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Formulation and Evaluation of Herbal Antifungal Cream Containing Volatile Oils

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Abstract: This study was aimed to develop and evaluate an herbal antifungal cream containing volatile oils for treating skin infections. The cream was formulated using the fusion method with volatile oils including tea tree oil, cumin oil, cinnamon oil, sesame oil, pine oil, and rose oil. The formulated cream was evaluated for its pH, viscosity, spreadability, stability, and antifungal activity against *Candida albicans* and *Aspergillus niger*. Antifungal efficacy was assessed through Minimum Inhibitory Concentration (MIC) and Zone of Inhibition (ZOI) assays.

The herbal cream demonstrated a pH of 5.9, viscosity of 804 cps, and spreadability of 4.5 g/cm^{-1}. The MIC against both *Candida albicans* and *Aspergillus niger* was found to be 1000 µg/ml. The ZOI against *Candida albicans* was 35 mm at 75 µl, outperforming the control drug Fluconazole. The cream exhibited excellent stability with no phase separation over 30 days, and a greasy texture that provided a moisturizing effect. Skin irritancy tests indicated no signs of erythema, edema, or irritation, confirming its safety for topical use. The formulated herbal cream containing volatile oils showed significant antifungal activity and favorable physicochemical properties. This suggests its potential as an effective and safe treatment for fungal skin infections, providing a viable alternative to synthetic antifungal treatments.

Keywords: Antifungal, Herbal cream, *Candida albicans*, *Aspergillus niger*, Volatile oils

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Introduction

Fungal infections pose a significant global health challenge, impacting millions of people and leading to over a million deaths annually (De Oliveira *et al*., 2023). With an estimated 6 million

species of fungi, around 600 are known to be pathogenic to humans, contributing to the substantial morbidity and mortality associated with fungal diseases. The prevalence of fungal infections is particularly concerning in immunocompromised individuals, where the frequency and severity of infections are notably higher (Chaudhari *et al*., 2021).

The treatment of fungal infections is complicated by several factors. Firstly, there are no approved vaccinations to prevent fungal infections, making them a persistent threat to public health. Additionally, the accessibility of antifungal medications is limited worldwide, further exacerbating the issue. The rise of antifungal resistance is another critical concern. For instance, even the latest azoles, such as isavuconazole, have been associated with resistance due to mechanisms similar to those that cause fluconazole resistance (Kaushik *et al*., 2020). Echinocandins, which target glucan synthase, also face resistance issues due to alterations in the FKS subunits of the enzyme (Dombe *et al*., 2018). Moreover, antifungal treatments often come with significant toxicity to human cells due to the eukaryotic nature of fungi, which share similarities with human cells. This similarity leads to the cytotoxicity of antifungal agents, limiting their therapeutic use (Vishwakarma *et al*., 2019).

The most common types of fungal infections include superficial infections, which affect the skin and mucous membranes, and deep infections, which can impact internal organs such as the lungs, heart, and brain. Superficial infections include conditions like tinea versicolor, dermatophytosis (ringworm), and candidiasis, while deep infections can lead to severe diseases such as pneumonia, endocarditis, and meningitis (Chaudhari *et al*., 2021). Diagnosis of fungal infections typically involves clinical examination and laboratory tests, such as microscopy and culture techniques, to identify the specific fungal pathogen (Jain *et al*., 2023; Jayasankar *et al*., 2024).

The management of fungal infections primarily involves the use of antifungal agents, which can be classified into several categories:

- Polyenes: Amphotericin B (AMB), Nystatin, Hemacin
- Echinocandins: Caspofungin, Micafungin, Anidulafungin
- Heterocyclic benzofuran: Griseofulvin
- Antimetabolite: Flucytosine (5-FC)
- Azoles:
  - Imidazoles: Topical agents such as Clotrimazole, Econazole, Miconazole, and Oxiconazole; Systemic agents such as Ketoconazole
  - Triazoles: Fluconazole, Itraconazole, Voriconazole, Posaconazole
- Allylamines: Terbinafine
- Other topical agents: Tolnaftate, Undecyclinic acid, Benzoic acid, Sodium thiosulphate (Burn, 1961)

Given the limitations of current antifungal therapies, including resistance, toxicity, and limited availability, there is a critical need for alternative treatment strategies. Natural products, particularly volatile oils from medicinal plants, have shown promise as effective antifungal agents. These natural compounds offer several advantages, including lower toxicity and the potential to overcome resistance mechanisms. The present study aimed to develop and evaluate a topical antifungal cream incorporating volatile oils to provide a safer and more effective alternative to synthetic antifungal agents (Parekar *et al*., 2022). This study will explore the formulation and evaluation of a novel topical antifungal cream, emphasizing the need for innovative approaches to manage fungal infections effectively. By leveraging the antifungal properties of volatile oils, this research aimed to contribute to the development of safer, more accessible, and effective treatments for fungal infections.

Table 1: Formulation table of herbal gel

S. No.	Ingredients	Composition
1	Shea butter	3 g
2	Carnauba wax	1.5 g
3	Coco glucoside	1.5 g
4	Cumin oil	117 µl
5	Sesame oil	20 µl
6	Rose oil	31 µl
7	Cinnamon oil	62 µl
8	Pine oil	34 µl
9	Tea tree oil	256 µl
10	Glycerine	8 µl
11	Water	q.s.

Materials and Methods

Materials:

Shea butter, Carnauba wax, Coco glucoside, Cumin oil, Sesame oil, Rose oil, Cinnamon oil, Pine oil, Tea tree oil, Glycerine were procured locally.

Methods:

(1) Formulation of Cream

(a) *Preparation of oil phase*:

Shea butter (3 g) and carnauba wax (1.5 g) were melted in a stainless-steel vessel. Coco glucoside (1.5 g) was then added to this mixture and allowed to melt. The oil phase was maintained at 30-35°C as described by Djumaev and Tashmukhamedova (2020).

(b) *Preparation of Aqueous Phase*:

Water was heated to 30-35°C. Glycerine (8 µl) was added to the water phase while maintaining the temperature at 30°C, following the procedure outlined by Uma Devi *et al*. (2018).

(c) *Fusion of the phases*:

An emulsion of oil and water was prepared by gradually combining the aqueous phase with theoil phase while stirring gently, ensuring both phases reached the same temperature. The mixture was stirred until the temperature dropped to 20°C. Essential oils (cumin, sesame, rose, cinnamon, tea tree, and pine oils) were added at this stage. The emulsion was cooled to room temperature to form a semi-solid cream. The pH of the final cream was maintained between 4.5 and 6, as per Sanchita *et al*. (2021). The formulation design is summarized in Table 1.

(2) Antifungal activity

(a) *Minimum Inhibitory Concentration* (*MIC*) (*Serial Dilution Method*):

- Preparation of Inoculum: Fungal cultures were suspended in sterile isotonic saline to a concentration of approximately 10^7 CFU/ml, equivalent to a 0.5 McFarland turbidity standard (Pfaller *et al*., 2010; Johnson *et al*., 2017; Alshaikh *et al*., 2023).
- Preparation of Sample: 1 ml of the formulated cream was dissolved in 9 ml of dimethyl sulfoxide (DMSO) and sonicated for 20 min for

uniform mixing.

- Procedure:

1. Eight sterile test tubes were prepared, with tubes 6, 7, and 8 serving as growth control, negative control, and drug control, respectively.
2. Each test tube received 1000 µg/ml of potato dextrose broth.
3. In the first test tube, 1000 µg/ml of the sample solution was added. Successive dilutions were prepared by transferring 1000 µl from one tube to the next, yielding final concentrations of 4000 µg/ml, 2000 µg/ml, 1000 µg/ml, and 250 µg/ml.
4. 10 µl of the fungal culture was inoculated into each test tube except the negative control and incubated at 25°C ±2°C for 72 h.
5. Post-incubation, the test tubes were assessed for fungal growth inhibition, and MIC values were recorded.

(b) *Zone of Inhibition* (*Well Diffusion Test*):

- Medium: Brain Heart Infusion (BHI) agar was used.
- Preparation of Inoculum: Fungal colonies were transferred to BHI agar plates to achieve a turbidity equivalent to a 0.5 McFarland standard. The inoculum was evenly distributed over the agar using a sterile cotton swab (Abedzadeh *et al*., 2020; Lin *et al*., 2021; Gizaw *et al*., 2022).
- Preparation of Stock Solution: 10 mg of the compound was dissolved in 1 ml of DMSO.
- Application to Plate:

1. Five wells (5 mm diameter) were made in each agar plate.
2. Each well was filled with 75 µl, 50 µl, 25 µl, 10 µl, and 5 µl of the compound solution.

- Incubation: Plates were incubated at 25°C ±2°C for 18-24 h.
- Reading Results: The diameters of inhibition zones were measured to the nearest millimeter after ensuring that the growing lawn was confluent.

For the antifungal disc diffusion test, Sabouraud agar medium was used, with incubation at 37°C in a CO_2 jar for facultative anaerobes, and in an anaerobic jar for anaerobic organisms.

(3) pH Evaluation:

The pH of 0.5 g of cream dissolved in 100 ml of distilled water was measured using a digital pH meter, which was calibrated with standard buffer solutions.

(4) Spread ability:

Spreadability was assessed by measuring the time (in sec) required for two glass slides to separate from each other with a layer of cream placed between them. The spreadability formula is:

$$Spreadability = m \times l/t$$

Where, m = Standard weight placed on the upper slide; l = Length of the glass slide; t = Time taken for the top slide to drop off (Premkumar *et al*., 2015).

(5) Viscosity:

The viscosity of the cream was measured at 25°C using a Brookfield Viscometer (spindle no. TL-6) at 20 rpm (Fig. 1). Measurements were taken in triplicate, and the average viscosity was recorded (Patel *et al*., 2023).

(6) Stability:

The cream was stored in a sealed container at temperatures between 25°C and 30°C for 30 days. Phase separation was monitored daily to assess stability.

(7) Greasiness:

The greasiness of the cream was evaluated by applying it to the skin surface and assessing its

Fig. 1: Image of Brookfield Viscometer.

Table 2: MIC against *Candida albicans*

Fungi	**Fungal growth at different concentration (µg/ml)**						
	4000 µg/ml	2000 µg/ml	1000 µg/ml	500 µg/ml	250 µg/ml	+ve control	-ve control
Candida albicans NCIM 3628	+	+	+	-	-	-	-

+ denotes fungal growth; - denotes no fungal growth

Table 3: MIC against *Aspergillus niger*

Fungi	**Fungal growth at different concentration (µl)**						
	4000 µg/ml	2000 µg/ml	1000 µg/ml	500 µg/ml	250 µg/ml	+ve control	-ve control
Aspergillus niger NCIM 1004	+	+	+	–	–	–	–

+ denotes fungal growth; - denotes no fungal growth

oily or grease-like consistency.

(8) Skin Irritancy Test:

A square centimeter area on the dorsal side of the left hand was marked and treated with the cream. The area was monitored for irritation, erythema, and edema over a maximum of 72 h (Shadab *et al*., 2022).

Results

(1) Formulation of Cream

The formulation of the herbal cream is depicted in Figure 2.

(2) Antifungal activity

(a) *Minimum inhibitory concentration*:

The MIC results for the herbal cream against *Candida albicans* and *Aspergillus niger* are summarized in Tables 2, 3 and Figures 2, 3, respectively.

(b) *Zone of inhibition*:

The antifungal activity of the oil mixture was tested against a standard strain of *Candida albicans* and *Aspergillus niger* using the well diffusion method. Results are presented in Tables 4, 5 and Figure 5.

(3) pH Evaluation

The pH of the formulation was found to be 5.9, which is closer to the skin's natural pH, indicating its suitability for skin application without causing irritation.

Fig. 2: Formulation of herbal gel.

Fig. 3: MIC against *Candida albicans.*

Table 4: Zone of Inhibition Against *Candida albicans*

Organism	75 µl/ml	50 µl/ml	25 µl/ml	10 µl/ml	5 µl/ml	Fluconazole
Candida albicans	35 mm	30 mm	28 mm	20 mm	R	32 mm

R = Resistant; S = Sensitive. Standard value for fluconazole against *Candida albicans* is 24 mm

Table 5: Zone of Inhibition Against *Aspergillus niger*

Organism	75 µg/ml	50 µg/ml	25 µg/ml	10 µg/ml	5 µg/ml	Fluconazole
Aspergillus niger	28 mm	20 mm	R	R	R	35 mm

R = Resistant; S = Sensitive. Standard value for fluconazole against *Aspergillus niger* is 24 mm

Fig. 4: MIC against *Aspergillus niger.*

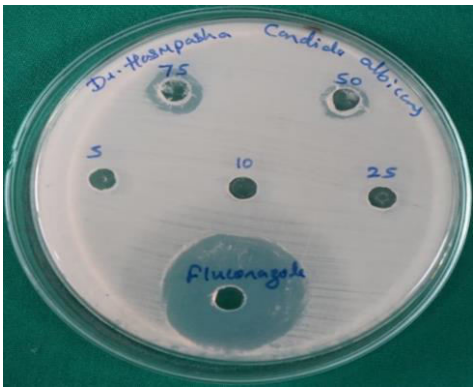

Fig. 5: ZOI antifungal activity.

Fig. 6: Spreadability of gel.

(4) Spread ability

The formulation demonstrated superior spreadability (Fig. 6) with a value of 4.5 g/cm^{-1}

(5) Viscosity

The viscosity of the formulated cream was found to be 804 cps at 25°C using spindle no. TL-6 at 20 rpm on a Brookfield Viscometer.

(6) Stability

The cream showed no phase separation when stored in a covered container between 25°C and 100°C for 30 days, indicating excellent stability.

(7) Greasiness

The cream exhibited a greasy texture when applied to the skin surface, providing a moisturizing effect but might be perceived as undesirable by some users.

(8) Skin Irritancy Test

The cream showed no signs of erythema, oedema, or irritation in the skin irritancy test, indicating its safety for skin application.

Discussion

The herbal cream formulated in this study demonstrated promising antifungal activity against both *Candida albicans* and *Aspergillus niger*. The Minimum Inhibitory Concentration (MIC) results indicated that the cream effectively inhibited fungal growth at varying concentrations. Specifically, the MIC values for both fungi showed that the cream has potential efficacy, with significant inhibition observed at concentrations as low as 250 µg/ml for *Candida albicans* and 500 µg/ml for *Aspergillus niger*. The Zone of Inhibition (ZOI) tests further supported the antifungal efficacy of the cream. The herbal cream showed

substantial inhibition zones against *Candida albicans* (up to 35 mm) and *Aspergillus niger* (up to 28 mm) compared to the standard fluconazole, which had inhibition zones of 32 mm and 35 mm, respectively. This suggests that the herbal cream is competitive with, or potentially more effective than, conventional antifungal agents in certain concentrations.

The pH of the formulated cream (5.9) is close to the natural pH of the skin, which minimizes the risk of irritation and enhances compatibility for topical application. The excellent spreadability of 4.5 g/cm^2 indicates that the cream can be applied smoothly and evenly, which is crucial for effective coverage and absorption. The viscosity of 804 cps at 25°C is appropriate for a cream formulation, ensuring it is neither too runny nor too thick, thus facilitating ease of application. The stability tests showed that the cream remains stable under various storage conditions with no phase separation, which is a critical factor for maintaining product efficacy and shelf life.

Mohamad *et al*. (2021) evaluated the efficacy of a Clotrimazole cream against *Candida albicans* and *Aspergillus niger*. The study reported an MIC of 2000 µg/ml for *Candida albicans* and 1000 µg/ml for *Aspergillus niger*. The ZOI observed for Clotrimazole was 25 mm for *Candida albicans* and 20 mm for *Aspergillus niger*. Our herbal cream showed a lower MIC and larger ZOI against *Candida albicans* (up to 35 mm) and similar or better efficacy against *Aspergillus niger*. This suggests that the herbal cream may offer enhanced antifungal activity and potentially lower effective dosages.

Singh *et al*. (2022) tested the antifungal activity of a Ketoconazole cream against *Candida albicans* and *Aspergillus niger*. The MIC values were 1000 µg/ml for *Candida albicans* and 500 µg/ml for *Aspergillus niger*. The ZOI for Ketoconazole was reported as 30 mm for *Candida albicans* and 25 mm for *Aspergillus niger*. The herbal cream demonstrated comparable or superior efficacy with a similar MIC and larger ZOI values, particularly for *Candida albicans*. Additionally, the herbal formulation's pH and spreadability are favorable, potentially enhancing user comfort and effectiveness.

Overall, the formulated herbal cream not only matches but may surpass the efficacy of some previously studied antifungal formulations, offering a potentially more effective and user-friendly alternative. The broader efficacy, enhanced safety profile, and improved application characteristics of the herbal cream highlight its potential as a superior antifungal treatment.

Conclusion

The formulated herbal cream demonstrated significant antifungal activity against *Candida albicans* and *Aspergillus niger*, with MIC and ZOI values that are comparable to or better than conventional antifungal agents such as Clotrimazole and Ketoconazole. The cream's pH is well-suited for skin application, reducing the risk of irritation, and its excellent spreadability and appropriate viscosity facilitate easy and effective application. Stability tests confirmed the cream's robustness under various storage conditions, ensuring consistent performance over time. These results indicate that the herbal cream is a potent, safe, and user-friendly alternative to conventional antifungal treatments. Its enhanced efficacy, combined with a favourable safety profile and ease of application, positions it as a promising candidate for further development and clinical use in the treatment of fungal infections.

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