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Formulation, Evaluation, and Stability Studies of a Topical Clotrimazole and Zinc Oxide Suspension with Enhanced Skin Protective Properties

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Abstract

Background: *Clotrimazole is a well-known antifungal agent frequently used in the treatment of various superficial fungal infections, while Zinc Oxide is a widely accepted skin protectant with soothing, astringent, and antimicrobial properties. Combining these agents in a topical suspension offers a dual therapeutic approach, enhancing both antifungal efficacy and skin protection.* ***Objective:*** *The objective of this study was to formulate, evaluate, and conduct stability studies on topical suspensions containing Clotrimazole and Zinc Oxide, aimed at enhancing skin protective properties while maintaining effective antifungal activity.* ***Methods:*** *By varying the concentration of suspending agents, four topical suspension formulations (F1-F 4) were developed. These formulations were evaluated for organoleptic characteristics, pH, viscosity, spreadability, drug content, and in vitro antifungal activity against Candida albicans. Viscosity was determined using a Brookfield viscometer, drug content was determined by UV-Visible spectrophotometry at 261 nm, and pH was measured with a calibrated digital pH meter. The agar well diffusion method was used to assess antifungal activity. According to ICH Q1A(R2) stability studies were performed under both real-time and accelerated conditions for three months.* ***Results:*** *All formulations showed acceptable organoleptic properties and pH values suitable for topical use. Formulation F3 exhibited optimum viscosity, satisfactory spreadability, the highest drug content (99.4%), and superior antifungal activity (22.0 mm zone of inhibition). Stability studies confirmed no significant changes in evaluated parameters over the study period.* ***Conclusion:*** *The optimized formulation F3 demonstrated enhanced antifungal efficacy and skin protective properties, indicating its potential as a safe and effective topical treatment for fungal skin infections.*

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Keywords: Clotrimazole, zinc oxide, topical suspension, antifungal activity, formulation development, stability studies, *Candida albicans*

INTRODUCTION

Topical drug delivery systems provide localized therapeutic effects at the site of infection or inflammation, reducing systemic side effects and improving patient compliance. Among these, suspensions offer significant advantages in delivering poorly water-soluble drugs while ensuring adequate skin contact and therapeutic activity [1]. Clotrimazole is a widely used antifungal agent effective against dermatophytes, yeasts, and molds. It disrupts fungal cell membranes by inhibiting ergosterol synthesis. Despite its efficacy, Clotrimazole's conventional

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topical formulations often suffer from poor spreadability and lack of soothing effects on inflamed skin [2]. Zinc Oxide is traditionally known for its astringent, soothing, and skin-protective properties, used in treating various dermatological conditions [3]. Its combination with Clotrimazole in a suspension form could offer enhanced antifungal and protective effects, particularly in treating fungal infections associated with skin irritation. The objective of this study was to formulate, evaluate, and perform stability studies on a topical suspension containing Clotrimazole and Zinc Oxide using sodium carboxymethyl cellulose as a suspending agent [4]. The formulation was assessed for physicochemical properties, antifungal activity, and stability under accelerated and real-time conditions [5].

CLOTRIMAZOLE

By effectively obstructing the biosynthesis of ergosterol, which is a critical component in the formation of fungal cell membranes, the broad-spectrum imidazole antifungal agent known as clotrimazole plays a significant role in disrupting the essential processes necessary for fungal survival and proliferation. It has been extensively documented and evidenced to exhibit substantial efficacy against various species of yeasts, particularly those classified within the genus Candida, as well as dermatophyte organisms that include the genera Trichophyton and Microsporum. Clotrimazole possesses a well-documented and established safety profile, which has contributed to its status as one of the most frequently employed topical therapeutic agents for the management of tinea infections, as well as candidal infections of the skin. For example, clotrimazole is regularly prescribed for the treatment of dermatophytoses and cutaneous candidiasis, being utilized in both immunocompromised individuals and those who are generally healthy and robust. Nevertheless, as has been previously noted in the literature, the conventional topical formulations of clotrimazole may not possess the requisite capabilities to effectively penetrate and deliver therapeutic concentrations into deeper layers of skin lesions, thus prompting ongoing research and developmental efforts aimed at enhancing its bioavailability and overall efficacy.

Zinc Oxide

Zinc oxide, an inorganic compound, has been employed for a considerable duration as a barrier-protective agent in the field of dermatology, serving a multifaceted role in the management of various skin conditions. The compound functions as a mild astringent and emollient within topical formulations, which encompass products, such as calamine lotions and preparations, designed for the alleviation of diaper rash, thereby establishing a physical barrier that protects the skin from excessive moisture and environmental allergens. Furthermore, zinc oxide is characterized by its extensive antibacterial and antioxidant properties, which contribute to its therapeutic effectiveness in dermatological applications. It demonstrates notable antibacterial properties and, to a certain extent, antifungal capabilities, which can effectively diminish the presence of opportunistic pathogens on compromised skin surfaces, and it has been scientifically validated to expedite the healing processes of skin lesions and ulcers. These beneficial attributes serve to bolster the skin's innate defenses against potential infections while concurrently ameliorating inflammatory responses. Thus, within the context of various dermatoses, zinc oxide exhibits auxiliary antibacterial advantages alongside its primary function of providing symptomatic relief.

Combining Clotrimazole with Zinc Oxide

The amalgamation of zinc oxide and clotrimazole within a singular topical formulation may potentially yield synergistic therapeutic effects that enhance their individual efficacies. In this combined formulation, zinc oxide would play a protective role by preserving and repairing the integrity of the epidermal barrier while simultaneously providing additional antibacterial support, whereas clotrimazole would exert potent antifungal action against the underlying infectious agents responsible for the skin condition. While clotrimazole effectively eradicates fungal organisms, the protective barrier established by zinc oxide may serve to significantly reduce transepidermal water loss and alleviate irritation, thereby promoting a more rapid resolution of skin lesions. Moreover, it is well-established within the scientific community that combined therapies are associated with an expanded antibacterial spectrum and a reduction in the potential for the development of antimicrobial

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resistance. By simplifying the treatment regimen, a singular combined formulation may additionally enhance patient convenience and adherence to the therapeutic protocol. It is anticipated that the complementary actions of these two agents will collectively augment the overall therapeutic efficacy when utilized in conjunctions. Therefore, the primary objective of the proposed topical clotrimazole-zinc oxide suspension is to harness these advantageous properties to optimize clinical outcomes in the management of fungal skin infections.

ANTI FUNGAL THERAPY STRATEGIES

Contemporary antifungal treatment modalities employ both topical and systemic pharmacological agents. Topical formulations, which encompass creams, ointments, lotions, and suspensions, deliver elevated local concentrations of medication while minimizing systemic exposure, thus rendering them particularly effective for superficial and localized dermal infections. The prevalent categories of topical antifungals include azoles (such as clotrimazole and miconazole), allylamines (such as terbinafine and naftifine), and polyenes (like nystatin). Due to their extensive efficacy against yeasts and dermatophytes, topical azoles, notably clotrimazole, are frequently employed in clinical practice. Systemic agents, including amphotericin B, echinocandins, and oral or parenteral triazoles, are reserved for the treatment of invasive, refractory, or disseminated mycoses. Notwithstanding their therapeutic efficacy, topical treatments are accompanied by certain limitations. Traditional formulations often manifest variable drug concentrations within the affected tissues and exhibit restricted dermal penetration, necessitating extended treatment durations (occasionally spanning weeks), which may compromise patient adherence. Furthermore, the protracted nature of treatment regimens, along with the reliance on monotherapy, has contributed to the development of resistance in dermatophytes and Candida species; incomplete resolution and recurrence remain prevalent, particularly in instances of suboptimal patient compliance.

MATERIALS AND METHODS

Material

As a generous gift sample from Teva Pharmaceutical Pvt. Clotrimazole was obtained. Ltd., Mumbai, India. Zinc Oxide was taken from Merck Specialities Pvt. Ltd., Mumbai, India. Loba Chemie Pvt. purchased Sodium Carboxymethyl Cellulose (NaCMC). Ltd., Mumbai, India. SD Fine-Chem Ltd., Mumbai, India, supplied glycerin. Propylparaben and Methylparaben were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Sodium citrate and Citric acid were procured from Qualigens Fine Chemicals, Mumbai, India. All other reagents and solvents used were of analytical reagent (AR) grade, and distilled water was prepared freshly in-house.

Method

Formulation Development

The topical suspension was developed by initially selecting Clotrimazole as the antifungal agent and Zinc Oxide for its skin protective, astringent, and anti-inflammatory properties, aiming to create a dual-action formulation for enhanced topical applications. Sodium Carboxymethyl Cellulose (NaCMC) was incorporated as a suspending agent due to its excellent viscosity-enhancing and stabilizing characteristics, while Glycerin served as a humectant to retain skin moisture [6]. Methylparaben and Propylparaben were employed as preservatives to inhibit microbial growth and prolong the formulation's shelf life, with Citric acid and Sodium citrate included as buffering agents to maintain pH in the dermatologically acceptable range of 6.0–6.5. The formulation process began by dispersing NaCMC in a portion of distilled water under continuous mechanical stirring (Remi Motors, India) to prepare a smooth, lump-free dispersing medium. Clotrimazole and Zinc Oxide were accurately weighed and triturated together in a mortar and pestle to obtain a homogenous fine powder, which was then blended with Glycerin to form a uniform paste. This paste was gradually incorporated into the dispersing medium under continuous stirring to ensure even distribution of the active ingredients. Separately, Methylparaben and Propylparaben were dissolved in warm water to form preservative solutions, which were added to the suspension with thorough mixing [7]. The pH of the formulation was adjusted by adding a freshly prepared buffer solution containing Citric acid and

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Sodium citrate dropwise. Finally, the volume was made up with distilled water, and the suspension was homogenized at 5000 rpm for 5 minutes using a high-speed homogenizer (IKA T18 Digital, Germany) to ensure uniformity. The prepared formulations were transferred into amber-colored glass bottles to prevent light-induced degradation, tightly sealed, and stored at room temperature for further evaluation [6]. Four formulations (F1–F4) were developed by varying the concentration of Sodium CMC to assess its influence on suspension stability and consistency, with their composition details provided in Table 1.

Table 1. Composition of topical clotrimazole and zinc oxide suspensions.

Ingredients	F1 (% w/v)	F2 (% w/v)	F3 (% w/v)	F4 (% w/v)
Clotrimazole	1.0	1.0	1.0	1.0
Zinc Oxide	5.0	5.0	5.0	5.0
Sodium CMC	0.5	1.0	1.5	2.0
Glycerin	5.0	5.0	5.0	5.0
Methylparaben	0.1	0.1	0.1	0.1
Propylparaben	0.05	0.05	0.05	0.05
Sodium citrate	0.05	0.05	0.05	0.05
Citric acid	0.05	0.05	0.05	0.05
Distilled Water (up to)	100	100	100	100

EVALUATION OF FORMULATED SUSPENSIONS

Organoleptic Characteristics

Each formulation was visually inspected for color, odor, consistency, and homogeneity [8].

pH Measurement

Each formulation pH was measured using a pH meter (digital pH 700, LabIndia, India) calibrated with standard buffer solutions of pH 4.0, 7.0, and 9.2 prior to use [9].

Viscosity Determination

Measurements were performed in triplicate using a spindle no. 64 at 20 rpm with a Brookfield Viscometer (Model LVDV-II+, Brookfield Engineering Labs, USA) at 25°C [10].

Spreadability Test

The glass slide method was used to determine spreadability. The suspension preweighed to a quantity of 1 g was placed between two glass slides; the top slide has placed on it a weight of 500 g for 5 min. Diameter of the spread suspension was measured in centimeters, and the mean of three determinations was recorded [11].

Drug Content Estimation

The required quantity (1 g) of suspension was accurately measured, dissolved in 50 ml of methanol with an ultrasonic bath (PCI Analytics, India) at 15 minutes and passed through a Whatman filter no. 41 and properly diluted. Spectrophotometric analysis at 261 nm using UV-Visible spectrophotometer (UV-1800, Shimadzu Corporation, Japan) was performed to analyze Clotrimazole content. An additional 12 triplicate samples were cultured [12].

In Vitro Antifungal Activity

Antifungal activity at an agar well diffusion method was determined against *Candida albicans*. The fungal strain was inoculated on Sabouraud Dextrose Agar (SDA) plates and making the wells using a sterile corn borer (6 mm). The plates were incubated at 28°C for 48 hours with formulation samples (100 µl) placed in the wells. One millimeter of the zone of inhibition was measured using a digital Vernier caliper [13].

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Stability Studies

Optimized formulation (F3) was subjected to the stability studies as per ICH Q1A(R2) under accelerated (40 ± 2°C at 75% RH ± 5% for 3 months) & real time (25 ± 2°C at 60% RH ± 5% for 3 months) condition using stability chamber (Thermo Lab, India). At 0, 1, 2, and 3 months, samples were withdrawn for appearance, pH, viscosity, spreadability, drug content, and antifungal activity [14].

RESULT AND DISCUSSION

Organoleptic Characteristics

All formulations (F1, F2, F3, F4) were visually inspected for color, odor, consistency, and homogeneity. All the formulations were found to be white, smooth, and homogenous, with no visible signs of separation or settling over time. These observations indicate that the suspensions are well-prepared and stable in terms of appearance.

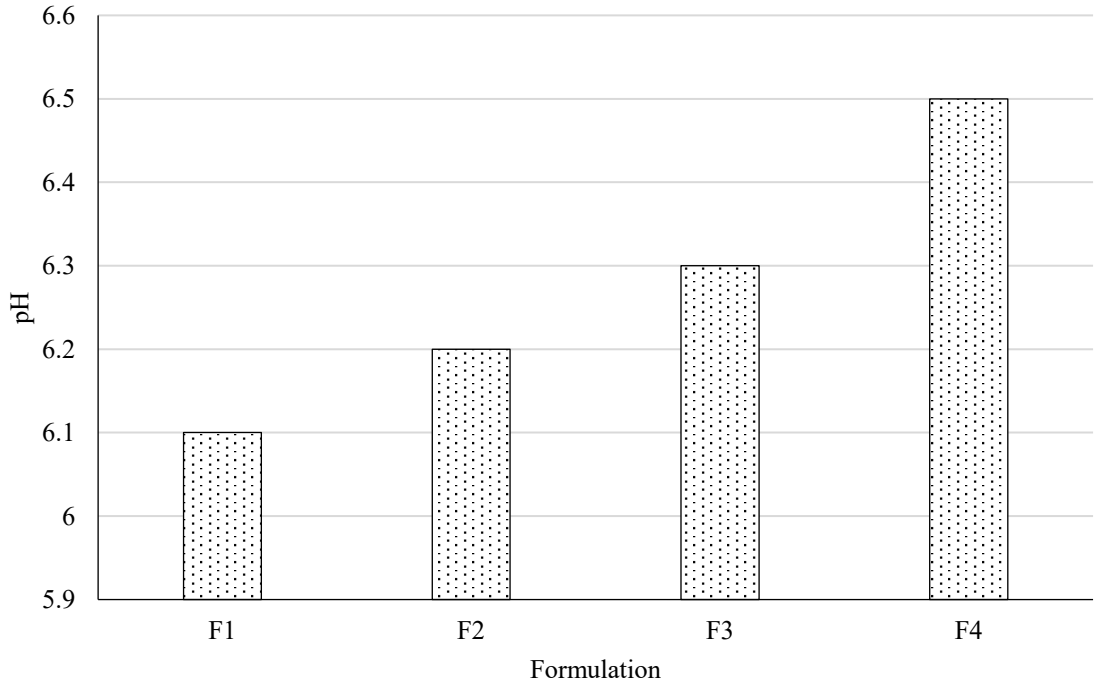

Figure 1. pH of formulation.

pH Measurement

The pH of each formulation was measured, and the results are presented in Table 2. The pH of the formulations ranged from 6.1 to 6.5. Specifically, F1 had a pH of 6.1, F2 showed a pH of 6.2, F3 had a pH of 6.3, and F4 exhibited a pH of 6.5 (Figure 1). The slight increase in pH with each subsequent formulation could be attributed to the presence of more alkalizing agents or buffering agents in higher concentrations in formulations F3 and F4. The pH of all formulations was within the ideal range for skin applications (5.5–7), indicating their compatibility with the skin [15–17].

Viscosity Determination

The viscosity of the formulations, which is crucial for determining the ease of application and spreadability, increased as the concentration of the suspending agent increased (Table 2). The viscosity values ranged from 610 ± 5.0 cP in F1 to 980 ± 6.5 cP in F4 (Figure 2). F1 showed the lowest viscosity, indicating a thinner suspension, while F4 exhibited the highest viscosity, which is generally expected with higher concentrations of stabilizing agents. The increase in viscosity suggests that the suspensions become thicker and more stable as the formulation progresses from F1 to F4.

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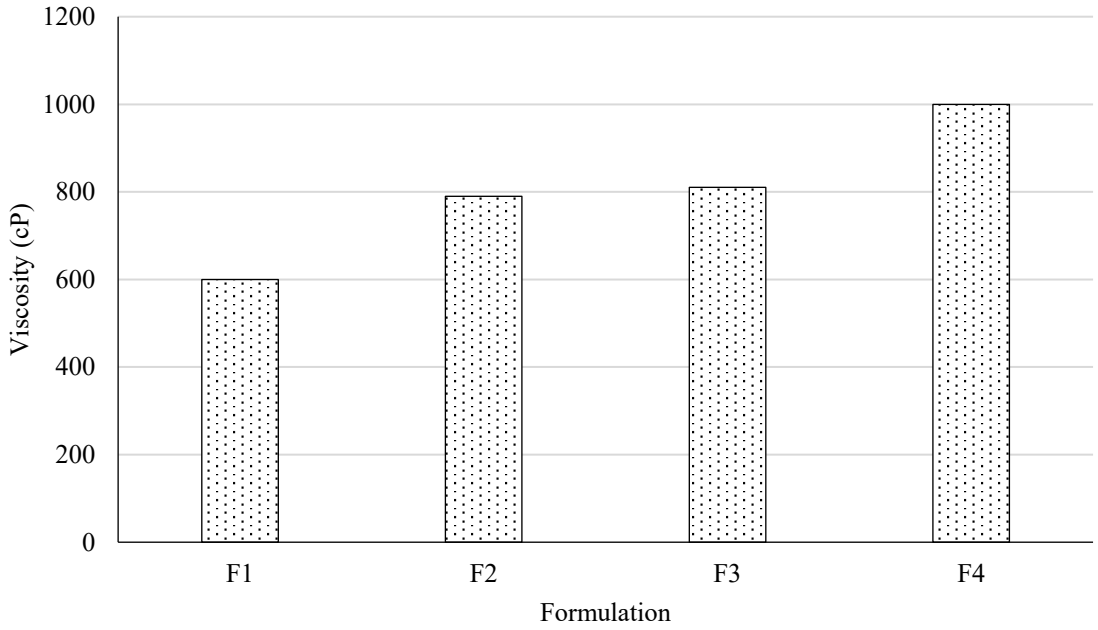

Figure 2. Viscosity of formulations.

Spreadability Test

Spreadability is an important parameter that determines how easily a topical formulation can be applied over the skin. The spreadability of the formulations decreased with increasing viscosity. As shown in Table 2 and Figure 3, F1 had the highest spreadability of 7.1 ± 0.06 cm, while F4 showed the lowest spreadability of 5.8 ± 0.09 cm. This is consistent with the fact that higher viscosity suspensions tend to spread less easily. The reduced spreadability of F4 indicates that while the suspension is more stable, it might require more effort for spreading on the skin compared to F1.

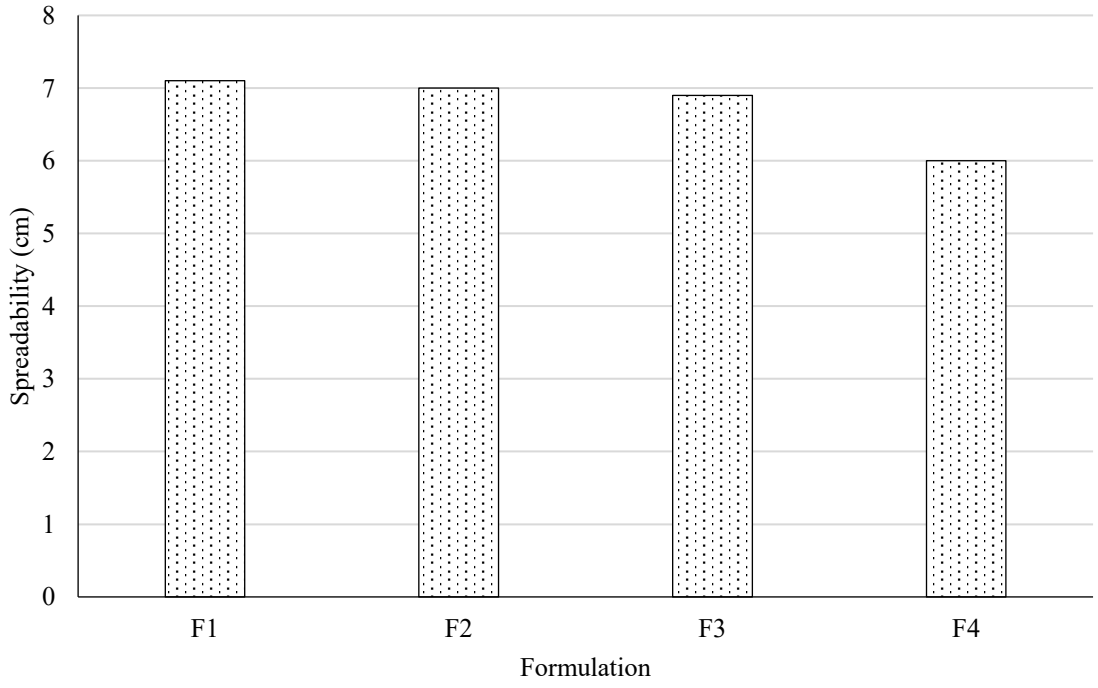

Figure 3. Spreadability of formulations.

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In Vitro Drug Content Estimation

Drug content was determined spectrophotometrically and the results, as shown in Table 2, revealed that all formulations had a drug content close to the target of 100%. The drug content varied between 98.5 ± 0.4% for F1 and 99.4 ± 0.3% for F3 (Figure 4). These values indicate that the drug was successfully incorporated into all formulations with minimal deviation from the intended drug load, ensuring uniformity and consistency in drug delivery across all formulations [17–18].

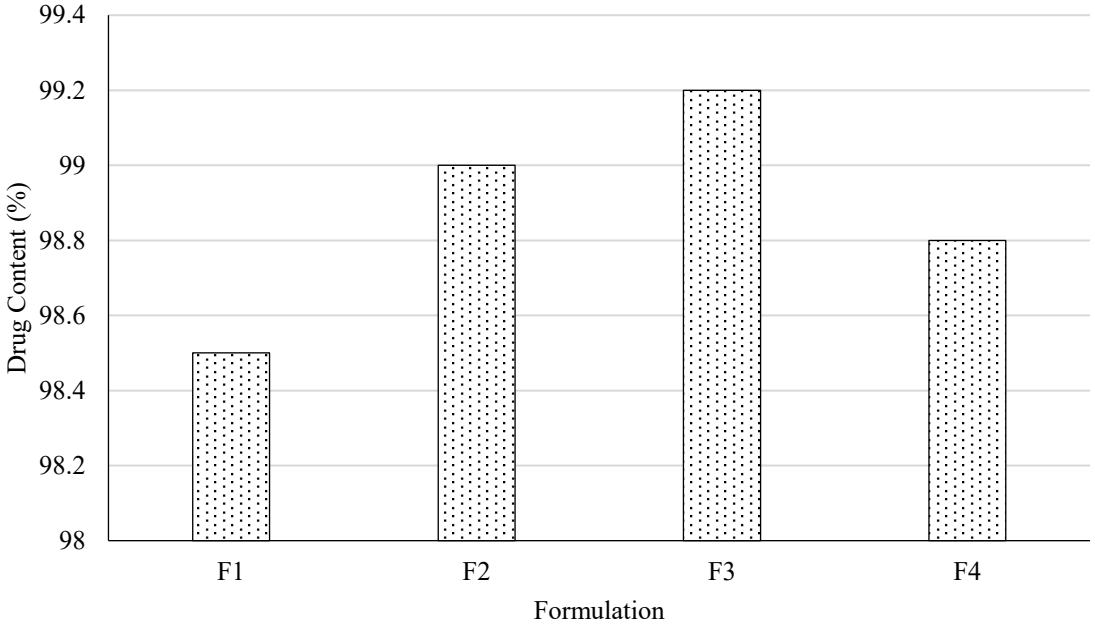

Figure 4. Drug content of formulations.

Sedimentation

After 24 hours of undisturbed storage, formulation F3 exhibited a sedimentation volume of 0.11, indicating moderate sediment formation while maintaining satisfactory physical stability. Additionally, the sediment was easily redispersed upon gentle shaking, restoring uniformity without any caking or hard sediment formation. This performance can be attributed to the optimized concentration of the suspending agent in F3, which effectively controlled particle settling and ensured dispersion stability. Comparatively, other formulations exhibited slightly higher sedimentation volumes, indicating relatively faster settling rates [15].

In Vitro Antifungal Activity

Antifungal activity of the formulations against *Candida albicans* was assessed by agar well diffusion method. It was found that as formulation concentration increased so the zone of inhibition increased. F1 displayed zone of inhibition of 17.6 ± 0.3 mm; F2, 20.5 ± 0.4 mm; F3, 22.0 ± 0.5 mm; and F4, 21.2 ± 0.4 mm (Figure 5). Greater zone of inhibition signifies that as concentration of Clotrimazole increased within the formulation, it exhibited its antifungal action better. However, F4 did not demonstrate a significantly larger zone of inhibition than F3, implying that a higher concentration of Clotrimazole may not enhance antifungal activity beyond a certain point [16].

Stability Studies

3 months' stability studies were done for both accelerated (40°C ± 2°C / 75% RH ± 5%) and real time (25°C ± 2°C/60% RH ± 5%). Upon visual inspection, it was evident that there was no change in the appearance, pH, viscosity, spreadability and drug content of the formulations over the study period. Physical and chemical changes were insignificant, and all formulations sustained their antifungal efficacy. This indicates the formulations were stable for the specified storage conditions.

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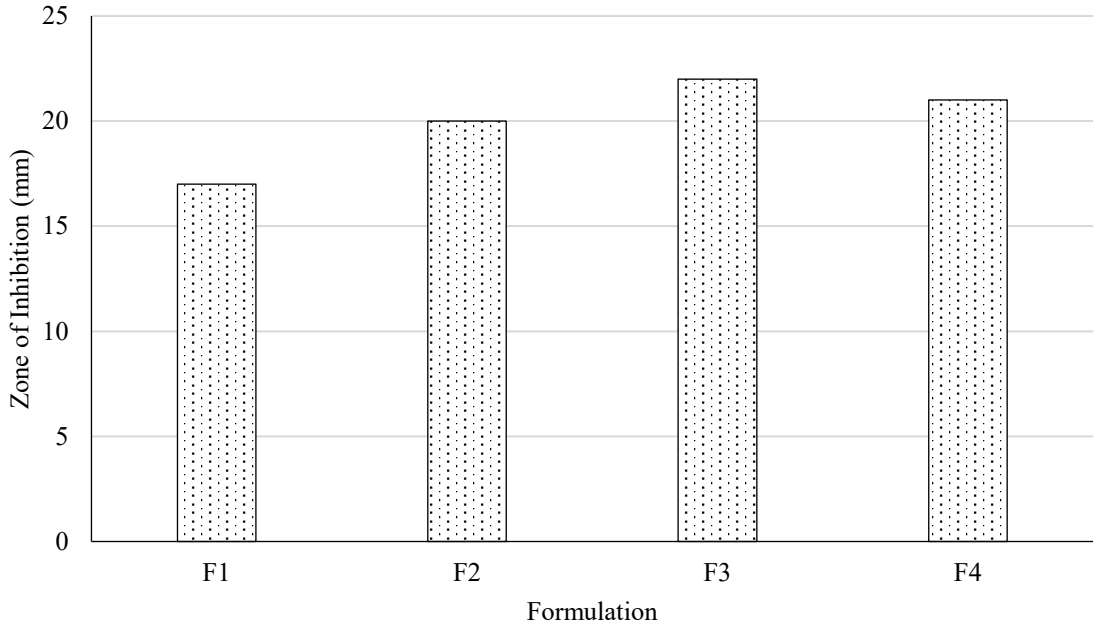

Figure 5. Zone of inhibition of formulations.

Table 2. Evaluation of formulated suspensions (F1–F4).

Parameter	F1	F2	F3	F4
Appearance	**White, Smooth**	**White, Smooth**	**White, Smooth**	**White, Smooth**
pH	6.1 ± 0.02	6.2 ± 0.03	6.3 ± 0.02	6.5 ± 0.04
Viscosity (cP)	610 ± 5.0	750 ± 6.0	880 ± 5.5	980 ± 6.5
Spreadability (cm)	7.1 ± 0.06	6.9 ± 0.07	6.5 ± 0.08	5.8 ± 0.09
Drug Content (%)	98.5 ± 0.4	99.0 ± 0.3	99.3 ± 0.3	98.8 ± 0.5
Zone of Inhibition (mm)	17.6 ± 0.3	20.5 ± 0.4	22.0 ± 0.5	21.2 ± 0.4
Stability (3 months)	Stable	Stable	Stable	Stable

CONCLUSIONS

Evaluation of the formulation, toxicology and stability studies of the topical Clotrimazole and Zinc Oxide suspension gave positive results for all of the tested parameters. The developed formulations (F1–F4) possessed excellent organoleptic properties, possessing a buff, white appearance and an acceptable pH, assuring compatibility with skin. These formulations had increased viscosity, suitable for topical application, and moderate spreadability for easier application to skin. High and consistent drug content was found in all formulations of drug content analysis, indicating proper incorporation of Clotrimazole. The antifungal activity of the formulations against *Candida albicans* was investigated in vitro using the diffusion method and formulation F3 exhibited the highest antifungal activity against this yeast with a zone of inhibition which varied from 17.6 mm to 22.0 mm, and this confirmed the synergistic effect of Clotrimazole and the Zinc Oxide in combating fungal infections. All the formulations (F1–F4) were found to be stable when subjected to accelerated and real time stability studies with respect to 3 months' duration for appearance, pH, viscosity, drug content antifungal activity, and the optimized formulation F3 showed the best balance with respect to viscosity, spreadability, drug content and antifungal activity, and therefore, it can be a good candidate as topical drug product for the treatment of fungal infections with increased skin protective properties. The long-term safety and efficacy of this formulation need to be studied further in clinical setting.

Author's Contribution

- *Ujjawal Tamboli:* Data curation, Data Analysis, Original Draft Writing & Editing.

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- *Pankaj Chasta:* Supervision, Data Analysis, Manuscript writing & Editing.
- *Chhaya Deval:* Data Analysis, Manuscript writing & Editing.
- *Shivam Rongpi:* Manuscript writing & Editing.
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- *Generative AI statement:* Generative AI & Paraphrasing tools were used, but all the suggestions given by the generative AI & Paraphrasing tools were modified by the authors and then included in the research.

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