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*Research Article*

# *In Vitro* Antifungal Evaluation of a Posaconazole-Loaded Menthosomal Hydrogel Against *Candida albicans*

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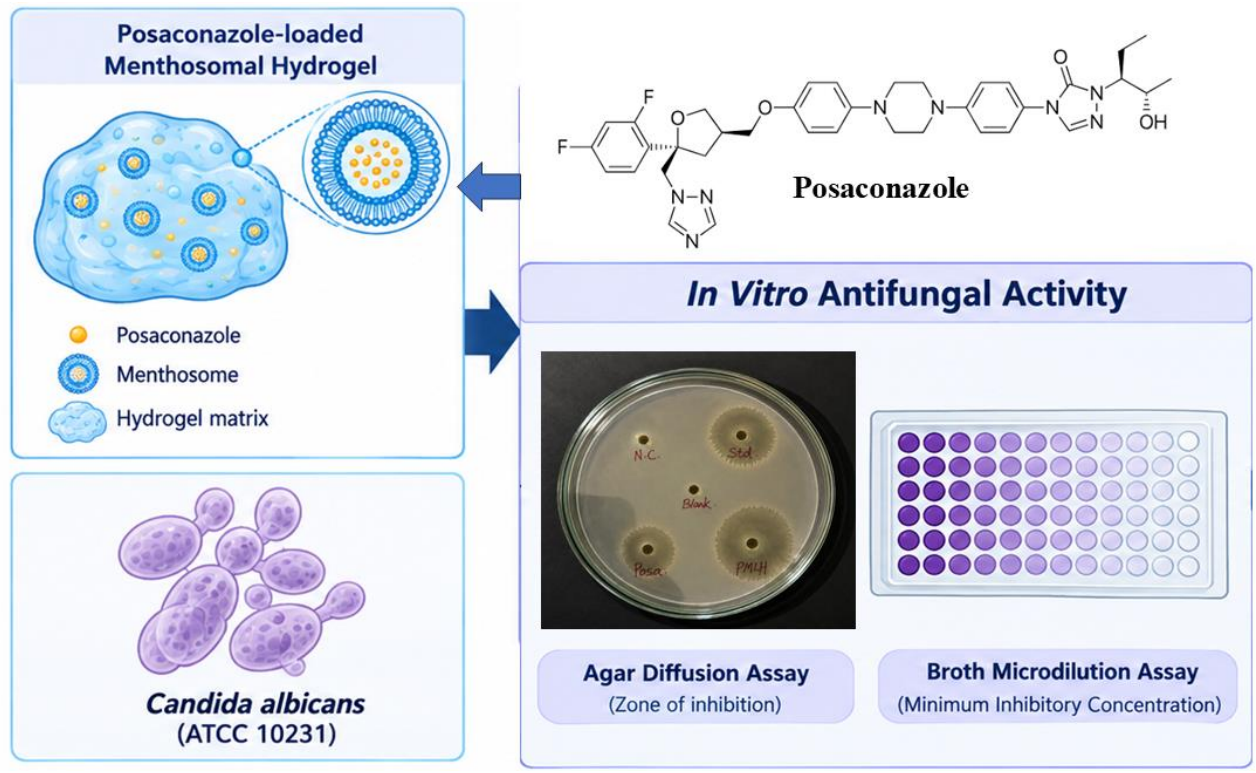
**Abstract:**
Posaconazole is a broad-spectrum triazole antifungal agent used against a range of pathogenic fungi. The present study aimed to evaluate the *in vitro* antifungal activity of an optimized Posaconazole-loaded Menthosomes hydrogel (PMLH) against *Candida albicans*. The antifungal activity was assessed using the agar well diffusion method, with the zone of inhibition (ZOI) used as the primary response. The optimized PMLH formulation was evaluated in comparison with a marketed Posaconazole formulation and Posaconazole active pharmaceutical ingredient (API). A drug-free hydrogel base and blank well were included as controls to determine the contribution of the formulation matrix and experimental setup to the observed antifungal activity. The optimized PMLH contained 90.20 ± 0.6% drug content and was tested at an equivalent Posaconazole concentration of 10 µg/mL. Following incubation, the ZOI was measured in millimetres. The PMLH exhibited a ZOI of 26.9 ± 1.7 mm, compared with 24.4 ± 1.5 mm for the marketed Posaconazole formulation and 18.8 ± 1.3 mm for Posaconazole API. No visible inhibition was observed for either the drug-free hydrogel base or blank. The larger inhibition zone produced by PMLH indicates enhanced apparent antifungal activity under the conditions of the agar diffusion assay and may be associated with improved drug dispersion and diffusion from the Menthosomal hydrogel matrix. However, the observed ZOI is also influenced by formulation viscosity, drug release, partitioning, and diffusion. Overall, the findings support the potential of PMLH as a promising formulation approach for improving the apparent antifungal performance of Posaconazole against *C. albicans*. Further standardized studies are warranted to confirm these findings.

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## Graphical Abstract




## 1. Introduction

Fungal infections represent an important global health concern, particularly among individuals with compromised immune function and those exposed to prolonged antimicrobial or immunosuppressive therapy. Among the clinically significant fungal pathogens, *Candida albicans* is one of the most frequently isolated opportunistic yeasts and is responsible for a broad range of superficial and invasive infections [1,2] . *C. albicans* commonly colonizes the skin and mucosal surfaces but can cause infection when host defense mechanisms are impaired or when the normal microbial balance is disrupted. The clinical manifestations range from superficial mucocutaneous infections to severe systemic candidiasis. The increasing incidence of antifungal resistance, together with limitations associated with conventional antifungal therapy, has created a need for improved drug-delivery approaches that can enhance local drug availability and therapeutic effectiveness.

Posaconazole is a broad-spectrum triazole antifungal agent that exhibits activity against several clinically important pathogenic fungi [2,3] . Its antifungal activity is primarily associated with inhibition of fungal lanosterol 14$\alpha$-demethylase, an enzyme involved in the biosynthesis of ergosterol, an essential component of the fungal cell membrane. Depletion of ergosterol and accumulation of abnormal sterol intermediates compromise membrane structure and function, thereby inhibiting fungal growth. Although Posaconazole possesses valuable antifungal properties, its physicochemical characteristics, particularly its high lipophilicity and limited aqueous solubility, can present challenges in developing effective topical formulations [4,5]. Therefore, incorporating posaconazole into an appropriate delivery system may improve its localization at the site of infection and enhance its antifungal performance.

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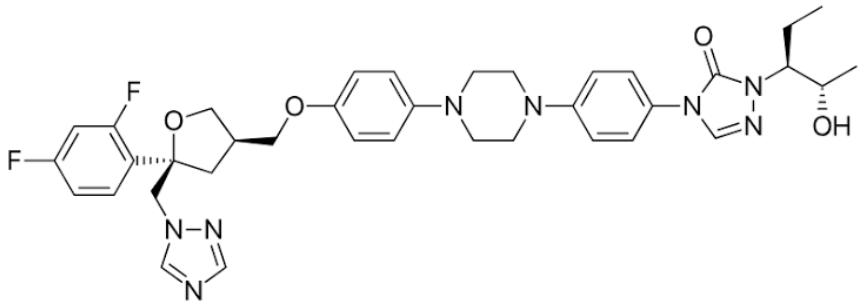


**Figure 1**. Chemical Structure of Posaconazole

Advanced topical drug-delivery systems have attracted considerable attention because they can improve drug retention, facilitate controlled release, and minimize unnecessary systemic exposure [6,7]. Menthosomes are vesicular drug-delivery systems that incorporate phospholipids and edge activators, with menthol serving as an important component. The presence of menthol may enhance vesicular flexibility and facilitate interaction with the skin barrier, potentially improving drug penetration and deposition within superficial tissues. Encapsulation of a lipophilic antifungal drug within such vesicular structures may also improve its dispersion and provide a more suitable microenvironment for drug delivery [8]. Consequently, Posaconazole-loaded Menthosomes may represent a promising approach for localized antifungal therapy.

Incorporation of drug-loaded vesicles into a hydrogel matrix can further improve the practical characteristics of topical administration [9]. Hydrogels are three-dimensional polymeric systems capable of retaining substantial quantities of water while providing a suitable semisolid platform for topical application. A hydrogel formulation may improve residence time at the application site, facilitate uniform spreading, and provide sustained release of the incorporated drug [10,11] . The combination of Menthosomal vesicles with a hydrogel matrix may therefore provide complementary advantages, including improved drug localization, prolonged contact with the infected site, and enhanced drug-release characteristics.

*In vitro* antifungal evaluation is an important preliminary step in assessing the efficacy of a newly developed topical antifungal formulation. *C. albicans* provides a relevant model organism for evaluating antifungal activity because of its clinical importance and widespread involvement in superficial candidal infections [12,13]. Assessment of the antifungal activity of a posaconazole-loaded Menthosomal hydrogel can provide information regarding its ability to inhibit fungal growth compared with the drug or formulation components alone. Parameters such as zone of inhibition, minimum inhibitory concentration, or other validated microbiological methods may be employed depending on the experimental design [14].

Therefore, the present study aimed to evaluate the in vitro antifungal activity of a Posaconazole-loaded Menthosomal hydrogel against *Candida albicans*. The study is intended to investigate whether incorporation of Posaconazole into a Menthosomal hydrogel system can provide effective antifungal activity and support the development of an improved topical delivery approach for localized management of *C. albicans* infections.

## 2. Materials and Methods

### 2.1. Previously Reported Posaconazole-Loaded Menthosomal Hydrogel

The optimized posaconazole-loaded menthosomal hydrogel (PMLH) used in the present study was developed and optimized in a previous study entitled "Formulation, Optimization, Physicochemical Characterization and Drug Release Kinetics Studies of a Posaconazole-Loaded Menthosomal Hydrogel." The optimized formulation was subsequently employed in the present investigation for further in vitro antifungal evaluation against Candida albicans using the agar well diffusion method. The drug content of the optimized formulation was 90.20 ± 0.6%, and the formulation was evaluated at an equivalent posaconazole concentration of 10 μg/mL.

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### 2.2 *In vitro* antifungal activity by agar well diffusion method

The *in vitro* antifungal activity of the developed Posaconazole-loaded Menthosomes hydrogel (PMLH) was evaluated against *Candida albicans* using the agar well diffusion method [15,16]. The antifungal activity was assessed by measuring the zone of inhibition (ZOI) produced by the tested formulations. A fresh culture of *C. albicans* was used to prepare a uniform fungal inoculum. Sterile Sabouraud dextrose agar (SDA) plates were prepared and uniformly inoculated with the fungal suspension [17]. After inoculation, wells of uniform diameter were aseptically prepared in the agar medium.

The test samples included marketed Posaconazole formulation (standard), Posaconazole API, (PMLH), Vehicle control (VC), and blank [18]. The marketed posaconazole formulation served as the reference preparation. Posaconazole API was prepared at a concentration of 10 µg/mL. The PMLH formulation was prepared at an equivalent concentration of 10 µg/mL Posaconazole, based on the experimentally determined drug content. The optimized PMLH formulation showed 90.20 ± 0.6% drug content, and the required quantity of hydrogel was calculated to obtain an equivalent Posaconazole concentration of 10 µg/mL [19].

The (VC) consisted of the drug-free hydrogel base containing only the hydrogel ingredients and no posaconazole and was used to evaluate whether the formulation base exhibited any antifungal activity [20]. The blank consisted of an empty well without addition of any formulation, drug, solvent, or chemical and represented the untreated condition.

An equal volume of each test preparation was carefully introduced into the respective wells. The plates were incubated for 24 hours at 35 ± 2$^0$C for the growth of *C. albicans*. After incubation, the plates were examined for clear inhibition zones surrounding the wells. The diameter of the (ZOI) was measured in millimetres using a calibrated scale. The inhibition-zone diameter was measured in two perpendicular directions, and the mean value was calculated.

**Table 1.** Samples used for *in vitro* antifungal activity

| S. No. | Sample | Recommended test concentration | Amount/concentration taken |
|---|---|---|---|
| 1 | Marketed Posaconazole formulation (Standard) | 10 µg/mL Posaconazole equivalent | Equivalent amount containing 10 µg Posaconazole |
| 2 | Posaconazole API | 10 µg/mL | 10 µg Posaconazole |
| 3 | Posaconazole-loaded Menthosomes hydrogel (PMLH) | 10 µg/mL Posaconazole equivalent | Quantity of hydrogel equivalent to 10 µg Posaconazole |
| 4 | Vehicle control (VC) | Drug-free hydrogel base | Hydrogel base without Posaconazole |
| 5 | Blank | — | Empty well without any formulation or chemical |

## 3. Results and Discussion

### 3.1 *In vitro* antifungal activity

The *in vitro* antifungal activity of the optimized Posaconazole-loaded Menthosomes hydrogel (PMLH) was evaluated against *Candida albicans* using the agar well diffusion method. The antifungal activity of PMLH was compared with that of the marketed Posaconazole formulation and Posaconazole API, while a drug-free hydrogel base and an empty well were included as controls. The observed zones of inhibition are presented in Table 2 and Figure 2. The revised results showed that the optimized PMLH produced a zone of inhibition (ZOI) of 26.9 ± 1.7 mm, whereas the marketed Posaconazole formulation produced a zone of 24.4 ± 1.5 mm and Posaconazole API exhibited a comparatively smaller zone of 18.8 ± 1.3 mm. The drug-free hydrogel base and blank well showed no visible inhibition.

The larger inhibition zone observed for PMLH compared with Posaconazole API indicates greater apparent antifungal activity under the conditions of the agar diffusion assay. PMLH produced approximately

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43.1% greater ZOI than Posaconazole API and approximately 10.2% greater ZOI than the marketed formulation. This enhanced zone may be associated with improved dispersion and diffusion of Posaconazole from the Menthosomal hydrogel matrix. However, because agar diffusion is influenced by formulation viscosity, drug diffusion, partitioning, and release characteristics in addition to intrinsic antifungal activity, the increased ZOI should be interpreted as an enhanced apparent antifungal effect in the diffusion assay, rather than direct evidence of increased intrinsic potency.

The relatively smaller ZOI produced by Posaconazole API may be related to its physicochemical properties and limited dispersion/diffusion through the agar medium. Incorporation into the Menthosomal system followed by incorporation into the hydrogel may facilitate distribution of the drug within the test medium and provide a more favorable release and diffusion profile. The visual appearance of the inhibition zones in Figure 2 is consistent with the numerical observations, with a visibly larger inhibition zone surrounding the PMLH sample compared with the Posaconazole API and marketed formulation.

The absence of inhibition around the drug-free hydrogel base indicates that the formulation matrix itself did not produce detectable antifungal activity under the experimental conditions. Similarly, the absence of inhibition around the blank well confirms that the observed inhibition zones were associated with the active test preparations rather than the agar well or experimental setup.

The results demonstrate that the optimized PMLH exhibited a larger (ZOI) against *C. albicans* than both Posaconazole API and the marketed formulation in the agar well diffusion assay. These findings support the potential of the Menthosomal hydrogel system to enhance the apparent antifungal performance and diffusion of Posaconazole. Nevertheless, additional standardized antifungal susceptibility and drug-release studies would be required to establish whether the formulation produces a genuine improvement in antifungal potency or simply enhanced diffusion through the agar medium.

**Table 2.** Zone of inhibition of (PMLH) against *Candida albicans*

| S. No. | Sample | Zone of inhibition (mm) |
|---|---|---|
| **1** | Marketed Posaconazole formulation (Standard) | 24.4 ± 1.5 |
| **2** | Posaconazole API | 18.8 ± 1.3 |
| **3** | Posaconazole-loaded menthosomes hydrogel (PMLH) | 26.9 ± 1.7 |
| **4** | Vehicle control (drug-free hydrogel base) | No inhibition |
| **5** | Blank | No inhibition |

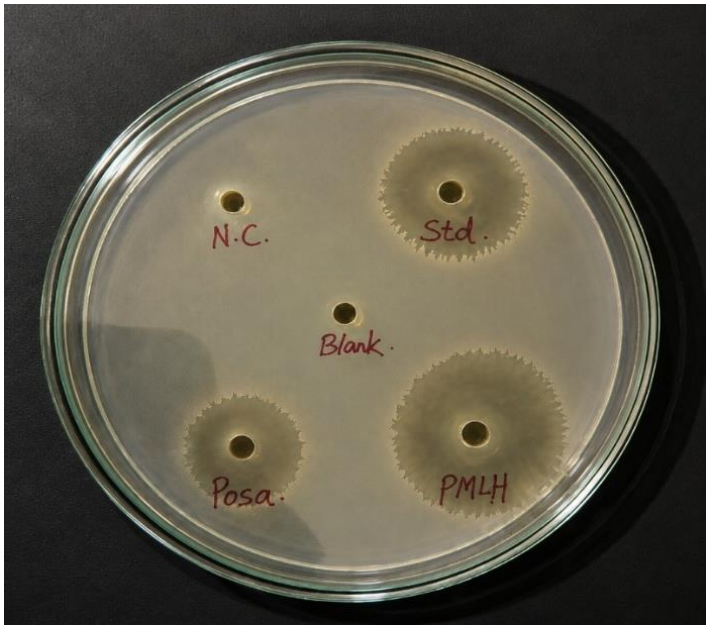



**Figure 2.** *In vitro* antifungal activity

## 4. Conclusion

The present study demonstrated the in *vitro* antifungal activity of the optimized Posaconazole-loaded Menthosomes hydrogel (PMLH) against *Candida albicans* using the agar well diffusion method. The optimized PMLH formulation exhibited a comparatively larger zone of inhibition (ZOI) than both the marketed Posaconazole formulation and Posaconazole API, indicating enhanced apparent antifungal performance under the experimental conditions. The absence of inhibition around the drug-free hydrogel base and blank confirmed that the observed antifungal activity was associated with the active Posaconazole-containing formulations rather than the hydrogel matrix or experimental setup. The improved inhibition observed with PMLH may be attributed to enhanced dispersion and diffusion of Posaconazole from the Menthosomal hydrogel system, potentially providing a more favorable drug-release and diffusion profile. However, the (ZOI) in an agar diffusion assay can be influenced by formulation viscosity, drug release, partitioning, and diffusion characteristics and therefore should not be interpreted as direct evidence of increased intrinsic antifungal potency. Overall, the findings support the potential of the Menthosomal hydrogel approach for enhancing the apparent antifungal performance of Posaconazole against *C. albicans*. Further standardized antifungal susceptibility and drug-release studies are required to confirm these findings and establish the therapeutic potential of the developed formulation.

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None

**Conflict of Interest**

The authors declare no conflict of interest.

**Authors' Contribution**

Conceptualization; methodology; software; validation; formal analysis; investigation; resources; data curation; writing—original draft preparation; writing—review and editing; visualization; supervision; project administration by N.S, D.K, and V.A. All authors have read and agreed to the published version of the manuscript.

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