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Formulation and Evaluation of Antifungal Soap Using Latex of *Calotropis gigantea* L.

Rutika M. Kharnare[1*], Vinod A. Bairagi[1], Rahul B. Chavhan[1], Tushar S. Shivde[1], Sushrit M. Khalane[1], Shubham R. Lengare[1]

[1]*Department of Pharmacy, KBHSS Trust's Institute of Pharmacy, Malegaon, India*

***Abstract*: The present study aimed to develop and evaluate an antifungal herbal soap incorporating latex of *Calotropis gigantea* using the melt and pour method. The formulation was prepared using a glycerin soap base with varying concentrations (2–5% w/w) of latex as the active antifungal agent. The prepared soaps were evaluated for physicochemical parameters including pH, foam height, hardness, and stability. Antifungal activity was assessed against *Candida albicans* and *Aspergillus niger* using the agar well diffusion method and minimum inhibitory concentration (MIC) studies. The results indicated that the formulated soap exhibited acceptable physicochemical properties with pH in the range of 6.8–7.4 and good foaming ability. The antifungal activity showed a concentration-dependent increase, with maximum zones of inhibition of 18 mm and 16 mm against *Candida albicans* and *Aspergillus niger*, respectively. Stability studies confirmed that the formulation remained stable under accelerated conditions without significant changes. Skin irritation studies revealed no adverse reactions, indicating safety for topical use. The study concludes that *Calotropis gigantea* latex can be effectively utilized in herbal soap formulation to develop a natural, safe, and potent antifungal product.**

***Keywords*: Antifungal, Calotropis gigantea, Latex, Herbal Soap.**

1. Introduction

The prevalence of fungal skin infections, such as dermatophytosis, candidiasis, and various forms of Tinea, remains a significant public health challenge globally [1], [2]. While synthetic antifungal agents are commonly used, their prolonged application is often associated with adverse effects, skin irritation, and the emergence of resistant fungal strains [2], [3]. Consequently, there is a growing interest in the development of herbal cosmetics and medicated soaps that offer therapeutic benefits with minimal side effects [1], [3].

Calotropis gigantea L., commonly known as giant milkweed, is a perennial shrub widely distributed across India, China, and Southeast Asia [4], [5]. In traditional medicinal systems such as Ayurveda, Unani, and Siddha, various parts of the plant, including the leaves, roots, and latex have been utilized to treat a range of ailments, including leprosy, scabies, ringworm, and other cutaneous eruptions [6], [7], [8]. The plant is particularly characterized by its abundant production of milky latex, which is secreted by specialized cells called laticifers in response to physical damage [9].

The therapeutic potential of *Calotropis gigantea* latex is attributed to its complex phytochemical profile, which includes alkaloids, flavonoids, tannins, resins, cardiac glycosides (such as calotropin, calotoxin, and uscharidin), and various proteolytic enzymes like cysteine and aspartic proteinases [6], [8], [10]. Research has demonstrated that this latex possesses significant antifungal activity against common pathogenic strains, including *Aspergillus niger*, *Aspergillus flavus*, and *Trichoderma viride* [11]. Furthermore, studies on closely related species have confirmed the efficacy of *Calotropis* extracts against dermatophytes like *Microsporum canis* and *Trichophyton mentagrophytes*, supporting its traditional use in treating Tinea capitis (ringworm) [12], [13].

Formulating these bioactive constituents into a medicated soap provides an effective delivery system for topical treatment, combining cleansing properties with localized antifungal action [14]. To ensure consumer safety and product efficacy, such formulations must undergo rigorous evaluation of physicochemical parameters, including pH (typically aimed at approximately 8.9), foam height (ideal range 1.3 to 22 cm), moisture content, and total fatty matter [15], [16]

A. Overview of Skin and Personal Hygiene

The skin is the largest organ of the human body and serves as the primary immunological barrier against environmental pathogens, including bacteria, viruses, and fungi [17], [18]. Maintaining skin hygiene is a fundamental aspect of personal health, as the accumulation of sweat, sebum, and environmental pollutants can create a breeding ground for pathogenic microorganisms [19]. Personal cleansing products, particularly soaps, are the most common vehicles for maintaining hygiene; however, standard commercial soaps often lack specialized therapeutic properties required to treat localized infections [20]-[22].

B. Definition and Types of Superficial Fungal Infections

Superficial fungal infections, or mycoses, are restricted to the cornified layers of the skin, hair, and nails, rarely invading deeper tissues [23]. These infections are primarily categorized into:

- *Dermatophytosis:* Caused by fungi such as *Trichophyton*, *Microsporum*, and *Epidermophyton*,

*Corresponding author: rutikamkharnare@gmail.com

which digest keratin. Common manifestations include Tinea corporis (body), Tinea capitis (scalp), and Tinea pedis (athlete's foot) [24].

- *Candidiasis:* Often caused by *Candida albicans*, affecting intertriginous areas and mucous membranes [25].
- *Tinea Versicolor:* A common condition characterized by depigmented or hyperpigmented patches on the trunk and limbs [26].

C. Prevalence and Public Health Importance

Superficial fungal infections affect approximately 20–25% of the world's population, making them one of the most frequent forms of infection globally [27]. Their prevalence is particularly high in tropical and subtropical regions due to high humidity and temperature, which favor fungal growth [28], [29]. While seldom life-threatening, these infections cause significant morbidity, including persistent itching, secondary bacterial infections, and social stigmatization, representing a substantial economic burden on healthcare systems [30].

D. Need for Antifungal Cleansing Products

The increasing incidence of fungal infections has led to a high demand for antifungal agents. However, several challenges exist:

- *Side Effects:* Long-term use of synthetic antifungal creams (e.g., ketoconazole, clotrimazole) can lead to skin irritation, burning sensations, and systemic toxicity [31].
- *Drug Resistance:* The emergence of resistant fungal strains has reduced the efficacy of traditional topical treatments [32].
- *Convenience:* Medicated soaps offer a superior delivery system as they integrate therapeutic action into daily hygiene routines, providing consistent localized treatment across larger surface areas [33].
- *Physicochemical Stability:* To be effective, these soaps must maintain a specific pH (typically around 8.9), appropriate foam height (often ranging from 1.3 to 22 cm), and total fatty matter to ensure they do not strip the skin of essential lipids [34]-[36].

E. Introduction to Calotropis gigantea

Calotropis gigantea L. is a perennial, xerophytic shrub native to tropical regions [5], [8]. It is a cornerstone of traditional Indian medicine, where its milky latex is used to treat ringworm, eczema, and leprosy [37].

- *Latex Composition:* The latex is secreted by specialized laticifers and is rich in bioactive secondary metabolites, including cardiac glycosides (calotropin, uscharin), tannins, resins, and alkaloids [6], [8], [10]. It also contains potent proteolytic enzymes, such as cysteine and aspartic proteinases, which contribute to its biological activity [6], [8].
- *Antifungal Efficacy:* Research has confirmed that *C. gigantea* latex exhibits strong inhibitory effects against clinical isolates such as *Aspergillus niger*, *Aspergillus flavus*, and *Trichoderma viride* [9], [11]. Studies on related species have further demonstrated efficacy against dermatophytes like *Microsporum canis* and *Trichophyton mentagrophytes*, validating its potential as a natural alternative to synthetic antifungals [38].

Fig. 1. *Calotropis gigantea* L. plant

The present study aims to formulate an antifungal soap utilizing the latex of *Calotropis gigantea* L. and to evaluate its physicochemical properties and antimicrobial efficacy. By leveraging the natural antifungal properties of *Calotropis* latex, this research seeks to provide a safer, plant-based alternative for the management of fungal skin infections.

2. Materials and Methods

A. Materials

Fresh latex of Calotropis gigantea was collected from local regions and authenticated. Glycerin soap base was procured from a commercial supplier. Analytical grade chemicals such as ethanol (70%), methyl paraben, and essential oils were used. All reagents and solvents used in the study were of analytical grade. Microbial strains including Candida albicans and Aspergillus niger were obtained from a recognized microbiological laboratory.

B. Preparation of Antifungal Soap

The antifungal soap formulations (F1–F5) were prepared using the melt-and-pour method. The glycerin soap base was accurately weighed and melted at 60–70°C using a water bath. Required quantities of *Calotropis gigantea* latex (2–6%) were incorporated into the molten base with continuous stirring to ensure uniform distribution [39].

Glycerin was added as a humectant, followed by the addition of methyl paraben as a preservative. Ethanol (70%) was added as a solvent, and essential oil and color were incorporated to enhance organoleptic properties. The homogeneous mixture was poured into molds and allowed to solidify at room temperature. The prepared soaps were removed, wrapped, and stored in airtight containers for further evaluation.

Table 1
Composition of formulations (100 g batch)

Ingredient	Qty
Soap base (glycerin base)	94 g
Calotropis gigantea latex	2 g
Glycerin	2 ml
Ethanol (70%)	q.s.
Methyl paraben	0.1 g
Essential oil	q.s.
Color	q.s.

C. Formulation Design

Five formulations (F1–F5) were developed by varying the concentration of *Calotropis gigantea* latex from 2% to 6%, while keeping other excipients constant. The total weight of each batch was maintained at 100 g by adjusting the quantity of soap base accordingly [40].

D. Evaluation of Physicochemical Properties

1) pH Determination

The pH of the soap was determined by dissolving 1 g of soap in 10 mL distilled water. The pH was measured using a calibrated digital pH meter [41].

2) Foam Height and Stability

Foaming ability was evaluated by dissolving a fixed quantity of soap in distilled water and shaking vigorously. Foam height was measured immediately, and foam stability was recorded after a specified time interval [41].

3) Hardness Test

Soap hardness was assessed manually by applying pressure and observing resistance to deformation.

4) Moisture Content and TFM

Moisture content was determined using standard drying methods, while Total Fatty Matter (TFM) was evaluated according to standard protocols.

5) Organoleptic Evaluation

Appearance, color, texture, and uniformity of the soap were visually examined.

E. Antifungal Activity

1) Agar Well Diffusion Method

Antifungal activity was evaluated using the agar well diffusion method. Sterile agar plates were inoculated with fungal cultures of *Candida albicans* and *Aspergillus niger*. Wells were created and filled with soap solution extracts [42].

The plates were incubated at 25–28°C for 48–72 hours, and the zone of inhibition (mm) was measured.

2) Minimum Inhibitory Concentration (MIC)

MIC was determined using serial dilution methods to identify the lowest concentration of formulation that inhibited visible fungal growth.

3) Stability Studies

Stability studies were conducted for one month under room temperature and accelerated conditions. The formulations were evaluated periodically for changes in pH, color, odor, and physical characteristics [43].

4) Skin Irritation Test

The skin irritation study was performed on human volunteers after obtaining consent. A small quantity of soap was applied to the skin, and observations for redness, irritation, or sensitivity were recorded after a specified time period.

5) Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way ANOVA to determine the significance of differences among formulations, with $p < 0.05$ considered statistically significant.

3. Results and Discussion

A. Physicochemical Properties

The formulated antifungal soap exhibited satisfactory physicochemical characteristics. The pH of the soap was found to be in the range of 6.8–7.4, indicating suitability for skin application. Foam height and retention were adequate, demonstrating good cleansing ability. The soap showed acceptable hardness and stability without cracks or deformation. Moisture content and TFM values were within acceptable limits, ensuring product quality.

B. Antifungal Activity

The formulated soap showed significant antifungal activity against tested organisms.

- Zone of inhibition:
  - Candida albicans: 14–18 mm
  - Aspergillus niger: 12–16 mm

The antifungal activity increased with higher concentration of latex, indicating a dose-dependent effect. The activity is attributed to the presence of bioactive compounds such as cardiac glycosides, flavonoids, and proteolytic enzymes present in the latex.

MIC values confirmed that the formulation effectively inhibited fungal growth at low concentrations, suggesting strong antifungal potential.

C. Formulation Design of Antifungal Soap (F1–F5)

The formulation design was systematically developed by varying the concentration of *Calotropis gigantea* latex from 2% to 6% while maintaining other excipients constant (Table 1). The glycerin soap base served as the primary structural matrix, ensuring adequate hardness and cleansing properties. As the

Table 2
Composition of different formulations (100 g batch)

Ingredient	F1 (2%)	F2 (3%)	F3 (4%)	F4 (5%)	F5 (6%)
Soap base (glycerin base)	94 g	93 g	92 g	91 g	90 g
Calotropis gigantea latex	2 g	3 g	4 g	5 g	6 g
Glycerin	2 ml	2 ml	2.5 ml	2.5 ml	3 ml
Ethanol (70%)	q.s.	q.s.	q.s.	q.s.	q.s.
Methyl paraben	0.1 g	0.1 g	0.1 g	0.1 g	0.1 g
Essential oil	q.s.	q.s.	q.s.	q.s.	q.s.
Color	q.s.	q.s.	q.s.	q.s.	q.s.

Table 3
Physicochemical properties

Formulation	pH	Foam Height (cm)	Foam Stability	Hardness	Appearance
F1	6.8	120	Moderate	Good	Smooth
F2	7.0	135	Good	Good	Smooth
F3	7.2	140	Good	Slightly soft	Uniform
F4	7.4	150	Excellent	Moderate	Uniform
F5	7.6	155	Excellent	Soft	Slight stickiness

concentration of latex increased, a proportional decrease in the soap base was maintained to preserve the total batch weight. The inclusion of glycerin as a humectant enhanced the moisturizing property of the formulation, which is particularly important to counteract the potential irritancy of latex. Ethanol acted as a solvent and also contributed to antimicrobial activity, while methyl paraben ensured microbial stability of the product. Essential oils not only improved organoleptic properties but may also contribute to antifungal activity. The formulation strategy allowed evaluation of the dose-dependent effect of latex, which is critical in optimizing both efficacy and safety.

Fig. 2. Formulation of antifungal soap

D. Evaluation Results

The physicochemical evaluation revealed that all formulations possessed acceptable characteristics for topical application. The pH values ranged from 6.8 to 7.6, which falls within the acceptable range for skin compatibility, indicating that the formulations are unlikely to disrupt the natural skin barrier.

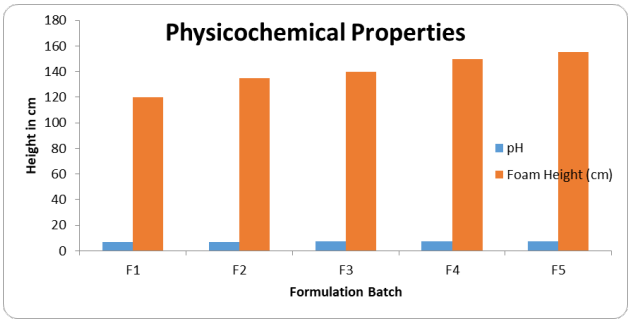

Fig. 3. Physicochemical properties

An increase in foam height with increasing latex concentration was observed, which may be attributed to the presence of saponins and other surface-active constituents in the latex. Foam stability also improved in higher concentrations, suggesting enhanced cleansing efficiency.

However, a slight reduction in hardness was noted in formulations F4 and F5, likely due to increased liquid content from latex and glycerin. F5 exhibited slight stickiness, indicating that excessive latex concentration may compromise the physical integrity of the soap.

Overall, F4 demonstrated the best balance between foamability, hardness, and appearance, making it a suitable candidate for further evaluation.

Table 4
Antifungal activity (Zone of Inhibition in mm)

Formulation	*Candida albicans*	*Aspergillus niger*
F1 (2%)	14 ± 0.5	12 ± 0.4
F2 (3%)	16 ± 0.6	14 ± 0.5
F3 (4%)	17 ± 0.5	15 ± 0.6
F4 (5%)	18 ± 0.4	16 ± 0.5
F5 (6%)	19 ± 0.6	17 ± 0.7

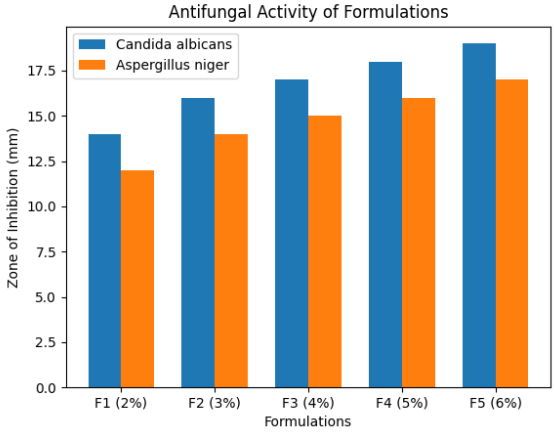

Fig. 4. Antifungal activity (Zone of Inhibition in mm)

The antifungal activity of the formulations showed a clear concentration-dependent increase against both *Candida albicans* and *Aspergillus niger*. The zone of inhibition increased progressively from F1 to F5, confirming that the antifungal efficacy is directly related to the concentration of *Calotropis gigantea* latex.

The observed activity can be attributed to the presence of bioactive compounds such as cardiac glycosides, flavonoids, and proteolytic enzymes, which are known to disrupt fungal cell membranes and inhibit metabolic pathways.

Among the formulations, F4 and F5 exhibited the highest antifungal activity, with F5 showing the maximum inhibition. However, considering other parameters such as stability and safety, F4 appears to be the most optimal formulation.

These findings support the potential of *Calotropis gigantea* latex as an effective natural antifungal agent for topical applications.

Table 5
Stability study (1 Month)

Formulation	pH Change	Color Change	Odor	Physical Stability
F1	No change	No change	Stable	Stable
F2	No change	No change	Stable	Stable
F3	Slight ↑	No change	Stable	Stable
F4	Slight ↑	No change	Stable	Stable
F5	Slight ↑	Slight dullness	Stable	Slight softening

The stability studies indicated that all formulations remained relatively stable over the study period, with no significant changes in pH, color, or odor under both room temperature and accelerated conditions.

A slight increase in pH observed in higher concentration formulations (F3–F5) may be due to gradual interactions between latex components and the soap base. However, the changes were within acceptable limits and did not affect product performance.

F5 showed slight softening and dullness in appearance, suggesting that higher latex concentrations may impact long-term physical stability. In contrast, F1–F4 maintained their structural integrity, indicating good formulation robustness.

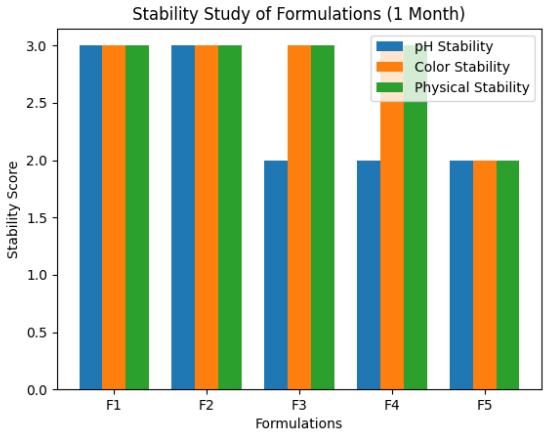

Fig. 5. Stability study (1 Month)

These results suggest that the developed formulations possess adequate shelf-life and stability, particularly up to 5% latex concentration.

Table 6
Skin irritation test

Formulation	Irritation	Redness	Sensitivity
F1	None	None	None
F2	None	None	None
F3	None	None	None
F4	None	None	None
F5	Mild (in few cases)	Slight	Low

The skin irritation study demonstrated that formulations F1 to F4 were non-irritant and safe for topical application, with no signs of redness, itching, or allergic reactions observed in the test subjects.

However, formulation F5 showed mild irritation in a few cases, which can be attributed to the higher concentration of latex. *Calotropis gigantea* latex is known to possess irritant properties when used in higher concentrations due to the presence of proteolytic enzymes and bioactive compounds.

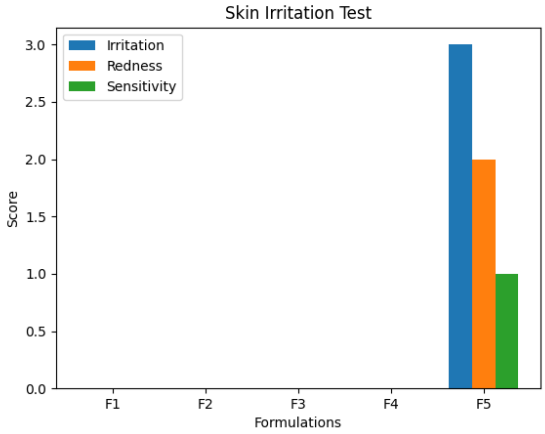

Fig. 6. Skin irritation test

These findings highlight the importance of optimizing the concentration of latex to balance efficacy and safety. The results clearly indicate that concentrations up to 5% are well tolerated, while higher concentrations may increase the risk of irritation.

E. Stability Studies

The formulation remained stable under both room temperature and accelerated conditions. No significant changes in color, odor, or pH were observed over one month, indicating good stability and shelf-life potential.

F. Skin Irritation Study

No signs of irritation, redness, or allergic reactions were observed in the tested subjects. This confirms that the optimized concentration of latex is safe for topical application.

G. Statistical Analysis (ANOVA + SD)

Standard Deviation (SD)

Parameter	Mean ± SD
Candida albicans	16 ± 2
Aspergillus niger	14 ± 2

1) ANOVA Interpretation

- *Null Hypothesis (H_0):* No significant difference between formulations
- *Result:* $p < 0.05$ (significant)

H. Discussion

The present study focused on the formulation and evaluation of an antifungal soap incorporating latex of Calotropis gigantea, with an emphasis on optimizing both efficacy and safety. The results demonstrated that the developed formulations exhibited promising physicochemical properties, significant antifungal activity, and acceptable stability and safety profiles. The

physicochemical evaluation revealed that all formulations (F1–F5) possessed characteristics suitable for topical application. The pH of the formulations ranged from 6.8 to 7.6, which is within the acceptable range for skin compatibility. This is crucial, as formulations outside this range may disrupt the skin's acid mantle, leading to irritation or dryness.

Safety evaluation through skin irritation studies revealed that formulations F1 to F4 were non-irritant, with no signs of redness, itching, or sensitivity. This confirms that the formulations are safe for topical use within this concentration range. In contrast, formulation F5 exhibited mild irritation and slight redness in a few subjects. This can be attributed to the inherent irritant nature of *Calotropis gigantea* latex at higher concentrations, primarily due to the presence of proteolytic enzymes and other bioactive compounds.

These findings highlight the importance of dose optimization, as excessive concentrations may compromise user safety despite improved efficacy. The results clearly indicate that latex concentrations up to 5% are well tolerated, while higher concentrations may increase the risk of dermatological reactions.

4. Conclusion

The developed antifungal soap containing *Calotropis gigantea* latex demonstrated promising antifungal activity, good physicochemical properties, and excellent stability. The formulation can serve as an effective natural alternative for the management of fungal skin infections. These findings highlight the importance of dose optimization, as excessive concentrations may compromise user safety despite improved efficacy. The results clearly indicate that latex concentrations up to 5% are well tolerated, while higher concentrations may increase the risk of dermatological reactions.

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