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Formulation and Comparison of the Anti-fungal Activity of Cream and Gel made from Methanolic Extract of *Lantana camara*

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Abstract: Toxic plants pose significant challenges to veterinary health, impacting cattle mortality rates and productivity. Among these, the *Lantana camara* stands out as a global menace due to its widespread proliferation and ornamental allure. This resilient shrub, with over 600 natural varieties, thrives across diverse ecosystems and geographic regions. Its phytoconstituents, including coumarins and flavonoids, contribute to its medicinal properties, offering therapeutic benefits such as hepatoprotection and anticancer activity. Notably, *L. camara* exhibits antifungal properties, making it a potential candidate for pharmaceutical formulations. Historically, antifungal agents like amphotericin B and triazoles have been pivotal in treating fungal infections. They target ergosterol synthesis and disrupt fungal cell walls, inhibiting nucleic acid and protein synthesis. However, the emergence of resistant strains necessitates the exploration of alternative sources. Natural antifungals, including those derived from plants, fungi, and bacteria, offer promising avenues for drug discovery. *L. camara*, with its diverse bioactive compounds, presents an intriguing option for herbal antifungal formulations. The formulation and evaluation of antifungal gel and cream using methanolic extracts of *L. camara* signify a step towards harnessing its therapeutic potential. These formulations offer novel approaches to combat fungal infections, providing alternatives to conventional antifungal medications. As the demand for effective antifungal treatments grows, exploring natural sources like *L. camara* becomes imperative. Herbal formulations, with their lower adverse effects and broad-spectrum activity, hold promise for the pharmaceutical industry. In conclusion, *L. camara* emerges as a valuable resource in the quest for novel antifungal agents. Its diverse pharmacological activities and ease of formulation into various delivery systems highlight its potential in combating fungal infections. Further research into the efficacy and safety of *L. camara*-based formulations is warranted to fully realize its therapeutic benefits in veterinary and human medicine.

Keywords: Antifungal, *Lantana camara,* Camarinic acid, Ergosterol, Methanolic extract, Gel, Cream

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Introduction

Veterinarians face challenges from toxic plants, which can cause increased mortality rates and reduced productivity in cattle. The toxicity level depends on plant species, ingested parts, toxins consumed, environmental conditions, and animal characteristics. *Lantana camara*, a toxic flora, is a prominent global threat (Kumbe *et al.*, 2024). *Lantana camara*, originating from India, has naturalized and spread across the Indian subcontinent. Its medicinal properties date back to Linnaeus's classification in 1753. With over 600 natural varieties, it offers aesthetic appeal (Negi *et al.*, 2019).

Lantana camara is an invasive species that spread across India, the Caribbean, Central, and Northern South America and 60 tropical and subtropical countries. Its natural habitat includes ecosystems in the Greater Antilles, Lesser Antilles, and coastal enclaves in the US and South America (Love *et al.*, 2009).

Morphologically, *Lantana camara* manifests as a resilient, multi-stemmed shrub adorned with thorny appendages, typically attaining heights of up to 6 feet. Characterized by its square stems adorned with bristly hair, the plant features simple, opposite leaves flaunting elongated petioles and oval blades exuding a distinctive aroma. Its diminutive yet vibrant flowers congregate in dense clusters, undergoing a chromatic metamorphosis post-blooming, while its diminutive fruits, bearing two nutlets, readily germinate in a variety of environments, particularly arid stony hills and black soils prevalent in central and southern India (Kato-Noguchi and Kurniadie, 2021).

Lantana camara is an adaptable species that thrives in diverse soil compositions and climatic conditions, thriving in areas with varying rainfall and temperatures. It thrives in disturbed habitats but faces challenges like frost, low temperatures, and saline soils (Tiwari *et al.*, 2021).

Plants contain phytoconstituents, which can provide various health benefits. *Lantana camara* oil contains biologically active substances like coumarins, lignans, flavones, and isoflavones. Camarinic acid and Camarilic acid are triterpenoid esters. Five compounds were found in *Lantana camara* oil, including beta-caryophyllene, alpha-humulene, zingiberene, and gamma-curcumin (Barik *et al.*, 2020).

Lantana camara, a plant known for its bioactive compounds and phytomolecules, has been used in traditional medicines for treating various illnesses. Its extracts have shown antifungal activity against *Aspergillus*, *Candida*, and *Colletrotrichum* species. The plant's antifungal properties are attributed to its acetone, chloroform, hot water, methanolic, and ethanolic components (Kulkarni *et al.*, 2019).

Amphotericin B was the only medication for fungal infections for 30 years. In the late 1980s and early 1990s, imidazoles and triazoles, including fluconazole, improved treatment efficiency. Fluconazole has treated over 16 million individuals, including 300,000 AIDS patients (Noël de Tilly and Tharmalingam, 2022).

Antifungal drugs, including azoles, polyenes, and allylamine/thiocarbamates, are primarily used to suppress ergosterol synthesis or contact with it. Amphotericin B (AMB), a polyene, interacts with ergosterol, causing fungicidal effects on *Aspergillus fumigatus*, *A. flavus*, and *Candida* species. Triazoles disrupt ergosterol biosynthesis, while echinocandins inhibit fungal cell wall production. Flucytosine (5-FC) influences protein and DNA synthesis (Hossain *et al.*, 2022).

Ergosterol is crucial for cell survival and fungal membrane fluidity. Antifungals target ergosterol by creating pores or blocking its production. Allylamines, like terbinafine and naftifine, inhibit fungal ergosterol production, reducing squalene and other sterol derivatives. Polyenes like nystatin and amphotericin B bind to ergosterol, rupture membranes, and release free radicals, reducing membrane permeability (Ivanov *et al.*, 2022).

Fungal cell wall components, including mannans, glycoproteins, glucans, and chitin, are crucial for adhesion and fungal disease. Antifungals inhibit glucan synthase, preventing chitin and β-glucan formation. Echinocandin class medications target glucan synthase, weakening cell wall. Chitin, a crucial part of the cell wall, is targeted by nikkomycin and polyoxins (Hasim and Coleman, 2019).

5-flucytosine, a compound found in RNA, can suppress nucleic acid synthesis, preventing DNA synthesis. It also inhibits fungal mitosis and slows cell proliferation. Antifungals like Itraconazole and AmB target reactive oxygen species (ROS), which can harm DNA, lipids, and proteins. Plant-derived antifungals, such as curcumin and resveratrol have antiviral, antibacterial, and antifungal activities. Fungal-derived antifungals, such as echinocandins and ibrexafungerp, are derived from naturally occurring chemicals produced by fungi. Actino-bacteria have antifungal characteristics and can generate up to 100,000 molecules with anti-bacterial activity. However, only polyenes are clinically relevant antifungals from Actinobacteria. Despite their potential, many *Bacillus* and *Pseudomonas* species are hazardous to hosts and have not been successful in developing clinically applicable antifungal drugs (Tong *et al.*, 2021).

Soil-derived microorganisms, also known as "microbial dark matter," make up 99% of natural ecosystems. They are classified into uncultured, *in situ* cultivable, and cultivated minority. Only 1% of bacteria are in the cultivated minority, where antibiotics originate. In situ cultivable bacteria require iChip isolation (Liu *et al*., 2022).

Azole resistance has not significantly impacted antifungal treatments, except for superficial Candidiasis infections. The growth of *Candida auris* is a concern, and finding new antifungals is crucial. Phytochemistry analysis of plant extracts suggests that phytochemicals may be more effective than artificial medications. Plants have been used as medicine since ancient times, and their chemical components induce physiological effects. Novel antifungal drugs with a wide range of action, fewer adverse effects, and economic benefits are needed. Herbal formulations have gained popularity due to their high activity and lower adverse effects (Argüelles *et al.*, 2024).

The present study investigates the formulation and evaluation of antifungal gel and cream using *Lantana camara*'s methanolic extract, comparing their antifungal activity and the wide range of formulations with their antifungal properties.

Materials and Methods

Materials

Distilled water, Carbopol 940P, Propylene glycol, Methylparaben, Methanolic extract of *Lantana camara*, Triethanolamine, Stearic acid, Cetyl alcohol, Potassium hydroxide, Liq. Paraffin, Glycerin, Methylparaben, and Lemon grass oil were used in this study.

Preparation of Lantana camara extract for Antifungal gel

The leaves of *Lantana camara* were collected, washed, and shade-dried for about a month to prevent chemical degradation and loss of volatile constituents. The dried leaves were then ground in a grinder, to obtain a coarse powder. To obtain homogeneity, the powder was passed through Sieve 80. The obtained powder was then used for extraction.

Methanol was used as a solvent for extraction. Extraction was carried out using the Soxhlet apparatus. The obtained extract was evaporated to dryness using a hot plate. The dried extract was collected and used for the preparation of the formulation.

Formulation of Antifungal Gel

50 ml of distilled water was taken in a beaker and 1 g of Carbopol 940P was added. The dispersion was kept aside for half an hour, to allow the Carbopol to swell. When the Carbopol swelled completely, the mixture was stirred vigorously to form a homogenous gel. In another beaker, the required quantity of methylparaben (0.3 g) was added to 5 ml of distilled water and heated in a water bath, to dissolve it. After the solution cooled

Table 1: Formulation of Antifungal Gel

Ingredients	Quantity
Lantana camara extract	1 g
Carbopol 940P	1.9 g
Methylparaben	0.3 g
Propylene glycol	5 ml
Triethanolamine	q.s. to adjust the pH
Perfume	q.s.
Distilled water	q.s. 100 ml

Table 2: Formulation of Antifungal Cream

Ingredients	Quantity
Stearic acid	13.5 g
Cetyl Alcohol	0.6 g
Potassium hydroxide	0.9 g
Liq. paraffin	4.5 g
Glycerin	7.5 g
Methylparaben	0.12 g
Lantana camara extract	27 ml
Distilled water	q.s. 100 ml

down 5 ml of propylene glycol was added. 1 g of dried methanolic Lantana camara extract was added to this mixture (Table 1). This complete mixture was then added to the Carbopol gel. The gel was mixed. To maintain the consistency of the gel, the remaining amount of Carbopol 940P was added with the remaining amount of distilled water to make up to 100 ml and allowed to rest. After some time, the gel was obtained, which was then continuously stirred using a mechanical stirrer to obtain the required consistency. Triethanolamine was added to maintain the optimum skin pH (6.8-7).

The *Lantana camara* extract was then added to the emulsion to make up the volume.

Preparation of Lantana camara extract for Antifungal Cream

The leaves of *Lantana camara* were collected, washed, and shade-dried for about a month to prevent chemical degradation and loss of volatile constituents. The dried leaves were then ground in a grinder, to obtain a coarse powder. To obtain homogeneity, the powder was passed through Sieve 80. The obtained powder was then used for extraction.

Methanol was used as a solvent for extraction. Extraction was carried out using the Soxhlet apparatus. The obtained extract was then filtered using Whatman filter paper. 18 ml of filtered extract was mixed with 36 ml of distilled water. This was then heated on a hot plate to evaporate the methanolic content. The obtained extract was then used in the preparation of the formulation.

Formulation of Antifungal Cream

Stearic acid was melted in a china dish at 70 °C, in a water bath with constant stirring. The other ingredients of the oil phase like cetyl alcohol, potassium hydroxide, and liq. paraffin was mixed in the melted stearic acid (Table 2). The components of the aqueous phase like glycerin were heated to about the same temperature in another beaker on the water bath. The aqueous phase was then added to the oil phase slowly, with continuous stirring to avoid the formation of lumps. Preservative methylparaben was then added after cooling at 40°C.

Evaluation of Antifungal Gel

Physical evaluation: Physical parameters such as color, odor, and appearance were checked.

Determination of pH: Using a digital pH meter, the gel's pH (Table 3) was measured by fully submerging the glass electrode in the gel system to cover the electrode. The measurement was done three times, and the mean of the three measurements was noted (Aiyalu *et al.*, 2016).

Spreadability test: The Spreadability (Table 3) test was performed using the Spreadability apparatus. Standard-sized glass slides were taken in two sets. One of the slides has the formulation for the herbal gel on it. The gel was sandwiched between the two slides in a region that was occupied by 7.5 cm along the slides when the second slide was positioned on top of the gel.

100 g weight was applied to the upper slides, pressing it evenly to create a thin layer between the two slides. After removing the weight, the extra gel that was sticking to the slides was scraped off.

The two slides in place were securely fastened to a platform so that only the upper slide could come off by itself when weight was applied to it. Carefully, a 90 g weight was fastened to the upper slide. It was recorded how long it took for the upper slide, acting as an effect of gravity, to move a distance and separate from the bottom slide. Three repetitions of the experiment were conducted, and the mean time was calculated.

Spreadability was calculated using the formula:
$S = M * L/t$

Where, S= Spreadability; M= Weight attached to the upper slide; L= Length of the slides; t= Time taken to move the distance.

Determination of Viscosity: Using a Brookfield viscometer (S-63, model LVDV-E) at 25 °C and a spindle speed of 10, 20, and 50 rpm, the viscosity of the gel (Table 3) was measured.

Evaluation of Antifungal Cream

Physical evaluation: Physical parameters such as color, odor, and appearance were checked.

Determination of pH: The standard buffer solution was used to calibrate the pH meter. With the use of a digital pH meter, the pH of 0.5 g of cream dissolved in 50 ml of distilled water was determined (Table 3) (Sharma *et al.*, 2019).

Spreadability test: Cream bases should not increase friction while rubbing and should spread easily without too much drag. The spreadability apparatus, consisting of a wooden board with a scale and two glass slides with a pan hung on a pulley, was used to calculate spreadability (Table 3).

To compress the excess material to a consistent thickness, it was sandwiched between the two glass slides and 100 g of weight was applied to the upper slide for 5 min. To the pan weight (90 g) was added. Spreadability was measured by recording the number of seconds needed to separate the two slides (Sabale *et al.*, 2011).

Spreadability was calculated using the formula:
$S = M * L/t$

Where, S= Spreadability; M= Weight attached to the upper slide; L= Length of the slides; t= Time taken to move the distance.

Determination of Viscosity: The cream's viscosity (Table 3) was assessed using a Brookfield viscometer at 10, 20, and 50 rpm and spindle number 63.

Comparison of Antifungal Activity: For the comparison of antifungal activity, the formulations were tested against fungus.

For this purpose, the culture media of Sabouraud Dextrose Agar was prepared. Culture media was prepared by dissolving 16.25 g of Sabouraud Dextrose Agar in 250 ml of distilled water. To this 2.5% agar was added. The mixture was heated till the powder completely dissolved.

The media was then autoclaved at 121 °C for 1 h. After autoclaving, the media was allowed to cool till the temperature reached 40-45 °C. The media

Table 3: Physical Characteristics of Antifungal Gel and Cream

Properties	Gel	Cream
Color	Greenish- brown	Light green
Odor	Lemon grass	Lemon grass
Appearance	Homogenous	Homogenous
pH	6.9±0.2	6.4±0.2
Spreadability (gcm/sec)	46.27±1.57	20.45±1.03
Viscosity		
@10 rpm	2400	9106
@20rpm	1458	5728
@50rpm	1200	2345

was then poured into the petri plates and allowed to settle at 37 °C for a day in an incubator.

A pure culture of the *Candida albicans* yeast fungus was obtained from a reliable source. To obtain isolated colonies, streaking of the *Candida albicans* culture was done onto the SDA plates with a sterile inoculation loop. The plates were incubated inverted at 37°C for 24-48 h to allow fungal development.

The test materials were prepared, which included antifungal gels and cream. To attain the necessary testing concentration, it was ensured that the test chemicals were diluted or suspended in an acceptable solvent or medium. Sterile water was used as a solvent.

The well diffusion assay was prepared once the *Candida albicans* cultures had grown. Using a sterile cork borer or pipette tip, uniformly spaced wells on the agar plates' surface were made. These wells acted as reservoirs for the test materials.

Using a sterile pipette, the test substances were carefully dispensed into the wells, ensuring that each well received an equal volume of the test solution. The test compounds were allowed to permeate through the agar for a few minutes.

After applying the test chemicals, the agar plates were incubated inverted at 37°C for 24-48 hours. This temperature and duration replicate the ideal growth conditions for *Candida albicans*, allowing enough time for test substance diffusion and consequent fungal growth suppression.

After the incubation period, the agar plates were carefully checked to see whether there were any zones of inhibition surrounding the test chemicals. Using a ruler or calipers, the diameter of each zone of inhibition was measured. The mean diameter of each test substance's zones of inhibition was calculated and established criteria were used to determine whether or not antifungal activity exists.

Results and Discussion

The experiment's outcomes comparing the efficacy of two formulations against *Candida albicans*, a well-known fungus species responsible for various diseases, revealed a significant discrepancy in performance. Despite predictions, the antifungal gel was ineffective against the *Candida albicans* strain tested. After careful study, no obvious zone of inhibition, the hallmark sign of antifungal efficacy, was found surrounding the gel application site. This lack of inhibition indicates a weakness in the gel's ability to limit the development or proliferation of the fungal species, showing its ineffectiveness as a viable treatment option for *Candida albicans* infection.

Conversely, the antifungal cream demonstrated promising antifungal activity against *Candida albicans* (Fig. 1). A clear zone of inhibition, 10 mm in diameter, surrounded the

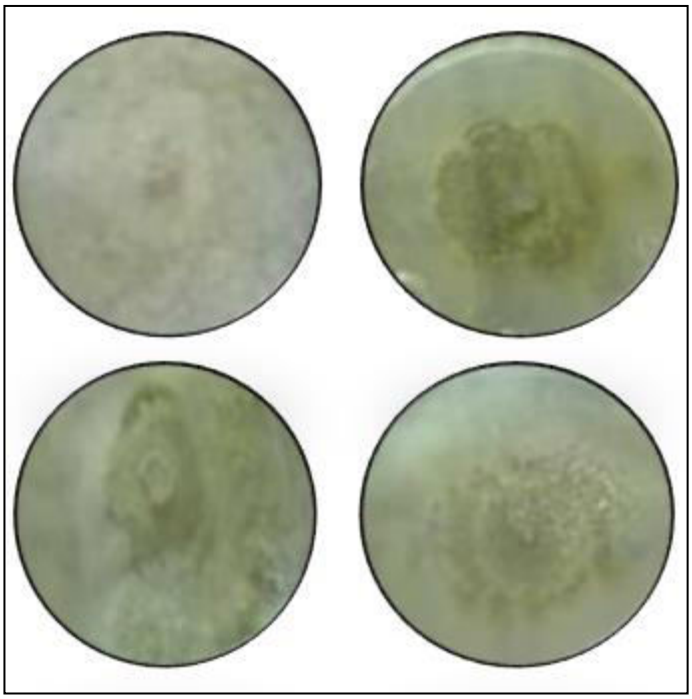

Fig. 1: Antifungal Activity of Cream and Gel made from Methanolic Extract of *Lantana camara.*

region where the cream was applied. This large zone of inhibition represents the cream's capacity to effectively block the growth and spread of the *Candida albicans* fungus. This strong antifungal reaction highlights the cream's potential as a feasible therapeutic intervention for treating infections caused by this specific fungus strain.

The distinction in outcomes between gel and cream formulations emphasizes the relevance of formulation design and ingredient selection in determining antifungal therapy success. Furthermore, our findings highlight the important necessity for extensive preclinical testing and evaluation of antifungal formulations to verify their efficacy and safety before clinical use.

The disparity in antifungal effectiveness seen between the cream and gel formulations against *Candida* species prompts several relevant questions. To begin, the compositional differences between the two formulations most certainly contributed significantly to the differential efficacy against fungus species. Creams are normally made up of a water phase distributed in an oil phase, which may include emulsifying agents and other active components. In contrast, gels are semisolid systems made up of a gelling ingredient dispersed in a liquid phase. These formulation changes can have an impact on the active antifungal agent's release and availability, as well as its ability to penetrate the fungal cell wall.

One probable explanation for the gel formulation's lack of antifungal action is insufficient drug solubility or inadequate drug release. Gels frequently have slower drug release kinetics than creams, which can lead to insufficient antifungal agent concentrations at the site of action. Furthermore, the gel matrix may restrict the drug's diffusion, reducing bioavailability and therapeutic efficacy. Further examination of the gel formulation's physicochemical features, such as drug solubility and release kinetics, shed light on its lack of efficacy against *Candida* species.

In contrast, the cream formulation's antifungal activity, as indicated by a zone of inhibition, indicates that the active antifungal substance effectively inhibited *Candida* species growth. A 10 mm zone of inhibition implies considerable

antifungal activity, while more formulation modifications may be required to increase efficacy. This could include increasing the active component concentration, changing the formulation to optimize medication release and penetration, or adding synergistic agents to boost antifungal efficacy.

The absence of antifungal activity in the gel formulation may be attributed to the loss of phytoconstituents responsible for the antifungal activity due to direct evaporation of the methanolic extract. On the contrary, the cream formulation showed activity due to the phytoconstituents being soluble in water too.

To summarize, the observed differences in antifungal activity between the cream and gel formulations emphasize the importance of formulation design and optimization in achieving therapeutic efficacy against fungal infections. More research is needed to understand the processes behind these discrepancies and improve the antifungal effectiveness of topical preparations against *Candida* species.

Conclusion

In the present work, the preparation of antifungal gel and cream from *Lantana camara* leaves extract using methanol as a solvent proved to be a promising approach. The process involved several key steps, including plucking and drying the leaves, crushing them, and then extracting the active components using a Soxhlet apparatus. The methanol extract was subsequently dried on a hot plate before being incorporated into both cream and gel bases.

Carbopol 940P was employed for gel preparation, ensuring a suitable consistency and texture. Evaluation tests were conducted to assess various parameters such as spreadability, viscosity, pH, physical characteristics, and antifungal activity.

Results indicated positive outcomes for the cream formulation, demonstrating its potential effectiveness in combating fungal infections, and exhibited notably higher antifungal activity compared to the gel. This variance could be attributed to differences in formulation and mode of application, with the cream likely providing better penetration and sustained release of the active compounds.

Despite the slightly lower efficacy of the gel, it still represents a viable option for antifungal therapy due to its ease of application and potential for targeted delivery. Further optimization of the gel formulation may enhance its antifungal activity, possibly through adjustments in concentration or the addition of synergistic compounds.

Overall, the development of an antifungal cream formulation from *Lantana camara* leaves extract presents a promising avenue for the treatment of fungal infections.

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