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Development and evaluation of topical antifungal products based on leaf extract of *Cassia alata* Linn (FABACEAE)

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Abstract

The study aims to validate the scientific use of *Cassia alata* Linn (FABACEAE) by developing topical antifungal products for managing skin infections. It is an important medicinal and ornamental flowering plant. In many countries, leaf decoctions have been used to treat various infectious skin diseases in humans and animals, including fungal infections such as *Pityriasis versicolor*, ringworm, and scabies. To conduct the research, the aqueous extract of the leaves was used to develop dermatological cream and soap containing active components. A set of methods was used to analyze the quality and efficacy. *In vitro* testing revealed that the aqueous extract exhibits high antifungal activity against *C. albicans* in a dose-dependent manner and has significant antioxidant properties. The outcome of the research on the formulated cream and soap demonstrated satisfactory physicochemical parameters, including antifungal efficacy, stability, spreadability, and homogeneity. There was no skin irritation upon application. This indicates compliance with quality control tests for active pharmaceutical ingredients and finished drug products, ensuring that they are safe and effective. Investigating novel drugs that offer high-quality, low-cost, and natural alternatives for treating skin infections aligns with a growing scientific interest in natural products for dermatological treatments. These findings are promising, but further large-scale clinical trials and investigation of molecular mechanisms are needed to validate them. This research supports the use of *C. alata* in complementary medicine to manage skin conditions and provides an alternative to synthetic antifungal drugs.

Keywords: *Cassia alata*; Antifungal; Topical; Formulation; Quality control

1. Introduction

The FABACEAE, or legume family, is the third-largest family of flowering plants. It is a globally distributed group of trees, shrubs, and herbaceous plants that are agriculturally and economically vital, though not the most evolved family in the Fabales order [1]. The family's woody species are often found in tropical regions, while its herbaceous members are common in subtropical and warm temperate climates. These herbaceous members contribute to soil fertility through nitrogen fixation and provide a wide range of products, such as food, fuel, and timber.

Cassia alata Linn belongs to the FABACEAE plant family. Also known as the “ringworm plant” or “candle bush”, *C. alata* is native to Southeast Asia, Africa, and Latin America but it can be found in diverse habitats throughout the world, including Madagascar Island. It is cultivated for its ornamental and medicinal properties. It has diverse uses in traditional medicine, particularly the leaves for skin infections like ringworm, as well as for conditions such as constipation and inflammation [2,3]. Several studies have shown that this species contains antimicrobial substances that may be responsible for its effectiveness against bacterial and fungal infections [4,5,6].

Escalating rates of drug-resistant human pathogens, combined with the harmful side effects of conventional antimicrobials, necessitate the development of safer, more effective new agents [7]. Poor hygiene, immunosuppression,

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and resistance to conventional antifungal therapies are leading to an increase in reports of superficial mycoses caused by dermatophytes (*Trichophyton, Microsporum*, *Epidermophyton*) and yeasts like *Candida albicans* [8]. Fungal infections are among the most prevalent and challenging skin ailments worldwide, especially in tropical and subtropical regions, where the heat and humidity promote the growth of pathogenic fungi [9].

Therefore, the goal of this study is to formulate and evaluate the effectiveness of dermatological products made from the aqueous extract of *C. alata* for managing fungal skin infections.

2. Material and methods

2.1. Materials

The plant material consists of *Cassia alata* Linn leaves, which were collected in May 2022 in Antananarivo, Madagascar. The sample was authenticated at the Botanical and Zoological Park of Tsimbazaza, Antananarivo. The collected leaves were air-dried at room temperature and ground into a powder (Figure 1a). The powdered plant material was stored in an airtight container prior to extraction.

Animal experiments were carried out on SWISS mice weighing between 20 and 30 grams, which were supplied by the IMVAVET (Institut Malgache des Vaccins Vétérinaires) animal facility. The mice had free access to tap water and were fed a standard diet (Figure 1b).

Figure 1 a) Plant material: *Cassia alata* Linn b) SWISS mice: *Mus musculus*

2.2. Preparation of plant extract

According to literature on the use of *Cassia alata*, plant extraction is done by decoction [10]. This process involves boiling dried leaf powder in distilled water at 100° C for 30 minutes under reflux. The mixture is cooled, and then the liquid is filtered using Whatman filter paper (No.1) to remove solid plant residues. Finally, vacuum evaporation is performed to concentrate the liquid extract into a residue (Figure 2).

The resulting dry residue containing the active ingredients is weighed to calculate the yield and is then incorporated into dermatological formulations to ensure the correct concentration of the active ingredient [11]. The yield of the obtained extracts is calculated using the following formula:

$$Yield\ (\%) = \frac{Quantity\ of\ extract\ obtanined}{Quantity\ of\ plant\ powder\ used}\ x\ 100$$

Figure 2 Plant extraction process: A: Decoction; B: Filtration; C: Evaporation

2.3. Thin Layer Chromatography (TLC)

Thin-layer chromatography (TLC) is a chromatographic technique that uses a thin stationary phase supported by an inert backing to separate the components of a mixture. TLC is a widely used analytical tool because it is simple, relatively inexpensive, highly sensitive, and fast. Like all chromatography methods, this technique is based on the principle that a compound will have different affinities for the mobile and stationary phases, which affects the speed at which it migrates. The goal is to obtain well-defined, well-separated spots [12].

The following procedure was used to perform TLC for the present study on the aqueous extract of *C. alata* leaves. First, 10 mg of the extract was dissolved in 1 mL of methanol to form a dilute solution of the extract. Then, 10 µL of the solution was deposited onto an Aluminum silica gel plate (Merck 60 F_{254}) that had been dried prior to being introduced into the migration tank. The eluent system, consisting of n-propanol (40) / Ethyl acetate (40) / Water (30) served as the migration solvent. The obtained TLC chromatograms were then observed under 254- and 365-nm wavelengths using an ultraviolet lamp. After spraying the plate with 10 % Sulfuric acid, the retention factor (Rf) values of all spots were determined.

2.4. DPPH free radical scavenging assay

The antioxidant activity of the aqueous extract was determined by the method of DPPH assay, based on the degradation of DPPH radical (2, 2 Diphenyl-1Picrylhydrazyl) with some modifications. The addition of an antioxidant reduces the DPPH radical, causing the mixture to discolor [13].

The qualitative assay was performed according to the method of Takao *et al*. (1994) and Ruiz-Terán *et al*. (2008) [14, 15]. Two milligrams of the dried extract were diluted with 1 mL of methanol. Then, 20 µL of each dilution was carefully loaded individually onto the baseline of the 20 cm x 10 cm TLC plates and allowed to dry [16]. The mobile phase was a mixture of n-propanol, ethyl acetate, and water in a ratio of 4/4/3 (v/v/v). Once the plate was dry, it was sprayed with a 4 % methanol solution of DPPH radicals. Compounds with radical-scavenging activity produced a yellow-on-purple spot due to DPPH discoloration.

2.5. Evaluation of antimicrobial activity

The antimicrobial activity of the plant extract was studied using the solid-state diffusion disk method with *Staphylococcus aureus* (ATCC 11632) and *Candida albicans* (ATCC 10231) germs obtained from stock cultures stored at the National Center for Pharmaceutical Research Applications (CNARP) in Antananarivo, Madagascar.

This method is often used for initial screening because it is fast, cost-effective, and can rapidly test multiple compounds [17]. The culture media (Mueller-Hinton Agar and Sabouraud-Dextrose Agar) were prepared according to the manufacturer's specifications. The prepared medium was sterilized by autoclaving at 121° C for 15 minutes. It was then allowed to cool before being used to cultivate microorganisms. 10 µL of the plant extract were applied to discs at specific concentrations (50, 100, 150, and 200 mg/ml). The plates were allowed to diffuse for one hour before being incubated at 37° C for 24 hours for *S. aureus* and at 25° C for 48 hours for *C. albicans* to test the zones of inhibition [18].

To quantify the activity of the plant extract, the ability to inhibit microbial growth was measured by the diameter of the zone of inhibition (DZI), expressed in millimeters, after the incubation period [19, 20]. The discs impregnated with Neomycin (30 µg /disc) and Ketoconazole (200 mg/ml) were used as positive controls.

2.6. Formulation of topical antifungal products

Galenic preparation is an essential step because its sole objective is to optimize the effectiveness of the treatment by taking the pathology and the active ingredient into account. This study aims to formulate a more environmentally friendly range of dermatological products. Thus, a dermatological cream and soap for external use were prepared, based on an aqueous extract and natural ingredients. The entire formulation process was carried out in accordance with European Pharmacopoeia recommendations for topical preparations [21].

2.6.1. Preparation of antifungal cream

The cream was made by adding a quantity of plant extract to an oil-in-water (O/W) cream base. The emulsifier, fat-soluble components, thickening agent, and emollient or lubricant were dissolved in the oil phase and heated to 75° C (Part A). The aqueous phase was prepared by dissolving the required amount of *C. alata* leaf extract in a glycerin-based solvent and water mixture. The preservatives and other water-soluble components used as humectants were dissolved in the aqueous phase and heated to 75° C. Then, the aqueous phase (Part B) was slowly added to the oil phase (Part A) while stirring continuously until the emulsifier cooled [22].

2.6.2. Preparation of dermatological soap

Soaps are considered the oldest hygiene products used by humans. They play an important role in hygiene and health by removing dirt from the body. For this study, a soap bar for cleansing the body has been prepared as a hygiene product. It contains *C. alata* extract, as well as other additives, essences, and aromas. This dermatological soap is the result of a cold saponification reaction [23, 24].

2.7. Quality controls of the formulated products

In accordance with European Pharmacopoeia standards for topical products, essential pharmacotechnical controls must be performed to ensure the quality, stability, and reproducibility of the finished product. The International Conference on Harmonization (ICH) guidelines describe the requirements for quality and stability testing of formulated creams and soaps under the heading "Stability Testing of New Pharmaceutical Substances and Products" [25,26]. The control procedures were carried out as indicated below.

2.7.1. Cream evaluation

- Physical evaluations of the cream were carried out over a 14 day period using the following organoleptic parameters: color, odor, appearance, and texture.
- Stability of the cream was determined by observing changes in its physicochemical parameters over 14 days.
- Spreadability of the formulations was determined by measuring the diameter of a 0.1 g sample spread between two horizontal glass plates after one minute. This spreadability was then compared to that of a commercially available cream.
- pH was determined using a digital pH meter on a 0.5 g sample of cream dispersed in 50 ml of distilled water.

2.7.2. Soap evaluation

- Washing ability of the herbal soap was tested with water.
- pH of the soap was measured using a pH meter.
- To determine the foaming capacity, approximately 1.0 g of soap was dissolved in 50 ml of distilled water using a graduated cylinder. The cylinder was agitated for about 10 minutes during the measuring cycle. After this time, the foam height was measured.
- Lather Retention Time: this is the length of time that a soap's lather persists before it collapses. It is determined by measuring its volume or height over a given period of time, typically a few minutes after formation. A longer lather retention time indicates a more stable and effective product, so this measurement helps assess the quality of a soap. It was measured for approximately 5 to 10 minutes after repeating the process.
- Stability testing was conducted by subjecting the formulated soap to a one-month stability test at 40° C, 2° C, 75 % RH and 5 % RH.
- The moisture content of the soap was calculated by measuring the percentage of water in the soap. For this process, the ‘wet weight’ (Ww) and the ‘dry weight’ (Wd) of the soap were measured before drying it in a dryer. Then, to calculate the percentage of water in the original wet soap sample, the following formula was used :

$$Mosture\ Content\ (\%) = \big((Ww) - (Wd)\big)x\ 100$$

2.8. Sterility test

Sterility testing is the study of the microbiological cleanliness of formulated products [27]. This testing is fundamental because products intended for application to the skin must be free from contamination. Topical semi-solid formulations generally have low levels of microbial contamination, but these levels increase with the presence of a continuous aqueous phase and an ineffective preservation system. Thus, monitoring consists of screening and counting any bacterial or fungi that may be present in the preparation.

Depending on the risk of contamination of the preparation, the test was performed periodically: after 3 days, 1 week, 2 weeks, 1 month, 2 months, and 6 months of storage. Microbial contamination was monitored using the germ count method [28]. To do this, surface inoculation was performed on each culture medium. Then, 0.1 ml of each product was aseptically placed on the surface of the agar contained in Petri dishes. Next, the inoculum was spread on the surface of the agar using a sterile rake. The dishes were incubated at 37° C in an incubator. After 24 hours, they were observed for Mueller-Hinton Agar (MHA) medium, and after 48 hours, for Sabouraud medium. The number of microorganisms in the ointment sample was calculated using the formula described by the French Association for Standardization (AFNOR) [29, 30]. The preparation is considered acceptable if the number of viable germs falls within the microbial contamination limits specified in the European Pharmacopoeia monographs [31].

2.9. Skin toxicity test

The tests were carried out in accordance with Organization for Economic Cooperation and Development (OECD) guidelines [32]. The 3Rs method (replacement, reduction and refinement) was used. All animals were acclimated in a cage at a temperature of ± 23° C for one week. The total duration of the study was 72 hours, starting from the day of application. This timeframe allows for an assessment and categorization of the product's effects on skin contact [33].

The experiment aimed to evaluate the product's potential skin toxicity by measuring its irritability index on mice using the Draize acute primary irritation test procedure [34]. Twenty-four hours before the experiment, the backs of 15 mice were shaved. Then, the extract was applied to a 1 cm^2 area on each animal. The test procedure for the skin toxicity in mice is summarized in Table 1. Data collected from this test indicates the product's potential to cause skin damage or irritation.

The application site was evaluated to score the reaction, namely erythema and edema, as show in Table 2 [35, 36]. Scores are assigned to erythema and edema based on severity, typically on a scale ranging from 0 (no reaction) to 4 (severe reaction). The Primary Irritation Index (PII) is obtained by summing the mean erythema and mean edema values for each animal over all observation periods after 24, 48, and 72 hours of application (Table 3).

The maximum possible PII value is 8, which is calculated from a maximum score of 4 for each of the two parameters (erythema and edema).

$$PII = Mean\ value\ (Edema + Erythema)$$

Table 1 Procedure of the skin toxicity test in mice

Bach No.	Treatments
1	Untreated (shaved skin)
2	Shaved back + product application
3	Shaved back + scarification+ product application
4	Shaved back + product application + bandage
5	Shaved back + scarification + product application + bandage

Table 2 Scoring system of skin reaction according to the Draize method

Type of lesion	Degree of Injury/Irritation	Score
Erythema and eschar formation	No erythema	0
	Very light (barely noticeable)	1
	Well defined	2
	Moderate to severe	3
	Severe with pressure sore formation	4
Edema formation	Very light (barely noticeable)	1
	Light (well-defined edge)	2
	Moderate (height about 1 mm)	3
	Severe (more than 1 mm in height and extends beyond the exposed surface)	4

Draize, 1944

Table 3 Scoring system of skin reaction according to the Draize method

PII value	Categories	PII value	Categories
PII < 0.5	Non-irritating	2.1 < PII < 5	Irritant (Moderate Reaction)
0.5 < PII < 2	Slightly irritating	5.1 < PII < 8	Very irritating (Severe reaction)

2.10. Antifungal study of the formulated cream and soap

The antifungal activity of the formulated products was evaluated *in vitro* using the agar well method. Four different concentrations (10, 25, 50, and 100 mg/ml) of the cream and soap were prepared.

For the antifungal cream and soap, commercially available Miconazole cream and Keto Plus soap served as positive controls, respectively. Product bases without active extract served as negative controls. The two solutions were then dissolved separately in 5 ml of DMSO and used to fill the wells. Then, 200 µl of cream and soap at the aforementioned concentrations were poured into 9-mm-diameter, 8-mm-high wells made on agar previously inoculated with a 0.5 MacFarland *C. albicans* culture. The plates were kept at room temperature for one hour, after which they were incubated at 25° C for 48 hours.

The diameter of inhibition zones formed was measured, and the average was calculated [37,38]. The extent of antifungal activity was interpreted according to Arenas *et al*. (2022) based on the recorded zone of inhibition (DZI) value as follows: + = very low activity (DZI: < 10 mm); + + = low activity (DZI: 10–13 mm); + + + = moderate activity (DZI: 14–19 mm ZOI), + + + + = very high activity (DZI: > 19 mm) [39].

2.11. Statistical Analysis

A Student t-test was performed to determine the significance level of the various bacterial zones of inhibition observed. P-value less than 0.05 were considered significant.

3. Results

3.1. Extraction result

A 24.15 % yield of *Cassia alata* aqueous extract was obtained in the form of a greenish-black paste with a characteristic odor.

3.2. Thin-Layer Chromatography profile of the aqueous extract

The TLC result is show in Figure 3. The positions and characteristics of the spots reveal the number of compounds in the plant extract. For the aqueous extract of *C. alata* leaves, the retention times for each chromatogram are as follows: Rf (7) = 0.97, Rf (6) = 0.91, Rf (5) = 0.86, Rf (4) = 0.79, Rf (3) = 0.74, Rf (2) = 0.69, Rf (1) = 0.54.

These results confirm the presence of several bioactive compounds in the aqueous extract.

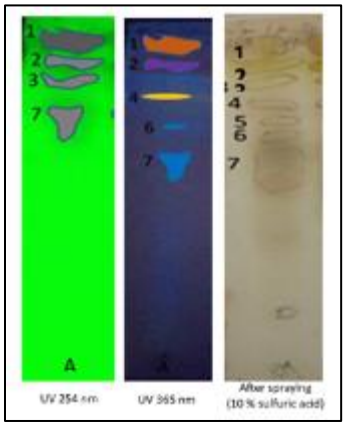

Figure 3 TLC of the aqueous extract (*C. alata* leaves**)**

3.3. Free radical scavenging capacity

The aqueous extract of *C. alata* leaves exhibited antioxidant activity. TLC -bioautography revealed the formation of an intense yellow spot (Figure 4). The yellow color change in the DPPH assay indicates a reduction in the DPPH radical, which is a key indicator of antioxidant power in plant extracts. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical that appears purple in its radical form. The presence of antioxidants in a sample causes them to react with the DPPH radical, reducing it to a colorless or yellow compound called diphenyl picryl hydrazine. This reaction highlights the antioxidants' potential to neutralize free radicals, protect cells from oxidative damage, and promote health. Thus, they are promising candidates for therapeutic use.

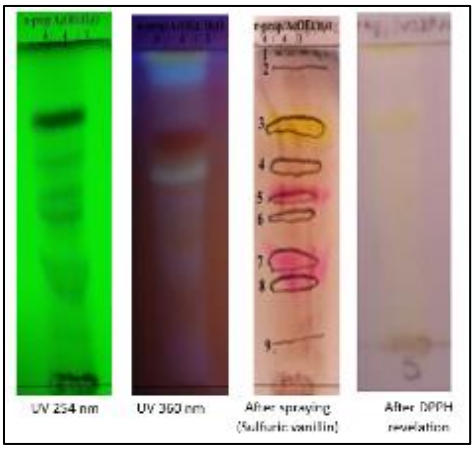

Figure 4 DPPH scavenging activity of aqueous extract (*C. alata* leaves)

3.4. Antimicrobial activity of the aqueous extract

The antifungal and antibacterial activities of the aqueous leaf extract of *Cassia alata* varied depending on the concentration used (20 to 200 mg/ml) when tested against *S. Aureus* and *C. albicans*.

There was a higher statistically significant difference in activity at 150 and 200 mg/ml of the extract than the positive control (Ketoconazole) on *C. albicans*. The zone of inhibition increased with the concentration of aqueous extract. Dose-dependent antifungal activity was observed. However, this study did not compare the extract and the positive controls

at the same concentration. Table 1 reports the antimicrobial activity of the aqueous leaf extract of *C. alata* in terms of average zones of inhibition in millimeters (mm) (*: significant difference at $p < 0.05$).

Table 4 Antimicrobial screening of the aqueous leaf extract of *Cassia alata*

Organism	**Mean zones of inhibition (mm)**			
	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml
Staphylococcus aureus	8 ± 0.01	11 ± 0.45	12.5 ± 0.65	14.5 ± 0.14
Candida albicans	9 ± 0.74	19.20 ± 0.68	29.40 ± 0.34*	36.80 ± 0.41
Neomycin (30 µg /disc)	23 ± 0			
Ketoconazole (200 mg/ml)	25.45 ± 0.3			

3.5. Formulation results

3.5.1. Characterization of the herbal cream

The herbal cream is an oil-in-water emulsion that contains 3 % *C. alata* aqueous extract. The cream exhibited good physicochemical properties. Its pH level of 5.1 is ideal for topical applications and is the recommended pH level for the skin. The herbal cream resists mechanical action well and has a good consistency. It has a smooth, even appearance. Stability tests showed no changes in color, odor, spreadability, texture, or pH over time, and no phase separation or coalescence was observed. Table 5 shows the results of the physicochemical characteristics of the formulated antifungal cream.

Table 5 Physicochemical parameters of the herbal formulated cream with *C. alata* extract

Parameters	**Results**
Homogeneity	Good
Appearance	Brown light
Odor	Good
Spreadability	Good
Texture	Emollient
Type of smear	Non-greasy
Removal	Easy
Stability	Stable
pH	5.1

3.5.2. Characterization of the dermatological soap

The herbal soap was evaluated for quality parameters. Table 7 shows the main characteristics of the soaps made with the aqueous extract of *C. alata* leaves. The pH value of this dermatological soap is found to be between 8 and 10. The results show that all physicochemical parameters of the herbal soap formulated with *C. alata* extract are in good accordance with the values in the standard literature. The pH level is an important parameter for soap quality because it acts as a barrier against bacteria and viruses and plays a role in the pathogenesis of some skin diseases.

Table 6 Physicochemical parameters of the dermatological soap based on *C. alata* leaves extract

Parameters	**Standards value**	**Observed value**
Color, Odor, Appearance	None	Brown, Pleasant, Smooth texture
pH	8-10	9.4
Foam height	1.3- 22 cm	8.11 cm
Washing ability	-	10.05 s

Foam retention	Over 5 min	4 min
Total Fatty Matter (TFM)	36.8 %	0.03 %
Moisture Content	10 %	2.01 %

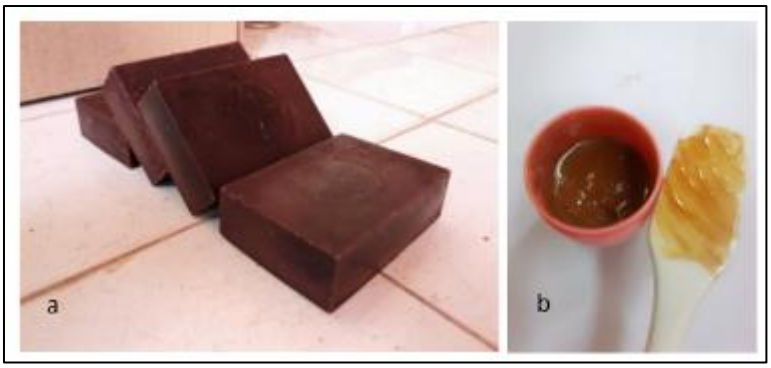

Figure 5 Topical antifungal soap (a) and cream (b) based on *C. alata* extract (leaves)

3.6. Skin tolerance

The purpose of the test was to identify possible signs of toxicity and skin irritation caused by applying the extract and formulated products to the skin. According to the Draize scoring system, the PII values obtained for the cream and soap were zero after 24 and 72 hours of application to the animals' skin. No signs of skin irritation were observed. Based on these results, the aqueous extract of the *C. alata* plant and its derivatives are considered non-irritating and non-toxic to the skin. Therefore, the resulting preparations are non-irritating and free of side effects.

The results above confirm that the *C. alata* aqueous extract, as well as the resulting cream and soap, are safe for skin application. They are neither irritating nor toxic. The absence of significant reactions in all animals indicates that the tested product is well tolerated by the skin.

3.7. Sterility of herbal cream and soap formulated

Sterility indicates that the cream and soap contain zero Colony-Forming Units (CFU) of bacteria, fungi, and yeast per gram. The tests found no live bacteria, fungi, or yeast in the cream and soap samples after six months of storage. The absence of microbial contamination ensures that the products are safe for consumers to use without risking infection or adverse reactions from microbial growth.

3.8. Antifungal activity of herbal cream and soap formulated

The antifungal efficacy of the herbal cream and soap formulations was assessed against *Candida albicans* using the Agar Well Diffusion method. A larger zone indicates higher antifungal efficacy. The product efficacy results revealed that the formulation based on *C. alata* leaf extract exhibited significant antifungal activity (Table 7).

Table 7 Efficacity of the cream and soap formulated in the growth inhibition of *C. albicans*

Formulation	**Zone of Inhibition (mm)- *Candida albicans***			
Concentrations	10 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Herbal Cream	13 ± 0.1	25.5 ± 0.3	30.04 ± 1.8	33.2 ± 0.7
Dermatological soap	10 ± 0.9	19.3 ± 0.5	22.6 ± 0.5	26.5 ± 2.5
Negative control				
Cream base	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Soap base	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Positive control				
Miconazol cream	13 ± 0.3	20 ± 1.2	28 ± 0.2	32 ± 0.6
Keto Plus soap	09 ± 0.04	15 ± 0.8	19 ±0.7	24 ±0.33

Inhibition of yeast growth in the different formulations (soap and cream) is dose-dependent. The diameter of the inhibition zone increases with increasing product concentration. The herbal cream was found to be the most effective formulation with significantly higher antifungal activity (DZI > 19 mm) than the commercially available cream (Miconazole). The soap was effective against *C. albicans* as well, but its activity was lower than that of the cream. In this trial, positive controls were also tested and inhibited *C. albicans* growth. In contrast, the negative control showed no inhibition.

Overall, the results suggest that the excipients used influence the antifungal inhibitory efficacy of the prepared cream and soap. Thus, the antifungal activity of the aqueous extract is reduced when combined with the soap base. Conversely, when included in an emulsion as a cream base, the antifungal activity against *C. albicans* is greater than that of the commercial cream. Therefore, it can be concluded that drug-excipient compatibility is present.

4. Discussion

The goal of this research was to develop and validate a dermatological antifungal cream and soap based on aqueous extract of C. alata leaves to treat skin diseases.

The aqueous extraction yield from the leaves of *C. alata* was approximately 25 %. This yield is notably higher than that of other plants in the same family, such as *Crotalaria cleomifolia*, which had a yield of 4.52 % from a similar extraction [40]. This high yield demonstrates *C. alata*'s significant potential for exploitation. Its widespread growth also ensures a sustainable supply, making it a promising candidate for various applications.

The results of the antimicrobial activity test on the aqueous extract of *C. alata* leaves agree well with those reported by other researchers. These results support the scientific fact that this plant has pharmacological properties, such as antioxidant, antifungal, and antibacterial properties [41,42]. This study demonstrated dose-dependent antibacterial (against *S. aureus*) and antifungal (against *C. albicans*) activities of the aqueous plant extract. The higher statistically significant difference in the activity of the leaf extracts at higher doses compared to Ketoconazole against *Candida albicans* could be due to the presence of some bioactive components in the extracts [43]. According to research conducted by Charlie Basset in 2011, the antifungal activity of *C. alata* may be due to the presence of molecules such as griseofulvin and nikkomycins [44]. Others have reported that this activity may be due to secondary metabolites, such as phytoalexins and phytoanticipins, that are present in the plant [45].

Based on these scientific data, a line of dermatological products in cream and soap form was developed using the aqueous extract of the plant's leaves. These products contain no synthetic preservatives, artificial fragrances, or ingredients of animal origin. The physicochemical, quality, toxicity, and sterility parameters of these products comply with the European Pharmacopoeia guidelines. The stability of the formulated cream and soap can be attributed to the antioxidant ability of the aqueous extract. These antioxidant metabolites can protect other ingredients from oxidation, which can cause changes in appearance, odor, and consistency. Thus, they contribute to the product's overall stability.

The present study demonstrates that topical antifungal creams and soaps made from the aqueous extract of *C. alata* leaves effectively inhibit the viability of *C. albicans*. These new antifungal products may serve as an alternative treatment for cutaneous infections, such as Candidiasis or ringworm. These *C. alata*-based products have the advantage of being sold at a lower cost than modern antifungals, such as ketoconazole.

5. Conclusion

These findings support the effectiveness of *C. alata* leaf-based herbal cream and soap as natural antifungal agents. The cream and soap demonstrated comparable antifungal activity to that of commercially available antifungal products. These results lend support to the traditional use of the leaves to treat skin diseases.

Compliance with ethical standards

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Disclosure of Conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

All the tests on animals were approved and in line with the standard established by Ethics Committee of the Pasteur Institute of Madagascar (IPM).

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