RESEARCH PAPER

FORMULATION AND ANTIMICROBIAL EVALUATION OF A NIOSOMAL GEL INCORPORATING KIGELIA AFRICANA EXTRACT

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Abstract

Topical delivery of herbal antimicrobials is often limited by poor stability and skin penetration of the free extract. This study aimed to develop and evaluate a niosomal gel loaded with the methanolic extract of *Kigelia africana* for topical antimicrobial application. Niosomes were prepared by the thin-film hydration (hand-shaking) technique using Span 60 and cholesterol in five surfactant-to-cholesterol ratios (F1–F5) and were characterized for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency and surface morphology. The optimized niosomal dispersion was incorporated into a Carbopol 934 gel base and evaluated for appearance, pH, viscosity, spreadability, drug content, in-vitro drug release, and antimicrobial activity against *Escherichia coli* by the agar well diffusion method. Formulation F2 (Span 60: cholesterol, 150:100 mg) was identified as optimal, exhibiting the smallest particle size (128.42 nm), lowest PDI (0.241), highest negative zeta potential (−30.6 mV) and greatest entrapment efficiency (89.18 ± 0.51%) among the five formulations tested. Scanning electron microscopy confirmed spherical, smooth, uniformly distributed vesicles. The corresponding niosomal gel was brown, smooth and homogeneous, with a pH of 6.7 ± 0.03, viscosity of 5989 ± 0.54 cps, spreadability of 10.59 ± 0.12 g·cm/s and drug content of 97.82 ± 0.41%, and it exhibited sustained drug release reaching 92.7 ± 0.39% at 12 h. In the agar well diffusion assay, the niosomal gel produced a larger zone of inhibition against *E. coli* (13 ± 0.4 mm) than the pure extract (9.0 ± 0.3 mm), approaching the activity of standard ciprofloxacin (18 ± 0.2 mm, 10 μg/mL). These findings demonstrate that niosomal encapsulation markedly enhances the physicochemical performance and antimicrobial efficacy of *Kigelia africana* extract, supporting the developed gel as a promising, stable, natural alternative for the topical management of microbial skin infections.

Keywords: *Kigelia africana*, niosomes, thin-film hydration, Span 60, herbal gel, antimicrobial activity, topical drug delivery.

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Introduction

The antimicrobial screening and characterization against the Gram-negative bacterium *E. coli* was carried out by the in-vitro model, while the antimicrobial activity combating *S. aureus* and *C. albicans* mentioned in the phytochemical rationale was not re-confirmed independently for the final gel formulation within the scope of the present study. Accelerated and long-term stability data, ex-vivo skin permeation data or in-vivo efficacy and safety data were not prepared. The entrapment efficiency and release studies were conducted by UV–visible spectrophotometry at one specific λ max which was chosen to be representative of the whole extract and not by a validated assay based on marker compounds, as this may not fully represent the release profile of the individual phytoconstituents with varying polarities.

The foregoing, need to be incorporated in subsequent investigations prior to preclinical development of the formulation. Key constituents such as kigelinone, pinnatal, verminoside, specioside, ferulic acid derivatives and lapachol analogues have been linked to antimicrobial, antioxidant, analgesic, cytotoxic, wound-healing, and anti-inflammatory effects. Research has shown that *Kigelia africana* extracts inhibit pathogens such as *C. albicans*, *E.coli*, *Staphylococcus aureus*, *P. aeruginosa* and various dermatophytic fungi, lending scientific support to its traditional use in treating infected wounds and skin disorders[12,13,14,15]. Despite this medicinal potential, direct incorporation

of *Kigelia africana* extract into conventional gels or creams is limited by poor stability, unpleasant texture, inadequate penetration and rapid loss from the application site. Encapsulation within niosomal vesicles provides a protective microenvironment for the active constituents, enhances skin permeation, ensures controlled release, and prolongs contact with infected tissue; incorporating the resulting niosomes into a gel base further improves ease of application, spreadability, texture and patient acceptability(16,17).

Among the key constituents are kigelinone, pinnatal, verminoside, specioside, ferulic acid derivatives, and lapachol analogues determined with antimicrobial, anti-inflammatory, analgesic, antioxidant, cytotoxic and wound-healing properties. The extract of *Kigelia africana* is inhibitory against the pathogens of *S.aureus, P.aeruginosa, C. albicans, E. coli,* and dermatophytic fungi, which provides scientific support applicative in the treatment of infected wounds and skin disorders(12,13,14,15). The medicinal potential was not exploited in direct incorporation in conventional gels or creams due to the poor stability, the unpleasant texture, the low penetration and the high rate of loss from the application site. The encapsulated active ingredients inside the niosomal vesicles offer protection from degradation and degradation by the skin, better permeation of the skin and controlled release, and extended contact time with infected tissue, while the inclusion of the niosomes in a gel base enhances ease of application, spreadability, texture and patient acceptability(16,17). In recent years, the problem of antimicrobial resistance has become a worldwide concern, which has led to an increased interest in antimicrobial substances derived from plants as adjuncts or alternatives to routinely used antibiotics for the cure of superficial soft-tissue and skin infections.

Delayed wound healing and recurrent skin infections are becoming more common with the use of multidrug resistant strains of S. aureus and *E. coli,* and there is a need for new antimicrobial topical agents with a good safety profile. Standardization of herbal extract is one approach currently under investigation to achieve consistency and potency of such natural antimicrobial agents: encapsulation can standardize the amount per dose, mask the undesirable organoleptic properties of the crude extract, and ensure that labile components are not degraded during storage and application5,8.

This study was therefore conceived and designed to formulate and evaluate the topical antimicrobial effect of the niosomal gel containing *Kigelia africana* extract. The individual objective consisted in establishing the optimal ratio of surfactant: cholesterol in the vesicles obtained by thin film hydration method. Vesicle will characterize by vesicle size, charge, entrapment efficiency and morphological characterization, entrapment optimal vesicular form in the gel formulation on the Carbopol 934 base and then to examine the in vitro evaluation of physicochemical parameters of gels as well as antimicrobial efficacy. If it can be developed successfully, this formulation could be a safe and effective alternative to control microbial skin infections, and it would be natural.

Materials and Methods

Materials

The methanolic extract of *K. africana* fruit was obtained from a certified herbal supplier and kept in an amber-colored air tight container at refrigerated temperature of 4 °C until use. Vesicle-forming agents were obtained: Span 60 (sorbitan monostearate) and cholesterol. Carbopol 934 was used as a gelling polymer, and triethanolamine as a neutralisation and pH adjustment polymer. While propylene glycol was used as a humectant and penetration enhancer, both propyl and methyl parabens were used as preservatives. Lipid film preparation was done using chloroform and methanol (analytical grade) and phosphate buffer (pH 5.5, 7.4) was prepared according to India Pharmacopoeia. Nutrient agar, Sabouraud dextrose agar and other microbial media were used for the antimicrobial assays. All chemicals and solvents were analytically pure (18,19).

Microorganisms

The formulation showed its antimicrobial activity against *E.coli* (Gram-negative, ATCC 25922), all of which are associated with skin and soft-tissue infections. Standard cultures were grown on nutrient agar slants, and subcultured before use.(20).

Extraction of plant material and phytochemical screening

The Kigelia africana fruit was shade dried and subsequently powdered before being extracted using Soxhlet extraction method in petroleum ether to remove impurities of a non-polar/fatty nature before methanol was used to extract the fruit. The percentage yield of both extracts was determined with reference to the dry plant material used (21,22). Preliminary qualitative phytochemical screening

was carried out for the presence of alkaloids (Dragendorff's test, Mayer's test, and Wagner's test), flavonoids (alkaline reagent test), phenolics (ferric chloride test), tannins, glycosides, saponins, carbohydrates and proteins/amino acids using standard methods.(23,24)

Preparation of niosomes

Non-ionic surfactant vesicles containing the methanolic extract of Kigelia africana were prepared using the most commonly used and reproducible thin-film hydration (hand-shaking) method, which was able to entrap high percentage of the extract with multilamellar vesicles(25,26). Five different ratios (Table 1) of accurately weighed amounts of Span 60 and cholesterol were placed in a dry 100 mL round-bottom flask. The lipids were completely dissolved in 10 mL chloroform to form a homogeneous and clear organic phase. Span 60 was chosen as the non-ionic surfactant due to its favourable phase transition temperature and in stable vesicles possessing excellent entrapped drug, cholesterol was added as a membrane stabilizer to increase the rigidity of the bilayers, decrease permeability, and to prevent leakage of the entrapped drug during storage(27).

Organic solvent was removed using a rotary vacuum evaporator (Buchi, Switzerland) at 50 ± 2 °C and 80–100 rpm until a thin uniform lipid film was obtained on the inner wall of the flask, then further evaporation was continued under vacuum for 15–20 min until all the chloroform was evaporated. The dried lipid film in the flask was then placed in a vacuum desiccator for about 1h to eliminate any traces of solvents and the risk of solvent contamination of the final formulation. This lipid film was then rehydrated with 10 mL of a phosphate buffer (pH 5.5) with an already known amount of methanolic extract. Hydration was performed at 60 ± 2 °C, which was maintained above the phase transition temperature of Span 60, using gentle rotation of the flask (60 rpm) for 2 h. The lipid film slowly stripped off the wall of the flask, and spontaneously formed multilamellar niosomal vesicles which encapsulated the plant extract28. The niosomal suspension thus prepared was then left to mature for 30 min at room temperature for complete vesicle formation. The suspension was subjected to bath sonication for 5–10 min to get a homogeneous vesicle dispersion and to minimise the aggregation of vesicles. The prepared niosomal formulation was then transferred to the air-tight, amber coloured glass vials and kept at 4 ± 2 °C until further characterization such as particle size analysis, zeta potential measurement, determination of encapsulation efficiency and scanning electron microscopy was performed along with incorporating in gel formulation.

Formulation	Extract (mg)	Span 60 (mg)	Cholesterol (mg)	Chloroform (mL)	PBS (mL)
F1	200	100	50	10	10
F2	200	150	100	10	10
F3	200	200	150	10	10
F4	200	250	200	10	10
F5	200	300	250	10	10

Table 1. Composition of niosomal formulations F1–F5, prepared by the thin-film hydration method.

Characterization of niosomes

Particle size, PDI and zeta potential. The particle size, PDI and zeta potential were measured by a Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire, UK) which is based on the principles of dynamic light scattering (DLS) and laser Doppler electrophoresis (LDE)(29). The freshly prepared niosomal suspension was diluted 10 times with Millipore-filtered distilled water (0.22 μm) to achieve a suitable scattering intensity and then diluted sample was agitated with sonicator for 5–10 min to ensure the homogeneous distribution and aggregation-free state of the vesicles. Each sample was transferred in disposable polystyrene cuvettes and the measurements were taken at 25 ± 0.5 °C. The average hydrodynamic diameter (nm) was obtained and the PDI was employed for uniformity in the size distribution of the vesicles, with a PDI value < 0.30 considered as a narrow and homogeneous particle size distribution suitable for stable vesicular formulations. The values of the zeta potential were obtained by measuring the potential in disposable folded capillary cells and given in millivolts; the magnitude of the zeta potential indicates the surface charge and physical stability of the niosomes; values above about ±30 mV is good

colloidal stability due to electrostatic repulsion(30,31).

The entrapment efficiency (EE%) of each of the formulations of the niosomes was measured by the centrifugation method. In brief, 2 mL of each freshly made niosomal suspension was centrifuged at 4 ℃ for 30 min at 15,000 rpm to isolate the entrapped from unentrapped drug. Free drug was measured using a UV-Vis spectrophotometry at the pre-determined max for the methanolic extract and unentrapped methanolic extract was then harvested by using plain supernatant layer and analysed. The amount of trapped drug was evaluated by: subtracting the amount of free drug in supernatant from the total amount of drug added using the equation:

Entrapment Efficiency (%) = [(Total Drug − Free Drug) / Total Drug] × 100

All determinations were carried out in triplicate and results were given as mean SD. A higher entrapment efficiency indicates greater incorporation of the extract within the niosomal vesicles, reflecting improved vesicle stability and drug-loading capacity(6).

Surface morphology (SEM). To characterize the shape and surface features of the optimized niosomes, SEM imaging was employed. The niosomal suspension was dropped onto a clean aluminium stud and dried in vacuum before the coating of a thin layer of gold (10-20 nm) was performed to increase the conductivity of the sample and to reduce possible charging effects. A Carl Zeiss EVO series scanning electron microscope was used to observe the samples at an accelerating voltage of 10–20 kV. Micrographs of high resolution were captured to assess the surface characteristics, integrity, and aggregation behaviour of vesicles, as well as the shape of these vesicles(32).

Preparation of niosomal gel

The optimized niosomal formulation with methanolic extract of Kigelia africana was prepared and added to Carbopol 934 gel base to make niosomal gel. Carbopol 934 was chosen as the gelling agent due to its superior bio-adhesive qualities, high viscosity, compatibility with a broad range of pharmaceutical ingredients and capacity to create a clear and stable gel which is appropriate for topical application (3). One percent w/v Carbopol 934 was slowly dispersed into the required volume of distilled water with vigorous stirring on a magnetic stirrer (600 rpm) to avoid clumps, the mixture was kept at room temperature for 2 hours to facilitate complete hydration and swelling of the polymer, resulting in a homogeneous gel base. The preservative, methyl paraben (0.2% w/v), was then dissolved in a small amount of warm distilled water (50-60 ℃) and stirred the hydrated Carbopol dispersion to ensure even distribution of the preservative. Propylene glycol 0.5% (v/v) was then added as a permeation enhancer and humectant to facilitate permeation of the entrapped phytoconstituents through the skin and make the formulation more moisturizing.

The optimized niosomal suspension (formulation F2) was then added to the gel base slowly and continuously mixed into base with gentle stir, so as to avoid the disruption of the niosomal bilayer and ensure uniform incorporation. The stirring was carried on until a uniform dispersion was achieved. Finally, the Carbopol dispersions were neutralized with slow addition of triethanolamine with vigorous agitation to obtain a niosomal gel with pH range of 6.8-7.0, suitable for the physiological pH of skin and the gel obtained was smooth and transparent with homogeneous structure. The formulation was kept24 h at room temperature, entrained air bubbles were removed and the formulation was completely stabilized in the gel; it was then put in the airtight, light-resistant containers and stored at 4 ± 2 ℃ until further testing.

Ingredient	Quantity
Carbopol 934	1 g
Propylene glycol	0.5 mL
Methyl paraben	0.2 g
Optimized niosomes (F2)	10 mL
Triethanolamine	q.s.
Distilled water	100 mL

Table 2. Composition of the optimized niosomal gel.

Evaluation of niosomal gel

The formulated niosomal gel was characterized. The physical appearance, homogeneity, pH, spreadability, viscosity, and drug content of the formulated niosomal gel as well as in-vitro drug release profile, were evaluated and characterised to ensure the quality, homogeneity and stability, and suitability for topical application of the formulated product.

pH: The gel was found to have a pH by using a calibrated digital pH meter (Equiptronics, India)

which was previously calibrated with standard buffers of pH 4.0, 7.0 and 9.2. About 1 g of gel was dissolved in 10 mL of distilled water and left to equilibrate for 2 h and then the glass electrode was inserted and a stable reading was obtained. The formulations were tested in triplicate and the mean ± SD was reported; the aim of pH of the gels was to keep values near the physiological pH of the skin (5.5-7.0) to reduce the irritation and to ensure patient acceptability.(19)

Viscosity. Viscosity was determined using about 50 g of gel (that was free from entrapped air bubbles) at 25 ± 1 ℃ and 100 rpm in a Brookfield digital viscometer with spindle no. 61. The measurements were repeated three times, and expressed in centipoise (cP); gel viscosity affects spreadability, consistency, skin-residence time and patient compliance3.

Spreadability. The spreadability was assessed using the glass-slide method that measures the gel's ability to spread across the skin surface. An amount of 1 g gel was clamped between two clean, equal size glass slides and 500g weight placed on top of the upper slide for 5 minutes to facilitate uniform spreading and to exclude air bubbles before another 50g weight was hung on the top slide by a pulley system. The timing of the upper slide in order to achieve a certain distance was measured and the spreadability was calculated as:

S = M × L / T

In the above, S is the spreadability (g·cm/s), M is the weight attached to the upper slide (g), L is the distance over which the slide moves (cm), and T is the time required (s). The greater the spreadability value, the easier it is to apply and the more evenly it's distributed on the skin(33).

Determination of λmax and preparation of calibration curve

The methanolic extract was dissolved in phosphate-buffered saline (PBS, pH 7.4) containing 0.01% methanol and scanned by UV–visible spectrophotometer, the extract had λmax at 225 nm which was used for all subsequent spectrophotometric studies with PBS as blank. Standard solutions (2, 4, 6, 8 and 10 µg/mL) were then prepared by suitable dilution of a stock solution in PBS (pH 7.4, 0.01% methanol) and a calibration curve constructed. The absorbance at 225 nm was measured in triplicate (n = 3) for each concentration and the mean absorbance was plotted against the concentrations; the linear regression equation and the correlation coefficient (R^2) were determined to evaluate the linearity of the method. The formulation was a standardized crude extract and not an isolated marker compound, allowing the construction of a calibration curve with the same analytical conditions used for the in-vitro release study, thereby allowing reliable spectrophotometric quantification of the amount of released extract during the course of the study (34,35)

Drug content: The weighed quantity of gel equivalent to 100 mg of extract was dispersed in 100 mL of phosphate buffer (pH 7.4) or suitable solvent (as mentioned above), stirred until solution was formed and filtered through a Whatman No. 1 filter paper. The diluted extract was analyzed using UV-visible spectrophotometer at max determined as above, and the concentration of drug was calculated with reference to the calibration curve established for the above drug, expressed as a percent of theoretical drug content in triplicate (36).

In-vitro drug release: Drug release from the optimized gel formulation was evaluated using a Franz diffusion cell apparatus fitted with a dialysis membrane (molecular weight cut-off: 12,000–14,000 Da). Prior to the experiment, the membrane was hydrated by soaking it overnight in phosphate buffer (pH 7.4). A precisely weighed quantity of the gel, corresponding to a fixed amount of extract, was placed in the donor compartment. The receptor compartment was filled with phosphate buffer (pH 7.4) maintained under sink conditions, with continuous stirring at 100 rpm and temperature controlled at 37 ± 0.5 °C throughout the study.Aliquots of 5 mL were withdrawn from the receptor compartment at predetermined intervals (0.5, 1, 2, 4, 6, 8, 10, and 12 h), with each withdrawn volume immediately replenished with an equal amount of fresh, pre-warmed buffer to maintain sink conditions. The collected samples were subsequently analyzed for extract content by UV–visible spectrophotometry at the previously established λmax(37,38).

Analysis of drug release kinetics

The optimized niosomal gel was optimized for release of the drug, the cumulative in-vitro release data were fitted with five kinetic models: zero-order, first-order, Higuchi, Hixson–Crowell and Korsmeyer–Peppas to elucidate the mechanism governing the release of the drug. The zero-order model is illustrated by plotting cumulative drug release versus time, where a release rate of n38 equal to a constant amount of drug per unit time is being observed for a drug delivery system. The first order

model is logarithmic plot of % remaining vs. time, representing concentration dependent release. The Higuchi model depicts the release of cumulative of the square root of time, which is applicable to diffusion-controlled release from a matrix system39. The erosion model of Hixson–Crowell describes the relationship between the cube root of the initial drug load and the cube root of the remaining drug load at time t as a linear function of time, which implies that a contribution of surface erosion to release40. The release data were then used to fit the semi-empirical Korsmeyer–Peppas model ($Mt/M\infty = Ktn$), where release exponent ($n < 0.45$ is indicative of Fickian (diffusion-controlled) release, $0.45 < n < 0.89$ is indicative of anomalous (non-Fickian) transport in which relaxation of the polymer and transport of vesicles are both combined with diffusion, $n = 0.89$ is indicative of Case-II (relaxation-controlled) transport, and $n > 0.89$ is indicative of super Case-II transport(41,42). The coefficient of determination (R2) from the linear regression of the transformed release data was used for each model to determine the model that fitted the experimental release profile best(43).

Antimicrobial activity

The optimized niosomal gel, pure methanolic extract and standard ciprofloxacin (10 µg/mL) were tested for their antimicrobial activity against Escherichia coli (ATCC 25922) by the agar well diffusion method, which is a common method used to assess the antimicrobial activity of topical formulations by observing the zone of inhibition created around wells containing the sample. Nutrient agar (28 g/L) was dissolved and then the pH was adjusted to 7.2 ± 0.2, followed by autoclaving (121 °C, 15 psi, 15 min) and then poured into sterile Petri dishes (20–25 mL) under laminar air flow while the medium was still at 45–50 °C. Plates were allowed to solidify and stored at 4 °C until use20. A pure culture of *E. coli* (ATCC 25922) was grown in nutrient broth for 18–24 h at 37 °C until the culture was at the turbidity of the 0.5 McFarland standard (≈ 1 × 108 CFU/mL) for uniform inoculum density(45,46). The sterile 6mm diameter wells were obtained using a sterile cork borer, and the well were filled with 100L of pure extract, optimized niosomal gel and standard ciprofloxacin solution (10 µg/mL) respectively and left for 10–15 minutes. The plates were left undisturbed for 30 min to allow the pre-diffusion of the test samples and then incubated inverted for 24 h at 37 ± 1 ℃. The diameter of the clear zone of inhibition around each well was measured in mean ± SD in triplicate with a digital Vernier caliper, with larger zone of inhibition signifying higher antibacterial activity(44).

Statistical analysis

Unless otherwise indicated, all experiments were carried out three times (n = 3) and the results represent mean ± standard deviation (SD). The data values of particle size, PDI, zeta potential, EE and the physicochemical and antimicrobial parameters of the gel were compared between the formulations by using one way analysis of variance (ANOVA) and the value of $p < 0.05$ was considered statistically significant. Data were analyzed using basic spreadsheet programs.

Results

Percentage yield of extract

The extraction of Kigelia africana fruit was done in series using petroleum ether and methanol. The dark brown semisolid mass of methanolic extract yielded significantly more than the light green sticky petroleum ether extract **(Table 3)** which had a characteristic aroma. Methanol gave the highest recovery indicating its ability to extract more phytoconstituents which are polar in nature such as phenolic compounds, flavonoids, glycosides and tannins and proved to be the best extraction solvent for further formulation studies.

Table 3. Percentage yield of successive solvent extraction of *K. africana* fruit.

S. No.	Solvent	Weight of drug (g)	Yield (g)	% Yield
1	Petroleum ether	300	2.15	0.72
2	Methanol	300	8.43	2.81

Preliminary phytochemical screening

The qualitative phytochemical screening of methanolic extract showed the presence of alkaloids, flavonoids, phenolics, tannins, glycosides, saponins and carbohydrates, and the absence of proteins and amino acids **(Table 4)**. The high concentration of flavonoids and phenolics indicates the antimicrobial and antioxidant properties of the extract, which could be beneficial for wound healing and skin protection, as well as for being applied in a topical system.

Table 4. Preliminary phytochemical screening of the methanolic extract of Kigelia africana fruit.

Phytochemical	Observation	Result

Alkaloids	Cream precipitate	Present (+)
Flavonoids	Yellow precipitate	Present (+++)
Phenolics	Dark green colour	Present (+++)
Tannins	White precipitate	Present (++)
Glycosides	Blood-red colour	Present (++)
Saponins	Persistent foam	Present (+)
Carbohydrates	Violet ring	Present (+)
Proteins	No colour change	Absent (−)

Determination of λmax and calibration curve

The UV scanning of methanolic extract in phosphate buffered saline (pH 7.4, 0.01% methanol) revealed the highest absorption peak at 225 nm which was employed for all further spectrophotometric estimations. A calibration curve was made between 2–10 µg/mL (Table 5) and plotted (Figure 1), which was a linear increase and had a correlation coefficient of R^2 = 0.9987. The developed UV spectrophotometric method was thus found to be suitable to estimate the amount of entrapped, released and retained extract in niosomal formulation and gel throughout the study, with the high correlation coefficient obtained and hence, very good linearity and close adherence to Beer–Lambert law were obtained in the selected concentration range.

Table 5. Calibration curve data for the methanolic extract of *Kigelia africana* in PBS (pH 7.4, 0.01% methanol) at 225 nm (y = 0.0516x − 0.0078, R^2 = 0.9987).

Concentration (µg/mL)	Absorbance at 225 nm (mean ± SD, n = 3)
2	0.083 ± 0.004
4	0.200 ± 0.005
6	0.303 ± 0.006
8	0.409 ± 0.005
10	0.506 ± 0.007

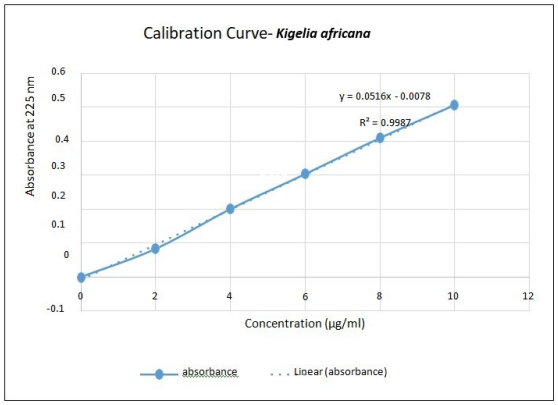

Figure-1. Calibration curve of the methanolic extract of *Kigelia africana* in phosphate-buffered saline (pH 7.4, 0.01% methanol) at 225 nm.

Optimization of niosomal formulations (F1–F5)

The optimized formulation was achieved by preparing the five niosomal formulations with different concentrations of Span 60 and cholesterol by the thin-film hydration technique and the various characteristics of the niosomes were evaluated, such as PDI, particle size, and zeta potential (Table 6). F2 had the most desirable physicochemical characteristics with the smallest particle size (128.42 nm), the lowest PDI (0.241) and the highest negative zeta potential (−30.6 mV) among all the formulations. The smaller size of the particles results in better skin penetration; the narrow particle size distribution, as suggested by the low PDI, ensures a very homogeneous distribution; and the greater negative zeta potential, which indicates good electrostatic stabilisation, decreases the tendency of vesicles to aggregate during storage. The particle size of formulations F1, F3, F4 and F5 was comparatively higher while the magnitude of the zeta potential was comparatively lower, which indicates lower stability and higher aggregation tendency. Thus, formulation F2 was chosen as optimized formulation for further characterization and consideration for the topical gel.

Table 6. Optimization of niosomal formulations F1–F5: particle size, PDI and zeta potential.

Formulation	Particle size (nm)	PDI	Zeta potential (mV)
F1	342.16	0.321	−18.2
F2	128.42	0.241	−30.6
F3	205.38	0.298	−24.4
F4	278.53	0.332	−20.7
F5	398.11	0.351	−17.3

Characterization of optimized niosomes (F2)

Particle size, PDI and zeta potential. Particle size, PDI and zeta potential. The optimized formulation (F2) was then determined by dynamic light scattering analysis, which showed an average particle size of 128.42 nm that is appropriate for topical drug delivery as nanometric vesicles have a larger surface area which will facilitate drug permeation through the skin. The PDI value of 0.241 showed a narrow size distribution and excellent homogeneity of the vesicles; PDI values < 0.30 are considered to represent a system of monodisperse nanoparticles. The zeta potential was found to be at −30.6 mV, which showed sufficient electrostatic repulsion between vesicles, resulting in long term physical stability **(Table 7, Figure 2).**

Table-7. Physicochemical characteristics of the optimized niosomal formulation (F2).

Parameter	Result
Particle size	128.42 nm
PDI	0.241
Zeta potential	−30.6 mV

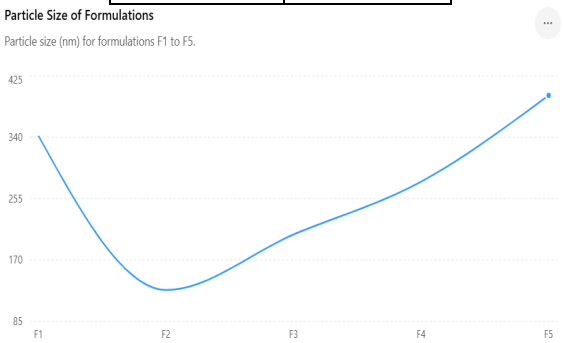

Figure 2. Particle size and zeta potential distribution of the optimized niosomal formulation (F2), determined by dynamic light scattering and laser Doppler electrophoresis.

Entrapment efficiency: The entrapment efficiency of the niosomal formulations prepared with different Span 60/cholesterol ratio **(Table 8)**. The formulation F2 had the highest entrapment efficiency (89.18 ± 0.51%), signifying the efficient entrapment of the methanolic extract in the niosomal bilayer which may be due to the optimum ratio of Span 60 and cholesterol that produced a stable niosomal membrane with minimum leakage of the drug. The entrapment efficiency decreased with a progressive decrease in the surfactant and cholesterol concentration (F3–F5), which may be due to the increase in bilayer rigidity and decreased space available for drug encapsulation. For further characterisation, preparation of the gel and release of the drug, and antimicrobial studies, formulation F2 was therefore chosen.

Table 8. Entrapment efficiency of niosomal formulations F1–F5.

Formulation	Entrapment efficiency (%)
F1	68.45 ± 0.54
F2	89.18 ± 0.51
F3	83.57 ± 0.43
F4	79.64 ± 0.71
F5	73.82 ± 0.62

Surface morphology (SEM). The SEM micrographs of the optimized formulation showed that the vesicles were mostly spherical with a uniform distribution of vesicles with smooth surface and no sign of aggregation **(Table 9, Figure 3)**. The morphology confirms successful formation of stable multilamellar niosomal vesicles with uniform particle size and spherical shape to enhance the drug encapsulation efficiency and controlled drug release.

Table 9. SEM observations for the optimized niosomal formulation (F2).

Parameter	Observation
Shape	Spherical
Surface	Smooth
Aggregation	Negligible
Distribution	Uniform

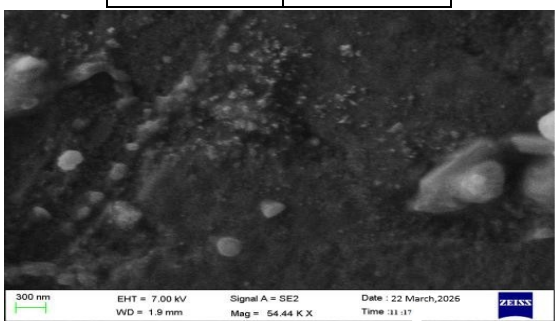

Figure 3. Scanning electron micrograph showing spherical, uniformly distributed niosomal vesicles of the optimized formulation (F2).

Evaluation of niosomal gel

The optimized niosomal formulation was then added to a Carbopol 934 gel and tested for physicochemical parameters (Table 10). The prepared gel was brown, smooth and homogeneous with no grittiness. The formulation's pH (6.7 ± 0.03) was in line with the normal skin pH, which also showed that the formulation was compatible with

normal skin pH, and the viscosity (5989 ± 0.54 cps) was suitable for topical application and was good to allow the formulation to stay at the application site. The spreadability (10.59 ± 0.12 g·cm/s) indicated ease of application and it also showed excellent consistency and homogeneity along with high drug content uniformity which indicates that the methanolic extract is distributed uniformly throughout the gel matrix **(Figure 4).**

Table 10. Evaluation of the optimized niosomal gel.

Parameter	Result
Appearance	Brown, smooth gel
Homogeneity	Homogeneous
pH	6.7 ± 0.03
Viscosity	5989 ± 0.54 cps
Spreadability	10.59 ± 0.12 g·cm/s
Drug content	97.82 ± 0.41%

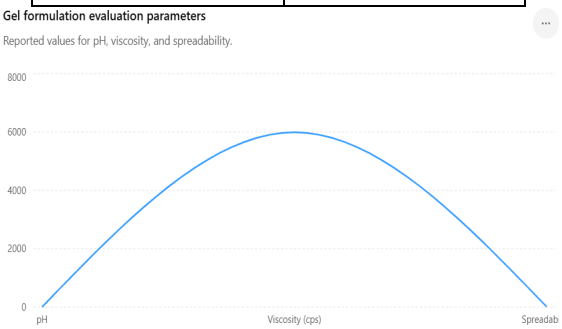

Figure 4. Appearance of the optimized Kigelia africana niosomal gel.

In-vitro drug release study

The Franz diffusion cell was used to evaluate the sustained drug-release pattern of the optimized niosomal gel which was found to be 12 h. (Table 11, Figure 5). It was seen that after 1 h, there was an initial release of 18.2 ± 0.34% and the release gradually increased thereafter and reached 92.7 ± 0.39% after 12 h. This sustained release phenomenon can be explained by the incorporation of the extract in the niosomal vesicles and by the barrier effect of the Carbopol gel. Controlled delivery is beneficial for topical products because fewer doses may be required and the presence of a drug at the application site will remain at therapeutic levels.

Table 11. In-vitro drug release profile of the optimized niosomal gel (F2).

Time (h)	% Drug release
0.5	9.82 ± 0.25
1	18.2 ± 0.34
2	29.4 ± 0.42
4	46.8 ± 0.56
6	61.5 ± 0.63
8	74.3 ± 0.58
10	84.6 ± 0.47
12	92.7 ± 0.39

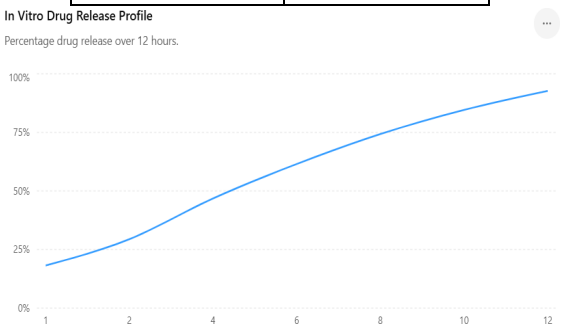

Figure 5. In-vitro drug release profile of the optimized niosomal gel (F2) over 12 hours.

Drug release kinetics of formulation F2

The optimized niosomal formulation (F2) was optimized by fitting the in-vitro release data to various kinetic models (zero-order, first-order, Higuchi, Hixson–Crowell and Korsmeyer–Peppas) to determine the mechanism of drug release as shown in Table 12 and Figure 6. The poorest fit was obtained with the zero order model (R^2 = 0.978) while the first order (R^2 = 0.995) and Higuchi (R^2 = 0.992) models provided progressively better fits; the Korsmeyer–Peppas model provided the best fit overall (R^2 = 0.997). The release exponent (n) calculated from the Korsmeyer- Peppas equation was 0.689, which falls between 0.45 and 0.89, indicating anomalous (non-Fickian) diffusion, and diffusion and vesicle/ relaxation of the polymer mechanism was therefore involved during the release of the methanolic extract from the niosomal vesicles. The relatively high correlation value shown for the first-order model further implies that the drug release rate was dependent on the concentration of the extract inside the niosomal vesicles, and the good linearity of the Higuchi plot also confirms that the drug release was mainly controlled by diffusion from the vesicles; the Higuchi–Crowell model fit (R^2 = 0.986) indicates that the surface erosion of the vesicles/gel matrix also contributed, but to a lesser extent, in addition to diffusion. All these findings suggest that formulation F2 gave a real controlled and sustained release of the methanolic extract of

Kigelia africana, suitable for prolonged topical drug delivery.

Table 12. Release kinetic parameters of the optimized niosomal formulation (F2).

Kinetic model	R^2	Interpretation
Zero-order	0.978	Moderate fit
First-order	0.995	Excellent fit
Higuchi	0.992	Diffusion-controlled release
Hixson–Crowell	0.986	Surface erosion contributes
Korsmeyer–Peppas	0.997 (n = 0.689)	Best fit; anomalous (non-Fickian) diffusion

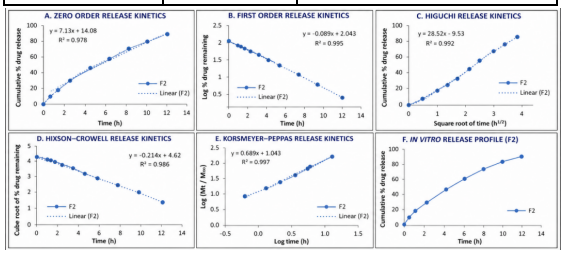

Figure 6. In-vitro drug release kinetics of the optimized niosomal formulation (F2) fitted to (A) zero-order, (B) first-order, (C) Higuchi, (D) Hixson–Crowell and (E) Korsmeyer–Peppas models, and (F) the cumulative in-vitro drug release profile.

Antimicrobial activity

The antibacterial activity of the formulation against Escherichia coli was evaluated by agar well diffusion method. The zone of inhibition obtained by the pure extract, niosomal gel and the standard drug (ciprofloxacin- 10ug/mL) is presented in **Table 13** and the representative agar well diffusion plate is presented in f**igure 7**. The niosomal gel showed a larger zone of inhibition (13 ± 0.4 mm) than the pure extract (9.0 ± 0.3 mm) with the highest zone of inhibition (18 ± 0.2 mm) shown by the standard drug. The increased activity of the niosomal gel could be explained by the added penetration and sustained release effect of the bioactive phytoconstituents from the niosomal carrier system, supporting that microbial activity of Kigelia africana extract was considerably enhanced by its niosomal encapsulation.

Table 13. Antimicrobial activity of pure extract, niosomal gel, and standard drug against Escherichia coli.

Sample	Zone of inhibition (mm)
Pure methanolic fruit extract	9.0 ± 0.3
Niosomal gel	13 ± 0.4
Standard drug (ciprofloxacin, 10 µg/mL)	18 ± 0.2

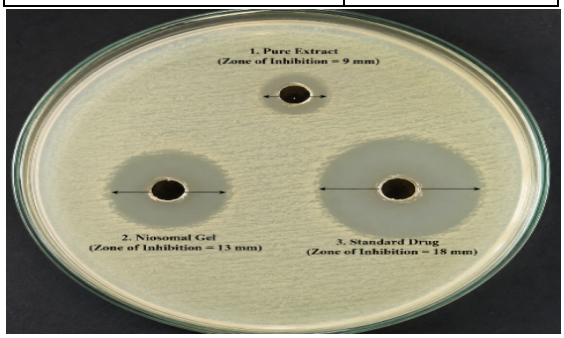

Figure 7. Agar well diffusion assay showing zones of inhibition against Escherichia coli: (1) pure extract (9 mm), (2) niosomal gel (13 mm), and (3) standard drug, ciprofloxacin 10 µg/mL (18 mm).

Discussion

The aim of the investigation was to develop and test the topical antimicrobial formulation of methanolic extract of Kigelia africana fruit incorporated in niosomes as a gel. The methanolic extract exhibited a very high percentage yield (2.81%) as compared to the petroleum ether extract (0.72), which means methanol is a more effective solvent for the extraction of polar phytoconstituents. The observation is in agreement with previous reports that the bioactive secondary metabolites like glycosides, tannins, phenolics, flavonoids and saponins present in the medicinal plants could be better recovered by polar solvents22,23. Preliminary phytochemical screening revealed the presence of alkaloids, phenolic compounds, tannins, flavonoids, glycosides, saponins and carbohydrates in methanolic extract, while proteins and amino acids were not detected. The results of this phytochemical profile are consistent with the traditional medicinal uses of Kigelia africana and corroborate earlier research which demonstrated the presence of various bioactive compounds with antimicrobial, antioxidant and wound healing activity.10 Flavonoids and phenolic compounds are of special significance due to their ability to disrupt the microbial membrane, inhibit microbial enzymes and interfere with the synthesis of nucleic acids, while tannins could have antimicrobial activity by precipitating proteins and causing the microbial adhesion disruption9,23. The spectrophotometric

quantification of extract by UV spectrophotometric method was developed at the wavelength (λmax) of 225 nm and the calibration curve was linear with the correlation coefficient (R^2) of 0.9987, which fulfils the criteria for spectrophotometric method validation for linearity34,35. The use of a standardized crude extract and thus quantifying the whole extract under the same analytical conditions applied in the release study, instead of an individual phytoconstituent, was the most defensible approach for tracking overall drug release and the good linearity results obtained support the reliability of the entrapment-efficacy, drug-content and release data reported throughout this study. Five niosomal formulations (F1–F5) were prepared by changing the ratio of span 60 to cholesterol to optimize the characteristics of the vesicles. Of these, F2 had the smallest particle size (128.42 nm), lowest polydispersity index (0.241) and highest negative zeta potential (−30.6 mV). The main advantages of nanometric vesicles, for topical drug delivery, are related to their high surface area, increased contact with the skin surface and increased capacity to penetrate the stratum corneum27. A PDI value of < 0.30 means that the vesicles are a relatively homogeneous population, and a greater negative value of zeta potential means better colloidal stability and less aggregation. The results are consistent with previous reports indicating that the ratio between the surfactant and cholesterol is very important in achieving stable niosomal vesicles with desirable size and dispersity47,5. The drug-loading capacity of vesicular delivery systems is important and entrapment efficiency is one of the important indicators. The formulation F2 showed the highest entrapment efficiency (89.18 ± 0.51%), which was considered as an efficient encapsulation of methanolic extract into niosomal bilayer. The enhanced entrapment may be explained by the optimal concentration of span 60 and cholesterol, which provides an appropriate rigidity–fluidity balance of the membrane, leading to reduced drug leakage and greater stability of vesicles. This has also been reported for niosomal systems made using Span 60, which has a relatively high phase-transition temperature and low HLB that supports good drug entrapment and stable vesicle formation27,47. The optimized formulation was found to be the spherical vesicles with smooth surface and uniform distribution without any aggregation by the scanning electron microscopy. This morphology has been confirmed to be successful in vesicle formation, hence it is a good morphology for extended drug retention and controlled drug release, and it is also good for the uniform distribution of vesicles which leads to formulation stability and reproducible drug delivery27,32. A niosomal formulation was optimized and incorporated successfully in a Carbopol 934 gel. The prepared gel had good physicochemical properties, as indicated by its smooth appearance, homogeneous consistency, skin compatible pH (6.7 ± 0.03), acceptable viscosity (5989 ± 0.54 cps), good spreadability (10.59 ± 0.12 g·cm/s) and high drug content (97.82 ± 0.41%). The drug formulation would be expected to enhance patient acceptability and give even drug distribution over the skin surface with the use of these characteristics for topical application. In-vitro drug release study showed that the drug release profile was sustained, with 92.7 ± 0.39% of the drug being released after 12 h. Controlled release is related to the presence of the extract in the interior of the vesicles formed and the diffusion barrier provided by the Carbopol gel matrix. The advantage of sustained release is for topical therapy: it extends the time that the drug stays at the site of application, decreases the number of doses required and helps to keep the drug in the body for longer1,38. From the observed release pattern, it can be concluded that the niosomal gel can possibly be utilized in the antimicrobial therapy for localized application in which the active components require prolonged contact with the target area. The release data was best fitted to the Korsmeyer–Peppas model (R^2 = 0.997) followed by the Higuchi model (R^2 = 0.992), the first order model (R^2 = 0.995) and the worst fit to the zero order model (R^2 = 0.978). The relatively good agreement with the first order model suggests the release rate was dependent upon the concentration of the extract in the vesicles, as its amount in the vesicles decreased, the release rate decreased, and the high correlation with the Higuchi model, which describes the amount of drug released as a function of the square root of time, confirms that the diffusion through the vesicular bilayer and gel matrix was a major rate controlling process39. The intermediate fit achieved using the Hixson–Crowell erosion model (R^2 = 0.986) suggests that a small amount of surface erosion of vesicles or gel matrix may have contributed to release, in addition to diffusion, but that diffusion would most likely be the dominant mechanism for release40. The release exponent (n = 0.689) was the most informative parameter for mechanistic interpretation and

indicates that the drug release was not diffusionally controlled, but rather vesicle/polymer-relaxation processes, in addition to Fickian diffusion, were involved in the drug release, i.e., diffusion was anomalous, non-Fickian transport41,42. This is also mechanistically consistent with a niosome-in-Carbopol-gel system, where for such a system, the exponent n values are located between the purely Fickian (n = 0.45) and Case-II (n = 0.89) transport behaviour in cross-linked or swellable polymeric matrices(43). The kinetic analysis taken together suggests that the niosomal gel is not merely a depot of antimicrobial agents, but a truly controlled-release system which is well suited for long lasting topical antimicrobial effect while reducing the frequency of administration.

The optimized niosomal gel had the largest zone of inhibition (13 ± 0.4 mm) against E. coli among the methanolic extract (9.0 ± 0.3 mm) while the standard drug, Ciprofloxacin had the highest zone of inhibition (18 ± 0.2 mm). The increased antibacterial efficacy of the niosomal gel could be due to better penetration of the encapsulated phytoconstituents, the protection of the bioactive components from degradation and the release of the bioactive ingredients in a controlled manner. Plant extracts administered in the form of vesicles can be expected to show better therapeutic efficacy due to increased solubility, stability and interaction with biological membranes than their administration in the non-vesicular form27,9. In conclusion results suggest that niosomal encapsulation has a significant effect on the antimicrobial activity of Kigelia africana extract and make it a potential topical herbal drug delivery system.

Conclusion

Successful design and assessment of niosomal gel formulation using the methanolic extract of Kigelia africana fruit (KAF) for use in the topical application as an antimicrobial agent. The methanolic extract was found to have a higher yield of extraction and contained different important phytoconstituents such as flavonoids, glycosides, saponins, phenolic compounds, tannins and alkaloids. On the basis of the results obtained from the five formulations prepared, formulation F2 was identified as the optimized niosomal formulation having a small particle size, low polydispersity index, high negative zeta potential and maximum entrapment efficiency. The optimized niosomal gel exhibited appropriate physicochemical properties, high drug loading and a prolonged release of the drug for 12 hrs. In addition, the antibacterial activity of the niosomal gel is higher than the methanolic extract itself against Escherichia coli, showing that niosomal encapsulation was more effective in enhancing the therapeutic activity of the herbal extract. In conclusion, it can be inferred from the results that the prepared niosomal gel provides a stable and potent drug delivery system to enhance antimicrobial activity of Kigelia africana and hence can be suggested as substitute for conventional topical herbal drug delivery system.

Future Scope

- In order to evaluate long term physical and chemical stability of the optimized niosomal gel under ICH stability conditions 48
- To evaluate the formulation in-vivo wound-healing and antimicrobial activity by appropriate animal models.
- To investigate skin permeation and retention characteristics of the optimized formulation in ex-vivo human/ animal skin. Identify and quantify the key bioactive compounds which exhibit antimicrobial activity by HPLC or LC–MS.
- To assess the activity of the formulation against a wider variety of Gram-positive and Gram-negative bacteria as well as Candida albicans.
- To further optimize the formulation by the addition of further permeation enhancers or alternative non-ionic surfactant.
- To carry out pre-clinical safety and clinical studies to evaluate the therapeutic efficacy and marketability of the developed niosomal gel.

Limitations of the Study

The antimicrobial screening and characterization against the Gram-negative bacterium Escherichia coli (E. coli) was performed in the in-vitro model, while the antimicrobial activity against Staphylococcus aureus (S. aureus) and Candida albicans (C. albicans) mentioned in the phytochemical rationale was not re-confirmed independently for the final gel formulation within the scope of the present study. Accelerated and long-term stability data, ex-vivo skin permeation data or in-vivo efficacy and safety data were not prepared. The entrapment efficiency and release studies were conducted by UV–visible spectrophotometry at one specific λmax which was chosen to be representative of the whole extract and not by a validated assay

based on marker compounds, as this may not fully represent the release profile of the individual phytoconstituents with varying polarities. The above features have to be taken into consideration while carrying future work before moving towards preclinical development of the formulation.

List of Abbreviations

Abbreviation	Full form
ATCC	American Type Culture Collection
cP / cps	Centipoise
CFU	Colony-forming unit
CLSI	Clinical and Laboratory Standards Institute
DLS	Dynamic light scattering
EE%	Entrapment efficiency (percentage)
HLB	Hydrophilic–lipophilic balance
ICH	International Council for Harmonisation
LDE	Laser Doppler electrophoresis
PBS	Phosphate-buffered saline
PDI	Polydispersity index
SD	Standard deviation
SEM	Scanning electron microscopy
UV	Ultraviolet
λmax	Wavelength of maximum absorbance

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data supporting the findings of this study will be made available by the corresponding author upon reasonable request.

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