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Original Article

Formulation, Optimization, and Antifungal Efficacy Evaluation of *Wrightia tinctoria* R.Br Latex-Loaded Niosomal *in situ* Gel

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ABSTRACT

Introduction: *Wrightia tinctoria* Latex has been found to have an antifungal effect but its therapeutic use is limited because of low stability and low bioavailability. **Aim:** To prepare a niosomal *in situ* gel formulation that enhances the antifungal efficacy and the stability of *Wrightia tinctoria* Latex. **Materials and Methods:** Niosomes were made from Span 60 and cholesterol, which contained *Wrightia tinctoria* Latex, and were added to an *in situ* gel containing Carbopol and HPMC. The physio-chemical characteristics of the formulation, the drug delivery profile and the antifungal effects of the formulation were determined. **Results:** The niosome formulation showed an entrapment efficiency of 73%, a prolonged drug release over 8 hr, and better antifungal activities on both *Candida albicans* and *Aspergillus niger* when compared to crude Latex. **Conclusion:** A niosomal *in situ* gel formulation has increased the stability, the drug release and the antifungal effects of *Wrightia tinctoria* Latex, providing an opportunity for the use of this product in a clinical setting as an anti-fungal agent for treating skin fungal infections.

Keywords: Antifungal efficacy, Bioavailability, Drug delivery system, Herbal drug, Nanotechnology, Niosomes, Phytochemicals, Skin infection.

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INTRODUCTION

The advancement of nanotechnology in health care system is an advanced method to develop diagnostics tools, drug therapeutics to diagnose and treat diseases, which is referred to as nanomedicine. This new technology enhances drug stability, improves drug solubility, bioavailability at lower doses, and reduces the toxic effects of drugs. Nanotechnology has been mainly utilized to enhance drug delivery systems that target healthy cells with low toxicity and high efficacy. Evolution has changed the form of drugs from micro to nano technology and this research is initiating the formulation of niosome-loaded *in situ* gels. A niosome is a microscopic lamellar structure formed through the mixing of cholesterol and non-ionic surfactants of many different types (e.g., tween 80, span 60), in which both hydrophilic and lipophilic drugs can be entrapped. An *in situ* gel is a polymer-based formulation that represents an innovative drug delivery system characterized by the unique ability to undergo a transition from a liquid (sol) to a solid (gel) (Khan and Irchhaiya, 2016; Ray *et al*., 2018; Saharawat and Verma, 2024). The combination of these two innovations in one formulation produces an enhanced formulation for anti-fungal activity, which is focused on either inhibiting or killing fungi, often referred to as antimycotic agents (Figure 1). Our research focuses on *Wrightia tinctoria* R.Br, a deciduous shrub or small tree that ranges from 3 - 15 M in height. It belongs to the "Apocynaceae" family and is typically found in Australia, India, Myanmar, Nepal, Timor, and Vietnam. The Latex of *Wrightia tinctoria* has been shown to possess significant antifungal properties, supporting its traditional application for the treatment of fungal infections (Figure 2). Bioactive compounds, such as triterpenoids, flavonoids, and phenolic compounds, which are present in the Latex of *Wrightia tinctoria* have exhibited inhibitory effects against various pathogenic fungi including *Candida albicans, Aspergillus niger*, and *Trichophyton* spp (Jamshed *et al*., 2019; Tomar *et al*., 2008). The antifungal properties support its historical application to treat skin infections, ringworm, and other dermatologic fungal infections. Topical applications of the Latex inhibit fungal growth and inflammation thereby enabling the healing of infected skin areas rapidly. Although the antifungal properties of *Wrightia tinctoria* Latex have been supported historically by tradition, additional scientific validation is necessary to

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define its mechanism of action and to maximize its therapeutic effectiveness against a larger array of fungal pathogens. Therefore, this research will provide a new drug delivery system (Aditya *et al*., 2005; Alghaith *et al*., 2021).

MATERIALS AND METHODS

Materials

The Latex of *Wrightia tinctoria* was collected from the Arumbathapuram-Thavalakuppam Road at the Thirukanchi Sri Kasi Vishalakshi Sametha Sri Kasi Vishvathar Temple in Othiyapet, Villianur, Puducherry, India. The other materials that were used in this study are: phosphate buffer pH 7.4; Span 60 a non-ionic surfactant; cholesterol; Carbopol 934; Hydroxypropyl Methyl Cellulose (HPMC); triethanolamine; glycerine; and methyl paraben (Rajesh *et al*., 2007; Tomar *et al*., 2008).

Methods

Preparation of Niosomes

The fresh Latex was dried (lyophilized) and tested for preliminary analytical properties. The Latex was then formed into niosomes via the thin-film hydration process. Span 60 as a non-ionic surfactant and cholesterol, in ratios of 1:1 or 2:1, were mixed together to create a thin layer on a round bottom flask. Upon formation of this layer, it was then hydrated with PBS (pH=7.4) that contained the Latex. The niosome-containing formulation was then frozen (lyophilized) once again to produce a powdered niosome product; which is easily loaded into an *in situ* gel (Table 1) (Miatmoko *et al*., 2021; Sezgin-Bayindir and Yuksel, 2012).

Loading Niosomes into *in situ* Gel

Carbopol 934 was measured out, dissolved in distilled water, and stirred for 2 hr to ensure total hydration. At the same time, HPMC was weighed, dissolved in distilled water, and heated to 40°C. Next, the hydrated Carbopol 934 was combined with the HPMC solution, the niosomal protein was then added to the resulting Carbopol-HPMC suspension. A small amount of triethanolamine was then slowly dripped into the suspension while it was being stirred to adjust its pH to between 4.5-5.5. Finally, a preservative was included and the suspension was filled to the required volume with distilled water (Table 2) (Guo, 1994; Shahin *et al*., 2011).

Quantitative Analysis of *Wrightia tinctoria* Latex

Determination of λ_{max} and Calibration Curve

The absorption maximum for the niosome suspension was determined by scanning the wavelength range of 200-400 nm with a UV-visible spectrophotometer using a 5 mg/mL *Wrightia tinctoria* Latex suspension that was prepared in a phosphate buffer (pH 7.4). The calibration curve was developed by dissolving 1 mL of the niosomal suspension in 100 mL of phosphate buffer (pH 7.4) to create a 50 µg/mL stock solution; 1 mL of the stock was then further diluted to 10 mL with phosphate buffer (pH 7.4) to create a 5 µg/mL stock solution. Five different aliquots (2, 4, 6, 8, 10 mL) of the stock solution were taken and placed into volumetric flasks and the volume adjusted to 10 mL. Absorbance measurements were conducted on each of the five solutions at 204 nm, and the absorbance values were plotted to develop the calibration curve (Kadam *et al*., 2024; Sadeghi *et al*., 2020; Said *et al*., 2024; Tomar *et al*., 2008).

Statistical analysis for *Wrightia tinctoria* Latex Loaded Niosomes

Vesicle Shape

Niosome shape and morphology were assessed utilizing an optical microscope with Dewinter Pro 4.1 Vision microscopic software. A droplet of the niosome preparation was placed onto a clean glass slide and viewed through a 100x lens. The images of the niosomes were then taken with a Dewinter digital camera to document and analyse the shape and size of the vesicles. In addition to analysing the surface morphology of the vesicles and their internal structures, SEM was used (Mor *et al*., 2021; Takayama *et al*., 1973).

Vesicle Size and Polydispersity Index

Vesicle average size and Polydispersity Index (PDI), of niosome formulations, were determined by utilizing a Malvern Zeta sizer (v7.1). Only a few drops of formulation were mixed with distilled H_2O and then placed in a disposable zeta cell. Samples were allowed to equilibrate at 25°C for 80 sec prior to measurements. Measurements were performed in triplicate, to provide for both accurate and reproducible data, and the mean size and PDI values were recorded (Sadeghi *et al*., 2020; Shukr, 2016).

Zeta Potential

Surface charges on niosomal vesicles were measured with a Zeta sizer (Version 7.1) through Laser Doppler Electrophoresis to measure the charge. The niosomal sample was diluted with distilled water and placed within the electrophoretic chamber and subjected to a 150 mV electric potential; The zeta potential results will provide an insight into how stable the vesicles are physically and whether they are likely to aggregate due to electrostatic repulsive forces as higher absolute zeta potential values would indicate greater electrostatic repulsion of one vesicle by another and thus reduce likelihood of aggregation and increase the physical stability of the vesicles (Al-Mahallawi *et al*., 2019; Bhattacharya *et al*., 2025; Singh *et al*., 2011).

Entrapment Efficiency

The Entrapment Efficiency (EE%) of the Latex loaded niosomes made from *Wrightia tinctoria* Latex were measured based on the amount of unentrapped drug present in the supernate after separation of the clear supernate from the niosomal suspension (*Wrightia tinctoria* Latex-loaded niosomes). First, a

predetermined volume (2 mL) of the niosomal suspension was placed in a centrifugal tube; 8 mL of phosphate buffer (pH 7.4) was then added to the tube. The solution was then centrifuged at 3000 rpm. for 30 min at ambient temperature. The clear supernate was removed and the amount of free (unentrapped) drug that remained in the supernate was analysed via a UV Spectrophotometer at 204 nm against a calibration curve of drug concentrations (Gupta *et al.*, 2011; Moghassemi *et al.*, 2016).

In addition to determining the free drug, the total drug content within the niosomal suspension was determined by lysing a portion of the original niosomal suspension by adding an equal volume of methanol to it, and then vortexing the solution for 10 min to break down the vesicles. The total drug content was also measured via UV Spectrophotometry at 204 nm. The EE% was determined as follows:

$$EE\ \% = \left(\frac{Total\ drug\ -\ Free\ drug}{\text{Total drug}}\right) X100$$

Antifungal Activity

Antifungal activity of Niosomal *Wrightia tinctoria* Latex was assessed by a Disc Diffusion Technique in an Agar Plate (Agar-Disc-Diffusion Test). *Candida albicans* and A*spergillus niger* (fungi) were cultured and inoculated onto a sterile Sabouraud Dextrose Agar (SDA) plate. Fungi were first suspended in saline solution and adjusted to 0.5 McFarland turbidity. Inoculums were then spread on the sterile SDA medium. Paper disks (diameter of 6 mm) were used as carriers of the test samples, positive control, and negative control. Each disk was loaded with Niosomal *Wrightia tinctoria*

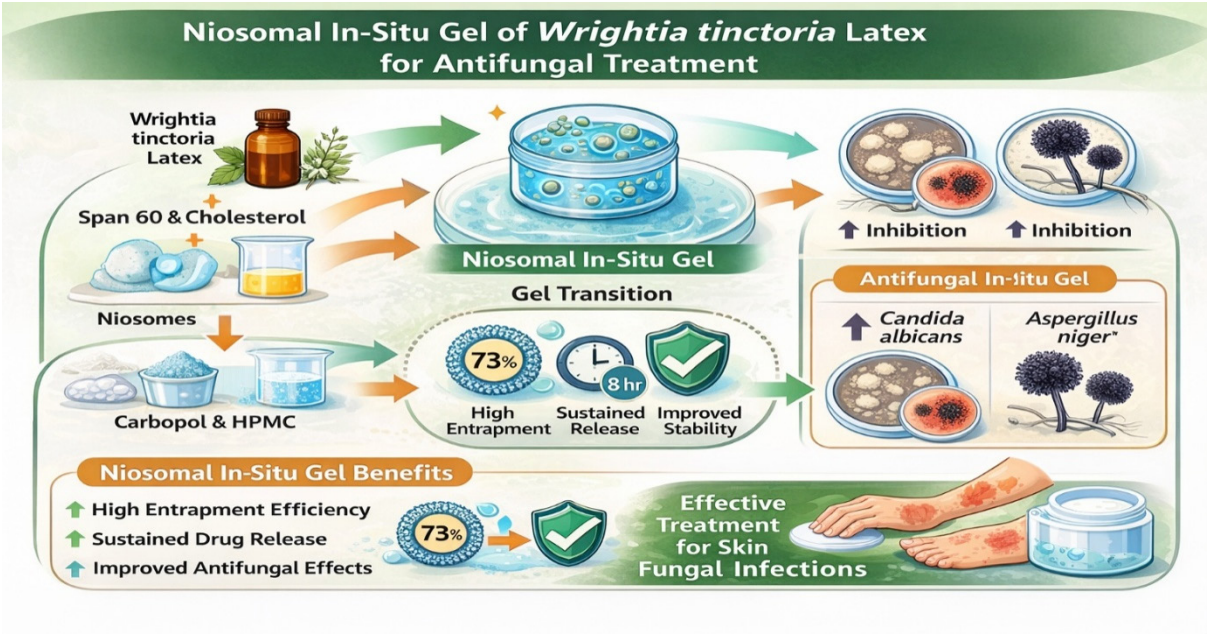

Figure 1: Graphical Abstract.

Table 1: Formulations of *Wrightia tinctoria* Latex loaded Niosome (Celdrán *et al.*, 2023).

Sl. No.	Formulation code	Chloroform (mL)	Methanol (mL)	Cholesterol (mg)	*Wrightia tinctoria* Latex (mg)	Span 60 (mg)	Phosphate buffer 7.4 (mL)
1.	A1	20	40	250	500	250	Q.S
2.	A2	40	60	250	500	500	Q.S
3.	A3	60	80	250	500	1000	Q.S

Table 2: Formulations of Niosome loaded *in situ* Gel (Gustiani *et al.*, 2020).

Sl. No.	Formulation code	Carbopol 934 (gm)	HPMC (gm)	Glycerine (mL)	Triethanolamine/ Dilute HCl (mL)	Noisome loaded WT latex (gm)	Methyl paraben (gm)	Distilled water
1.	F1	0.2	1	3	0.3-0.5	10	0.02	Q.S
2.	F2	0.4	1.5	3	0.4-0.6	10	0.02	Q.S
3.	F3	0.8	2	3	0.5-0.7	10	0.02	Q.S

Latex, Fluconazole (positive control) and Phosphate Buffer (Negative Control). The disks were laid down on inoculated SDA plates and kept in an incubator under temperature of 28-30°C for 48-72 hr. After incubation, the presence of the inhibition zone around the test disks and standard disks indicated that the test products exhibited antifungal properties. Sizes of the inhibition zones formed around the test and standard disks were determined in millimetre units. Each test was repeated three times to ensure reproducibility (Garg *et al.*, 2021; Haque *et al.*, 2017).

Evaluation of *Wrightia tinctoria* Latex Loaded Niosomal *in situ* Gel

Appearance and Clarity

Visual inspection of the formulation was conducted on white and black backgrounds to examine colour, uniformity, and clarity. Any visible particulate matter, sedimentation, or phase separation in the formulation was documented to evaluate formulation homogeneity.

Gelling Time

Gelling time was evaluated using the tube inversion technique. 1 mL of the niosomal formulation was pipetted into a test tube that contained 5 mL of pre-warmed simulated physiological solution (phosphate buffer, pH = 7.4) at 37°C. After the tube was lightly agitated; the time required for the gel to set, as defined by the inability of the gel to flow upon inversion of the tube, was recorded using a timer. This procedure was repeated three times, and the mean gelling time is reported (Asthana *et al.*, 2016).

Gelling Capacity

The gelling capacity of each formulation was tested using 5 mL of the phosphate buffer solution, pH 7.4, that had been warmed to 37°C. To this, 1 mL of the formulation was added to the solution contained within a test tube and the transformation from liquid to gel was observed visually. Each sample's gelation capability was rated as follows: (+) = slow forming gel that will dissolve very quickly; (++) = an immediate gel formation that is stable for a short time (a few hours); (+++)= an instantaneous gelation that will be stable for a longer period of time (Mahajan *et al.*, 2020; Shastri *et al.*, 2010).

Figure 2: *Wrightia tinctoria* (this image is obtained from creative common licenses internet source).

pH

The pH of an *in situ* gel formulation was determined using a calibrated digital pH meter. After adding 1 g of the gel to 10 mL of deionized water and allowing the mixture to reach equilibrium at room temperature for 60 min, the pH of the solution was measured (Zhang and Zhou, 2018; Zhu *et al.*, 2017).

Viscosity

Viscosity measurements were performed on the formulation using a Brookfield viscometer. First, a known volume of the formulation was added to a beaker and allowed to reach 37±1°C. Then the spindle was placed in the sample, and the viscosity was recorded at each of four different rotational speeds (10 rpm, 20 rpm, 50 rpm, 100 rpm) in order to determine the rheological properties of the sample. Each measurement was made in triplicate, and the average viscosity of the sample was calculated from the three separate measurements (Ghica *et al.*, 2016).

In vitro Drug Release

The *in vitro* release of drugs from formulations were tested as drug diffuses through an *in vivo* like diffusion barrier, the eggshell membrane. Firstly, the eggshell membrane is separated from the eggshell by placing the eggshell into dilute nitric acid for a short period of time. Secondly, the eggshell membrane is then placed in the middle of the diffusion apparatus's two chambers (donor and receiver). Thirdly, the formulation is added to the donor chamber and phosphate buffer (pH 7.4) at 37°C is added to the receiver chamber along with a stirrer. Lastly, at specified time periods (15, 30, 60, 120, 240 and 480 min), 2 mL of the solution in the receiver chamber is removed and analysed using a UV spectrophotometer at 204 nm to determine how much drug had been released over the course of that time interval (El-Badry *et al.*, 2014; Kim *et al.*, 2021).

Release Kinetics

In order to understand the release mechanisms of drugs from these formulations, the release profiles from the *in vitro* studies were analysed by means of four kinetic models: Zero-Order, First-Order, Higuchi, and Korsmeyer-Peppas. Correlation Coefficient (R^2) Values were determined to assess how well each

Table 3: Solubility profile of *Wrightia tinctoria* Latex.

Solvent	Solubility Observation
Distilled water	Insoluble
Ethanol	Partially Soluble
Dimethyl sulfoxide	Soluble
Other organic solvents (Chloroform)	soluble

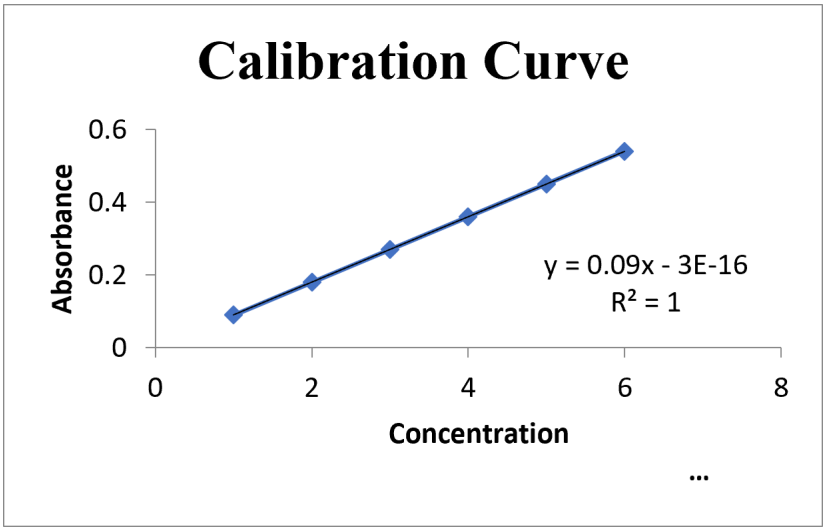

Figure 3: Calibration curve.

Table 4: **Phytochemical screening of *Wrightia tinctoria* Latex.**

Secondary Metabolites	Test Performed	Results
Steroids	Liberman-Burchard's Test	+
Flavonoids	Alkaline Reagent Test	+
Amino acids	Ninhydrin test	-
Carbohydrates	Molisch's Test	+
Tannins	Lead acetate test	+
Terpenoids	Salkowski's Test	+
Glycosides	Keller-Killiani Test	+
	Borntrager's Test	+
Reducing sugars	Fehling's test	+
Saponins	Froth Test	+
Alkaloids	Mayer's test	+
Proteins	Biuret Test	+
Phenols	Ferric Chloride Test	+

of the above-mentioned models described the *in vitro* release data. A high R^2 Value indicates a better fit and therefore the most likely release mechanism (Haghiralsadat *et al.*, 2017).

Ethical Statement

None.

RESULTS

Qualitative Analysis of *Wrightia tinctoria* Latex

Solubility Analysis

The Solubility Characteristics of *Wrightia tinctoria* Latex were investigated by visually examining the dissolution characteristics of the latex in various solvents at laboratory scale. The *Wrightia tinctoria* Latex did not dissolve in Distilled Water; dissolved partially in Ethanol; and dissolved completely in Dimethyl Sulfoxide (DMSO); and in Chloroform. The results of this study are provided in detail in (Table 3).

Preliminary phytochemical analysis

Phytochemical analysis on Latex from *Wrightia tinctoria* revealed the existence of numerous bioactive compounds; specifically, the preliminary data showed that *Wrightia tinctoria* Latex contains alkaloids, cardiac glycoside, anthraquinone glycosides, flavonoids, saponins, carbohydrates, reducing sugar, steroids, terpenoids, tannin, phenol, and proteins. Amino acid was reported as absent (Table 4). Many of the above-mentioned compounds have been documented to show antifungal activity, including alkaloids, flavonoids, tannins, phenols, and saponins. Therefore, the large variety of identified secondary metabolites from Latex of *Wrightia tinctoria* indicate that it has a considerable pharmacological importance to support the traditional application of *Wrightia tinctoria* Latex in treating fungal skin infections.

Quantitative analysis of *Wrightia tinctoria* Latex

Determination of λ_{max} and calibration curve

The UV-vis spectroscopic measurements of the Latex from *Wrightia tinctoria* indicated an absorption band at 204 nm, which was identified as λ_{max} and therefore the most probable wavelength for UV-vis spectroscopic measurements to be made between 200 and 400 nm. Calibration curves were generated from standards solutions (Figure 3).

Vesicle Shape

The surface morphology of the prepared *Wrightia tinctoria* Latex loaded niosomes were studied by Scanning Electron Microscopy (SEM). SEM pictures demonstrated that the formed vesicles had a spherical shape, a smooth surface and a uniform particle size. The observations illustrated the niosomal structures were successfully produced in a very stable form (Figure 4). Spherical shapes are favourable because they lead to improved structural stability of

the particles; provide a uniform incorporation of the drug into the vesicles; and reduce the risks of particle aggregation when stored. These results clearly show that niosome formulations based on a mixture of surfactants and cholesterol will produce nanostructures of a high degree of stability that can be used in the pharmaceutical field.

Vesicle Size

The vesicle size of the developed wild type *Wrightia tinctoria* Latex loaded niosomes was determined via a zeta analyser (DLS), Dynamic Light Scattering; this analysis showed that the niosome formulation was at optimal size (average of 117.0 nm) and had a Polydispersity Index (PDI) of 0.285. As the PDI is under 0.3, this is representative of a very narrow distribution of particle sizes; and therefore a homogeneous population of particles. It has been suggested that lower PDI values are beneficial for drug carriers as they demonstrate that the particles will have consistent sizes. Consistent particle sizes will lead to the ability to predict the pharmacokinetics and stability of the formulation, and ultimately be able to reproduce the formulations. The particle size and the distribution of the developed niosomal suspensions indicate that these systems will be suitable for use as drug delivery systems that can provide controlled release.

Zeta Potential

The zeta-potential of *Wrightia tinctoria* latex loaded into the formulated niosomes was determined with a zeta sizer, to determine the amount of electrical charge at the surface of the niosome, and to evaluate the stability of the prepared niosomal suspension. as seen from Figure 4, the measured zeta-potential is -13.9 mv and therefore falls in the acceptable zone of moderate stability (from +10 to -30 mv) generally shown in non-ionic surfactants based vesicles. since it has a negative zeta-potential the vesicle is covered by an uniform electrical double layer which results in sufficient electrostatic repulsive forces among the vesicles, this repulsion will prevent the aggregation of the vesicles during the storage time and ensure good physical stability of the niosomal suspension. therefore, the zeta-potential of the formulated niosomes shows that they have appropriate physical stability for their use in the pharmaceutical field (Figure 5).

Entrapment Efficiency

The Entrapment Efficiency (EE) of the formulated niosome encapsulated with phytochemicals derived from the latex of *Wrightia tinctoria* was 73± 0.58%. That means a substantial amount of the phytochemicals are encapsulated in the double-layered vesicle. Entrapment efficiency is an important parameter to evaluate the suitability of vesicular carriers because high entrapment efficiency would ensure high therapeutic load and good bioavailability of the active ingredient. The reason why the entrapment efficiency is so high could be due to the optimal surfactant and cholesterol ratios used in the formulations that enable stable bilayer formations and reduce the amount of active ingredients leaking out. In general hydrophilic components are trapped in the aqueous core of the vesicles while lipophilic components are trapped in the lipid bilayer of the vesicles. Since phytochemicals have both hydrophilic and lipophilic properties they should be effectively trapped in the vesicles. Thus, the high

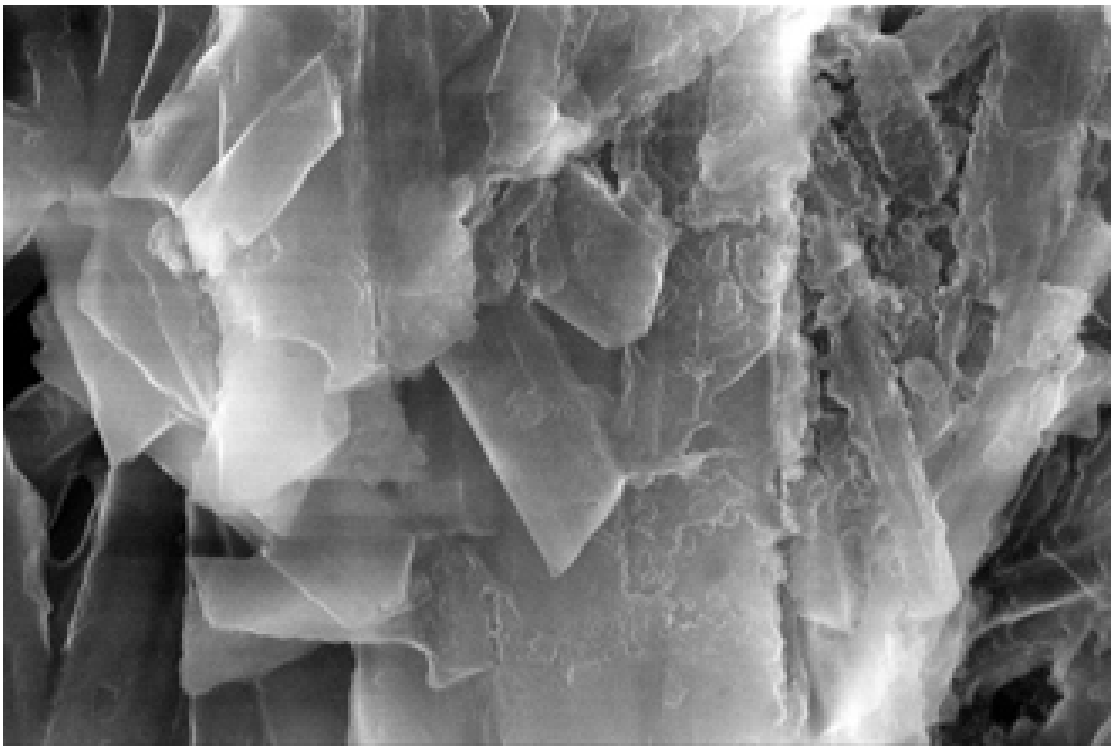

Figure 4: Vesicle shape (SEM image).

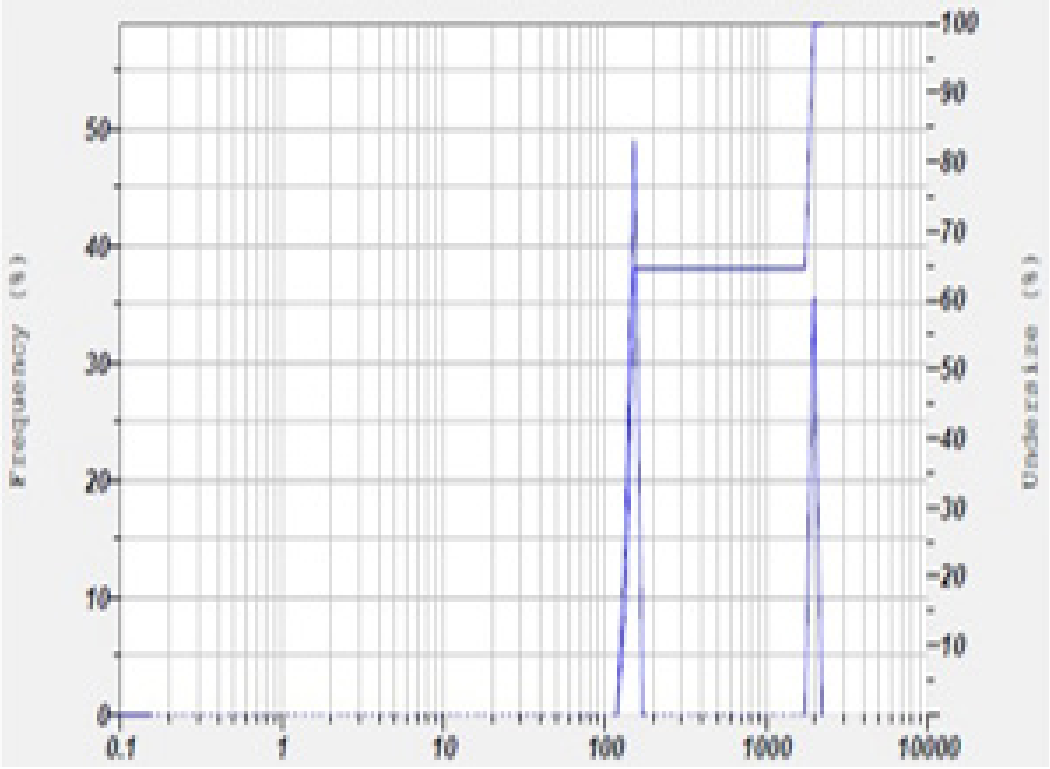

Figure 5: Zeta Potential.

entrapment efficiency of this formulation demonstrates that it has potential as a carrier for effective drug delivery and therefore serves as a good starting point for further studies on *in vitro* release characteristics etc.

Antifungal Activity

An assessment of the antifungal properties of the niosomal formulation of *Wrightia tinctoria* latex with respect to *Candida albicans* and *Aspergillus niger* was conducted using the agar disc diffusion method. Zone of inhibition data indicated that the niosomal formulation (T) demonstrated a zone of inhibition around the disc, supporting the conclusion that it has good antifungal effects. The zone of inhibition for the standard fluconazole (S), compared to T, was greater, thereby demonstrating high antifungal activity. No zone of inhibition was found for the Control (C) disk containing phosphate buffer at pH 7.4, thus eliminating the possibility of nonspecific inhibition (Figure 6).

The niosomal formulation inhibited *Candida albicans* with clearly defined zones of inhibition (14-16 mm) while fluconazole was able to produce zones of inhibition (approximately 18-20 mm). The niosomal formulation also exhibited zones of inhibition (12-14 mm) against *Aspergillus niger*, as opposed to the standard fluconazole which was able to produce larger zones of inhibition (17-19 mm) (Figure 6). Overall, these data indicate that niosomal encapsulated *Wrightia tinctoria* Latex is more effective than unencapsulated crude latex as an antifungal agent; this enhanced activity may be due to the delivery of drugs through the vesicular system resulting in better drug delivery and longer duration of drug release.

Evaluation of Niosomal *in situ* gel

Appearance and clarity

Depending upon the concentration of Carbopol, there were noticeable differences in the physical appearance and clarity of the *Wrightia tinctoria* Latex loaded Niosomal *in situ* Gel Formulations as determined by visual inspection. As indicated by the results in Table 6, the F1 formulation exhibited a milky white opaque yet uniform dispersion which reflected the good homogenization of the formulation without any evidence of particulate matter. Similarly, the F2 formulation displayed similar properties to the F1 formulation in terms of color and opacity; however, it demonstrated an increased density compared to F1 and a uniform dispersion throughout. Finally, the F3 formulation was visually characterized as a thick milky white opaque dispersion demonstrating the effect of increased polymer concentration on viscosity and gel density.

A uniform and homogeneous appearance for all formulations indicates the absence of particle agglomeration or phase separation which is indicative of the effective inclusion of niosomes into the Carbopol matrix. A uniform and homogeneous appearance is a critical Quality Attribute for topical/transdermal gel formulations as it will directly impact both patient acceptability and the long-term stability of the product (Table 5).

pH

The pH levels of the prepared *Wrightia tinctoria* Latex loaded in niosomes *in situ* gels were analysed to determine whether they can be applied topically. All of the tested formulations fell in the physiological pH range of 6.0-7.0. The pH level of the F1 formulation was measured at 6.8. The pH level of the F2 formulation was measured at 6.5. The pH of the F3 formulation was measured at 6.2. It was also noted that there was an inverse relationship between pH and Carbopol concentrations. This result would support the idea that as Carbopol concentrations increase the pH of the formulation decreases due to the inherent acidic nature of Carbopol. Overall, these data suggest that all three of the tested formulations have pH ranges that are suitable for use on the skin, and thus minimize the potential for irritation (Table 6).

Gelling Time

The gelling times of *in situ* gels loaded with niosomes containing *Wrightia tinctoria* latex were measured to evaluate how quickly they could undergo sol-gel transformation on contact with skin. The F1 formulation formed a gel in 70±3 sec; the F2 and F3 formulations transformed much sooner, at 55±3 and 40±3 sec, respectively. Increased gelling times observed as Carbopol concentrations increased indicate an increase in the rate of polymeric cross-linking and therefore an increase in viscosity which ultimately results in quicker gel formation. A quick gelation would provide the desired stability of the formulation and longer duration of the formulation's residence at the intended site of action.

Gelling Capacity

The gelling potential of the *Wrightia tinctoria* Latex loaded niosomal *in situ* gel formulations were evaluated visually for assessment of the gelling capacity (strength and duration) of the formed gel. F1 exhibited moderate gelling properties (++), where there is an indication that gelation occurs within a short time frame; however, the gel structure is less rigid than would be desired for good adherence and sustained topical application. Conversely, both F2 and F3 formulations exhibited superior gelling capacity (+++), as evidenced by rapid gelation upon mixing and long-term stability of the gel with no evidence of gel dissolution. The enhanced polymer concentration in the latter

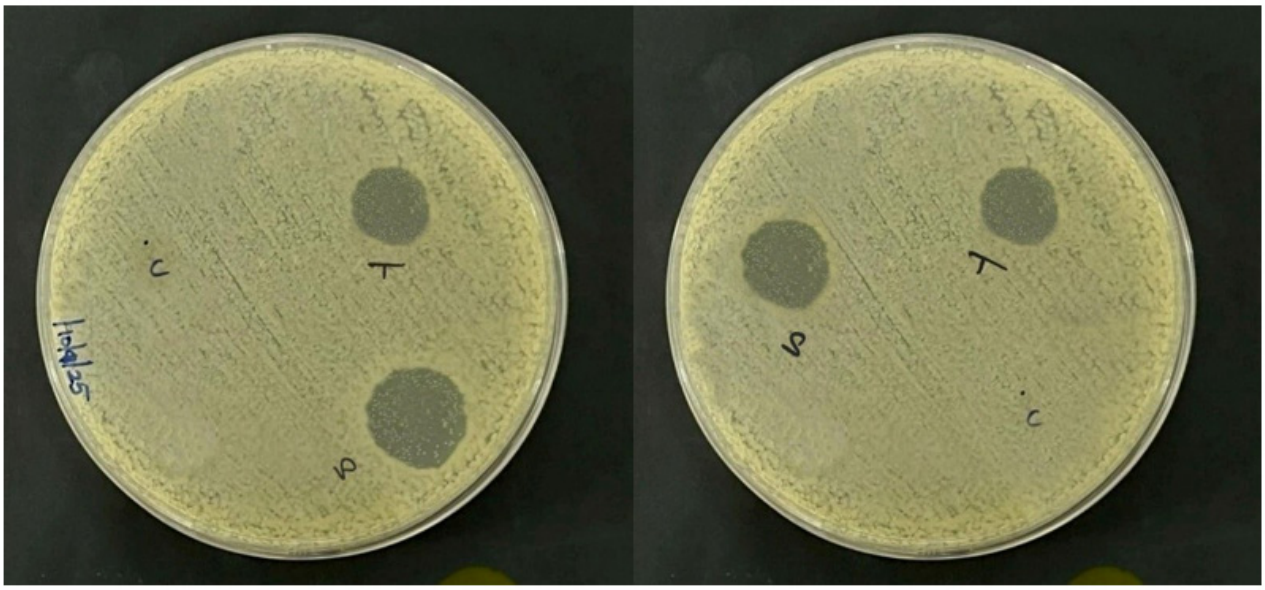

Figure 6: Antifungal Activity (Zone of inhibition).

Table 5: Overall Evaluation of Niosomal *in situ* Gel.

Formulation	Appearance	Clarity	pH	Gelling time (sec)	Gelling capacity	Viscosity (cP)	
						Viscosity before gelation	Viscosity after gelation
F1	Milky white, uniform dispersion	Opaque	6.8	70±3	++	500±15 cP	1800±28 cP
F2	Milky white, slightly more dense, uniform dispersion	Opaque	6.5	55±3	+++	640±18 cP	2500±35 cP
F3	Thick milky white, Uniform dispersion	Opaque	6.2	40±3	+++	880±20 cP	3200±42 cP

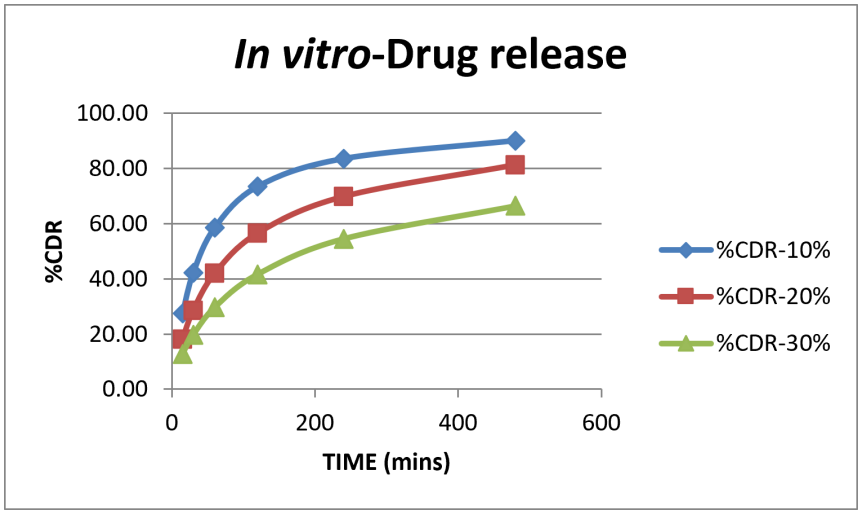

Figure 7: *In vitro* Drug Release.

two formulations resulted in increased gel viscosity and density, which significantly improved the adherence characteristics and extended the topical residence time of the formulations.

Viscosity

The data on the viscosity of the solutions show an increase based on concentration of the various *Wrightia tinctoria* latex loaded niosomal *in situ* gels; this is especially evident from the F1 formulation, which was found to have a solution viscosity of 500±15 cP, but the same formulation had a viscosity of 1800±28 cP after gelation. This high increase in viscosity would allow for the easy application of these formulations as they would be able to form a soft gel network upon gelation. The solution viscosity of the F2 formulation was found to be 640±18 cP, while the same formulation had a viscosity of 2500±35 cP after gelation. This large increase in solution viscosity indicates improved gel strength and retention. Finally, the F3 formulation demonstrated the largest increase in solution viscosity of all three formulations. Specifically, the F3 formulation was found to have a solution viscosity of 880±20 cP, but it had a viscosity of 3200±42 cP after gelation. These results demonstrate that increasing the concentration of polymers will result in a better sol to gel transition, an increase in gel network strength and retention (Table 6).

In vitro Drug Release

Drug release from *Wrightia tinctoria* latex loaded niosomal *in situ* gels was studied using an egg shell membrane diffusion test. Release of drugs from formulations F1, F2 and F3, which have different amounts of Carbopol, is significantly affected by polymer amount, as illustrated in Table 6 and Figure 6.

In the first samples taken after 15 min, the Cumulative Drug Release (CDR%) was measured to be 27.5% (F1), 18.1% (F2) and 12.8% (F3). CDR% then continued to increase until 120 min when it reached 73.5% (F1), 56.6% (F2) and 41.7% (F3) and finally at 480 min, the highest release percentage values of 91.8% (F1), 81.9% (F2) and 66.4% (F3) were obtained. This demonstrates that the drug release decreased as the amount of Carbopol used in the formulations increased.

The faster release rate seen in the F1 formulation could be explained by the less viscous and therefore more porous gel network allowing easier diffusion of drug through the system, whereas the F3 formulation had a more compact, more viscous gel matrix; therefore providing greater resistive force against diffusion and resulting in a slower release of the drug. The F2 formulation has a mid-point between these two extremes; therefore providing both a balance of rapid drug delivery and the sustained release of the drug.

The trends in this study are similar to those previously reported in studies concerning niosomal and polymeric gel-based systems, in that increasing the amount of gelling agents resulted in reduced diffusion rates of the drug into the matrix due to increased matrix density and viscosity. Therefore, the F1 formulation will result in the most rapid delivery of the drug, while the higher Carbopol content of the F3 formulation will provide a more sustained release of the drug over time, providing a longer lasting therapeutic effect (Figure 7).

Release Kinetics

In order to determine the drug release mechanism, several kinetic models (First-order, Higuchi, Korsmeyer-Peppas, and Zero-order) were used to fit the *in vitro* drug release data. The Zero-order plot (cumulative percentage drug released vs time) indicated some linearity with a coefficient of determination (R^2) of 0.7587, suggesting only partial compliance to zero-order kinetics. In contrast, the First-order plot (log % drug remaining vs time) had a very high coefficient of determination (R^2=0.9222) demonstrating that drug release from the formulation most closely followed first-order kinetics. The Higuchi plot (cumulative

Table 6: *In vitro* Drug Release.

Time (min)	Cumulative drug release %		
	F1-10%	F2-20%	F3-30%
15	27.49	18.13	12.87
30	42.23	28.54	19.72
60	58.59	41.99	29.71
120	73.50	56.58	41.7
240	83.57	69.86	54.54
480	90.12	81.36	66.48

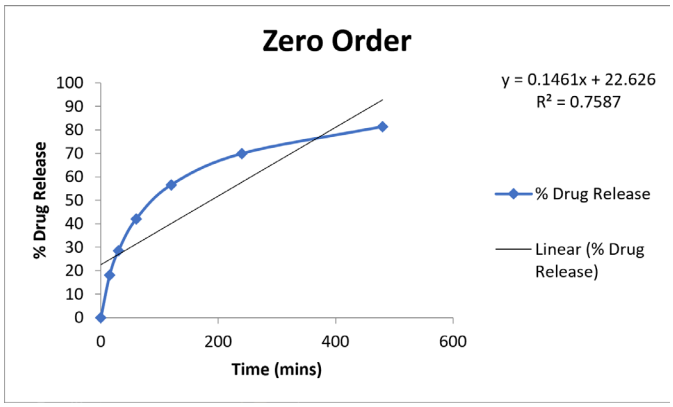

Figure 8: Zero Order Plot.

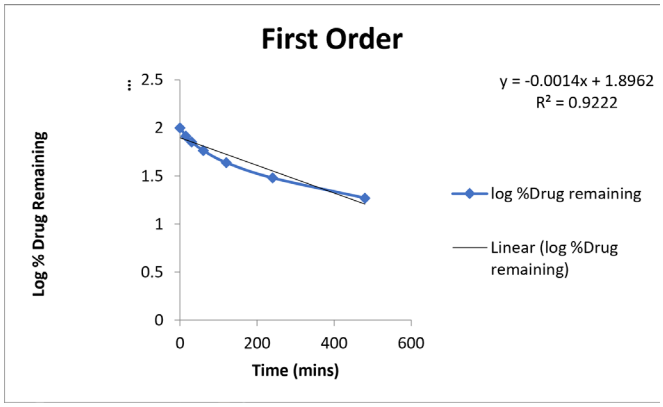

Figure 9: First Order Plot.

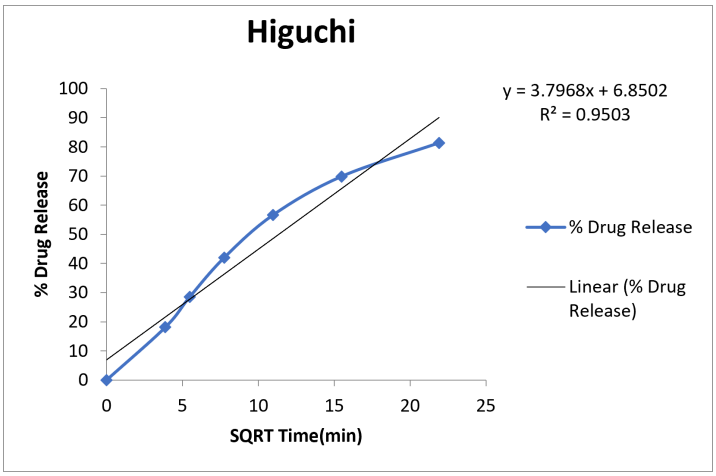

Figure 10: Higuchi Plot.

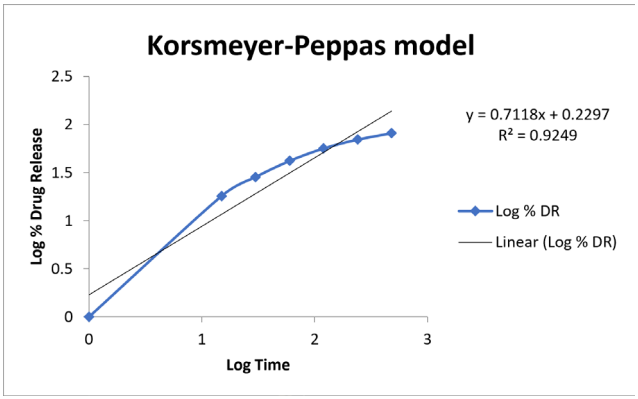

Figure 11: Korsmeyer-Peppas Plot.

drug released vs the square root of time) also showed high linearity (R^2=0.9503), showing that the release of the drug was controlled by diffusion. The Korsmeyer-Peppas model was also found to have a high coefficient of determination (R^2=0.9249) that supported the idea that the release of the drug was controlled by diffusion as well as possibly influenced by polymer relaxation and/or erosion.

Therefore, when comparing the high values of the coefficients of determination for both the First-Order and Higuchi models to those of the Zero-order model; it can be concluded that the drug release from the developed formulation, based on the overall correlation coefficients of the First-Order and Higuchi models, is controlled by a non-Fickian (anomalous) transport mechanism that is primarily driven by diffusion and therefore does not follow zero-order release (Figures 8-11).

DISCUSSION

This study was able to formulate and evaluate a niosomal *in situ* gel containing the latex from *Wrightia tinctoria* as an innovative method of antifungal drug delivery. Phytochemical screening of *W. tinctoria* latex confirmed the presence of several known bioactive compounds, i.e., alkaloids, flavonoids, terpenoids, tannins, and phenolic compounds; these compounds contribute to the antifungal properties of *W. tinctoria* Latex. In addition, the optimized formulation of niosomes had a well-defined spherical shape, narrow particle size distribution, excellent stability and a good encapsulation efficiency (73±0.58%). The niosomes were incorporated into a gelling agent based on Carbopol and HPMC, forming a thermosensitive gel formulation that is compatible with the pH of the skin (pH 6.2-6.8) and has an acceptable viscosity for topical use. *In vitro* release studies showed the niosomal *in situ* gel released the active ingredients over a period of time up to 8 hr. The release kinetics of the niosomal *in situ* gel followed both first order and Higuchi kinetic models, showing that the release of the active ingredients occurred through a diffusion mechanism (Akbari *et al.*, 2021; Gugleva *et al.*, 2022).

The results of antifungal testing against *Candida albicans* and *Aspergillus niger* confirmed that the inhibitory action of the latex loaded in niosomal *in situ* gel were greater than those obtained using the crude latex of *W. tinctoria*. Overall, the niosomal *in situ* gel containing latex from *W. tinctoria* represents a new, stable and biocompatible topical formulation with prolonged antifungal activity, suggesting that it may have great potential for use in future clinical and pharmaceutical treatments for the management of fungal skin infections.

CONCLUSION

Based on the results from this study, the latex loaded niosomal *in situ* gel system of the latex of *Wrightia tinctoria* shows considerable promise as a method for delivering antifungals. The inclusion of the niosomes into the formulation greatly increased the stability of the compound, increased the amount of the compound that was absorbed by the body, and provided a sustained release of the compounds so the overall antifungal activity was greater when compared to *Candida albicans* and *Aspergillus niger*. The formulation was also characterized with good physical-chemical properties, which included fast gelation times, appropriate pH values, and viscosity values were consistent with those expected for topical formulations. The entrapment efficiencies of the niosome ranged between 63% to 83% (average=73%) and the controlled release of the compounds can provide a stable and controlled release system, and therefore has the potential to be a candidate for future clinical trials for treating various dermatological fungal infections; however, further clinical studies are required to confirm the efficacy of the formulation in humans.

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None.

ABBREVIATIONS

WT: *Wrightia tinctoria*; **SDA:** Sabouraud Dextrose Agar; **SDS:** Sodium Dodecyl Sulfate; **HPMC:** Hydroxypropyl Methyl Cellulose; **UV:** Ultraviolet; **DLS:** Dynamic Light Scattering; **SEM:** Scanning Electron Microscopy; **PDI:** Polydispersity Index; **EE:** Entrapment Efficiency; **λ_{max}:** Maximum Absorption Wavelength; **R^2:** Coefficient of Determination; **cP:** Centipoise; **McFarland:**

McFarland Turbidity Standard; **DMSO:** Dimethyl Sulfoxide; **PBS:** Phosphate Buffer Solution.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The research was conceptualized by Sakthiganapathi Meenachisundaram, who also authored the manuscript. Kamesh Sendhil Murugan assisted in formulating and optimizing the gel. The analysis of *Wrightia tinctoria* latex was performed by Hema Munusamy; Shameera Banu Kafar Sharieff assisted with the interpretation of the data and manuscript preparation.

SUMMARY

The formulation of *Wrightia tinctoria* Latex-loaded niosomal *in situ* gels shows promising potential for clinical application as an effective antifungal drug delivery system. By encapsulating the bioactive compounds of *Wrightia tinctoria* in stable niosomal structures, this formulation enhances the bioavailability and controlled release of the antifungal agents, improving therapeutic efficacy against common fungal pathogens like *Candida albicans* and *Aspergillus niger*. The niosomal *in situ* gel demonstrated good physical properties, including a favorable pH range, fast gelation time, and sustained drug release over several hours. The high entrapment efficiency and biocompatibility suggest that this innovative drug delivery system could serve as a stable and efficient alternative to traditional antifungal treatments. This research paves the way for further clinical trials to validate its therapeutic potential, ultimately contributing to more effective management of dermatological fungal infections.

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