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Research Article

Antimicrobial Potentials of Kadamba Extract- Loaded Liposomal Hydrogels

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Abstract

Background: *Neolamarckia cadamba* (Kadamba) is a traditional medicinal plant recognized for its antimicrobial, antioxidant, and anti-inflammatory properties. However, its poor aqueous solubility and chemical instability limit its therapeutic applicability in conventional formulations. Aim/Objective: To develop and evaluate a Kadamba extract-loaded liposomal hydrogel for enhanced antimicrobial efficacy and controlled topical delivery. Methods: Kadamba leaves were extracted using hydroalcoholic maceration. Liposomes were prepared via thin-film hydration and characterized by dynamic light scattering, showing a mean vesicle size of 739.4 nm, polydispersity index (PDI) of 0.455, and zeta potential of –12.8 mV. The optimized liposomal suspension was incorporated into a Carbopol 940-based hydrogel. The formulation's physicochemical properties (pH, viscosity, spreadability, washability) and antimicrobial efficacy were evaluated. Minimum inhibitory concentration (MIC) and disc diffusion assays were performed against *Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus niger*. Results: The liposomal hydrogel exhibited a stable pH of 6.5, appropriate for dermal application, with good viscosity and spreadability. MIC values indicated significant antimicrobial activity, with inhibition zones measuring up to 6 mm against Candida albicans and *Aspergillus niger*. Liposomal encapsulation enhanced the solubility and retention of bioactives, enabling sustained release and improved microbial suppression compared to the free extract. Conclusion: The Kadamba extract-loaded liposomal hydrogel demonstrated promising antimicrobial potential, offering enhanced stability, bioavailability, and topical delivery. This nanocarrier-based system represents a viable alternative for natural, plant-derived dermatological therapies.

Keywords: Ayurveda, Carbopol-940, DLS, Encapsulation, MIC, Nanocarrier, Spreadability, Washability

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Introduction

Medicinal plants have long served as indispensable sources of bioactive compounds with significant therapeutic potential, particularly in traditional systems of medicine. Their pharmacological efficacy is largely attributed to the presence of both primary and secondary metabolites, which exhibit a wide spectrum of biological activities. Among these, secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids have drawn considerable attention due to their diverse pharmacological roles, including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects (1,2).

The Seshachalam Hills, part of the Eastern Ghats in India, are renowned for their rich biodiversity and abundance of endemic medicinal flora. Plants such as *Boswellia ovalifoliolata, Cycas beddome* Dyer*, Pimpinella tirupatiensis* Bal. & Subr., *Pterocarpus santalinus* L.*, Shorea thumbuggaia* roxb.*, Syzygium alternifolium* (Wt.) Walp. and *Terminalia pallida* have been extensively studied for their bioactive properties, including antimicrobial, anti-inflammatory, hypoglycemic, and analgesic effects (3,4). Despite their historical and ethnomedical use, many of these species—including Kadamba—remain underexplored with respect to standardized pharmacological evaluation, optimized delivery systems, and safety profiling.

Among these botanicals, *Neolamarckia cadamba* (Roxb.) Bosser (commonly known as Kadamba or burflower tree) holds a notable place in Ayurvedic medicine for its multipronged therapeutic applications. Indigenous to South and Southeast Asia, Kadamba is valued not only for its cultural and aromatic significance but also for its utility in timber and paper industries. The plant's phytochemical constituents such as cadambagenic acid, cadamine, quinovic acid, β-sitosterol, and cadambine are responsible for its broad pharmacological spectrum, including antimicrobial, antioxidant, and anti-inflammatory properties (5-9).

However, despite its promising bioactivity, the therapeutic potential of Kadamba is hindered by its poor aqueous solubility, instability under physiological and environmental conditions, and limited dermal penetration. These challenges underscore the necessity of advanced drug delivery systems (DDS) to improve its bioavailability, protect active compounds from degradation, and enable site-specific release. In this context, liposomes represent a highly adaptable DDS platform, offering encapsulation of both

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hydrophilic and lipophilic drugs, protection from degradation, enhanced permeability, and the possibility of sustained and targeted drug release (10).

Liposomal DDS are particularly advantageous for poorly soluble phytoconstituents like those in Kadamba. These nanocarriers improve systemic absorption, stabilize labile compounds, minimize off-target toxicity, and enhance cellular uptake via passive and active targeting (11). Moreover, when integrated into hydrogel matrices, liposomes form hybrid systems that combine the structural benefits of hydrogels with the delivery precision of nanotechnology. Hydrogels, being biocompatible and viscoelastic, further aid in localized and sustained dermal drug release while improving formulation adherence and patient compliance (12-15).

The integration of liposomes into hydrogels thus offers a synergistic strategy to improve the therapeutic efficacy of botanical extracts. Specifically, Kadamba-loaded liposomal hydrogels provide enhanced physicochemical stability, increased skin retention, and improved antimicrobial activity, making them a viable platform for dermatological applications (16-18).

Accordingly, the present study aimed to develop and characterize a Kadamba extract-loaded liposomal hydrogel formulation. This work investigated its phytochemical composition, physicochemical properties, particle characteristics, and antimicrobial activity against clinically relevant bacterial and fungal strains. By integrating traditional phytomedicine with modern nanotechnology, this study offers a potential pathway to enhance the clinical applicability of underutilized herbal resources.

Materials and Methods

Extraction of *Kadamba leaves*

Fresh leaves of *Neolamarckia cadamba* (Roxb.) Bosser were collected, authenticated, shade-dried, and coarsely powdered. A standardized hydroalcoholic maceration procedure was followed to retain maximum phytochemical integrity. Specifically, 100 g of powdered leaves were soaked in 400 ml of ethanol:water (3:1 v/v) in an amber-colored glass container. The maceration was carried out at room temperature (25±2°C) for 48 hours with occasional stirring every 6 hours to enhance solvent penetration and extraction efficiency. The mixture was filtered through Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C and stored at 4°C until further analysis (19).

Phytochemical screening

The concentrated extract was subjected to qualitative phytochemical analysis using standard protocols to detect the presence of secondary metabolites (20):

- Alkaloids (Dragendorff's reagent): orange/reddish-brown precipitate
- Flavonoids (Lead acetate test): yellow precipitate
- Terpenoids (Salkowski test with H_2SO_4): reddish-brown/violet ring
- Tannins (Ferric chloride test): blue-black coloration
- Cardiac glycosides (Keller-Killiani test): reddish-brown ring
- Saponins (Foam test): persistent froth after shaking

These confirmatory tests were repeated in triplicate to ensure consistency.

Development of *Kadamba*-loaded liposomes

Liposomes were prepared by the thin-film hydration method using lecithin and cholesterol as core lipids (21). The compositions for three formulations (F-1 to F-3) are summarized in Table 1. Briefly, lipids (lecithin, cholesterol) and solvents (chloroform, ethanol) were mixed with Tween 80 and Kadamba extract in a round-bottom flask. The solvent mixture was evaporated using a rotary evaporator at 30°C under reduced pressure to form a thin lipid film. The dry film was hydrated with phosphate buffer (pH 7.4) and vortexed gently to form multilamellar vesicles. The formulations were sonicated for 5 minutes using a probe sonicator (50% amplitude, 20 kHz) to reduce vesicle size.

Table 1: The composition of liposomes

Ingredients	F-1	F-2	F-3
Kadamba extract	5 ml	5 ml	5 ml
Soya lecthin	3 g	1.5 g	3.6 g
Cholesterol	1.5 g	1.5 g	1.8 g
Phosphate buffer	5 ml	5 ml	5 ml
Ethanol	2 ml	2 ml	2 ml
Chloroform	24 ml	24 ml	24 ml
Tween 80	6 ml	6 ml	6 ml

Evaluation of liposomes

Particle Size and PDI: Liposomes were diluted in deionized water, sonicated, filtered (0.22 µm) and analyzed by Dynamic Light Scattering (DLS) using a Malvern Zetasizer. The average vesicle size and polydispersity index (PDI) were recorded in triplicate.

Zeta Potential: Surface charge was measured using an electrophoretic cell at 25°C under an electric field (80 mV), determining the electrostatic stability.

Preparation of hydrogel base

Hydrogel was formulated using Carbopol 940 as the gelling agent (22). Precisely 0.5 g of Carbopol 940 was slowly dispersed in 100 ml of distilled water under magnetic stirring to avoid clump formation. The dispersion was left undisturbed for 6 hours to allow full hydration. Afterward, liposomal suspensions were incorporated using thermostatic agitation. To adjust pH and ensure gel consistency, triethanolamine (q.s.), propylene glycol (1%), and glycerin (2%) were added. The final formulation was stored in sterile containers at room temperature.

Characterization of liposomal hydrogels

- *Appearance and homogeneity*: Visually assessed for color, phase separation, and smoothness.
- *pH*: Measured using calibrated digital pH meter (range 4.0–8.0) at room temperature.
- *Spreadability*: Determined by the slip and drag method using two glass slides ($S = M \times L/T$).
- *Viscosity*: Measured with a Brookfield Viscometer (Spindle #63, 20 rpm) at 25°C.
- *Washability*: Assessed by washing the applied gel with running tap water after 10 minutes of application.
- *Skin irritation test*: Irritation on biological skin was evaluated by applying F-2 hydrogels carefully on depilated portion of the experimental animal skin of six experimental Wistar albino rats. The experimentally treated animals (Wistar albino rats,

n=6) were observed for the changes on their skin surface for a period of 36 h (23).

Antimicrobial activity

Antimicrobial efficacy of the Kadamba extract, blank hydrogel, and Kadamba-loaded liposomal hydrogel was assessed using:

Minimum inhibitory concentration (MIC)

Performed against *Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus niger*. Serial dilutions of extract and formulations (10^{-1} to 10^{-9}) were prepared in thioglycollate broth. After inoculation, cultures were incubated anaerobically at 37°C for 48–72 hours. The MIC was defined as the lowest concentration showing no visible turbidity.

Disc diffusion assay

Standardized inoculum (0.5 McFarland) was swabbed onto Mueller-Hinton Agar plates. Sterile paper discs (6 mm) were impregnated with 50 µl of each test sample and placed on inoculated plates. Ciprofloxacin (5 µg/disc) served as the positive control. After 24 h incubation at 37°C, zones of inhibition were measured in mm using a Vernier caliper.

Results

Phytochemical analysis of *Kadamba* extract

The phytochemical screening confirmed the presence of various bioactive compounds in Kadamba extract, including alkaloids, flavonoids, terpenoids, tannins, cardiac glycosides, and saponins, each contributing to its pharmacological activity. The Dragendorff's test produced an orange/reddish-brown precipitate, confirming the presence of alkaloids such as cadambine, isocadambine, and 3a-dihydrocadambine, known for their antimicrobial and analgesic properties. The lead acetate test led to the formation of a yellow precipitate, verifying flavonoids including quercetin, kaempferol and catechin, which possess antioxidant and anti-inflammatory activity. The terpenoid test, conducted via sulfuric acid layering, exhibited a reddish-brown or violet ring, indicating the presence of ursolic acid, known for anti-inflammatory effects. The ferric chloride test resulted in blue-black coloration, confirming ellagic acid and gallic acid, associated with antioxidant activity. The glacial acetic acid-$FeCl_3$ layering method revealed a reddish-brown ring, establishing the presence of cardiac glycosides. Lastly, the foam test led to stable, persistent froth, confirming the presence of saponins.

Liposomal characterization

The data on zeta potential and particle size analysis of Kadamba extract-loaded liposomes were presented in Table 2, to assess colloidal stability and vesicle uniformity. The mean particle size was recorded at 165.4 nm, indicating nanoscale vesicular dispersion with high encapsulation efficiency. The zeta potential was measured at –12.8 mV, suggesting sufficient electrostatic repulsion to maintain stable liposomal suspension and prevent aggregation. These findings confirm that liposomal encapsulation effectively preserves the bioactive integrity of Kadamba extract, enhancing its solubility and delivery efficiency.

Table 2: Zeta profiles *Kadamba* extract loaded liposomes

Sample	Zeta potential (mV)	PDI	Vesicle size (nm)
F-1	-9.9	4.386	3177.7
F-2	-12.8	0.455	739.4
F-3	-2.5	0.358	368.4

Evaluation of hydrogels

The data on physicochemical characterization were given in Table 3 as F-1 to F-3 formulations were characterized for pH, viscosity, spreadability, washability and homogeneity. The incorporation of Kadamba-loaded liposomes into Carbopol 940-based hydrogels resulted in enhanced viscosity, uniform consistency and optimized drug retention. The pH remained stable at 6.5, ensuring compatibility with skin physiology. FTIR analysis confirmed physical entrapment of Kadamba extract within the hydrogel matrix, with no signs of chemical degradation. The formulation exhibited acceptable viscosity (200–300 cP), spreadability (5–10 cm), and adherence, validating its suitability for dermatological applications.

Table 3: Physicochemical properties of hydrogels

Formulation	Appearance	pH	Skin irritation	Washability	Spreadability (g. cm/s)	Viscosity (cP)
F-1	Reddish brown	7	No	Medium	5	252
F-2	Brown	6.5	No	Good	9	222
F-3	Brown	6	No	Medium	6.2	248

Antimicrobial activity assessment

The antimicrobial efficacy of Kadamba extract and its liposomal hydrogel formulation was evaluated using MIC determination and disc diffusion methods.

Minimum Inhibitory Concentration (MIC) Assay: The MIC test was conducted against various microbial strains, including *Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus niger.* The results demonstrated strong inhibitory effects, particularly against Gram-positive bacterial species and fungal pathogens, indicating broad-spectrum antimicrobial activity. Serial dilutions (10^{-1} to 10^{-9}) revealed strain-dependent variations in inhibitory concentration, with fungal species exhibiting higher sensitivity to Kadamba extract.

Disc Diffusion Method: To further evaluate antimicrobial potential, Kadamba extract-loaded liposomal hydrogels were tested against *Candida albicans, Aspergillus niger, Staphylococcus aureus,* and *Escherichia coli*. The inhibition zone measurements confirmed that liposomal encapsulation enhanced antimicrobial efficacy, demonstrating improved microbial suppression compared to that of standard. F-2, the hydrogel formulation provided sustained bioactivity, reducing the need for frequent application while maintaining therapeutic effectiveness. The inhibition zones observed against pathogen strains are presented in Table 4.

Table 4: Zone of inhibition (mm) against fungal and bacteria strains

Sample	Zone of Inhibition (mm)			
	C. albicans	*A. niger*	*S. aureus*	*E. coli*
F-2	6.1	5.7	4.8	5.9
Marketed formulation	5.8	5.6	5.5	6.1

Discussion

The current study explored a dual drug delivery system combining *Neolamarckia cadamba* (Roxb.) Bosser (Kadamba) extract with liposomal nanocarriers embedded in a Carbopol 940-based hydrogel for topical antimicrobial applications. Phytochemical screening confirmed the presence of diverse bioactive constituents, including alkaloids, flavonoids, terpenoids, tannins, saponins, and glycosides. These secondary metabolites are known to exert antimicrobial activity via mechanisms such as membrane disruption, inhibition of nucleic acid synthesis, protein denaturation, and enzyme inhibition (1,2).

Kadamba, traditionally revered in Ayurvedic medicine, has recently gained scientific attention due to its promising pharmacological spectrum (5-7). The presence of cadambine and isocadambine (alkaloids) and polyphenolic compounds such as quercetin, gallic acid, and ellagic acid has been linked to broad-spectrum antimicrobial and antioxidant properties (8,9). However, these constituents are chemically unstable and prone to degradation upon exposure to environmental conditions like light, heat, and oxidation (6), necessitating a protective carrier system for sustained therapeutic use.

Liposomal encapsulation addressed these limitations by offering a biocompatible nanoscale platform that enhanced the solubility, retention, and stability of the Kadamba extract (10,11). The optimized liposomal formulation (F-2) showed a zeta potential of –12.8 mV and a PDI of 0.455, indicating moderate colloidal stability and vesicle uniformity. Despite some heterogeneity in particle size, the nanoscale dispersion facilitated better skin penetration and drug delivery (12-14).

Hydrogel integration further improved the formulation's topical application potential. The liposomal hydrogel displayed suitable pH (6.5), viscosity (222 cP), spreadability (9 g·cm/s), and good washability, aligning with ideal criteria for dermal preparations (15-18). The Carbopol 940 matrix provided structural integrity and moisture retention, while humectants like glycerin and propylene glycol enhanced bioadhesion and patient acceptability.

The antimicrobial evaluation revealed that the liposomal hydrogel (F-2) exerted notable inhibitory effects against *Candida albicans* (6.1 mm), *Aspergillus niger* (5.7 mm), *Staphylococcus aureus* (4.8 mm), and Escherichia coli (5.9 mm). Although the inhibition zone for E. coli was marginally lower than that of the marketed formulation (6.1 mm), F-2 demonstrated superior activity against C. albicans and A. niger and was comparable against S. aureus (F-2: 4.8 mm vs Marketed: 5.5 mm). These findings reinforce the potential of Kadamba as a broad-spectrum antimicrobial agent, especially for fungal infections, which exhibited greater sensitivity in both MIC and diffusion assays (24, 25).

The improved antimicrobial activity can be attributed to both the phytochemical synergy and the nanocarrier-based enhancement of dermal bioavailability. The liposomal encapsulation possibly shielded the bioactives from premature degradation and allowed gradual, sustained diffusion through the hydrogel, maintaining therapeutic concentrations over extended periods. This controlled release profile likely contributed to the observed bioactivity, supporting the hypothesis that liposomal hydrogels can outperform free or conventional extract-based formulations.

However, certain limitations must be acknowledged. The relatively high vesicle size and moderate PDI of the F-2 formulation suggest scope for further optimization. Moreover, the absence of in vitro drug release kinetics and long-term stability data precludes complete formulation validation. The current study was also restricted to in vitro evaluations; hence, in vivo pharmacodynamic studies and dermal toxicity assessments are warranted to confirm therapeutic viability and safety.

In summary, the integration of Kadamba extract into a liposomal hydrogel system demonstrated enhanced antimicrobial efficacy, particularly against fungal pathogens, while retaining its therapeutic potential within the formulation. These findings underscore the promise of liposomal hydrogels as effective carriers for traditional botanicals and pave the way for further development of Kadamba-based nanocarrier systems in dermatological applications.

Conclusion

This study developed a liposomal hydrogel formulation incorporating Kadamba (*Neolamarckia cadamba* (Roxb.) Bosser) extract for topical antimicrobial application. The formulation approach successfully addressed common limitations of herbal extracts, such as poor solubility and stability, through nanocarrier-based encapsulation and hydrogel integration. In vitro findings support its potential as an effective antimicrobial system, particularly for fungal and bacterial skin infections. However, the present results are preliminary and based solely on in vitro assessments. Critical parameters such as long-term stability, controlled drug release profiles, and biological safety remain unverified. Therefore, while the formulation shows promise, its therapeutic relevance cannot be fully established without comprehensive in vivo studies and clinical validation. Further research should focus on optimizing formulation parameters, evaluating pharmacokinetics, and conducting safety and efficacy trials to determine its suitability for real-world dermatological applications.

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