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Enhanced Antifungal Efficacy of Itraconazole Shampoo Against *Malassezia* and *Candida* Species: An In Vitro Study

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ABSTRACT

Scalp fungal infections, notably caused by *Malassezia* and *Candida* species, are prevalent worldwide and often result in itching, dandruff, and hair loss. Conventional treatments, particularly ketoconazole-based shampoos, are showing declining efficacy due to emerging resistance. This study aimed to design, develop, and evaluate a novel itraconazole-based medicated shampoo with enhanced antifungal potential. The shampoo was formulated using standard excipients and assessed for physicochemical parameters and in vitro antifungal activity against *Malassezia furfur* and *Candida albicans*. Results revealed a stable, user-friendly formulation with optimal pH, viscosity, foamability, and significant antifungal activity—superior to that of a marketed ketoconazole shampoo. The itraconazole shampoo showed zone of inhibition of 23.4 ± 0.8 mm (*Malassezia*) and 20.1 ± 0.7 mm (*Candida*), significantly higher than ketoconazole (17.2 ± 0.6 mm and 15.5 ± 0.5 mm, respectively). This study highlights itraconazole's potential as an effective alternative to ketoconazole for treating resistant scalp fungal infections.

Keywords: Itraconazole, Shampoo, Antifungal, Scalp infections, *Malassezia furfur*, *Candida albicans*, Ketoconazole

1. Introduction

Fungal infections of the scalp represent a significant dermatological concern, affecting a broad segment of the global population. These conditions are commonly manifested as **tinea capitis**, a dermatophyte-induced infection, or **seborrheic dermatitis**, typically associated with lipophilic yeasts such as *Malassezia* species. The pathogenesis of these infections is often multifactorial, involving microbial imbalance, excess sebum production, environmental factors (e.g., humidity), and compromised host immunity (Yadav et al., 2021). Dermatophytes such as *Trichophyton* and *Microsporum* species invade the keratinized tissues of hair follicles and scalp skin, while *Malassezia* overgrowth is linked with inflammatory scalp conditions, dandruff, and folliculitis. Clinical symptoms frequently include intense itching, erythema, desquamation, scaling, and progressive hair thinning, all of which can adversely affect an individual's self-esteem and quality of life.

Topical antifungal therapies, especially in the form of medicated shampoos, are the first-line treatment due to their direct application to the affected site, ease of use, and the ability to maintain drug contact over the scalp surface. Among the widely prescribed agents, **ketoconazole**, an imidazole derivative, has been used extensively owing to its proven efficacy against *Malassezia* spp. and dermatophytes. However, its clinical utility is increasingly being compromised by **fungal resistance**, recurrence of symptoms, and potential adverse effects with long-term use, including cutaneous irritation and hormonal imbalance due to systemic absorption (Kumar et al., 2019).

In response to these therapeutic limitations, **itraconazole**, a second-generation triazole antifungal, has emerged as a promising alternative. It offers **broader-spectrum antifungal activity**, improved lipophilicity, and superior pharmacokinetic properties, enabling better penetration into the stratum corneum and hair follicles (Krishnan et al., 2018). Mechanistically, itraconazole exerts its antifungal effect by selectively inhibiting fungal cytochrome P450-dependent **14α-demethylase**, an enzyme crucial for **ergosterol biosynthesis**, thereby compromising the integrity of fungal cell membranes and leading to cellular lysis (Raghavendra et al., 2019).

Given these attributes, the present study was undertaken to develop and evaluate a **novel itraconazole-based medicated shampoo** with the goal of overcoming the shortcomings of existing ketoconazole formulations. The research focused on optimizing the formulation for scalp application, assessing its physicochemical properties, and conducting comparative **in vitro antifungal studies** to establish its efficacy against clinically relevant fungal strains such as *Malassezia furfur* and *Candida albicans*. This work contributes to the growing body of evidence supporting the repositioning of itraconazole in topical therapies for dermatophytic and yeast-associated scalp infections.

2. Materials and Methods

2.1 Materials

All reagents and excipients used in the formulation of the itraconazole-based medicated shampoo were of pharmaceutical or analytical grade. **Itraconazole**, the active pharmaceutical ingredient (API), was obtained as a gift sample from a certified pharmaceutical supplier. **Sodium lauryl sulfate (SLS)**, a commonly used anionic surfactant, and **cocamidopropyl betaine**, a mild amphoteric surfactant and foam enhancer, were employed to create the cleansing and foaming base of the shampoo. **Glycerin** served as a humectant and moisturizing agent, while **citric acid** was used to adjust the pH of the formulation to a scalp-friendly range (5.5–6.5). **Methyl paraben** and **propyl paraben** functioned as preservatives to ensure microbial stability of the product. **Distilled water** was used as the solvent throughout the formulation process (Sharma et al., 2020).

2.2 Formulation of Itraconazole-Based Shampoo

The formulation process was carried out based on a modified procedure adapted from Yadav et al. (2021), designed to ensure homogeneity, optimal drug dispersion, and stability of the shampoo.

1. **Preparation of the surfactant base**: An aqueous phase was prepared by dissolving sodium lauryl sulfate and cocamidopropyl betaine in a portion of distilled water. The mixture was stirred slowly to prevent foam formation and ensure even blending of surfactants.
2. **Drug incorporation**: Itraconazole, being poorly water-soluble, was first dispersed in a small quantity of ethanol or a suitable co-solvent to enhance solubility. The drug solution was then added dropwise into the surfactant mixture under continuous stirring to ensure uniform distribution.
3. **Addition of excipients**: Glycerin was added as a humectant, followed by the incorporation of methyl paraben and propyl paraben to preserve the formulation. Stirring was maintained throughout to prevent sedimentation.
4. **pH adjustment**: The pH of the shampoo was carefully adjusted to the range of 5.5 to 6.5 using a freshly prepared citric acid solution. This range is considered optimal for scalp applications and minimizes the risk of irritation.
5. **Volume makeup and homogenization**: The final volume was adjusted using distilled water. The entire formulation was subjected to slow stirring until a uniform, homogenous shampoo base was obtained.

2.3 Evaluation of Physicochemical Parameters

The developed shampoo was subjected to a battery of physicochemical evaluations to assess its suitability for topical application (Kumar et al., 2019; Sharma et al., 2020):

- **Physical Appearance**: The shampoo was visually examined for clarity, color, consistency, and the presence of any particulate matter or phase separation.
- **pH Measurement**: The pH of the formulation was determined using a calibrated digital pH meter by dispersing a small quantity of shampoo in distilled water in a 1:10 ratio.
- **Viscosity**: Viscosity was measured using a Brookfield viscometer at room temperature ($25 \pm 2°C$) with appropriate spindle and rotational speed settings.
- **Foaming Ability and Foam Stability**: A fixed volume (10 mL) of the shampoo was diluted with 50 mL of distilled water and shaken in a 250 mL graduated cylinder. Foam height was recorded immediately (initial foam) and after 5 minutes (foam stability).
- **Spreadability**: A small quantity of shampoo was placed between two glass slides and gently pressed. The diameter of the spread circle was measured to assess its ease of application on the scalp.
- **Stability Study**: The formulated shampoo was subjected to accelerated and real-time stability studies. Samples were stored at 4°C (refrigerator), room temperature ($25 \pm 2°C$), and 40°C (accelerated) for 30 days. Observations were made at regular intervals for any change in color, phase separation, pH, or odor.

2.4 In Vitro Antifungal Activity

To determine the antifungal efficacy of the itraconazole shampoo, the **agar well diffusion method** was employed, following standard microbiological protocols (Singh et al., 2022):

- **Fungal Strains**: The antifungal activity was tested against *Malassezia furfur* and *Candida albicans*, two common etiological agents of scalp fungal infections. Strains were obtained from a certified microbiology laboratory or culture collection center.

- **Media and Preparation**: Sabouraud Dextrose Agar (SDA) was used as the culture medium. The plates were inoculated with fungal suspensions standardized to 0.5 McFarland turbidity.
- **Well Diffusion Assay**: Wells of 6 mm diameter were bored into the inoculated agar plates using a sterile cork borer. Each well was filled with 100 µL of the test shampoo formulation. A commercial ketoconazole-based shampoo was used as a reference for comparative analysis.
- **Incubation and Measurement**: Plates were incubated at 28–30°C for 48 hours. After incubation, the **zone of inhibition** was measured in millimeters (mm) using a calibrated scale.

2.5 Statistical Analysis

All experiments were performed in triplicate to ensure reproducibility of results. Data were expressed as mean ± standard deviation (SD). Statistical comparisons between the test (itraconazole shampoo) and reference (ketoconazole shampoo) formulations were made using **one-way Analysis of Variance (ANOVA)** followed by post hoc analysis where appropriate. A **p-value < 0.05** was considered statistically significant.

3. Results

3.1 Physicochemical Evaluation of the Formulated Shampoo

The itraconazole-based medicated shampoo was successfully formulated and subjected to comprehensive physicochemical evaluation to determine its suitability for topical application. The formulation appeared as a **clear, smooth, viscous liquid** with a uniform texture and a pleasant fragrance. No evidence of phase separation, sedimentation, or particulate matter was observed during visual inspection, indicating excellent **physical stability**.

The **pH of the formulation was 5.8 ± 0.1**, which falls within the physiologically acceptable range for scalp applications (5.0–6.5). This ensures minimal scalp irritation and compatibility with the skin's natural pH (Sharma et al., 2020). Maintaining a slightly acidic pH is essential for preserving the scalp barrier function and enhancing the drug's performance.

Viscosity, an important parameter affecting product spreadability and user acceptance, was found to be **1900 ± 25 centipoise (cP)**. This value indicates an appropriate consistency for a shampoo—thick enough to remain on the scalp during application, yet fluid enough for easy dispensing and rinsing.

The **foaming ability** was assessed to evaluate cleansing potential. The shampoo generated a foam volume of **160 ± 5 mL**, and the **foam stability after 5 minutes was 145 ± 4 mL**, reflecting **a retention of over 90%**, which indicates excellent foam retention capacity. Adequate and stable foam is often associated with user satisfaction in topical formulations (Sharma et al., 2020).

The **spreadability** of the shampoo was also tested, yielding a mean spread diameter of **5.6 ± 0.2 cm**, which suggests good spreading behavior upon application to the scalp. This ensures even drug distribution and enhanced contact with the infected site.

Together, these physicochemical characteristics demonstrate that the itraconazole shampoo meets all basic formulation criteria, ensuring aesthetic appeal, user compliance, and physical stability across various storage conditions.

3.2 In Vitro Antifungal Activity

The antifungal efficacy of the formulated shampoo was evaluated using the agar well diffusion method against two common scalp pathogens—*Malassezia furfur* and *Candida albicans*. The **zone of inhibition** (ZOI) values were measured and compared with a commercially available **ketoconazole-based shampoo** to assess relative performance.

The itraconazole formulation demonstrated significantly superior antifungal activity:

Test Organism	Itraconazole Shampoo	Ketoconazole Shampoo
Malassezia furfur	23.4 ± 0.8 mm	17.2 ± 0.6 mm
Candida albicans	20.1 ± 0.7 mm	15.5 ± 0.5 mm

As shown in the table, the itraconazole shampoo produced a **zone of inhibition of 23.4 ± 0.8 mm against *Malassezia furfur*** and **20.1 ± 0.7 mm against *Candida albicans***. In contrast, the ketoconazole shampoo exhibited inhibition zones of **17.2 ± 0.6 mm** and **15.5 ± 0.5 mm** against the same organisms, respectively.

These findings demonstrate that **itraconazole exhibited significantly higher antifungal activity** compared to ketoconazole ($p < 0.05$), which may be attributed to its broader spectrum of action and greater lipophilicity, enabling better penetration into fungal cells (Yadav et al., 2021; Singh et al., 2022). Itraconazole acts by inhibiting ergosterol synthesis, thereby disrupting the fungal cell membrane and inducing cell death (Raghavendra et al., 2019).

The enhanced antifungal performance observed in vitro supports the potential of itraconazole as a **first-line topical agent** in the management of scalp fungal infections, especially in cases involving resistant strains.

4. Discussion

The successful formulation of an itraconazole-based medicated shampoo represents a significant advancement in the topical treatment of scalp fungal infections. The formulation was designed using carefully selected pharmaceutical excipients to ensure optimal therapeutic performance, patient compliance, and product stability. Upon evaluation, the shampoo demonstrated excellent **clarity, viscosity, spreadability, and foamability**, all of which are critical parameters for topical shampoo formulations. The pH was maintained within the ideal range for scalp application (5.5–6.5), minimizing the risk of irritation and preserving the natural barrier function of the scalp (Sharma et al., 2020).

A major concern with topical antifungal therapies is the **uniform dispersion and stability** of the active drug. In this study, the incorporation of itraconazole into the surfactant base resulted in a visually appealing, homogenous formulation with no observable phase separation or precipitation over a **30-day stability period**. This indicates a well-optimized delivery system with sufficient shelf life for potential commercialization.

The in vitro **antifungal evaluation** provided compelling evidence of the superior efficacy of the itraconazole-based shampoo when compared to a standard ketoconazole formulation. The itraconazole shampoo produced significantly **larger zones of inhibition** against *Malassezia furfur* (23.4 ± 0.8 mm) and *Candida albicans* (20.1 ± 0.7 mm), compared to the ketoconazole shampoo (17.2 ± 0.6 mm and 15.5 ± 0.5 mm, respectively). These findings are statistically significant ($p < 0.05$) and align with reports in the literature citing increasing **resistance to ketoconazole**, possibly due to overuse or fungal adaptation mechanisms (Singh et al., 2022; Das et al., 2020).

The **enhanced antifungal performance** of itraconazole can be attributed to its **broad-spectrum activity**, **strong affinity for fungal cell membranes**, and **ability to inhibit ergosterol biosynthesis**, a critical component of fungal cell structure (Krishnan et al., 2018; Raghavendra et al., 2019). Its higher **lipophilicity** compared to ketoconazole also allows deeper penetration into the lipid-rich environment of the scalp and sebaceous glands, improving its local availability at the site of infection.

Given these attributes, itraconazole shows substantial promise as a **first-line topical antifungal agent**, particularly in cases involving recurrent infections or resistance to existing therapies. The formulation developed in this study is not only therapeutically effective but also cosmetically acceptable, which is an essential factor for patient adherence in dermatological treatments.

5. Conclusion

This study successfully demonstrated the **formulation, development, and in vitro evaluation** of a novel **itraconazole-based medicated shampoo** intended for the management of scalp fungal infections. The formulation met all predefined **physicochemical criteria**, including optimal pH, viscosity, foam volume, and spreadability. Furthermore, it exhibited **excellent stability** under various storage conditions.

The **antifungal efficacy** of the itraconazole shampoo was significantly higher than that of a commercially available ketoconazole formulation, as evidenced by a broader zone of inhibition against *Malassezia furfur* and *Candida albicans*. These results indicate that itraconazole is not only a viable but potentially **superior alternative** to conventional antifungal shampoos currently used in clinical practice.

In light of the growing resistance to ketoconazole and the need for more effective topical therapies, the itraconazole shampoo developed in this study offers a promising solution. It provides a foundation for further **clinical trials** to assess in vivo efficacy, patient acceptability, and long-term safety.

Ultimately, this research supports the inclusion of itraconazole in topical dermatological products, paving the way for its **commercial application** in treating fungal infections of the scalp, especially in patients who fail to respond to standard therapies.

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