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Research Article

Comparative *In Vitro* Evaluation of Berberine–Chlorogenic Acid and Its Phytosome-Based Hydrogel Against *Candida albicans*

Namita Gajanan Narvekar[1]*, Simachal Panda[1], Vijay Arjun Jagtap[2]

[1]*School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India.
[2]Department of Pharmaceutical Chemistry, Yashwantrao Bhonsale College of Pharmacy, Sawantwadi, Sindhudurg, Maharashtra, India.

*Corresponding author: namitagnarvekar@gmail.com

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Abstract:
Candida albicans is an important fungal pathogen associated with superficial and opportunistic infections, highlighting the need for effective topical antifungal formulations. The present study aimed to evaluate the *in vitro* antifungal activity of an optimized berberine–chlorogenic acid (B+CA) phytosome-based hydrogel against *C. albicans* and compare its performance with the pure B+CA combination and ketoconazole. The optimized F1 formulation contained berberine and chlorogenic acid in a 1:1 ratio and was evaluated using the agar well diffusion method. Ketoconazole served as the standard antifungal drug, while normal control and blank hydrogel were included as controls. Antifungal activity was assessed by measuring the zone of inhibition (ZOI) following incubation. The results demonstrated that the B+CA phytosome-based hydrogel produced a ZOI of 22.90 mm, whereas the pure B+CA combination exhibited a ZOI of 18.50 mm. The optimized F1 formulation showed an approximately 23.8% greater inhibition-zone diameter than the pure combination, indicating improved antifungal performance following phytosome-based incorporation into the hydrogel matrix. Ketoconazole demonstrated the highest antifungal activity, with a ZOI of 31.44 mm. Neither the normal control nor the blank hydrogel produced a measurable inhibition zone. The enhanced activity of F1 may be associated with improved dispersion and presentation of the active constituents and the contribution of the hydrogel matrix to formulation retention and diffusion. In conclusion, the findings support the potential of the B+CA phytosome-based hydrogel as a promising topical antifungal formulation. Further safety, skin compatibility, and *in vivo* studies are required to establish its therapeutic suitability.

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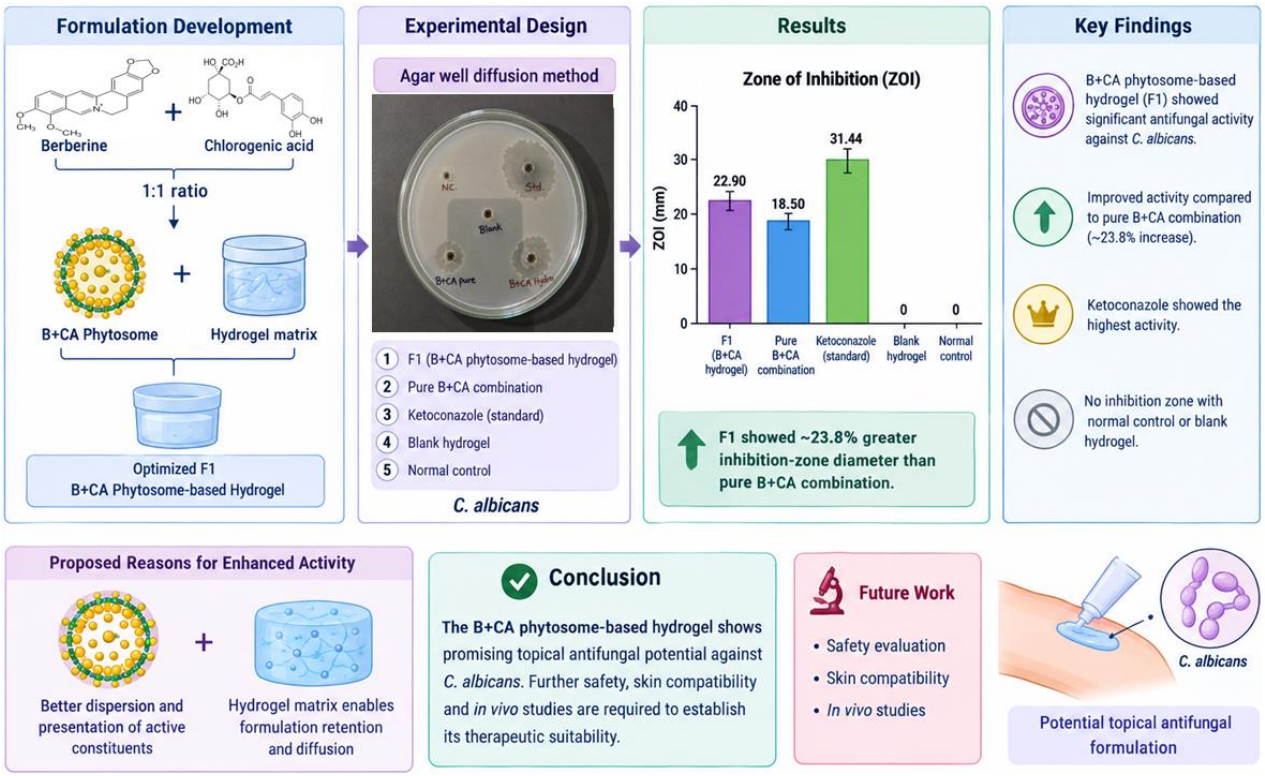
Graphical Abstract



1. Introduction

Candida albicans is an opportunistic fungal pathogen that commonly inhabits human mucosal surfaces but can cause infections when the host immune system or normal microbial balance is disrupted [1]. It is associated with a broad range of fungal infections, including oral candidiasis, vulvovaginal candidiasis, and cutaneous candidiasis [2]. The increasing incidence of recurrent fungal infections, the emergence of antifungal resistance, and the limitations associated with conventional antifungal treatments have encouraged the investigation of alternative therapeutic agents. Although several antifungal drugs are available, their clinical effectiveness may be limited by adverse effects, reduced susceptibility among fungal isolates, and challenges associated with achieving adequate drug concentrations at the site of infection [3]. Consequently, the exploration of plant-derived bioactive compounds and advanced topical drug delivery systems represents a promising approach for developing effective antifungal formulations.

Berberine is a naturally occurring isoquinoline alkaloid found in several medicinal plants and is recognized for its diverse pharmacological properties, including antimicrobial, anti-inflammatory, and antioxidant activities [4]. Its potential antifungal effects have attracted considerable interest because of its ability to interfere with cellular processes involved in microbial survival. Chlorogenic acid, a naturally occurring polyphenolic compound widely distributed in various plants, also exhibits antimicrobial, antioxidant, and anti-inflammatory properties [5]. Its biological activities have been associated with interactions involving microbial cell membranes, oxidative stress regulation, and other cellular mechanisms. The combination of berberine and chlorogenic acid may provide complementary antifungal effects; however, the effectiveness of such a combination depends on factors including the concentration of each constituent, their compatibility, and their availability at the site of action [6]. Therefore, evaluating the combined activity of these compounds against *C. albicans* is important for determining their potential application in antifungal therapy.

Despite their promising biological properties, the pharmaceutical application of plant-derived compounds may be restricted by limitations in solubility, stability, permeability, and delivery to the intended site of action [7]. Phytosome technology offers a potential strategy to address some of these challenges.

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Phytosomes are phospholipid-based complexes in which bioactive phytoconstituents interact with phospholipids, potentially improving their physicochemical characteristics and facilitating their interaction with biological membranes. Incorporating phytosome-based systems into topical formulations may further support localized drug delivery, improve formulation performance, and enhance contact between the active constituents and the infected tissue. Hydrogels are particularly suitable for topical applications because of their semisolid consistency, ease of application, spreadability, and ability to maintain contact with the application site [8]. A phytosome-based hydrogel containing berberine and chlorogenic acid may therefore represent a promising formulation approach for topical antifungal applications.

The evaluation of antifungal activity using *in vitro* methods provides an initial means of investigating the effectiveness of bioactive compounds and their pharmaceutical formulations [9]. The agar well diffusion method, for example, allows the assessment of fungal growth inhibition by measuring the zone of inhibition surrounding the tested sample. Comparative evaluation of the unformulated berberine–chlorogenic acid combination and its phytosome-based hydrogel can provide preliminary information regarding the influence of the delivery system on antifungal performance [10]. Such comparisons are important because formulation incorporation may affect the release, diffusion, and availability of active constituents within the test medium. Interpretation of inhibition zones should therefore consider the physicochemical properties of the tested preparations and should not be used alone to establish clinical superiority.

The present study aims to comparatively evaluate the *in vitro* antifungal activity of berberine–chlorogenic acid and its phytosome-based hydrogel against *Candida albicans*. By investigating the antifungal potential of the combined bioactive constituents alongside their formulated delivery system, this study seeks to determine whether incorporation into a phytosome-based hydrogel influences the observed antifungal response. The findings may contribute to understanding the potential of plant-derived bioactive combinations and phospholipid-based topical formulations as candidates for further development in antifungal drug delivery. Further studies involving formulation characterization, drug-release assessment, antifungal susceptibility testing, stability evaluation, and appropriate safety investigations would be required to establish their suitability for practical therapeutic applications.

2. Materials and Methods

2.1. Previously Reported Berberine–Chlorogenic Acid Phytosomal Hydrogel

The optimized berberine–chlorogenic acid (B+CA) phytosome-based hydrogel (formulation F1) used in the present study was developed and optimized as part of the formulation research entitled "Berberine–Chlorogenic Acid Phytosomal Hydrogel: Design, Optimization, Physicochemical Characterization, and Release Kinetics Studies." The optimized formulation contained berberine and chlorogenic acid in a 1:1 ratio, with 100 mg of each active constituent incorporated into the phytosomal formulation. The F1 formulation was selected for the present investigation to evaluate its *in vitro* antifungal activity against *Candida albicans* using the agar well diffusion method. Its antifungal performance was compared with that of the pure B+CA combination and ketoconazole as the standard antifungal drug, while blank hydrogel and normal control were included as controls**.**

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

The *in vitro* antifungal activity of the optimized berberine + chlorogenic acid (B+CA) phytosome-based hydrogel (F1) was evaluated against *Candida albicans* using the agar well diffusion method [11]. The selected F1 phytosomes contained berberine and chlorogenic acid in a 1:1 ratio, with 100 mg of each active constituent used in the phytosome formulation.

A fresh culture of C. albicans was used to prepare a uniform fungal inoculum [12]. Sterile Sabouraud dextrose agar (SDA) plates were uniformly inoculated with the prepared microbial suspension. After inoculation, wells of uniform diameter were aseptically prepared in the agar medium.

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The test samples included ketoconazole (standard antifungal drug), B+CA pure, B+CA phytosome hydrogel, blank, and normal control (NC) [13]. Ketoconazole was used at a concentration of 10 µg/mL. The B+CA pure sample was prepared at a total concentration of 10 µg/mL, containing 5 µg/mL berberine and 5 µg/mL chlorogenic acid [14]. The B+CA phytosome hydrogel was prepared at an equivalent concentration of 10 µg/mL total B+CA, based on the experimentally determined drug content of the hydrogel [15]. The reported drug content of the F1 hydrogel was 96.42 ± 0.6% for berberine and 95.88 ± 0.7% for chlorogenic acid.

The normal control (NC) consisted of the C. albicans inoculum without any test antifungal formulation and was used to confirm normal fungal growth in the absence of treatment [16]. The blank hydrogel consisted only of the drug-free hydrogel base without berberine or chlorogenic acid [17].

An equal volume of each test preparation and control was carefully introduced into the respective wells. The plates were incubated under suitable conditions for C. albicans. Following incubation, the plates were examined for clear inhibition zones around the wells [18]. The zone of inhibition (ZOI) was measured in millimetres using a calibrated scale. Measurements were taken in two perpendicular directions, and the mean diameter was calculated.

Table 1. Test samples, concentrations, and quantities used for in vitro antifungal activity.

Sample	Recommended test concentration	Amount for 1 mL
Ketoconazole (standard)	10 µg/mL	10 µg
B+CA pure	10 µg/mL total	5 µg berberine + 5 µg chlorogenic acid
B+CA phytosome hydrogel	10 µg/mL total B+CA equivalent	Equivalent to 10 µg total B+CA

3. Results and Discussion

3.1 *In vitro* antifungal activity

The in vitro antifungal activity of the developed berberine + chlorogenic acid (B+CA) phytosome-based hydrogel (F1) was evaluated against *Candida albicans* using the agar well diffusion method. Antifungal activity was assessed based on the diameter of the zone of inhibition (ZOI) produced by the standard antifungal drug, B+CA pure, and B+CA phytosome hydrogel, with normal control (NC) and blank hydrogel serving as controls. The experimental procedure and measurement of ZOI were performed as described in the methodology.

As presented in Table 1 and Figure 1, ketoconazole exhibited the largest inhibition zone, with a ZOI of 31.44 mm, confirming its pronounced antifungal activity against *C. albicans*. The B+CA pure combination produced a ZOI of 18.50 mm, demonstrating that the combination of berberine and chlorogenic acid possessed appreciable antifungal activity. In comparison, the B+CA phytosome hydrogel (F1) produced a ZOI of 22.90 mm, which was substantially higher than that observed for B+CA pure. The F1 formulation therefore produced an approximately 23.8% greater inhibition-zone diameter than the pure B+CA combination, indicating improved antifungal performance following incorporation into the phytosome-based hydrogel system.

The enhanced ZOI observed with the F1 phytosome hydrogel may be attributed to the improved formulation characteristics imparted by phytosome formation. Incorporation of B+CA into the phytosomal system may improve the dispersion and effective presentation of the active constituents, thereby facilitating their interaction with the fungal cells. In addition, the hydrogel matrix may provide better retention of the formulation at the application site and facilitate sustained diffusion of the incorporated constituents into the agar medium. These formulation-related effects may collectively contribute to the greater inhibition zone observed with F1 compared with the pure B+CA combination.

The NC and blank hydrogel exhibited no measurable inhibition zone (0.0 mm), indicating normal growth of *C. albicans* in the absence of antifungal treatment and confirming that the drug-free hydrogel base itself did not possess appreciable antifungal activity. Thus, the inhibition observed with F1 can reasonably be attributed to the incorporated B+CA rather than the hydrogel vehicle.

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Although the F1 formulation demonstrated improved activity compared with B+CA pure, its ZOI remained lower than that of ketoconazole (22.90 vs. 31.44 mm). This indicates that, under the present experimental conditions, the optimized phytosome hydrogel exhibited considerable antifungal activity but did not surpass the activity of the conventional reference antifungal drug. The findings demonstrate that phytosome-based incorporation of B+CA into a hydrogel system enhanced its in vitro antifungal activity against *C. albicans* compared with the pure B+CA combination, supporting the potential of the developed F1 formulation for further antifungal evaluation.

Table 2. *In vitro* antifungal activity of B+CA formulations against *Candida albicans*, expressed as zones of inhibition (mm).

S. No.	Treatment	Zone of inhibition (mm)
1	Normal control (NC)	0.0
2	Blank hydrogel	0.0
3	Standard antifungal drug (Ketoconazole)	31.44
4	B+CA pure	18.50
5	B+CA phytosome hydrogel (F1)	22.90

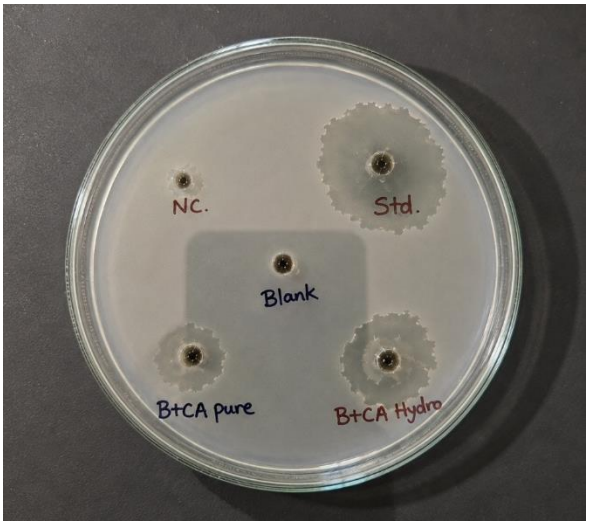

Figure 1. Comparative in vitro antifungal activity of ketoconazole, pure berberine–chlorogenic acid (B+CA), and B+CA phytosome hydrogel (F1) against *Candida albicans*. Zones of inhibition were 31.44, 18.50, and 22.90 mm, respectively, while the normal control and blank hydrogel showed no inhibition (0.0 mm).

4. Conclusion

The present study demonstrated the in vitro antifungal activity of the optimized berberine–chlorogenic acid (B+CA) phytosome-based hydrogel (F1) against *Candida albicans* using the agar well diffusion method. The F1 formulation exhibited a greater zone of inhibition than the pure B+CA combination, indicating improved antifungal performance following incorporation into the phytosome-based hydrogel system. This enhancement may be associated with improved dispersion and presentation of the active constituents, along with the potential contribution of the hydrogel matrix to formulation retention and drug diffusion. The absence of measurable inhibition zones with the normal control and blank hydrogel confirmed that the observed antifungal effect was primarily associated with the incorporated phytoconstituents rather than the drug-free vehicle. However, the antifungal activity of F1 remained lower than that of ketoconazole, the standard antifungal drug, under the experimental conditions. Overall, these findings support the potential of the B+CA phytosome-based hydrogel as a promising topical antifungal formulation. Further investigations, including additional antifungal assays, formulation safety assessment, skin compatibility studies, and in vivo evaluations, are warranted to establish its therapeutic potential and suitability for topical antifungal applications.

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NA

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

NGN: Conceptualization, methodology, investigation, data curation, formal analysis, and writing—original draft preparation. SP: Investigation, data curation, validation, and writing—review and editing. VAJ: Supervision, methodology, validation, and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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