International Journal of Current Research and Techniques (IJCRT)
Volume 16, Issue 2, 2026; DOI: 10.5281/ZENODO.20083961
Original Research Article

Phytochemical Screening and Antifungal Evaluation of *Calotropis gigantea* Leaf Extracts It in Topical Cream Formulation

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Manuscript Received: May 05, 2026; Revised: May 07, 2026; Published: May 08, 2026

Abstract: The study focuses on the development and evaluation of a herbal antifungal cream using *Calotropis gigantea* leaf extract. The plant contains bioactive compounds such as flavonoids, tannins, and glycosides, which contribute to antimicrobial, anti-inflammatory, and wound-healing properties. The formulation also includes supportive herbal ingredients like aloe vera, neem, and turmeric to enhance effectiveness. The cream was prepared through systematic processes including extraction, formulation, and in-vitro testing against *Candida albicans*.
The developed formulations showed acceptable physical characteristics, stability, skin safety, and no microbial contamination. Among the formulations, F3 exhibited the highest antifungal activity with a 16 mm zone of inhibition. The study concludes that the herbal cream is a safe, cost-effective, and promising alternative to conventional antifungal treatments with fewer side effects and good patient acceptability.

Keywords: - Calotropis gigantea, Phytochemical screening, Antifungal activity, Herbal cream formulation, Candida albicans, Fungal infections, Topical drug delivery, Bioactive compounds.

1. Introduction

Fungal infections are among the most common health problems affecting millions of people worldwide. These infections range from superficial skin infections to severe systemic conditions, particularly in immunocompromised individuals such as patients with HIV/AIDS, cancer, or those undergoing organ transplantation. Among various fungal pathogens, *Candida albicans* is one of the most prevalent opportunistic organisms responsible for infections of the skin, mucous membranes, and internal organs. The increasing incidence of fungal infections, along with the emergence of drug-resistant strains, has created a significant challenge in modern healthcare systems. (1,2)
Conventional antifungal therapies, including azoles, polyenes, and echinocandins, are widely used to treat fungal infections. However, these drugs often have limitations such as toxicity, adverse side effects, high cost, and the development of resistance with prolonged use. These drawbacks highlight the urgent need for safer, cost-effective, and more efficient alternatives. In recent years, there has been growing interest in the use of herbal medicines due to their natural origin, fewer side effects, and better patient compliance. (3)

Medicinal plants have been used for centuries in traditional systems of medicine such as Ayurveda, Unani, and traditional Chinese medicine. These plants are rich sources of bioactive compounds including flavonoids, alkaloids, tannins, glycosides, and phenolic compounds, which possess various pharmacological activities such as antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties. The use of plant-based formulations has gained popularity as they provide a holistic approach to treatment and are generally considered safe for long-term use. (4,5,6)
Calotropis gigantea, commonly known as giant milkweed or "Arka" in Ayurveda, is a medicinal plant widely distributed in tropical and subtropical regions of India. It belongs to the family Apocynaceae and has been extensively used in traditional medicine for the treatment of various ailments such as skin diseases, wounds, inflammation, and infections. The plant contains a variety of phytochemical constituents such as flavonoids, tannins, glycosides, terpenoids, and

phenolic compounds, which are responsible for its therapeutic activities. Several studies have reported its antimicrobial and antifungal potential, making it a suitable candidate for developing herbal formulations. (7)

In addition to *Calotropis gigantea*, other herbal ingredients such as aloe vera, neem, and turmeric are also known for their medicinal properties. Aloe vera possesses soothing, moisturizing, and wound-healing effects; neem exhibits strong antimicrobial and antifungal activity; while turmeric is well known for its anti-inflammatory and antiseptic properties. The combination of these herbal components can enhance the overall efficacy of the formulation and provide a synergistic effect in treating fungal infections. (6,8,9)

Topical drug delivery systems, such as creams, are widely preferred for the treatment of skin infections because they allow direct application of the drug to the affected area, ensuring localized action, reduced systemic side effects, and improved patient compliance. Cream formulations are easy to apply, non-greasy, and provide better spread ability and absorption of active ingredients into the skin. Herbal creams, in particular, are gaining attention due to their safety, effectiveness, and compatibility with different skin types. (6)

Therefore, the present study aims to formulate and evaluate a herbal antifungal cream containing *Calotropis gigantea* leaf extract. The study involves phytochemical screening, formulation development, and in-vitro antifungal evaluation against *Candida albicans*. The objective is to develop a safe, effective, and economical herbal formulation that can serve as an alternative to conventional antifungal therapies and contribute to the advancement of natural medicine in dermatological applications.

2. Literature review

a. Soriano *et.al.*, (2024): -Provided a comprehensive overview of invasive fungal diseases, highlighting their increasing global burden, particularly among immunocompromised individuals. The study emphasized that fungal infections such as candidiasis, aspergillosis, and mucormycosis have become major contributors to morbidity and mortality. It discussed the challenges in early diagnosis due to non-specific symptoms and limited diagnostic tools. The author also pointed out the growing concern of antifungal resistance, which complicates treatment strategies. Furthermore, the review stressed the importance of awareness, early detection, and improved therapeutic approaches. Soriano concluded that there is a critical need for the development of safer and more effective antifungal agents, including plant-based alternatives, to overcome current limitations in conventional antifungal therapy. (1)

b. **Pophale *et.al.*, (2023): -**Presented a comprehensive review on *Calotropis gigantea*, highlighting its pharmacological and therapeutic properties. The study reported that the plant exhibits a wide range of activities, including antifungal, antibacterial, anti-inflammatory, antioxidant, and wound-healing effects. The authors attributed these properties to the presence of various phytochemicals such as cardiac glycosides, flavonoids, and terpenoids. The review also discussed the traditional uses of the plant in treating skin diseases and infections. Additionally, the study emphasized the importance of *Calotropis gigantea*in modern herbal medicine and its potential for drug development. The authors concluded that the plant is a valuable medicinal resource and warrants further scientific investigation. (10)

c. **Jayavedi *et.al.*, (2023): -** Evaluated the antifungal activity of *Calotropis gigantea*leaf extract using standard in-vitro methods such as agar well diffusion. The study demonstrated significant inhibition of fungal growth, particularly against *Candida albicans*. The results indicated that the plant extract contains bioactive compounds responsible for antifungal activity. The authors suggested that phytochemicals such as flavonoids, tannins, and glycosides may play a key role in disrupting fungal cell structures. The study supported the traditional use of *Calotropis gigantea*in treating skin infections and emphasized its potential as a natural antifungal agent. Overall, the findings provided scientific validation for the use of this plant in herbal formulations and encouraged further research into its therapeutic applications. (3)

d. **Reddy *et.al.*, (2022): -** Discussed the pathogenesis, immune response, and treatment approaches for fungal infections. The authors explained how fungal pathogens evade host immune defences through mechanisms such as biofilm formation and morphological changes. The study highlighted commonly used antifungal drugs, including azoles, polyenes, and echinocandins, along with their mechanisms of action. However, it also addressed major limitations such as toxicity, high cost, and increasing resistance among fungal species. The review emphasized the urgent need for alternative treatment strategies, particularly plant-based compounds with antifungal properties. The authors concluded that natural products could serve as promising candidates for developing safer and more effective antifungal therapies, supporting further research into medicinal plants like *Calotropis gigantea*. (2)

2 Materials and methodology

2.1 Materials

- Neem oil - Antifungal, antibacterial, reduces skin infections
- Calotropis gigantea leaf extract - Antifungal, anti-inflammatory, antimicrobial
- Tea tree essential oil - Strong antifungal and antiseptic activity
- Coconut oil - Emollient, anti-inflammatory, improves skin barrier
- Beeswax - Thickening agent, protective barrier, stabilizes cream
- Kokum butter - Deep moisturizer, skin repair, improves texture
- Emulsifying wax - Helps mixing oil and water phases, forms stable cream
- Distilled water / Rose water - Solvent, hydration, soothing effect
- Aloe vera gel - Soothing, hydrating, healing, anti-irritant
- Turmeric extract - Antifungal, wound healing, anti-inflammatory
- Vitamin E - Antioxidant, skin healing, increases shelf life

2.2 Methodology

- **Extraction method**

A. Maceration (Cold Extraction)

Maceration is a simple and conventional extraction technique widely used for isolating bioactive compounds from plant materials. It involves soaking plant tissues in a suitable solvent for a specific period, allowing the solvent to penetrate the cellular structure, soften the tissues, and dissolve the desired constituents. During this process, the compounds diffuse from the plant matrix into the surrounding liquid, forming an extract. This method is considered economical, easy to perform, and suitable for extracting thermolabile (heat-sensitive) compounds, although it generally requires longer extraction time and may have lower efficiency compared to advanced techniques. (10)

➢ **For Calotropis Gigantea Leaves Extract**

Principle: Extraction by soaking the powdered drug in a suitable solvent at room temperature

Procedure

1) Leaves of *Calotropis gigantea* were collected and washed several times with distilled water.
2) Further washing was done using deionized distilled water to remove dust, debris, and impurities.
3) The cleaned leaves were kept under shade for 15 days at room temperature (25°C) for drying.
4) After complete drying, leaves were ground into fine powder.
5) About 10 g of powdered sample was taken for extraction.

Fig. No. 1 C. Gigantea Extract

6) The powder was extracted with 100 mL ethanol solution (7:3 v/v ethanol: distilled water)
7) Extraction process was carried out for 50 minutes
8) The obtained mixture was filtered using filter paper.
9) Store at cool place. (11)

Fig. No. 2 Turmeric Extract

➢ **For Turmeric Extract**

1) Cold maceration was carried out using a magnetic stirrer at 450–500 rpm.
2) Extraction was performed at room temperature.
3) 25.00 g of plant material was used
4) The material was extracted with 150 mL ethanol.
5) The mixture was stirred continuously for 3 hours.
6) The solution was filtered using MN-751 filter paper
7) Filtration separated the solid residue from the liquid extract
8) The filtrate was concentrated using a rotary evaporator (rotavapor)
9) Evaporation was done at 40°C
10) The final extract was stored in a freezer. (12)

Fig. No. 3 Neem Extract

➢ **For Neem Extract**

1) Dried and fresh leaves were cut into small pieces
2) The plant material was powdered using mortar and pestle
3) Powder was mixed in 1:10 ratio with different solvents: ethanol, methanol, ether, acetone, and distilled water separately
4) Extraction was carried out by continuous grinding using mortar and pestle
5) The mixture was filtered using Whatman No. 1 filter paper
6) Filtrates were collected separately for each solvent
7) Filtrates were vacuum-dried using a rotary evaporator
8) The concentrated extracts were stored at 4°C for further studies. (13)

B. Procedure for Preparation of Herbal Cream.

Step 1: Preparation of Oil Phase

- Take a clean beaker.
- Add coconut oil, neem oil, beeswax, kokum butter, and emulsifying wax
- Heat the mixture on a water bath (70–75°C) until all ingredients melt completely
- Stir continuously to get a uniform oily phase.

Step 2: Preparation of Aqueous Phase

- In another beaker, take distilled water / rose water.
- Add aloe vera gel and mix properly.
- Heat this mixture to the same temperature (70–75°C) to match the oil phase.

Step 3: Emulsification Process

- Slowly add the aqueous phase into the oil phase with continuous stirring.
- Stir continuously using a glass rod or mechanical stirrer.
- Maintain temperature and stirring to form a stable oil-in-water (O/W) emulsion.

Step 4: Cooling Phase

- Allow the emulsion to cool gradually to room temperature.
- Continue gentle stirring during cooling to avoid phase separation.

Step 5: Addition of Active Ingredients

- Once cooled below 40°C, add:
  - Calotropis gigantea extract
  - Turmeric extract
  - Vitamin E
  - Tea tree essential oil
- Mix thoroughly to ensure uniform distribution.

Step 6: Homogenization

- Stir or homogenize the cream to obtain a smooth and uniform consistency.
- Ensure no lumps or phase separation.

Step 7: Filling and Storage

- Transfer the prepared cream into a clean, dry container.
- Label properly and store in a cool and dry place. (14)

3. Formulation Table

Table. no. 1 Formulation Table for Herbal Antifungal Cream

Sr.no	Name of ingredients	F1	F2	F3	Purpose
1)	*Calotropis gigantea* leaf extract	1ml	1.5 ml	2 ml	Antifungal, antimicrobial
2)	Coconut oil	2g	2 g	2 g	Emollient, moisturizing agent
3)	Neem oil	1ml	1 ml	1 ml	Antibacterial, antifungal
4)	Beeswax	1g	1 g	1 g	Thickening agent, stabilizer
5)	Kokum butter	1g	1 g	1 g	Skin conditioning, emollient
6)	Emulsifying wax	1.2g	1.2 g	1.2 g	Emulsifier
7)	Aloe vera gel	1g	1.5 g	2 g	Soothing, moisturizing
8)	Distilled water / Rose water	q.s to 20ml	q.s to 20 ml	q.s to 20 ml	Hydration
9)	Turmeric extract	0.4ml	0.4 ml	0.4 ml	Anti-inflammatory, antioxidant
10)	Vitamin E	0.2g	0.2 g	0.2 g	Antioxidant, skin protection
11)	Tea tree essential oil	0.1ml	0.1 ml	0.1 ml	Antifungal, antimicrobial

4. Evaluation parameter

a) pH test: The pH of a cream is determined by dispersing a small amount of the cream in distilled water and measuring it using a calibrated pH meter.

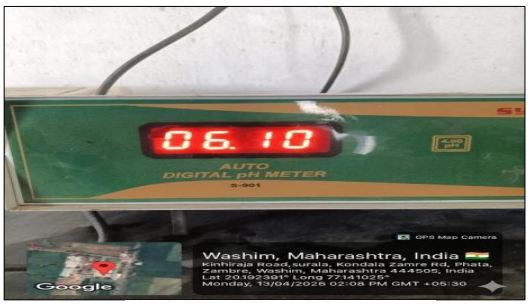

Fig. No. 4 pH Test

Result: The pH of the cream was found to be F1(5.9), F2 (6.10), F2(6.3).
Inference: Suitable for skin application and within normal skin pH range.

b) Spread ability Test: The device used to measure spread ability consisted of a wooden block with a pulley at one end. This approach examined spread ability based on the ointment's slide and drag properties. On the Ground slides, an excess of the ointment under investigation (about 2 grams) was applied. After that, the ointment was positioned with the hook between this glass slide and another one that had the same dimensions as a fixed ground slide. For five minutes, a one kilogram weighted was placed on top of the slides to release air and create a consistent layer of ointment between the slides. The excess ointment was removed by scraping off the edges. The top plate was then subjected to pull of 80 gm. With the help of string attached to the hook and the time

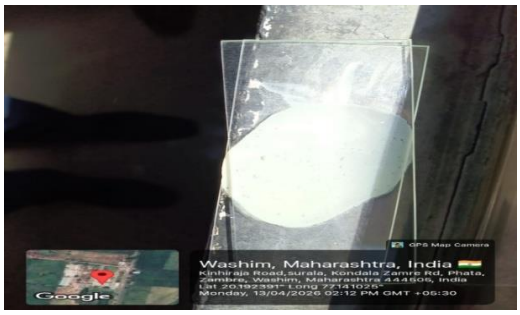

Fig. No. 5 Spread Ability Test

Result: The formulation showed good spread ability with smooth and uniform application.
Inference: Easy to apply and ensures proper distribution on skin.

c) Washability Test: 0.5 gm of prepared formulation was applied on the skin. And it was washed with warm water. The time taken for removal of preparation was noted.

Fig. No. 6 Washability Test

Result: The cream was easily washable with lukewarm water observe in three batches.
Inference: Non-greasy and user-friendly.

d) Irritancy test: Mark an area of 1sq cm on the left had dorsal surface. The cream was applied to the specified area and time was noted. Irritancy erythema. Edema was checked if any for regular intervals up to 24 hrs and reported.

Result: No erythema, edema, or irritation observed after 24 hours in all three batches.
Inference: Safe for topical use.

Fig. No. 7 Irritancy Test Before

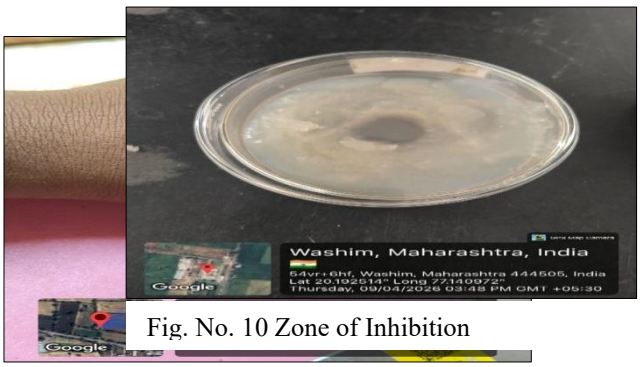

Fig. No. 8 Irritancy Test After

Fig. No. 10 Zone of Inhibition

Test for microbial growth in formulated: Creams: Using the streak plate method, the prepared creams Were inoculated into agar medium plates, and a control was created by leaving out the cream. The plates were put in the incubator and left there for a whole day at 37 OC. Plates were removed from the incubator after the incubation period to assess microbial growth by contrasting it with the control.

Observation: No microbial growth observed after incubation in all three batches.

Inference: The formulation possesses good antimicrobial properties.

e) Zone of Inhibition

The antifungal activity was evaluated using the agar well diffusion method against *Candida albicans*. The prepared cream was applied into wells on agar plates inoculated with the microorganism and incubated at 37°C for 24 hours.

Fig. No. 9 Microbial Growth Test

Result: A clear zone of inhibition of about F1(12mm), F2(14mm), F3(16mm) mm was observed around the well.

Inference: The formulation shows effective antifungal activity, indicating its suitability for treating fungal skin infections

f) Organoleptic Properties

Table. No. 2 Organoleptic Properties

5. Result And Discussion

Result: All formulations (F1, F2, F3) showed acceptable organoleptic properties, good pH, stability, and no skin irritation, indicating safety for topical use. The creams exhibited good spread ability, easy washability, and no microbial growth, confirming quality and antimicrobial nature. Among them, F3 showed the best performance with highest spread ability and antifungal activity (16 mm zone of inhibition), making it the most effective formulation. This are shown in below table

Table No. 3 Result Table for Herbal Antifungal Cream

Parameter	**Observation**	**Inference**
Colour	Pale white to light yellow	Acceptable and uniform appearance
Odour	Mild, characteristic herbal smell	Pleasant and acceptable
Texture	Smooth and homogeneous	Good formulation quality
Consistency	Semisolid	Suitable for topical application
Appearance	Non-greasy, glossy	Good aesthetic appeal
Feel on application	Soft and easily spreadable	Good patient compliance

Parameters	**F1**	**F2**	**F3**	**Inference**
Organoleptic Properties (Colour, Odour, Texture)	Pale white, mild odour, smooth	Pale white, pleasant odour, smooth	Slight yellow, pleasant herbal odour, smooth	Acceptable sensory characteristics
Physical Appearance	Smooth, pale white, semisolid	Smooth, slightly thick	Smooth, semisolid	All batches acceptable
pH	5.9	6.1	6.3	Within skin pH range (5–7)
Spread ability	Good	Very good	Excellent	Easy application
Washability	Easily washable	Easily washable	Easily washable	Non-greasy nature
Skin Irritation	No irritation	No irritation	No irritation	Safe for use
Microbial Growth	No growth	No growth	No growth	Good antimicrobial property
Zone of Inhibition	12 mm	14 mm	16 mm	Effective antifungal activity

5.1 Discussion

The present study highlights the successful formulation and evaluation of a herbal antifungal cream containing *Calotropis gigantea* leaf extract, demonstrating its effectiveness as a natural alternative to conventional antifungal agents. The results confirm that the prepared formulations exhibit significant antifungal activity along with acceptable physicochemical properties suitable for topical application.

The antifungal activity observed in this study can be attributed to the presence of phytochemical constituents such as flavonoids, tannins, glycosides, and phenolic compounds in *Calotropis gigantea*. These bioactive compounds are known to disrupt fungal cell membranes, inhibit spore germination, and induce oxidative stress, thereby inhibiting fungal growth. The study showed effective activity against *Candida albicans*, a common pathogen responsible for skin infections.

Among the three formulations (F1, F2, and F3), formulation F3 exhibited the highest antifungal activity with a zone of inhibition of 16 mm. This enhanced activity is likely due to the higher concentration of plant extract in F3, indicating that antifungal effectiveness is directly related to the concentration of active constituents.

All formulations showed good organoleptic properties such as smooth texture, uniform appearance, and pleasant odor. The pH of the formulations was within the normal skin range (5–7), ensuring compatibility and minimizing the risk of irritation. Additionally, the creams demonstrated good spreadability and washability, making them easy to apply and user-friendly.

The absence of skin irritation and microbial growth further confirms the safety and stability of the formulation. The inclusion of other herbal ingredients like neem, aloe vera, and turmeric enhanced the formulation through synergistic effects. Neem contributed antifungal and antibacterial activity, aloe vera provided soothing and healing properties, and turmeric added anti-inflammatory benefits.

These findings are consistent with previous studies supporting the use of plant-based formulations as safer and cost-effective alternatives to synthetic antifungal drugs. However, the study is limited to in-vitro evaluation, and further research including in-vivo studies and clinical trials is required.

In conclusion, the developed herbal cream shows promising antifungal potential and can be considered a safe, effective, and economical option for treating fungal skin infections.

6. Conclusion And Summary

6.1 Conclusion:

The present study successfully formulated and evaluated a herbal antifungal cream containing *Calotropis gigantea* leaf extract, demonstrating promising therapeutic potential. The developed formulations were found to be safe, stable, and effective against fungal pathogens, particularly Candida albicans. Among all formulations, F3 showed superior performance in terms of antifungal activity, spreadability, consistency, and overall stability, making it the optimized formulation.

The incorporation of natural ingredients such as neem, aloe vera, and turmeric significantly enhanced the antifungal, anti-inflammatory, and skin-healing properties through synergistic effects. Furthermore, the absence of skin irritation and microbial contamination confirms the safety and suitability of the formulation for topical application and prolonged use. The use of herbal components not only minimizes side effects but also provides a cost-effective and eco-friendly alternative to synthetic antifungal agents, highlighting the increasing relevance of plant-based medicines in modern healthcare.

However, to establish its full therapeutic potential and commercial viability, further research is required. Future studies should focus on advanced phytochemical isolation and characterization of active constituents using techniques such as HPLC, GC-MS, and FTIR. In-vivo studies, including animal and skin infection models, are necessary to validate efficacy beyond in-vitro conditions. Additionally, well-designed clinical trials are essential to assess safety, irritation potential, and effectiveness in human subjects. Further formulation optimization, including enhancement of stability,

shelf life, and the use of advanced delivery systems like nanoemulsions and liposomes, may improve drug penetration and therapeutic efficiency.

Overall, this study provides a strong foundation for the development of a safe, effective, and natural antifungal topical formulation with significant potential for future pharmaceutical applications.

6.2 Summary: The study investigates the increasing problem of fungal infections and explores a natural treatment approach by evaluating the antifungal potential of *Calotropis gigantea* leaf extracts through phytochemical screening and formulation into a topical herbal cream, where the plant's bioactive compounds such as flavonoids, tannins, and glycosides, along with supportive herbal ingredients like Aloe vera, neem, and turmeric, contribute to antimicrobial, anti-inflammatory, and wound-healing effects, and through systematic procedures including extraction, formulation, and in-vitro testing against *Candida albicans*, the developed cream formulations demonstrated acceptable physical properties, stability, skin safety, absence of microbial contamination, and significant antifungal activity, with formulation F3 showing the highest effectiveness (16 mm zone of inhibition), thereby concluding that the herbal cream is a safe, cost-effective, and promising alternative to conventional antifungal treatments with fewer side effects and good patient acceptability.

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