RESEARCH PAPER

BENZOYL PEROXIDE–CURCUMIN NANOSTRUCTURED PRONIOSOMAL GEL FOR THE TREATMENT OF SKIN INFECTION: FORMULATION OPTIMIZATION, CHARACTERIZATION AND IN-VITRO ANTIFUNGAL EVALUATION

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ABSTRACT

The development of localized topical drug delivery systems offers a promising approach for the management of cutaneous fungal infections. The present study aimed to develop and characterize a benzoyl peroxide–curcumin nanostructured proniosomal gel for topical application and evaluate its in-vitro antifungal activity against *Candida albicans*. Benzoyl peroxide and curcumin proniosomal gels were prepared separately using the coacervation phase separation method, followed by the development of an optimized combination formulation. The prepared formulations were evaluated for drug–excipient compatibility and characterized for entrapment efficiency, drug content, pH, viscosity, spreadability, vesicle size, polydispersity index, zeta potential and in-vitro drug release. The optimized benzoyl peroxide proniosomal gel (BPPG5) and curcumin proniosomal gel (CuPG5) exhibited entrapment efficiencies of 91.07 ± 1.19% and 88.51 ± 0.74%, respectively, with drug contents of 93.82 ± 0.73% and 92.05 ± 0.97%, respectively. The optimized combination formulation (BPPG5 + CuPG5) exhibited a pH of 6.7 ± 0.84, viscosity of 8524 ± 2.97 cP and spreadability of 44 ± 1.33 mm. The combination formulation had a mean vesicle size of 597.3 nm, a polydispersity index of 0.306 and a zeta potential of −33.1 mV. In-vitro drug release studies demonstrated cumulative release of 93.21 ± 1.64% for benzoyl peroxide and 92.08 ± 2.42% for curcumin over 24 h. The in-vitro antifungal activity was evaluated against *C. albicans* using the agar well diffusion method. The optimized BPPG5, CuPG5 and BPPG5 + CuPG5 formulations exhibited inhibition zones of 15 mm, 15 mm and 16 mm, respectively. The results indicated that the curcumin-containing individual and combination formulations demonstrated greater inhibition than the benzoyl peroxide formulation alone under the experimental conditions. Overall, the developed benzoyl peroxide–curcumin proniosomal gel exhibited suitable physicochemical characteristics, sustained drug release and measurable in-vitro antifungal activity against *C. albicans*, suggesting its potential as a topical drug delivery system for further investigation.

Keywords: Benzoyl peroxide; Curcumin; Proniosomal gel; Nanostructured vesicles; *Candida albicans*; Antifungal activity; Topical drug delivery.

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INTRODUCTION

Skin infections are an important public health concern because of their substantial disease burden, treatment limitations, and increasing antimicrobial resistance. *Candida albicans* is an opportunistic fungal pathogen associated with cutaneous and systemic infections. The continuing emergence of antifungal resistance and limitations in available antifungal treatments emphasize the need to

investigate alternative bioactive agents and innovative drug delivery systems (1).

Cutaneous fungal infections commonly affect the skin, hair, and nails and may be caused by dermatophytes and yeasts, including *Candida* species. *C. albicans* is capable of morphological transition, adhesion, and biofilm formation, which contribute to its persistence and pathogenicity (2). Although topical therapy provides localized drug administration, inadequate residence time, variable drug release, and limitations in drug deposition may influence therapeutic performance. Therefore, vesicular topical drug delivery systems that can improve formulation retention and modify drug-release characteristics are of considerable interest (3).

Benzoyl peroxide (BP) is a known topical antifungal and keratolytic agent widely used in dermatological formulations, particularly for the treatment of acne. Its antifungal activity is primarily associated with the generation of reactive oxygen species (ROS), which contribute to microbial cell damage (4). However, its topical performance can be influenced by formulation characteristics, drug release, and localization within the skin. In the present study, BP was investigated as an established topical antifungal component in combination with a phytoconstituent to explore the potential of a vesicular formulation approach for topical application (5).

Curcumin is a naturally occurring polyphenolic phytoconstituent obtained from the rhizomes of *Curcuma longa L*. It has attracted considerable research interest because of its diverse pharmacological properties, including antioxidant, anti-inflammatory, and antimicrobial activities. Curcumin has also been investigated for its antifungal potential against different fungal species, including *Candida albicans*. These biological properties support its consideration as a bioactive component in topical antifungal formulation development. However, the pharmaceutical application of curcumin is limited by its poor aqueous solubility, low dissolution rate, and susceptibility to degradation under certain environmental conditions. These physicochemical and biopharmaceutical limitations may affect its topical delivery and therapeutic performance, highlighting the need for an appropriate drug delivery system (6).

Proniosomal gel is a semisolid precursor system that generates niosomal vesicles upon hydration. These systems are composed mainly of non-ionic surfactants, cholesterol, and other membrane-forming components. Proniosomal formulations have attracted interest because of their formulation flexibility, drug-loading capacity, and potential to improve the stability and delivery characteristics of vesicular systems. Proniosomal gel systems are particularly suitable for topical administration because the resulting vesicles can be incorporated into semisolid bases, combining the advantages of vesicular drug delivery with the practical benefits of topical application (7). Niosomal and proniosomal systems have been investigated for topical and transdermal drug delivery and may influence drug localization, retention, and release characteristics. Carbopol-based gels provide suitable consistency and rheological properties for topical administration and can serve as vehicles for vesicular formulations (8).

The combination of BP and curcumin represents a formulation approach involving an established topical antifungal agent and a naturally occurring phytoconstituent with reported antifungal potential. Incorporating these components into a nanostructured proniosomal gel may provide a suitable topical delivery platform and facilitate the evaluation of their physicochemical characteristics, drug-release behavior, and in-vitro antifungal activity. Furthermore, the incorporation of optimized proniosomal formulations into a Carbopol 934 gel base may provide suitable semisolid characteristics for topical application. Further investigation is required to establish the formulation characteristics and in-vitro antifungal performance of the BP–curcumin combination proniosomal gel against *C. albicans* (9).

The present study aimed to develop and characterize a Benzoyl Peroxide–Curcumin nanostructured proniosomal gel using cholesterol, soya lecithin, Span 60, and Tween 80, followed by incorporation of the optimized proniosomal formulations into a Carbopol 934 gel base. The prepared formulations were evaluated for drug–excipient compatibility using Fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). Further characterization included entrapment efficiency, drug content, pH, viscosity, spreadability, vesicle size, polydispersity index, zeta potential, surface morphology, and in-vitro drug-release studies. The optimized formulations were additionally evaluated for in-vitro antifungal activity against *Candida albicans* using the agar well diffusion method to assess their formulation performance and antifungal potential.

MATERIALS AND METHODS

Drug and Chemicals

Benzoyl peroxide and curcumin were used as the active pharmaceutical ingredients. Cholesterol, soya lecithin, Span 60 and Tween 80 were used for proniosome preparation, while Carbopol 934 was used as the gelling polymer. Methanol, phosphate-buffered saline (PBS), methyl paraben, propyl

paraben, propylene glycol and triethanolamine were used for formulation and evaluation. All other chemicals and reagents were of analytical grade. *Candida albicans* was used to evaluate the in-vitro antifungal activity of the optimized proniosomal gel formulations.

Preformulation Studies

Benzoyl peroxide and curcumin were evaluated for appearance, solubility and melting point before formulation. The solubility was qualitatively assessed in methanol, ethanol and PBS. The melting point was determined using a digital melting point apparatus (10).

UV-Visible Spectrophotometric Analysis

Standard solutions of benzoyl peroxide and curcumin were prepared separately in methanol and scanned against methanol as blank. Benzoyl peroxide was scanned in the wavelength range of 200–400 nm, whereas curcumin was scanned in the range of 200–500 nm. The respective wavelengths of maximum absorbance (λmax) were recorded. Stock solutions were prepared by dissolving 10 mg of each drug separately in methanol. Suitable aliquots were diluted to obtain concentrations ranging from 5–25 µg/mL. Calibration curves were prepared by plotting concentration against absorbance, and the regression equation and coefficient of determination (r^2) were calculated (11).

FTIR Studies

FTIR spectra of benzoyl peroxide, curcumin, their physical mixture and the optimized formulation were recorded over 4000–400 cm^{-1} to identify characteristic functional groups and assess possible drug–excipient interactions (12).

Differential Scanning Calorimetry (DSC)

DSC thermograms of benzoyl peroxide, curcumin and the optimized formulation were recorded to evaluate their thermal behaviour and assess possible changes in characteristic thermal transitions. Samples were sealed in aluminum pans and heated at a constant rate over a suitable temperature range. The thermograms were examined for changes in melting endotherms (13).

Preparation of Benzoyl Peroxide Proniosomal Gel

Benzoyl peroxide proniosomal gels (BPPG1–BPPG8) were prepared using the coacervation phase separation method. The formulations were prepared using two levels of three variables: cholesterol, soya lecithin and a combination of surfactants (Span 60 and Tween 80 in a 1:1 ratio). Benzoyl peroxide and excipients were dissolved in methanol and heated at 60–65°C for 5–10 min at 40 rpm. Preheated PBS was then added gradually with continuous stirring to obtain the proniosomal gel. The formulations were stored in airtight containers protected from light exposure (14).

Table 1. Composition of Benzoyl Peroxide proniosomal gel formulations

Formulation	BP (mg)	Cholesterol (mg)	Soya lecithin (mg)	Span 60 + Tween 80 (mg)
BPPG1	25	90	180	90 + 90
BPPG2	25	90	180	45 + 45
BPPG3	25	90	90	90 + 90
BPPG4	25	90	90	45 + 45
BPPG5	25	45	180	90 + 90
BPPG6	25	45	180	45 + 45
BPPG7	25	45	90	90 + 90
BPPG8	25	45	90	45 + 45

Preparation of Curcumin Proniosomal Gel

Curcumin proniosomal gels (CuPG1–CuPG8) were prepared using the coacervation phase separation method. The formulations were prepared using two levels of three variables: cholesterol, soya lecithin and a combination of surfactants (Span 60 and Tween 80 in a 1:1 ratio). Curcumin and the excipients were dissolved in methanol and heated at 60–65°C for 5–10 min at 40 rpm. Preheated PBS was added gradually with continuous stirring to obtain the proniosomal gel. The formulations were stored in airtight containers protected from light exposure (15).

Table 2. Composition of Curcumin proniosomal gel formulations

Formulation	Curcumin (mg)	Cholesterol (mg)	Soya lecithin (mg)	Span 60 + Tween 80 (mg)
CuPG1	10	90	180	90 + 90
CuPG2	10	90	180	45 + 45
CuPG3	10	90	90	90 + 90
CuPG4	10	90	90	45 + 45
CuPG5	10	45	180	90 + 90
CuPG6	10	45	180	45 + 45
CuPG7	10	45	90	90 + 90
CuPG8	10	45	90	45 + 45

Preparation of Benzoyl Peroxide–Curcumin Combination Formulation

The optimized BPPG and CuPG formulations were incorporated into a 1.5% Carbopol 934 gel and mixed gently to obtain a homogeneous benzoyl peroxide–curcumin proniosomal gel. The final combination formulation was specified to contain benzoyl peroxide (2.5% w/w) and curcumin (1 % w/w). The required quantities of the respective optimized proniosomal gels and 1.5% plain Carbopol 934 gel were mixed thoroughly to ensure uniform distribution of the drug-loaded vesicles. Methyl paraben and propyl paraben were incorporated as preservatives. The formulation was allowed to stand under vacuum overnight to remove entrapped air bubbles and was stored in an airtight, light-protected container (16).

Characterization of Proniosomal Gel

The formulations were evaluated for appearance, entrapment efficiency, drug content, pH, viscosity and spreadability. Optimized formulations were further evaluated for vesicle size, PDI, zeta potential, surface morphology and in-vitro drug release.

Entrapment Efficiency and Drug Content

For entrapment efficiency, approximately 1 g of the formulation was hydrated with PBS (pH 7.4) and centrifuged at 18,000 rpm for 40 min at 4°C. The free drug in the supernatant was quantified using a suitable drug-specific analytical method. Entrapment efficiency was calculated using the following equation:

EE (%) = [(Total drug − Free drug) / Total drug] × 100

Drug content was determined after methanolic extraction. Accurately weighed 1 g of proniosomal gel was transferred into methanol and sonicated for 15 min. The volume was adjusted with methanol, filtered through Whatman filter paper No. 1 and suitably diluted. The amount of each drug was determined from its calibration curve and expressed as percentage drug content. Triplicate determinations were performed (17).

Vesicle Size and Polydispersity Index

The vesicle size and PDI of the optimized formulation were determined by dynamic light scattering (DLS) using a Zetasizer after suitable dilution with distilled water at room temperature. The average vesicle size and PDI were recorded (18).

Zeta Potential

The zeta potential of the optimized formulation was determined by electrophoretic light scattering using a Zetasizer after suitable dilution with distilled water. Measurements were performed at room temperature (19).

Surface Morphology by Scanning Electron Microscopy

The surface morphology of the optimized formulation was examined using scanning electron microscopy (SEM) after suitable sample preparation and mounting. The micrographs were examined for vesicle shape, surface characteristics and aggregation (20).

In-vitro Drug Release

In-vitro drug release was evaluated using a Franz diffusion cell with a dialysis membrane at 37 ± 0.5°C under continuous stirring. The receptor compartment was filled with a suitable release medium. Samples were withdrawn at predetermined intervals and replaced with fresh receptor medium to maintain sink conditions. The withdrawn samples were analyzed for benzoyl peroxide and curcumin using a UV Spectrophotometry, and cumulative drug release was calculated (21).

Antibacterial Activity against *Candida albicans*

The antibacterial activity of the optimized formulations was assessed against Candida albicans using the agar well diffusion technique. The prepared formulations were filled into wells made in agar plates previously inoculated with *Candida albicans*. The plates were incubated under appropriate microbiological conditions for 24 hours. Following incubation, the diameter of the zone of inhibition (ZOI) surrounding each well was measured in millimetres (mm) to evaluate the antimicrobial activity of the formulations (22).

RESULTS AND DISCUSSION

Preformulation Studies

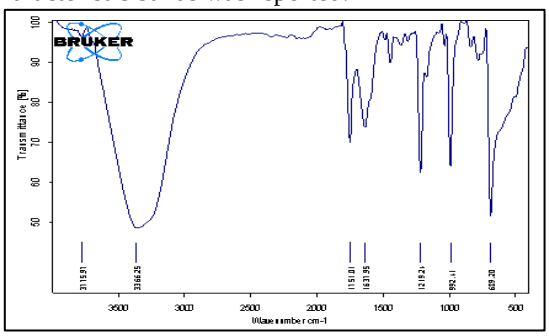
Benzoyl peroxide appeared as a white crystalline powder, whereas curcumin was observed as a yellow-orange crystalline powder. Benzoyl peroxide was soluble in methanol and slightly soluble in phosphate-buffered saline (PBS), while curcumin was soluble in methanol and practically insoluble in PBS. The observed melting points were 105 ± 0.93°C for benzoyl peroxide and 182.20 ± 0.59°C for curcumin, consistent for formulation development.

UV-Visible Spectrophotometric Analysis

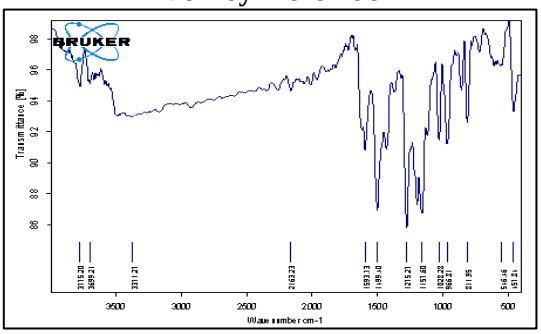
Benzoyl peroxide showed maximum absorbance at 235 nm, while curcumin exhibited maximum absorbance at 435 nm. Calibration curves were prepared over 5–25 µg/mL. The absorbance increased with increasing concentration for both drugs.

FTIR Studies

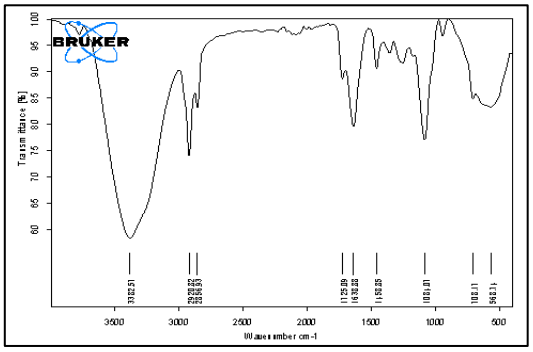
The FTIR spectrum of BPPG showed characteristic absorption bands associated with hydroxyl stretching, aliphatic C–H stretching, carbonyl stretching and C–O stretching. In the BPPG + CuPG combination, the source chapter reported bands at 3382, 2920, 2856, 1725, 1638 and 1084 cm^{-1}, assigned to O–H, aliphatic C–H, carbonyl, aromatic/conjugated C=C and C–O vibrations, respectively. No major shift or disappearance of characteristic bands was reported.



Benzoyl Peroxide



Curcumin



BPPG5 + CuPG5 formulation

Figure 1. FTIR spectra for Benzoyl Peroxide, Curcumin, and the optimized BPPG5 + CuPG5 formulation

Differential Scanning Calorimetry

Pure benzoyl peroxide exhibited an endothermic peak at 112.59°C with an enthalpy of fusion of −1122.69 J/g, while curcumin showed an endothermic transition at 172.12°C with an enthalpy of fusion of −89.79 J/g. The optimized BPPG5–CuPG5 combination exhibited a thermal transition with an onset at 103.77°C, peak at 108.00°C and end set at 121.15°C.

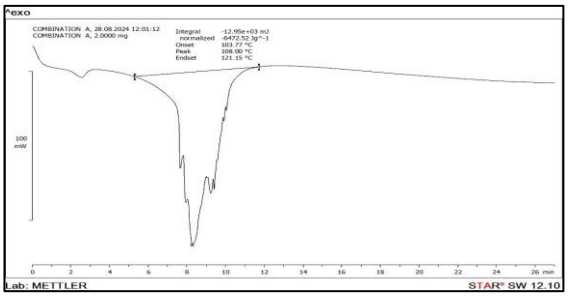


Figure 2. DSC thermogram of the optimized BPPG5 + CuPG5 combination formulation

Formulation Development and Optimization

Benzoyl peroxide proniosomal formulations (BPPG1–BPPG8) and curcumin proniosomal formulations (CuPG1–CuPG8) were prepared by the coacervation phase separation method. BPPG5 showed the highest entrapment efficiency (91.07 ± 1.19%) and drug content (93.82 ± 0.73%) among the BP batches. CuPG5 showed the highest entrapment efficiency (88.51 ± 0.74%) and drug content (92.05 ± 0.97%) among the curcumin batches. Therefore, BPPG5 and CuPG5 were selected as the optimized individual formulations. Values are mean ± SD (n = 3).

Physicochemical Characterization

The optimized BPPG5 + CuPG5 combination formulation exhibited a pH of 6.7 ± 0.84, viscosity of 8524 ± 2.97 cP and spreadability of 44 ± 1.33 mm. The rate of spontaneity was reported as 12 ± 0.96.

Vesicle Size, PDI and Zeta Potential

The optimized BPPG5 + CuPG5 formulation exhibited a mean hydrodynamic diameter of 597.3 nm, PDI of 0.306 and zeta potential of −33.1 mV. The source chapter reported a particle-size distribution extending approximately from 100 to 800 nm. The PDI indicates a moderately broad size distribution, while the negative zeta potential describes the surface-charge characteristics of the vesicles.

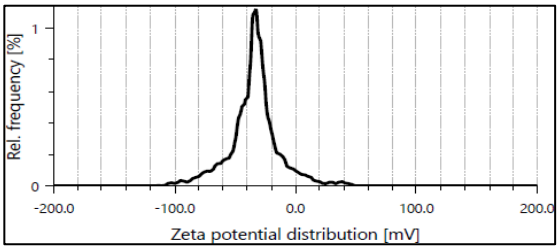


Figure 3. Zeta-potential distribution of the optimized BPPG5 + CuPG5 formulation.

Surface Morphology by Scanning Electron Microscopy

SEM examination of the optimized BPPG5 + CuPG5 formulation revealed discrete, predominantly spherical vesicular structures with distinguishable boundaries.

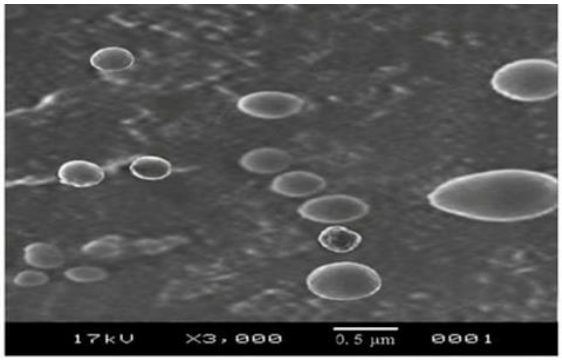
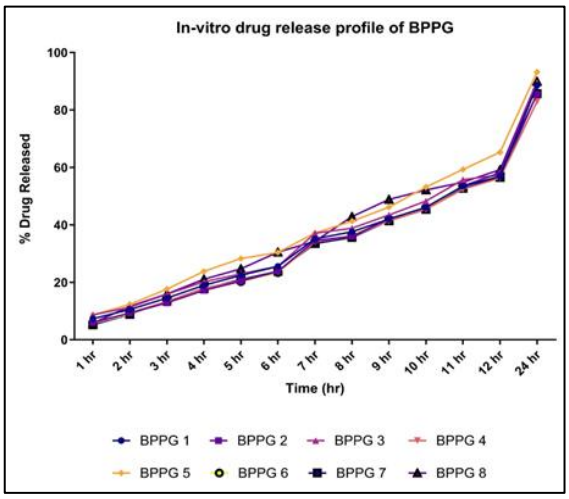
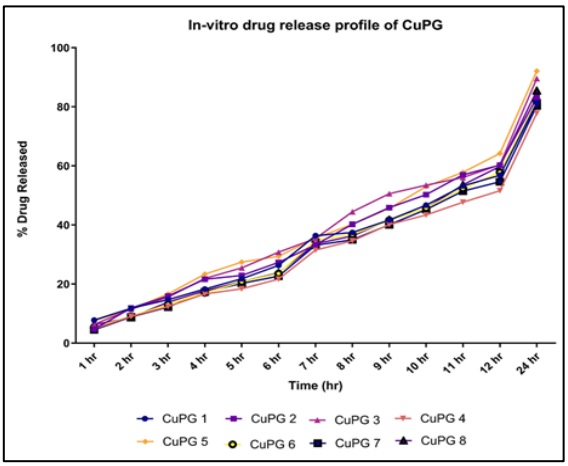


Figure 4. SEM image of the optimized BPPG5 + CuPG5 formulation

***In-vitro* Drug Release**

The optimized BPPG5 formulation released 93.21 ± 1.64% of benzoyl peroxide at 24 h, whereas CuPG5 released 92.08 ± 2.42% of curcumin at 24 h.





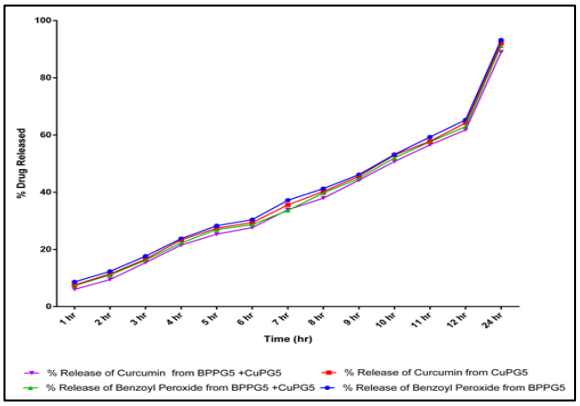
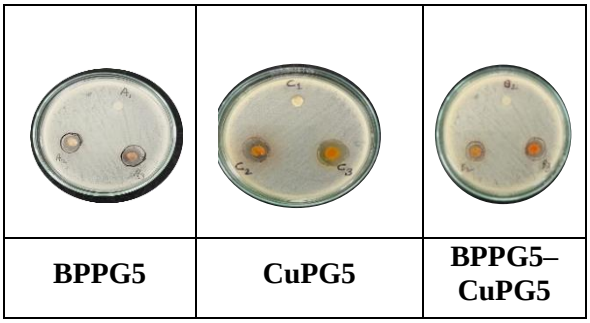


Figure 5. *In-vitro* cumulative drug-release profiles of BPPG5 + CuPG5 formulation over 24 hrs.

Antifungal Activity against *Candida albicans*

The optimized formulations were evaluated against *Candida albicans* using the agar well diffusion method. BPPG5, CuPG5, and BPPG5–CuPG5 showed inhibition zones of 15, 16, and 16 mm, respectively. The combination formulation showed a larger inhibition zone than the individual formulations, indicating enhanced in vitro antifungal activity.



BPPG5	CuPG5	BPPG5–CuPG5

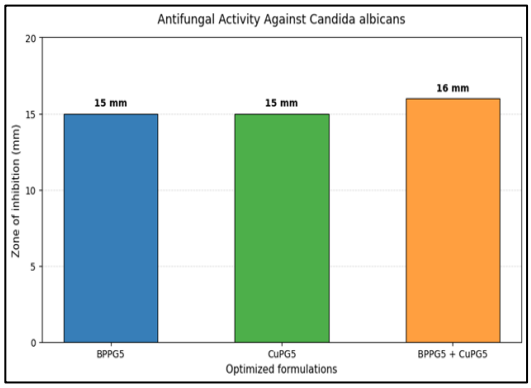


Figure 6. *In-vitro* antifungal activity of optimized BPPG5, CuPG5, and BPPG5–CuPG5 formulation against *Candida albicans*.

The present study successfully developed a Benzoyl Peroxide–Curcumin nanostructured proniosomal gel for topical delivery and evaluated its physicochemical characteristics and in-vitro antifungal activity against *Candida albicans.*

Optimization of the individual formulations identified BPPG5 and CuPG5 as the optimized formulations, exhibiting high entrapment efficiencies of 91.07 ± 1.19% and 88.51 ± 0.74%, respectively, with drug contents of 93.82 ± 0.73% and 92.05 ± 0.97%, respectively. The favorable composition of cholesterol, soya lecithin, and Span 60/Tween 80 may have contributed to efficient vesicle formation and drug entrapment.

The optimized BPPG5–CuPG5 combination formulation exhibited a pH of 6.7 ± 0.84, viscosity of 8524 ± 2.97 cP, and spreadability of 44 ± 1.33 mm, indicating suitable physicochemical characteristics for topical application. The mean vesicle size of 597.3 nm, polydispersity index (PDI) of 0.306, and zeta potential of −33.1 mV indicated the formation of nanosized vesicles with a moderately distributed size profile and favorable surface-charge characteristics. SEM analysis further supported the formation of discrete vesicular structures.

The optimized formulations demonstrated sustained in-vitro drug release over 24 h, with cumulative release values of 93.21 ± 1.64% for benzoyl peroxide and 92.08 ± 2.42% for curcumin. In the antifungal study, BPPG5, CuPG5, and BPPG5–CuPG5 exhibited inhibition zones of 15, 15, and 16 mm, respectively, against *Candida albicans*. The comparatively larger inhibition zone produced by the combination formulation suggests enhanced apparent in-vitro antifungal activity compared with the individual formulations. The optimized BPPG5, CuPG5, and BPPG5–CuPG5 formulations produced inhibition zones of 15, 15, and 16 mm, respectively, against *Candida albicans*. The combination formulation exhibited the highest apparent *in-vitro* antifungal activity among the tested formulations.

CONCLUSION

The present study successfully developed a benzoyl peroxide–curcumin nanostructured proniosomal gel for topical application. BPPG5 and CuPG5 exhibited high entrapment efficiencies of 91.07 ± 1.19% and 88.51 ± 0.74%, respectively. The optimized combination formulation showed suitable topical characteristics, including pH of 6.7 ± 0.84, viscosity of 8524 ± 2.97 cP, spreadability of 44 ± 1.33 mm, vesicle size of 597.3 nm, PDI of 0.306, and zeta potential of −33.1 mV. The formulations demonstrated drug release over 24 h, with cumulative release of 93.21 ± 1.64% for benzoyl peroxide and 92.08 ± 2.42% for curcumin. In-vitro antifungal evaluation against *Candida albicans* revealed inhibition zones of 15, 15, and 16 mm for BPPG5, CuPG5, and BPPG5–CuPG5, respectively. The combination formulation exhibited the highest apparent in-vitro antifungal activity among the tested formulations. Overall, these findings support the potential of the BP–curcumin proniosomal gel as a topical vesicular formulation with promising in-vitro antifungal activity.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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