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Research Article

Liposomal Hydrogel of *Piper longum* for Enhanced Antifungal Therapy: Formulation and Evaluation

Prasanna Raju Yalavarthi*, Rekha Devi Allagadda, Yuvasri D, Poojitha S, Ramyasri Ponna M, Mounika S, Harichandan Sai KVVS

1. Pharmaceutics Division, Seven Hills College of Pharmacy (Autonomous), Venkatramapuram, Tirupati, IN-517561.

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Abstract

Background: Fungal and skin infections represent a significant burden on global public health, exacerbated by the emergence of antifungal resistance and the limitations of conventional topical therapies. *Piper longum* L., a phytomedicinal plant rich in piperine, has demonstrated promising antifungal, anti-inflammatory, and antimicrobial properties. However, its therapeutic potential was not explored for newer or novel drug delivery systems. Aim and objective: To develop, characterize, and evaluate a liposomal-based hydrogel system encapsulating *Piper longum* L. extract for effective antifungal dynamics. Methods: *Piper longum* L. was extracted using water:ethanol (3:1) solvent system and incorporated into liposomes (PLL) prepared via the thin-film hydration technique using varying ratios of soya lecithin and cholesterol. The optimized liposomes were integrated into a Carbopol 940-based hydrogel. Formulations were assessed for physicochemical properties (vesicle size, zeta potential, pH, viscosity, spreadability, and homogeneity), skin irritation and antifungal activity against *Candida albicans*, *Rhizopus*, *Actinomyces*, and *Saccharomyces* species. Vesicle characterization was performed using optical microscopy and dynamic light scattering. Results and discussion: The optimized liposomal vesicles displayed a uniform nanometric size distribution (700 nm–3 µm) with a low polydispersity index (<1) and moderate zeta potential (1.2–10.7 mV), confirming colloidal stability. LBH formulations exhibited neutral pH (7.0–7.4), non-irritating properties, and desirable rheological characteristics. Of the tested formulations, PLL2 exhibited the most significant antifungal activity, showing inhibition zones up to 6 mm against *C. albicans*. Hydrophilic Carbopol 940 matrix facilitated enhanced spreadability and occlusive retention, while liposomal encapsulation ensured sustained bioactive compounds release and targeted epidermal delivery. Conclusion: The *Piper longum*-loaded liposomal hydrogel represents a scientifically rational, biocompatible and effective nanocarrier system for topical antifungal therapy. The synergistic interplay between vesicular phospholipids, bioactive piperine and hydrogel polymers substantially improved dermal delivery and antifungal performance.

Keywords: Carbopol 940, Mycoses, Nanocarrier, Phospholipid, Piperine, Polymer-matrix, Zeta potential

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Introduction

Topical drug delivery systems offer a valuable route for the administration of therapeutic agents, especially in treating localized diseases. This approach circumvents the gastrointestinal tract, thereby avoiding associated irritation and first-pass hepatic metabolism. Moreover, topical formulations allow direct access to the site of infection or inflammation, ensuring higher local drug concentration and minimized systemic side effects (1).

Depending on the desired pharmacological outcome, topical medications can be categorized into four types: (i) agents not intended for systemic absorption (e.g., sunscreens, metal-based protectants); (ii) drugs targeting the skin surface or epidermis (e.g., for acne or eczema); (iii) drugs aiming at deeper tissues such as muscles or joints (e.g., analgesics and anti-inflammatories); and (iv) drugs intended for transdermal systemic absorption (e.g., hormone or insulin patches) (2).

Recent advancements in topical delivery have highlighted hydrogel systems as promising carriers due to their biocompatibility, ease of application, and ability to release drugs in a controlled manner. These systems are particularly suitable for both local and systemic effects depending on formulation design. This study focuses on the use of naturally derived compounds, specifically from *Piper longum* L., for the treatment of superficial fungal infections through a topical hydrogel system.

Piper longum L. (long pepper), a species belonging to the Piperaceae family, has a long-standing role in traditional medicine systems, particularly Ayurveda, due to its wide range of therapeutic properties. It is also used in culinary practices. The plant contains various bioactive phytochemicals such as piperine, piperlongumine, sylvatin, sesamin, diaeudesmin, pipermonaline,

*** Corresponding Author:**
Prasanna Raju Yalavarthi
Pharmaceutics Division,
Seven Hills College of Pharmacy (Autonomous),
Venkatramapuram, Tirupati, IN-517561. India.
Email Id: kanishka9002@gmail.com

and piperundecalidine, which contribute to its medicinal value (3,4).

Piperine ($C_{17}H_{19}NO_5$), the principal alkaloid of *Piper longum* L., is a hydrophobic molecule with low aqueous solubility but good solubility in organic solvents like DMSO, ethanol, and methanol. It has been extensively studied for its pharmacological activities, including antifungal, antimicrobial, anti-inflammatory, antiasthmatic, antidiabetic, hypocholesterolemic, hepatoprotective, and anticancer effects (5,6).

On the other side, fungal infections continue to pose a global health challenge, affecting the skin, nails, and mucosal surfaces. More than one billion individuals are estimated to suffer from fungal infections annually, with *Candida albicans* being a predominant pathogen. Candidiasis can manifest as superficial infections or evolve into life-threatening systemic conditions. Superficial dermatophytoses and other mycoses are commonly treated using azole-class antifungals, but issues such as resistance and adverse effects call for safer alternatives (7–9).

In recent years, studies have shown that *Piper longum* L. and its active constituents, particularly piperine and piperlongumine, possess notable antifungal activity on par with traditional literature (10). These compounds act by disrupting fungal cell membranes, inhibiting ergosterol biosynthesis, and generating reactive oxygen species (ROS) that interfere with fungal metabolism (11). Their natural origin and safety profile make them suitable candidates for topical antifungal applications.

Topical drug delivery is especially suited for fungal skin infections since the epidermis serves as the primary site of fungal colonization and proliferation. Effective treatment requires formulations capable of sustaining therapeutic drug levels within the stratum corneum, while avoiding systemic absorption. This has led to growing interest in nanocarrier-based delivery systems such as liposomes, which enhance drug solubility, stability, and skin penetration (12,13).

Liposomes, derived from the Greek words "lipos" (fat) and "soma" (body), were first described by Dr. Alec D. Bangham in 1961. These are spherical vesicles composed of phospholipid bilayers capable of encapsulating both hydrophilic and hydrophobic drugs. Liposomes are typically classified into multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), and large unilamellar vesicles (LUVs), based on their size and structure (14,15).

As drug carriers, liposomes offer several advantages: improved drug bioavailability, sustained and controlled release, reduced dosing frequency, and enhanced localization at the site of action. Clinically, liposomes have been utilized in therapies for cancer, fungal infections, and viral diseases (16). Incorporating *Piper longum* extract into liposomes could potentially enhance its antifungal activity and minimize toxicity.

To further optimize dermal delivery, liposomes can be embedded in hydrogel matrices, forming liposomal-based hydrogels. Hydrogels are three-dimensional, hydrophilic polymer networks that can absorb significant quantities of water. Their porous structure allows efficient drug entrapment and controlled release. When designed appropriately, hydrogels enhance skin adhesion, penetration, and user compliance (17,18).

Despite certain limitations such as reduced mechanical strength and production scalability, hydrogels remain widely used in biomedical applications including wound healing, drug delivery and cosmetics. Polymers like Carbopol 940, an anionic polyacrylate, are commonly employed to formulate hydrogels due to their gelling efficiency, safety and compatibility with other ingredients (19,20).

To date, limited work has been done on integrating *Piper longum* L. extract into liposomal hydrogels for fungal therapy, making this approach novel and significant, the present study was aimed on *Piper longum*-loaded liposomal hydrogel system for topical antifungal therapy. Using the thin-film hydration method, liposomes were prepared and incorporated into a Carbopol-based hydrogel. The formulation was characterized for its physicochemical, rheological, and biological properties, and its antifungal efficacy was tested against common pathogenic fungal strains.

Materials and methods

Materials

Piper longum L. (long pepper) fruits were procured from local herbal markets in Tirupati, Andhra Pradesh, India. Ethanol, chloroform, triethanolamine, propylene glycol, tween 80, carbopol 940, lecithin and cholesterol were obtained from M/s. SD Fine Chem. Pvt. Ltd., Chennai, India. All chemicals used were of analytical grade.

Plant identification

The botanical identity of *Piper longum* L. was authenticated by local Ayurvedic experts in Tirupati, Chittoor district, based on morphological characteristics, with reference to Ayurvedic Pharmacopoeia standards.

Extraction of active compounds from *Piper longum* L.

Extraction was performed using a standard solvent extraction method. Dried *Piper longum* L. powder (500 g) was macerated in a 3:1 ratio of water and ethanol (w/v) for 24 hours in a dark environment to prevent degradation of light-sensitive constituents. The mixture was then filtered through muslin cloth, and the filtrate was subjected to rotary evaporation at 30–40°C and 92 rpm until a viscous extract was obtained. The resulting concentrated extract was stored in an airtight container for further use.

Table 1: The composition of liposomes

Ingredients	F1	F2	F3	F4	F5
Bioactive P.L. extract	5 ml	5 ml	5 ml	5 ml	5 ml
Soya lecithin	1000 mg	600 mg	500 mg	700 mg	1200 mg
Cholesterol	500 mg	400 mg	500 mg	300 mg	600 mg
Phosphate buffer (7.4)	5 ml	5 ml	5 ml	5 ml	5 ml
Ethanol	2 ml	2 ml	2 ml	2 ml	2 ml
Chloroform	8 ml	8 ml	8 ml	8 ml	8 ml
Tween 80	2 ml	2 ml	2 ml	2 ml	2 ml

Preparation of liposomes

Liposomes were prepared using the thin-film hydration technique. As presented in Table.1 Various formulations were developed by dissolving different ratios of soya lecithin (0.5–1.2 g) and cholesterol (0.4–0.6 g) in 8 ml of chloroform and 2 ml of ethanol. This lipid solution was transferred into a 250 ml round-bottom flask and rotated at 80 rpm using a rotary evaporator at 40°C to form a thin lipid film on the inner walls. The film was hydrated with 10 ml of phosphate buffer (pH 6.8) under continuous

agitation for 2 hours, resulting in the formation of milky liposomal suspensions. These were centrifuged at 10,000 rpm for 30 minutes to separate unencapsulated extract and obtain a uniform dispersion (21).

Preparation of liposomal-based hydrogel

Selected liposome formulation, based on preliminary characterization of zeta profile was incorporated into a hydrogel matrix. Carbopol 940 (0.5 g) was dispersed in 100 ml of double-distilled water and allowed to swell for 6 hours with intermittent stirring to obtain a uniform gel base. The liposome suspension was then incorporated under gentle mechanical stirring. Triethanolamine (2 ml) was added gradually to adjust the gel pH to 7.4. To enhance skin hydration and penetration, 1 ml of propylene glycol and an appropriate amount of glycerin were included in liposomal-based hydrogels (PLL-1 to 4) (22).

Evaluation of *Piper longum* L. extract

Physical characteristics

The extract was evaluated for colour, odour, consistency and viscosity

Evaluation of liposomes

Vesicle size and morphology

Liposome vesicles were observed under optical microscopy to determine shape and size distribution. A thin smear of the sample was prepared on a glass slide for imaging and analysis.

Organoleptic properties

Formulations were assessed visually for appearance, clarity, and homogeneity.

Particle size and zeta potential

To prepare the liposome samples, 1–2 mg of them were mixed with 10–20 ml of deionized water and sonicated for 5–10 minutes to ensure they were well dispersed. The mixture was then filtered using a 0.22 μm syringe filter to remove unwanted particles. Next, the particle size and distribution were measured using a device called ZetaSizer HAS 3000. The stability of the liposomes was also checked by measuring their zeta potential, using disposable zeta cells. Finally, the polydispersity index (PDI), which indicates how varied the particle sizes are, was calculated using the formula $PDI = M_w/M_n$, where M_w is the weight-average molar mass and M_n is the number-average molar mass.

Evaluation of hydrogel formulations

Appearance and homogeneity

Visual inspection was conducted to assess clarity, texture and absence of lumps.

pH measurement

The pH of the formulations was determined using a calibrated digital pH meter. Ideal pH for dermal application was considered to be between 6.0–7.4.

Viscosity measurement

Viscosity was evaluated using a Brookfield synchro-electric viscometer (Model LVDV, spindle #64) at rotational speeds of 0.5, 1, 2.5 and 5 rpm. Readings were recorded at each speed.

Spreadability

Spreadability was measured using a glass slide apparatus. A fixed quantity of gel was placed between two slides; a weight was applied, and the time taken for the upper slide to separate was recorded. Spreadability was calculated as:
$S = (m \times l)/t$, where, S = Spreadability, m = weight applied, l = slide length, t = time to slide separation.

Washability

A small quantity of gel was applied to the skin and rinsed with warm water to assess ease of removal.

Skin irritation test

The hydrogel formulations were tested on Wistar albino rats. Formulations were applied to shaved skin areas and observed for 36 hours for signs of irritation (erythema, edema).

In-vitro antifungal activity

The antifungal efficacy of selected liposomal-based hydrogel formulations (PLL-2 and marketed formulation) was evaluated against pathogenic strains: *Actinomyces*, *Candida albicans*, *Rhizopus* and *Saccharomyces*. Fungal strains were cultured according to ATCC protocols and incubated for 24 hours to promote growth. A sterile Petri dish was prepared by pouring solid agar medium and allowing it to solidify. Once set, the fungal inoculum was evenly spread across the surface using a sterile glass spreader to ensure uniform coverage. Two wells were carefully created in each dish using a sterile cork borer, then filled with PLL-2 and marketed formulation. The plates were incubated for 72–96 hours, allowing the antifungal agents to take effect. Antifungal activity was assessed by measuring the diameter of the inhibition zones, which indicated the extent of fungal growth suppression (23,24).

Results

Evaluation of Piper longum extract

Physical appearance

The water-ethanol extract of *Piper longum* L. was observed to be light brown to dark brown in color, with a distinct pungent and peppery aroma, characteristic of its major bioactive compound, piperine. The extract exhibited a spicy, hot and slightly bitter taste, consistent with phytochemical expectations of phenolic, alkaloidal constituents and volatile oils.

Consistency and viscosity

The consistency of the extract varied from watery to moderately thick depending on the final concentration of solutes and the rate of solvent evaporation. On controlled evaporation at 35°C using a rotary evaporator, the extract became increasingly viscous.

Evaluation of liposomal formulations

Vesicle size and morphology

Optical microscopy revealed that the liposomes were spherical and uniformly distributed. The vesicle size was formulation-dependent, with smaller vesicles forming at higher lecithin-to-cholesterol ratios. Uniformity in size distribution reflects the reproducibility of the thin-film hydration technique and stability of the lipid bilayer during hydration.

Organoleptic properties

All formulations displayed a milky-white appearance with translucent to slightly opalescent dispersion, attributable to the Tyndall effect from nanoscale vesicles. The formulations were homogenous with no sedimentation or phase separation observed during storage, suggesting good physical stability.

Zeta potential and particle size analysis

Dynamic light scattering (DLS) analysis showed that the average particle size of liposomes ranged between 700 nm to 3 µm**,** with a polydispersity index (PDI) below 1**,** indicating a narrow size distribution and high homogeneity of vesicle populations. Among all, formulation PLL-2 exhibited the largest average particle size, attributed to its higher oil phase content, which may lead to the formation of larger lipid domains. As demonstrated in Table 2, zeta potential values ranged from +1.2 to +10.7 mV, suggesting moderate stability of the colloidal system.

Table 2: Zeta profiles PL loaded liposomal hydrogels

Sample	Zeta potential (mV)	PDI	Vesicle size (nm)
PLL-1	-4.1	0.320	716
PLL-2	-10.3	0.324	806.8
PLL-3	-2.6	0.553	1488.7
PLL-4	-8.6	0.603	3041.8

Evaluation of liposomal-based hydrogels

Appearance and homogeneity

As data shown in Table 3, the hydrogels were visually smooth, transparent and free from phase separation or undissolved particles. No grittiness was observed, confirming the proper dispersion of liposomes within the gel matrix. The even texture suggests optimal compatibility between the liposomes and the Carbopol polymer network.

Table 3: Results of Physicochemical tests of hydrogels

Formulation	Appearance	pH	Washability	Spreadability (g. cm/s)	Skin irritation	Viscosity (cP)
PLL-1	Light Brown	6.9	Medium	0.55	No	180
PLL-2	Light Brown	7.1	Good	0.7	No	195
PLL-3	Brown	6.8	Medium	0.6	No	185
PLL-4	Brown	6.7	Medium	0.62	No	190

pH measurement

The pH of the hydrogel formulations was found to be in the range of 7.0 to 7.4 (Table 3)**,** which falls within the ideal range for dermal applications and minimizes the risk of skin irritation. The neutral pH is favorable for patient compliance and maintains the bioactivity of both liposomal vesicles and the encapsulated drug.

Viscosity

The results are given in Table 3 and viscosity was found to exceed 180 centipoise (cP)**,** indicating good gel consistency and shear-thinning behavior desirable in topical applications. The hydrogel maintained its structure across varying shear rates, reflecting its robustness and ease of application.

Spreadability

As shown in Table 3, the PLL-2 formulation exhibited excellent spreadability, enabling uniform application across the skin surface as it is a crucial parameter for ensuring effective drug contact and absorption at the site of infection.

Washability

Observed in Table 3 data, the hydrogels were easily removed from the skin surface using lukewarm water, confirming the formulation's user-friendly nature. High washability ensures that residues do not accumulate on the skin, reducing the risk of clogging pores or inducing allergic reactions.

Skin irritation test

No erythema, edema, or other signs of irritation were observed on the depilated skin of Wistar albino rats over a 36-hour observation period. This confirms the dermatological safety of the hydrogel formulations and supports their potential use in human subjects.

In vitro antifungal activity

Antifungal efficacy of the liposomal-based hydrogel formulations (PLL-1 to 4) was tested against pathogenic fungal strains, including *Candida albicans*, *Actinomyces*, *Rhizopus*, and *Saccharomyces* species. After 72 hours of incubation at 37°C, zones of inhibition were measured. As demonstrated in Table 4, the optimized formulation PLL-2 demonstrated the largest inhibition zone (~6 mm) against *Candida albicans*, suggesting superior antifungal activity compared to other formulations. The activity of PLL-2 was found to be comparable or superior to a marketed antifungal formulation**,** highlighting the potential of liposome-loaded herbal gels in replacing or supplementing conventional treatments.

Table 4: Zone of inhibition (mm) against Candida albicans

Sample	Zone of Inhibition(mm)
Formulation PLL-2	6
Marketed formulation	5.8

Discussion

The study encompassed the extraction of *Piper longum* L. and to overcome the permeability challenges associated with piperine and associated bioactive compounds possesses antifungal properties by entrapping them in phospholipid based liposomes and incorporating these into a hydrophilic polymeric gel for dermal delivery. The extraction process enriched the formulation with piperine, and thin-film hydration enabled its effective encapsulation within liposomal bilayers composed of lecithin and cholesterol**,** both of which contribute to vesicle flexibility and stability. Formulation strategies were optimized based on vesicle size, zeta potential, pH, viscosity, and homogeneity, leading to the selection of the most promising hydrogel formulations (PLL-1–4), with PLL-2 exhibiting the most favorable therapeutic profile.

It was attributed that piperine's antifungal activity via multiple mechanisms, including disruption of fungal cell membranes**,** inhibition of ergosterol synthesis**,** and induction of oxidative stress through reactive oxygen species (ROS) generation. However, its low aqueous solubility and rapid degradation limit its bioavailability in traditional formulations. Entrapment in liposomes preserved the functional integrity of piperine and enhanced its targeted delivery through the stratum corneum**,** thus elevating its antifungal potency.

The liposomes formulated in this study demonstrated nanometric vesicle sizes (700 nm -3 µm) and low polydispersity index (PDI < 1.0), indicating uniform vesicle distribution. These attributes are critical for effective skin permeation. The zeta potential values (1.2–10.7 mV), while moderate, indicated colloidal stability, likely maintained through steric hindrance provided by Tween 80, a non-ionic surfactant that minimized vesicle aggregation via steric repulsion rather than electrostatic charge.

The small vesicle size contributed to deep epidermal penetration**,** enhancing local drug concentrations at the infection site while minimizing systemic exposure. Moreover, the use of cholesterol enhanced the rigidity of the bilayer, preventing premature leakage of the encapsulated bioactives.

The liposomal suspensions were embedded in Carbopol 940-based hydrogels, a crosslinked polyacrylic acid polymer known for its high water content**,** biocompatibility and mucoadhesiveness**.** Upon hydration, Carbopol underwent rapid swelling, forming a three-dimensional matrix capable of holding dispersed vesicles and sustaining drug release. The pH of the hydrogel formulations was maintained at 7.0–7.4 using triethanolamine, which ensures skin compatibility and avoids irritation.

The resulting gels exhibited desirable rheological properties like viscous yet spreadable, washable and non-irritating, which were confirmed through pH analysis, viscosity profiling (>180 cP) and *in vivo* skin irritation tests in Wistar rats. The absence of erythema or edema validated the safety of these formulations for dermal use.

In-vitro antifungal assays against *Candida albicans*, *Actinomyces*, *Rhizopus*, and *Saccharomyces* demonstrated significant zones of inhibition (up to 6 mm)**,** particularly with formulation PLL-2. The enhanced fungicidal activity of PLL-2 can be attributed to efficient encapsulation and sustained release of piperine, improved vesicular delivery through the lipid-rich layers of the epidermis, optimal physicochemical properties, including vesicle uniformity and stability, synergistic effects of liposomes and the hydrophilic gel matrix facilitating enhanced drug permeation and retention at the site of infection (25,26)**.**

These findings are in agreement with previous studies reporting the effectiveness of plant-extract-loaded liposomes in improving antifungal outcomes through enhanced dermal penetration and controlled drug release. The observed antifungal effect can be attributed to the enhanced permeation of bioactives of *Piper longum* L. delivered through liposomes into the fungal cell wall, leading to membrane disruption, oxidative stress, and inhibition of ergosterol synthesis (27,28).

Overall, the liposomal-based hydrogel formulation successfully addressed the inherent limitations of piperine delivery by utilizing phospholipid nanocarriers and a biocompatible hydrogel matrix**.** The combined system demonstrated thermodynamic stability**,** dermal tolerability and superior antifungal activity**,** positioning it as a promising candidate for further preclinical and clinical investigation.

Conclusion

Piper longum L., a plant deeply rooted in Indian traditional medicine, is a promising source of phytoconstituents with notable antifungal, antimicrobial, antioxidant and anti-inflammatory properties. Extracted bioactive components were incorporated into a liposomal hydrogel delivery system using Carbopol 940 as a hydrophilic polymer matrix. PLL-1–4 formulations exhibited favorable physicochemical attributes, including optimal viscosity, clarity, neutral pH, and dermal non-irritancy. Notably, formulation PLL-2 demonstrated superior antifungal efficacy against clinically relevant fungal strains, suggesting its potential for effective localized treatment of superficial mycoses. The application of liposomal nanocarriers enhanced the stability, skin permeability and sustained release profiles, thereby improving its therapeutic potential. These findings underscore the viability of integrating plant-derived bioactives into advanced delivery systems for antifungal therapy. Further investigations should focus on pharmacokinetics, dermal absorption studies using Franz diffusion cells and evaluation on human skin models to substantiate the clinical relevance of the formulation and regulatory considerations will be critical for advancing this formulation toward translational or therapeutic applications.

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