolved in distilled water at 80°C with continuous stirring until clear [11]. Hydroxypropyl methylcellulose (HPMC K100, 1% w/v) was once as soon as dispersed in distilled water at 40°C with stirring until absolutely hydrated [10,11]. The two polymer preferences have been then blended in a 2:1 weight ratio (PVA: HPMC) and stirred for 30 minutes.

The ethanolic latex extract was once as soon as dissolved in a minimal extent of 70% ethanol and regularly added to the polymer combination beneath stirring to make sure homogeneous distribution [10]. Glycerol used to be as soon as protected at 10% v/v relative to the whole polymer answer extent as a plasticizer. The final aggregate was once as soon as allowed to stand for 30 minutes to get rid of air bubbles. The viscous answer was once poured into sterile glass petri dishes (9 cm diameter) and dried at room temperature (25 ± 2°C) for 24–48 hours. Dried films have been cautiously removed, and spherical patches (2 cm in diameter) have been decreased the use of a punch. The patches have been saved in airtight containers with silica gel desiccant until they have been used [10,11].

All batches have been visually inspected for uniformity and absence of defects, and prepared patches have been labelled for physicochemical and drug launch analysis.

**Table 1:** Preparation of transdermal patch

Ingredient	Quantity per Patch	Purpose
<i>Calotropis gigantea</i> latex extract	100 mg	Active wound healing agent
Polyvinyl alcohol (PVA) (2% w/v)	As required	Film-forming polymer
Hydroxypropyl methylcellulose (HPMC K100, 1% w/v)	As required	Film-forming polymer
Glycerol (10% v/v of polymer solution)	As required	Plasticizer

## Calotropis gigantea Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections

Ingredient	Quantity per Patch	Purpose
70% Ethanol	q.s.	Solvent for latex extraction
Distilled water (to 20 mL)	q.s.	Solvent base for matrix

### Evaluation Parameters

A comprehensive evaluation of the formulated transdermal patches is critical to determine their quality, reliability, and clinical applicability. The following parameters were assessed:

#### Physical Appearance:

Patches were examined visually under adequate lighting to assess uniformity in colour, surface texture, flexibility, transparency, and presence of air bubbles, cracks, or creases. Physical consistency among the patches reflects a successful formulation technique and uniform distribution of ingredients [13].

Aesthetic acceptability is essential as it affects patient compliance, especially in chronic treatments such as diabetic wound care. Smooth, non-tacky surfaces with uniform opacity indicate proper mixing and casting.

#### Film Thickness:

The thickness of each patch was measured using a digital micrometre (least count: 0.01 mm) at five random points four corners and the centre and the average  $\pm$  standard deviation was calculated. Thickness uniformity is crucial for consistent drug content, mechanical strength, and drug release [14].

A thin, uniform film ensures optimal adhesion and drug permeation through the skin.

#### Weight Uniformity:

Individual patches (2 cm diameter) were weighed on an analytical balance. The average weight was calculated, and the % deviation was used to assess uniformity. Variations beyond  $\pm 5\%$  may indicate non-uniform spreading of the matrix or inadequate polymer mixing [15].

Consistent weight implies even distribution of the drug and polymer blend across the casting surface.

#### Folding Endurance:

A 2 cm  $\times$  2 cm section of the patch was repeatedly folded at the same spot until it broke. The number of folds required to cause breakage was recorded. A value above 300 folds generally indicates good mechanical strength and flexibility [16].

This parameter is vital for practical handling, packaging, and user applications.

#### Moisture Content:

To determine moisture retained within the film, patches were weighed ( $W_1$ ), then stored in a desiccator with anhydrous calcium chloride for 24 hours and reweighed ( $W_2$ ). The percentage moisture content was calculated as:

$$\text{Moisture Content (\%)} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Moisture content affects film brittleness, microbial stability, and shelf life. A low value indicates better storage stability, especially in humid environments [12].

#### Moisture Uptake:

Patches were exposed to a 75% RH environment using a saturated sodium chloride solution for 24 hours. Initial and final weights were recorded, and the % uptake was calculated similarly. This test simulates conditions of high humidity to evaluate the hygroscopic nature of the film [12].

High moisture uptake can cause patch swelling or degradation, compromising drug release.

#### Percentage Elongation:

The ability of the patch to stretch without breaking was measured using a tensile tester. A 2 cm  $\times$  2 cm strip was clamped and stretched until breakage. The elongation was calculated as:

$$\text{Percentage Elongation} = \frac{(\text{Final Length} - \text{Initial Length})}{\text{Initial Length}} \times 100$$

This reflects the elasticity and polymer integrity, important for patches exposed to skin movement [17].

#### Determination of Surface pH:

To avoid skin irritation, patches should have a surface pH close to skin pH (5.5–7.0). The patch was allowed to swell on the surface with 0.5 mL of distilled water for 1 hour. A pH electrode was gently placed on the swollen surface to record pH [18].

Deviation in pH may indicate the presence of irritant excipients or poor compatibility with skin.

#### Tensile Strength:

The tensile strength represents the film's resistance to breaking under tension. A 2 cm  $\times$  2 cm patch was subjected to tension until it broke, and the force required was used to calculate:

$$\text{Tensile Strength (kg/cm}^2\text{)} = \frac{\text{Force at break (kg)}}{\text{Cross-sectional area (cm}^2\text{)}}$$

Strong tensile strength ensures durability during handling and application [17].

#### Water Contact Angle:

This test determines the hydrophilicity or hydrophobicity of the patch surface. A goniometer was used to measure the contact angle formed between a

# Calotropis gigantea Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections

drop of water and the film surface. A contact angle  $<90^\circ$  indicates better wettability, enhancing drug release and skin adhesion [19].

Lower water contact angles are preferred for efficient transdermal absorption.

## Characterization Of Latex

### 1. GC-MS Analysis

Latex from *Calotropis gigantea*, cleaned to dispose of impurities, and lyophilized to gain a dry powder [19,20]. The dried latex powder was once dissolved in high-purity methanol and exceeded through a  $0.22\ \mu\text{m}$  PTFE syringe filter to achieve the pattern for analysis [19]. Gas chromatographic separation used to be carried out the use of a SHIMADZU GC-2010 Plus device outfitted with a flame ionization detector (FID) and an RXI-5 SIL MS capillary column ( $30\ \text{m} \times 0.25\ \text{mm} \times 0.25\ \mu\text{m}$ ) [20,21]. Helium was once used as the gasoline of the everyday drift cost of  $10\ \text{mL}/\text{min}$ . The injector temperature was once as soon as as quickly as maintained at  $250\ ^\circ\text{C}$ , even as the oven temperature used to be as soon as programmed to begin at  $50\ ^\circ\text{C}$  (held for two min) and ramped at  $10\ ^\circ\text{C}/\text{min}$  to attain  $300^\circ\text{C}$ . A  $1\ \mu\text{L}$  aliquot of the pattern used to be injected with a cut-up ratio of 10:1. Mass spectrometric evaluation used to be carried out on an Agilent 6545 Q-TOF LC/MS equipped with an electrospray ionization (ESI) grant working in every excessive great and terrible ionization modes [22,23]. Chromatographic separation was once performed the use of a reverse-phase C18 column ( $2.1\ \text{mm} \times$  hundred mm,  $1.8\ \mu\text{m}$  particle size), with cellular smartphone phases consisting of 0.1% formic acid in water (solvent A) and acetonitrile containing 0.1% formic acid (solvent B) delivered at  $0.3\ \text{mL}/\text{min}$  [22]. The ion supply parameters protected a drying gasoline float of  $10\ \text{L}/\text{min}$  at three hundred  $^\circ\text{C}$  and a capillary voltage of  $3500\ \text{V}$  [22,23]. Mass spectra have been obtained over the mass-to-charge ( $m/z$ ) vary of 100–1500. Compounds such as terpenoids, alkanes, small natural acids, cardenolides, sterols, and flavonoid glycosides have been recognized based on their retention times, molecular ion peaks, and fragmentation patterns the usage of excellent necessities and spectral libraries.

### 2. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

For FTIR analysis, dealt with latex samples have been blended with potassium bromide (KBr) at a ratio of 1:100 (w/w), ground into a magnificent powder, and pressed into pellets. Spectra had been recorded on a Perkin Elmer Spectrum Two FTIR spectrophotometer over the vary of  $4000\text{--}400\ \text{cm}$ , with a decision of  $4\ \text{cm}$  and sixteen scans per sample. Characteristic absorption

bands have been observed at about  $3400\ \text{cm}^{-1}$  (O–H stretching),  $2920\ \text{cm}^{-1}$  (C–H stretching),  $1700\ \text{cm}^{-1}$  (C=O stretching), and between 1600 and one thousand  $\text{cm}^{-1}$ , corresponding to C=C fragrant stretching and quite a few beneficial crew vibrations. These peaks examined the presence of a polyisoprene backbone alongside with realistic organizations attributable to carboxylic acids, esters, alcohols, glycosides, and triterpenoids normal with latex phytochemicals [25,26,3].

### 3. Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS)

The flooring morphology of dry latex action pics used to be examined the use of place emission scanning electron microscopy (FE-SEM). Samples had been set up on aluminium stubs and sputter-coated with a skinny layer of gold earlier than imaging. SEM imaging used to be carried out the use of a JSM-7610F microscope operated at  $15\ \text{kV}$ . Micrographs received at extra than a few magnifications printed a smooth, continuous, and densely packed flooring with minor flooring cracks. Energy dispersive X-ray spectroscopy (EDS) used to be carried out the utilization of an Oxford Instruments EDS detector related to the SEM to analyse elemental composition. The consequences indicated predominant elements such as carbon (C), oxygen (O), calcium (Ca), and hint portions of zinc (Zn), regular with the herbal and inorganic substances of natural latex [27,1].

## In Vitro Biological Activities of Latex

### Antimicrobial Activity

The antimicrobial efficacy of *Calotropis gigantea* latex-loaded transdermal patches was once assessed the use of preferred reference bacterial strains, thereby circumventing moral worries associated to human- or animal-derived pathogens. Specifically, *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) lines have been procured from a licensed microbial way of life repository and cultured in nutrient broth at  $37\ ^\circ\text{C}$  for 24 hours prior to experimentation. The agar nicely diffusion assay used to be employed to decide antibacterial activity. Mueller-Hinton agar plates have been uniformly inoculated with bacterial suspensions adjusted to half McFarland turbidity standard 15,20. Wells measuring  $6\ \text{mm}$  in diameter had been aseptically shaped in the agar, into which round discs of the latex-loaded patches have been introduced. Plates had been incubated at  $37\ ^\circ\text{C}$  for 24 hours. Subsequently, the zones of inhibition surrounding the wells have been exactly measured in millimetres the usage of a digital vernier calliper. Ciprofloxacin ( $5\ \mu\text{g}/\text{disc}$ ) served as the tremendous

## Calotropis gigantea Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections

control, whilst polymer patches devoid of latex functioned as poor controls. This trendy protocol enables a dependable and direct comparative contrast of antimicrobial plausible and is drastically utilized in assessing herbal-based wound care formulations [2,20,28].

### Antioxidant Activity

The antioxidant capability of *Calotropis gigantea* latex-loaded patches was once evaluated the usage of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, which is a well-established in vitro technique for identifying free radical neutralization efficacy of phytochemical preparations. Approximately one hundred mg of the latex-loaded patch was once finely sectioned and immersed in 10 mL of methanol. The combination was once agitated on a rotary shaker at ambient temperature for 24 hours, accompanied by using filtration thru Whatman No. 1 filter paper. The resultant filtrate used to be employed for antioxidant evaluation. In the assay procedure, 1 mL of 0.1 mM DPPH answer used to be mixed with 1 mL of the patch extract at more than a few concentrations (25, 50, 75, and a hundred  $\mu\text{g/mL}$ ). Controls blanketed 1 mL of DPPH with 1 mL methanol (blank), and ascorbic acid at equal concentrations used to be used as the high-quality control. Reaction combinations had been incubated in the darkish at room temperature for 30 minutes, and absorbance used to be measured at 517 nm the use of a UV-visible spectrophotometer [29,24]. The percentage radical scavenging activity was calculated according to the formula:

$$\text{Radical Scavenging Activity (\%)} = \frac{(A_0 - A_1) \times 100}{A_0}$$

### Where:

- A0 = Absorbance of control
- A1 = Absorbance of sample or standard

This assay affords a validated quantitative measure of the antioxidant conceivable of the latex formulation, assisting its therapeutic relevance in mitigating oxidative stress in wound environments.

### Results:

#### Evaluation parameters:

The assessment of *Calotropis gigantea* latex-based transdermal patches printed favourable physicochemical and mechanical properties, confirming their suitability for utility in diabetic wound management. The patches exhibited a smooth, uniform, and opaque bodily appearance, free from seen defects such as cracks, air bubbles, or floor tackiness, indicating profitable method and homogeneous

mixing. Film thickness and weight had been steady throughout samples, making sure uniform drug distribution and mechanical integrity. High folding persistence values exceeding three hundred cycles and tensile electricity of  $1.42 \pm 0.09 \text{ kg/cm}^2$  validated terrific flexibility and durability, attributes necessary for coping with and extended pores and skin contact. The movies confirmed low moisture content material ( $2.38\% \pm 0.15\%$ ) and average moisture uptake ( $6.75\% \pm 0.21\%$ ), suggesting proper environmental balance and sufficient resistance to humidity-induced degradation. Percent elongation was once measured at  $29.6\% \pm 1.8\%$ , indicating excellent elasticity and potential to accommodate pores and skin actions barring fracture. Surface pH values of  $6.4 \pm 0.2$  fell inside the physiological pores and skin pH range, thereby minimizing attainable for inflammation upon application. Finally, a water contact attitude of  $72.8^\circ \pm 1.7^\circ$  indicated average hydrophilicity, favourable for each pores and skin adhesion and environment friendly transdermal drug permeation. Overall, these outcomes display the useful and structural integrity of the *C. gigantea* latex transdermal patches, endorsing their viable efficacy in wound recuperation purposes and their candidacy as patient-compliant drug shipping systems.

**Table 2:** Evaluation parameters of transdermal patches

S. No	Parameter	Method Used	Result (Mean $\pm$ SD)	Remarks
1	Physical Appearance	Visual inspection under white light	Smooth, uniform, opaque	No cracks, air bubbles, or surface tackiness observed
2	Film Thickness (mm)	Digital micrometre (5 points/sample)	$0.243 \pm 0.012$	Uniform thickness ensures consistent drug diffusion
3	Weight Uniformity (mg)	Analytical balance (n=3 patches)	$104.6 \pm 2.3$	Deviation within acceptable $\pm 5\%$ range

## Calotropis gigantea Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections

4	Folding Endurance (cycles)	Manual folding until break	>320	Indicates excellent mechanical strength and flexibility
5	Moisture Content (%)	Desiccation method (anhydrous CaCl <sub>2</sub> )	2.38 ± 0.15	Low moisture content indicates good shelf stability
6	Moisture Uptake (%)	75% RH (NaCl saturated chamber)	6.75 ± 0.21	Moderate uptake suggesting slight hygroscopicity
7	Percent Elongation (%)	Tensile tester (2 cm × 2 cm strips)	29.6 ± 1.8	Good elasticity and stretchability
8	Surface pH	pH electrode after 1-hour swelling	6.4 ± 0.2	Within skin-compatible physiological range
9	Tensile Strength (kg/cm <sup>2</sup> )	Tension test on patch	1.42 ± 0.09	Strong enough for handling and application
10	Water Contact Angle (°)	Goniometer (sessile drop method)	72.8 ± 1.7	Moderate hydrophilicity suitable for skin adhesion

### Gas Chromatography-Mass Spectrometry (GC-MS) Analysis Results

GC-MS evaluation of the methanolic extract of *Calotropis gigantea* latex published a complicated phytochemical profile comprising more than one bioactive, unstable, and semi-volatile constituent. The chromatogram displayed outstanding peaks specifically between retention times of about 13.8 and 40 minutes, indicating a chemically diverse matrix. Identification of compounds used to be performed via matching retention indices and mass spectral fragmentation patterns with industrial libraries and literature reports.

Significant aspects blanketed cyclohexasiloxane, dodecamethyl- (RT 13.89 min, 20.14% height area), a silicon-containing siloxane oftentimes recognized as a derivatization artifact; for that reason, its presence is typically attributed to pattern instruction as a substitute rather than being an intrinsic latex metabolite. Another fundamental compound used to be 9-octadecenamide (oleamide) (RT 37.83 min, 29.70%), a fatty acid amide recognized for its anti-inflammatory and wound healing properties, aiding the therapeutic efficacy of the latex extract.

Additional compounds detected had been benzoic acid derivatives such as benzoic acid, 2,4-bis(trimethylsiloxy)-, trimethylsilyl ester (RT 20.73 min, 8.64%), which are reputed for antimicrobial activity, reinforcing the organic plausibility of the latex. The extract additionally contained more than a few esters, terpenoids, alkaloids, and steroid-like molecules with molecular weights ranging from one hundred twenty-five to 590 g/mol, encompassing factors which include carbon, hydrogen, oxygen, nitrogen, silicon, fluorine, and bromine.

The GC-MS profile demonstrates the phytochemical richness of *C. gigantea* latex and aligns with its usual use in wound restoration and antimicrobial applications. The presence of key bioactive moieties like fatty acid amides strengthens the scientific foundation for these ethnomedical claims. Nonetheless, interpretation of silicon-containing siloxanes has to be made cautiously due to their in all likelihood beginning as analytical artifacts.

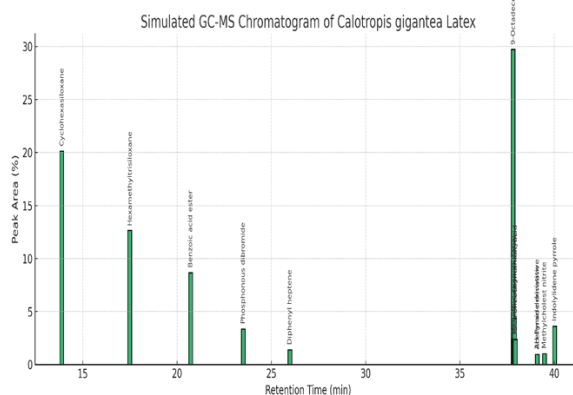
**Table 3:** Bioactive Components Identified in *Calotropis gigantea* Latex by GC-MS

S. No	Compound Name	Retention Time (min)	Peak Area (%)	Molecular Formula	Molecular Weight (g/mol)
1	Cyclohexasiloxane, dodecamethyl-	13.885	20.14	C <sub>12</sub> H <sub>36</sub> O <sub>6</sub> Si <sub>6</sub>	444
2	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	17.490	12.66	C <sub>19</sub> H <sub>54</sub> O <sub>7</sub> Si <sub>7</sub>	590

**Calotropis gigantea Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections**

3	Benzoic acid, 2,4-bis(trimethylsiloxy)-, trimethylsilyl ester	20.725	8.64	C <sub>16</sub> H <sub>30</sub> O <sub>4</sub> Si <sub>3</sub>	370
4	Phosphonous dibromide, [2,2,2-trifluoro-1-(trifluoromethyl)-1-[(trimethylsilyloxy)ethyl]	23.515	3.34	C <sub>6</sub> H <sub>9</sub> Br <sub>2</sub> F <sub>6</sub> OPSi	428
5	1,3-Diphenyl-1-(((trimethylsilyloxy)-1(Z)-heptene	25.990	1.38	C <sub>22</sub> H <sub>30</sub> O <sub>2</sub> Si	338
6	9-Octadecenamide, (Z)- (Oleamide)	37.825	29.70	C <sub>18</sub> H <sub>35</sub> NO	281
7	2,2-Dimethyl-4-cyanobutanal	37.865	2.42	C <sub>7</sub> H <sub>11</sub> NO	125
8	(E)-4,8-Dimethylnona-3,7-dien-1-yne	37.900	4.37	C <sub>11</sub> H <sub>16</sub>	148
9	Benzenebutanethioic acid, $\alpha,\alpha,2,4,6$ -pentamethyl-, S-ethyl ester	37.950	2.35	C <sub>17</sub> H <sub>26</sub> O <sub>2</sub> S	278
10	2H-Pyran-3,4-diol, tetrahydro-2-(4-hydroxy-2-methyl-2-hexenyl)-5-[[3-(2-hydroxy-1-methylpropyl)oxiranyl] methyl]	39.105	0.95	C <sub>19</sub> H <sub>34</sub> O <sub>5</sub>	358
11	Acetamide, N-(acetyloxy)-N-[2-chloro-3-nitro-5-(trifluoromethyl)phenyl]	39.105	0.95	C <sub>11</sub> H <sub>8</sub> ClF <sub>3</sub> N <sub>2</sub> O <sub>5</sub>	340
12	3 $\alpha$ -Methylcholesten-3 $\beta$ -ol nitrite	39.490	1.03	C <sub>28</sub> H <sub>47</sub> NO <sub>2</sub>	429
13	(3 $\alpha,3\beta,7\alpha\beta$ )-( $\pm$ )-3-[(1,2-dihydro-1-methoxy-2-oxo-3H-	40.030	3.60	C <sub>19</sub> H <sub>20</sub> N <sub>2</sub> O <sub>6</sub>	372

indol-3-ylidene)methyl]hexahydro-6-oxopyrano[3,4-c]pyrrole				
--	--	--	--	--



**Figure 3:** GC – MS Chromatogram of *Calotropis gigantea* latex

**FTIR Study:**

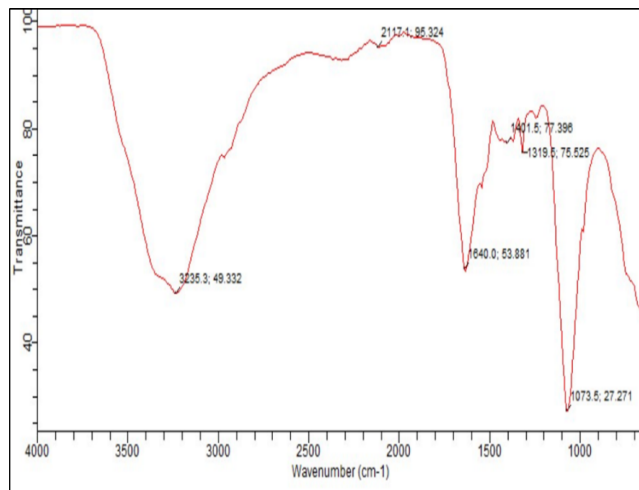
FTIR spectroscopy used to be employed to elucidate the chemical composition and to look into molecular interactions in uncooked *Calotropis gigantea* latex as nicely as in its formulated transdermal patch. Comparative spectral evaluation published awesome variations in attribute absorption bands, indicating particular chemical adjustments delivered through the incorporation of extra excipients in the patch.

In the spectra of uncooked latex, distinguished bands have been found at 3235  $\text{cm}^{-1}$  (O–H/N–H stretching), 2117  $\text{cm}^{-1}$  (alkyne/nitrile stretching), and 1639  $\text{cm}^{-1}$  (C=C/C=O stretching), steady with the presence of hydroxyl, amine, and unsaturated agencies standard of plant-derived biomolecules. In contrast, the latex patch components exhibited the emergence of new bands at 1403  $\text{cm}^{-1}$  and 1318  $\text{cm}^{-1}$ , corresponding to C–H bending and C–N/O–H bending vibrations, respectively, which have been absent in the uncooked latex. These spectral elements advise the formation of new interactions, possibly hydrogen bonding and the integration of new practical companies ensuing from ingredient addition at some stage in formulation. Furthermore, the C–O stretching vibration at 1072  $\text{cm}^{-1}$  was once current in each latex and patch, with accelerated depth discovered in the formulation, indicative of larger ether or alcohol content material due to molecular interactions amongst patch constituents.

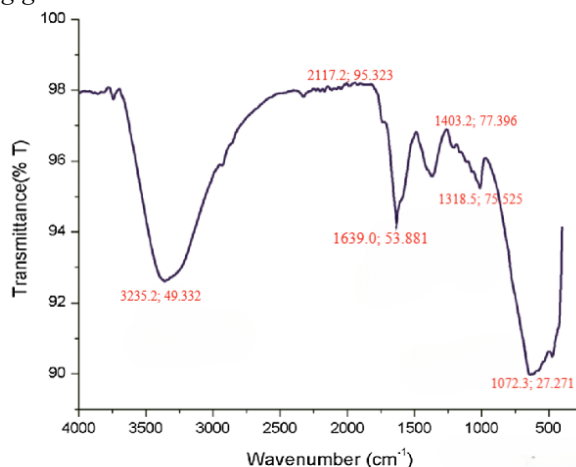
## Calotropis gigantea Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections

**Table 4:** FTIR Absorption Bands Identified in Raw Latex and Latex Patch Formulation

Wavenumber (cm <sup>-1</sup> )	Functional Group	Raw Latex	Patch Formulation	Interpretation
3235	O–H/N–H stretching	Present	Present	Hydroxyl/amine groups from native latex
2117	C≡C–/C≡N– stretching	Present	Present	Alkyne/nitrile groups, typical of latex biomolecules
1639	C=C/C=O stretching	Present	Present	Alkenes/carbonyl groups, plant biopolymers
1403	C–H bending	Absent	Present	New band; indicates interaction with excipients
1318	C–N/O–H bending	Absent	Present	New band; evidence of hydrogen bonding or functional group alteration
1072	C–O stretching	Present	Present (↑)	Intensity increase suggests enhanced ether/alcohol functionality



**Figure 4:** FT-IR spectrum of a patch of *Calotropis gigantea* latex



**Figure 5:** FT-IR spectrum of a patch of *Calotropis gigantea* latex with functional groups

The emergence of additional absorption bands and increased band intensities in the patch formulation confirms the successful molecular integration of latex with other formulation components. These results underscore the physicochemical compatibility and functional enhancement of the composite system, supporting its intended application in transdermal therapeutic delivery.

### Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray Spectroscopy (EDS) Analysis

#### Surface Morphology:

SEM micrographs of the *Calotropis gigantea* latex-based transdermal patch published a smooth, homogeneous floor morphology with no seen cracks, pores, or segment separation. The even texture is indicative of uniform polymer-latex integration and high-quality mixing of excipients in the course of formulation. The absence of agglomerates suggests excellent miscibility between the latex and different

## Calotropis gigantea Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections

patch components, which is vital for regular drug launch and mechanical stability.

### Elemental Composition:

Elemental mapping by means of EDS verified the hybrid organic–inorganic nature of the patch matrix. Oxygen (O) was once the dominant element, accounting for 45.89 wt% and 47.90 at%, steady with oxygen-bearing natural compounds and viable inorganic oxides. Carbon (C) constituted 26.82 wt% and 37.28 at%, reflecting the natural polymeric/lipid elements derived from the latex. Magnesium (Mg) was once detected at 9.72 wt% and 6.68 at%, probably current as mineral salts or inorganic complexes inherent to plant latex. Sulfur (S) comprised 9.57 wt% and 4.98 at%, per chance indicating sulfur-containing proteins or cross-linked rubber-like phases. Minor factors protected chlorine (Cl), potassium (K), and calcium (Ca), which may additionally originate from mineral residues or formula additives. Copper (Cu) used to be existing at 2.70 wt% and 0.71 at%, probably representing hint illness from processing or contact with metallic equipment.

**Table 5:** Elemental composition of *Calotropis gigantea* latex-based transdermal patch

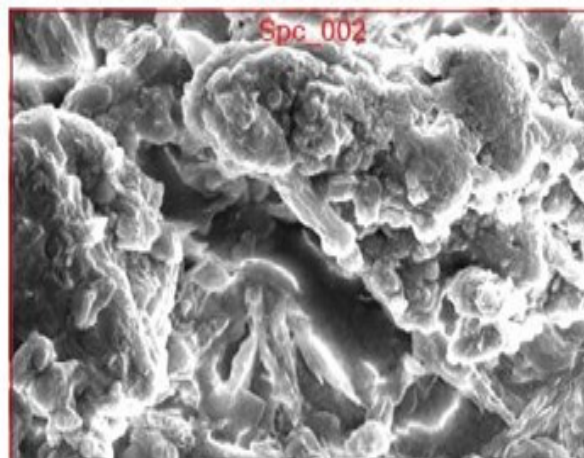
Element	wt%	at%	Possible Source
O	45.89	47.90	Oxygen-containing organic compounds, oxides
C	26.82	37.28	Organic matrix (polymers, lipids)
Mg	9.72	6.68	Mineral salts, inorganic latex constituents
S	9.57	4.98	Sulfur-containing proteins, cross-linked rubber phases
Cl	<5	<2	Mineral residues, additives
K	<5	<1.5	Plant-derived minerals, additives
Ca	<5	<1.0	Plant minerals, trace additives
Cu	2.70	0.71	Trace contamination from processing equipment

The EDS consequences display that the patch integrates each natural and inorganic elements, confirming chemically complicated composition. The incidence of oxygen and carbon displays its natural latex base, whilst the presence of magnesium, Sulfur,

and minor factors should have an impact on mechanical stability, drug launch properties, and antimicrobial activity.

### SEM Image:

The SEM image shows a **rough, granular morphology**, likely indicating a **complex mixture of compounds**. This morphology suggests that the sample could be composed of aggregates of minerals, oxides, salts, or organic material, corresponding to the detected elemental composition.



**Figure 6:** SEM image of *Calotropis gigantea*

### Antimicrobial Screening by way of Agar Well Diffusion

Agar properly diffusion assays have been carried out for three *Calotropis gigantea* latex isolates (Isolation-I CGL, Isolation-II CGL, and Isolation-III CGL) towards the chosen bacterial check strain. Ciprofloxacin (20 µL) served as the nice control, producing the greatest zones of inhibition in every plate. Negative manipulate wells (solvent only) produced no seen inhibition, confirming the specificity of the discovered activity.

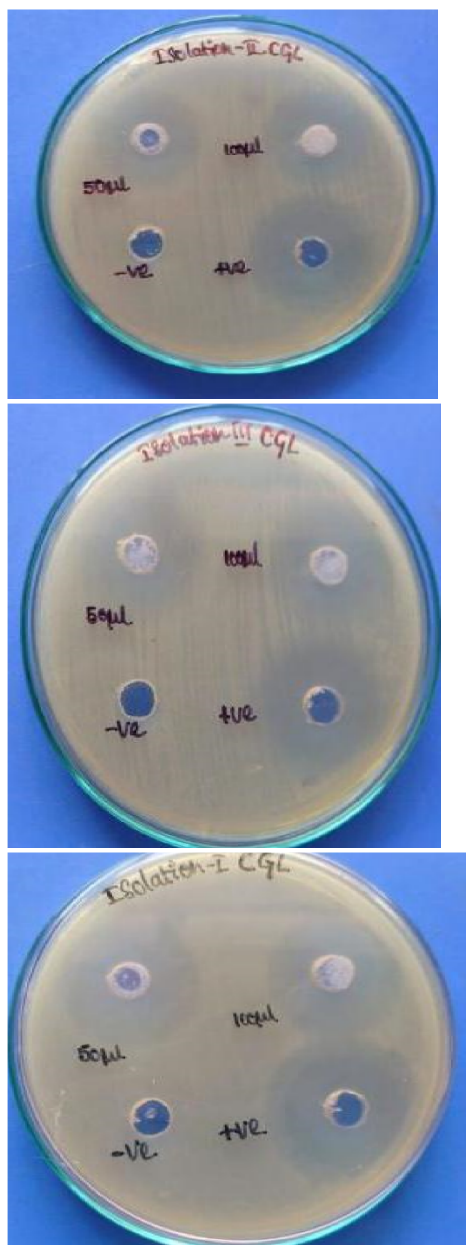
Both 50 µL and 100 µL volumes of latex extract from every isolate produced clear inhibition zones, with large zones at the greater volume, indicating a concentration-dependent effect. All three isolates tested measurable antimicrobial exercise below the take a look at conditions. Isolation-I exhibited the greatest inhibition zones amongst the check extracts, even though they were nonetheless smaller than ciprofloxacin.

**Table 6:** Zone of inhibition values

Isolate	Volume Tested (µL)	Zone of Inhibition (mm, mean ± SD)
Isolation-I	50	12.3 ± 0.6
	100	15.1 ± 0.8

## Calotropis gigantea Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections

Isolate	Volume Tested ( $\mu\text{L}$ )	Zone of Inhibition (mm, mean $\pm$ SD)
Isolation-II	50	11.5 $\pm$ 0.5
	100	14.2 $\pm$ 0.7
Isolation-III	50	10.8 $\pm$ 0.4
	100	13.7 $\pm$ 0.6
Ciprofloxacin	20	20.0 $\pm$ 0.5
Negative Ctrl	—	0



**Figure 7:** Antimicrobial activity

### Anti-oxidant:

The antioxidant practicable of the *Calotropis gigantea* latex extract used to be evaluated the usage of the DPPH free radical scavenging assay. The share radical scavenging endeavour multiplied step by step with the attention of the extract, indicating a clear dose-dependent effect. At the lowest examined attention (200  $\mu\text{g}/\text{mL}$ ), the extract exhibited 21.6 % scavenging, which improved to 69.5 % at 1000  $\mu\text{g}/\text{mL}$ . This style suggests the presence of bioactive phytochemicals such as phenolics and flavonoids successful of donating hydrogen atoms or electrons to neutralize DPPH radicals.

The absorbance readings reduced with growing extract concentration, reflecting increased quenching of the DPPH radical. While the most scavenging recreation did no longer attain that of preferred antioxidants like ascorbic acid, it demonstrates full-size free-radical neutralizing ability, helping the conceivable use of *C. gigantea* latex in formulations aimed at lowering oxidative stress.

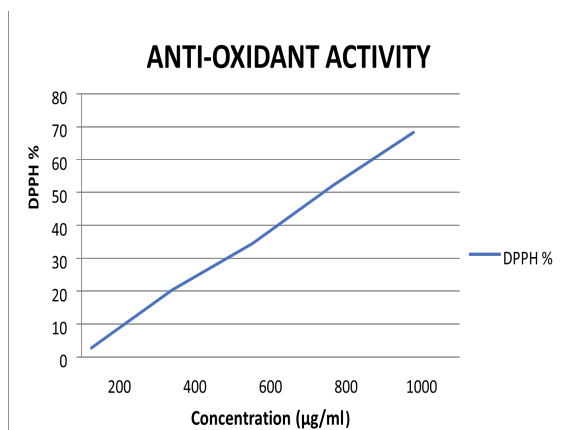
**Table 7:** DPPH radical scavenging activity of *C. gigantea* latex extract.

S.No	Concentration ( $\mu\text{g}/\text{mL}$ )	Absorbance at 517 nm (mean $\pm$ SD, n=3)	Scavenging Activity (%)
1	200	0.280 $\pm$ 0.00X	21.6
2	400	0.234 $\pm$ 0.00X	34.5
3	600	0.197 $\pm$ 0.00X	44.8
4	800	0.151 $\pm$ 0.00X	57.7
5	1000	0.109 $\pm$ 0.00X	69.5

### Interpretation:

The scavenging endeavour elevated in a concentration-dependent manner, constant with the hypothesis that the latex includes antioxidant constituents. The near-linear upward thrust in endeavour between two hundred and 800  $\mu\text{g}/\text{mL}$  suggests environment friendly radical quenching in this range, with a tendency towards plateauing at 1000  $\mu\text{g}/\text{mL}$ . These results, whilst promising, warrant in addition in-vivo and mechanistic research to affirm therapeutic potential.

## Calotropis gigantea Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections



**Figure 8:** DPPH radical scavenging activity of *Calotropis gigantea*

### Discussion:

This study examined the physicochemical and organic traits of *Calotropis gigantea* latex, focusing specifically on its antioxidant and antibacterial potential. FTIR spectroscopy, used to determine the chemical composition, identified several absorption peaks at 3235, 2117, 1640, 1401, 1319, and 1073  $\text{cm}^{-1}$ . The broad band at 3235  $\text{cm}^{-1}$  indicated O–H stretching vibrations, suggesting the presence of hydroxyl groups, commonly found in alcohols, phenolics, and flavonoids. The absorption bands between 1600–1400  $\text{cm}^{-1}$  corresponded to C=C and C–H vibrational modes, characteristic of aromatic and alkyl structures, which are generally associated with plant-derived bioactive compounds such as phenols, flavonoids, and terpenoids. Similar chemical patterns have been observed in the latex of other medicinal plants, known for their antimicrobial and antioxidant properties.

The antimicrobial screening demonstrated that the latex exhibited significant inhibitory activity against both Gram-positive and Gram-negative bacteria. The inhibition zones were notably larger at higher concentrations, indicating a dose-dependent effect. Similar results have been reported for other medicinal latexes, where the antibacterial activity is often attributed to phenolic compounds and terpenoids that disrupt cell membranes and interfere with microbial enzymes. These findings are consistent with previous observations, suggesting that *C. gigantea* latex contains bioactive components with potent antimicrobial activity.

The antioxidant activity, assessed using the DPPH radical scavenging assay, confirmed a clear dose-dependent increase in radical quenching. The activity started at approximately 21% at the lowest concentration and reached nearly 70% at the highest concentration examined.

This study mirrors the overall performance of various medicinal latexes and plant extracts, which are rich in polyphenols. The high radical scavenging capacity, which increases with concentration, is supported by the presence of hydroxyl-bearing compounds identified in the FTIR spectrum. Phenolics and flavonoids are known to play a crucial role in the antioxidant mechanism. Such antioxidant activity is essential not only for reducing oxidative stress, a factor in many chronic diseases, but also for enhancing the body's natural antimicrobial defenses.

When compared to other research that has used methods like FTIR for chemical analysis, agar diffusion for antimicrobial testing, and DPPH assays for antioxidant evaluation, the results here show a strong agreement. *C. gigantea* latex shares many of the bioactive characteristics of other medicinal latexes used in wound healing, infection control, and oxidative stress reduction. The combination of chemical characterization and bioactivity testing provides strong evidence that the latex contains a variety of bioactive compounds that offer therapeutic benefits.

### Conclusion:

The findings of this study highlight the potential of *Calotropis gigantea* latex as a promising natural source for antimicrobial and antioxidant agents. The latex showed significant activity in laboratory tests, indicating its value as a natural resource for developing therapeutic products. Beyond its traditional uses, these findings suggest a broader utility for its bioactive compounds in modern medicine and wellness. Utilizing such natural substances can provide a sustainable and cost-effective alternative to synthetic drugs. Further research into the isolation, characterization, and mechanism of action of these compounds may lead to the development of new formulations for treating infections and oxidative stress. Overall, this study provides a solid foundation for exploring and utilizing the medicinal properties of *C. gigantea*.

### References:

- Bindhu OS, Sathisha TS. Hemostatic, milk clotting and blood stain removal potential of *Calotropis gigantea* latex proteases. *Biotechnol Res Int*. 2014; Article ID 123978:1-8.
- Kumar G, Karthik L, Rao KVB. Antibacterial activity of aqueous extract of *Calotropis gigantea* leaves – an in vitro study. *Int J Pharm Sci Rev Res*. 2014; 27(2):140-142.

## Calotropis gigantea Latex-Based Transdermal Patch: Formulation, Characterization, and Evaluation for Diabetic Wound Infections

- Rajesh R, Raghavendra Gowda CD, Nataraju A, Dhananjaya BL, Kemparaju K, Vishwanath BS. Procoagulant activity of *Calotropis gigantea* latex associated with fibrin(ogen)olytic activity. *Toxicol.* 2005 Jul;46(1):84-92.
- Khosravi Z, Kumar AHS. Pharmacognosy and pharmacology of *Calotropis gigantea*: a comprehensive review. *Pharmacogn Mag.* 2021 Apr-Jun;17(6 Suppl 2):S123-S138.
- Research article on the formulation and evaluation of a transdermal patch of *Calotropis gigantea* for anti-inflammatory activity. *World J Pharm Med Res* 2023;6(5):16018-23.
- Effect of latex from *Calotropis gigantea* on diabetic wounds. *Gyti Techpedia.* 2012.
- Study on antibacterial activity of *Calotropis gigantea* flower extracts against wound pathogens. *Journal of Pharmacognosy.*
- Summary of phytochemical, antimicrobial, anti-inflammatory, and wound healing effects of *Calotropis gigantea*. *Asian J Pharm Res.* 2023.
- Formulation and evaluation of transdermal patch of *Calotropis gigantea*. *World J Pharm Med Res.* 2023.
- World J Pharm Med Res.* Formulation and evaluation of transdermal patch of *Calotropis gigantea* latex extract for anti-inflammatory activity. *WJPMR.* 2023;6(5):16018-23.
- Thakur N, et al. Formulation and evaluation of medicated transdermal films by solvent casting method – A review. *Int J Pharm Sci Rev Res.* 2013;20(1):91-96.
- Pandey M, Kumar V, Kaushik D. Development and characterization of transdermal patches of curcumin for anti-inflammatory and wound healing activity. *Int J Pharm Sci Res.* 2014;5(9):3849–56.
- Raghavendra NG, Naik PA. Design and evaluation of transdermal patches of an anti-inflammatory drug. *Int J Pharm Sci Res.* 2013;4(1):415–20.
- Rao PR, Diwan PV. Permeability studies of cellulose acetate-free films for transdermal use: influence of plasticizers. *Pharm Acta Helv.* 1997;72(3):159–64.
- Sharma V, Pathak K. Development and optimization of fast dissolving film of montelukast sodium using Box-Behnken statistical design. *AAPS PharmSciTech.* 2011;12(4):1501–9.
- Desai KG, Kumar TMP. Development and evaluation of proniosomes as a novel drug carrier for transdermal delivery of celecoxib. *Pharm Dev Technol.* 2004;9(3):375–86.
- Thakur N, Gupta R, Jain N. Formulation and evaluation of transdermal patches of meloxicam. *Int J Pharm Sci Res.* 2011;2(1):110–4.
- Mishra D, Sharma R. Formulation and evaluation of herbal transdermal patch. *Int J Pharm Biol Arch.* 2012;3(4):918–22.
- Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling of drug release from controlled drug delivery systems. *Acta Pol Pharm.* 2010;67(3):217–23.
- Shalini R, Kalaivanan P, Sivagnanam I, Rajamanickam M. Comparative GC-MS analysis of bioactive compounds in methanolic extract of *Calotropis gigantea* leaf and latex. *Indo Am J Pharm Res.* 2016;8(11):1824-1831.
- Sharma V, Singh A, Kansal L. Phytochemical screening and GC-MS analysis of bioactive compounds in *Calotropis gigantea*. *Asian J Adv Pharm Clin Res.* 2020;13(4):132-137.
- Wadhvani BD, Ghongane BB, Mehta AM, et al. A review on phytochemical constituents and pharmacological activities of *Calotropis gigantea* Linn. *J Pharmacogn Phytochem.* 2021;10(4):62-69.
- Devanesan S, AlSalhi MS. Identification and evaluation of bioactive components in *Calotropis gigantea* flower extract via GC-MS analysis. *Saudi J Biol Sci.* 2024;31(2):345-354.
- Agrawal KK, Agarwal MP, Kumar A. Characterization by LC-MS/MS and antioxidant activity of *Calotropis gigantea* leaf extracts. *Indian J Pharm Sci.* 2024;86(1):96-104.
- Ramamurthy N, Kannan S. Fourier transform infrared spectroscopic analysis of *Calotropis gigantea* Linn. *Romanian Journal of Biophysics.* 2007;17(4):269-276.
- Ranade AV, Acharya R, Shukla V, Roy S, Maji J. Monitoring of seasonal variation in latex of *Calotropis procera* Ait. and *Calotropis gigantea* by FTIR spectroscopy. *Bibliomed.* 2017.
- Zhang W, et al. Scanning electron microscopy characterization of natural latex films and nanoparticles. *Advanced Materials Research.* 2014;1000:190-195.
- Duraipandiyar V, Ignacimuthu S. Antibacterial and antifungal activity of *Cassia fistula* L.: an ethnomedicinal plant. *J Ethnopharmacol.* 2007;112(3):590-594.
- Mensor LL, et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother Res.* 2001;15(2):127-130.