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FORMULATION AND EVALUATION OF NIOSOMAL GEL OF *NEOLAMARCKIA CADAMBA* LEAVES EXTRACT AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

The study aimed to formulate and evaluate a niosomal gel incorporating *Neolamarekia cadamba* leaves extract for its potential antimicrobial activity. Niosomes, as vesicular systems, enhance the bioavailability and stability of phytochemicals. *Neolamarekia cadamba*, known for its rich array of bioactive compounds, presents promising antimicrobial properties. This research encapsulated the methanolic extract of *Neolamarekia cadamba* leaves into niosomes using a thin-film hydration technique. The niosomal formulations were optimized based on vesicle size, and zeta potential. The optimized niosomes exhibited an average particle size of 511.6 nm for all formulations, and a zeta potential of average from -0.4 to -4.4 mV, indicating good stability. The resulting niosomal gel was characterized for pH, viscosity, and spreadability. The gel maintained a pH of 6.7, suitable for topical application, with satisfactory viscosity and spreadability, ensuring ease of application and patient compliance. Antimicrobial activity was evaluated against common pathogenic strain including *Escherichia coli* using the Well diffusion method. The niosomal gel demonstrated significant antimicrobial activity, with zones of inhibition comparable to standard antibiotics. This novel formulation can be a potential candidate for treating microbial infections, offering an alternative to conventional antibiotics with added benefits of improved stability and bioavailability of the phytoconstituents. Future studies should focus on *in vivo* evaluations and long-term stability assessments to further substantiate these findings.

Keywords: Niosomal gel, *Neolamarekia cadamba*, Leaves extract, Antimicrobial activity, Formulation, Evaluation, Bioavailability.

INTRODUCTION

The formulation and evaluation of niosomal gels containing extracts from *Neolamarckia cadamba* leaves aim to harness the medicinal properties of this plant for enhanced antimicrobial activity. Neolamarckia cadamba, commonly known as the Kadam tree, is traditionally used in various parts of the world for its medicinal properties, including antimicrobial, anti-inflammatory, and wound-healing activities (Bhatnagar *et al.,* 2014). Niosomes, non-ionic surfactant-based vesicles, offer a promising delivery system for natural extracts. These vesicles can encapsulate both hydrophilic and lipophilic drugs, enhancing the stability and bioavailability of the encapsulated substances. The benefits of using niosomes include improved drug delivery, controlled release, and reduced toxicity (Marianecci *et al.,* 2014). In this study, the leaves of *Neolamarckia cadamba* were extracted using appropriate

solvents, and the extracts were incorporated into niosomal gels. The niosomal gels were characterized for their physical properties, particle size, zeta potential, and entrapment efficiency. Furthermore, the antimicrobial activity of the formulated gels was evaluated against common microbial strains to assess their potential as effective antimicrobial agents.

MATERIALS AND METHODS

Material

The formulation and evaluation of the niosomal gel of *Neolamarckia cadamba* leaves extract involved the use of various reagents and chemicals from reputable companies. The chemicals used included petroleum ether and copper sulfate from Ranken, methanol from Molychem, sodium hydroxide, concentrated sulfuric acid, lead acetate, mercuric chloride, ammonium sulfate, ferric chloride, hydrochloric acid, diethyl ether, HPMC, ethyl cellulose, and propylene glycol from Merck. Sodium nitrite was sourced from Sunchem, ninhydrin and potassium sodium tartrate from Himedia, and sodium citrate and sodium carbonate from s.d.fine-CHEM Ltd. Additionally, Folin-Ciocalteu's reagent was procured from Merck, while Carbopol 934 was obtained from Sulab, and triethanolamine from Loba. Methyl paraben and acetone were also supplied by Merck. These high-quality reagents and chemicals were essential for the successful extraction, formulation, and characterization of the niosomal gel, ensuring the reliability and efficacy of the final product.

Plant collection

Neolamarekia cadamba, a medicinal herb weighing 300 grams, was gathered. Following cleaning, plant components (leaves) were dried for three days at room temperature in the shade and for a further three days at 45°C in an oven. To prevent contamination and deterioration, dried plant leaf portions were kept in airtight glass containers in a dry, cool environment.

Verification of the identity and purity of a particular traditional plant - A plant taxonomist verified the identity and purity of the medicinal plant *Neolamarekia cadamba.*

Extraction of plant material

Plant material was extracted for the current investigation utilizing the Soxhlet apparatus and a continuous hot percolation process. *Neolamarekia cadamba* powder was added to a soxhlet apparatus thimble. Soxhlation was carried out at 60°C with a non-polar solvent such as petroleum ether. The plant material that had been exhausted (marc) was dried and then extracted again using methanol. Each solvent's soxhlation was continued until no discernible color change was seen in the siphon tube, and the extraction's completion was verified by the absence of any solvent residue upon evaporation. The obtained extracts were evaporated at 40°C in a Buchi-type rotating vacuum evaporator. Weighing the dried extract, we calculated the % yield for each extract using the following formula:

$$\%\ \text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Phytochemical investigation

By performing a thorough qualitative phytochemical analysis, the experiment was designed to determine whether or not various phytoconstituents were present. The precipitate formation or color intensity was utilized to gauge how the body would react to

various tests. Standard operating procedures were employed (Hegdekar *et al.,* 2023).

Formulation of Niosomes

The thin film hydration method was used to create niosomes, as indicated in Table. Ten milliliters of chloroform were used to dissolve a precise quantity of cholesterol and span 60 surfactant. After that, the mixture was heated to 50°C for thin film hydration till the thin film was discovered. For three hours, the film was gently shaken while being hydrated with 10 milliliters of pH 5.5 phosphate buffer containing *Neolamarekia cadamba* leaf extract (Sangkana *et al.,* 2024; Singh *et al.,* 2008).

Characterization of Niosomes

Particle size

One of the most crucial parameters for characterizing a niosome is its particle size. The Malvern Zeta sizer (Malvern Instruments) was used to measure the size of niosomes. The sample was put in a disposable sized cuvette after the dispersions were diluted with Millipore filtered water to the proper scattering intensity at 25°C (Đorđević *et al.,* 2015).

Zeta potential

In order to ascertain the particle charge and movement velocity of the particles in an electric field, the zeta potential was measured. In the current study, Zetasizer Malvern equipment were used to examine a niosome that had been diluted ten times with distilled water. A 5-to 15-minute sonication was applied to each sample prior to zeta potential measurements (Anwer *et al.,* 2019).

Scanning Electron Microscopic (SEM)

The morphological properties of the extract-loaded niosomes were obtained using the electron beam from a scanning electron microscope. A sputter coater operating under vacuum was used to coat the niosomes with a thin coating (2–20 nm) of metal, such as platinum, palladium, or gold. Following the preparation, the specimen was exposed to an electron beam, which caused secondary electrons known as auger electrons to develop. Only the electrons scattered at a 90° angle were chosen from this interaction between the electron beam and the specimen's atoms, and these were then processed using Rutherford and Kramer's Law to obtain the surface topography photographs (Abbas *et al.,* 2019).

Formulation of niosomes loaded gel

First, carbopol-934 was mixed evenly using a magnetic stirrer set at 600 rpm after being submerged in 50 mL of warm water (A) for two hours. To create a stiff gel, 50 milliliters of warm water (B) was combined with carboxymethyl cellulose and methyl paraben in a different container and continuously agitated. Continuous stirring was used to combine mixtures A and B. After adding triethanolamine (dropwise) to the dispersion to bring the pH level down, the improved formulation's noisome was added to create gel. Propylene glycol, a permeability enhancer, was introduced at this point. After the final dispersion was worked out, a lump-free, smooth gel was produced (Silpa *et al.,* 2019).

Characterization of Niosome loaded Gel

Physical appearance

The prepared Gel formulation was evaluated for appearance, Color, Odor, and homogeneity by visual observation (McGlynn; 2003).

pH

pH of the formulation was determined by using Digital pH meter (EI). The meter was allowed to stabilize as necessary and properly calibrated, begin by rinsing the probe with deionized or distilled water and blotting the probe dry with lint-free tissue paper (Monica and Gautami; 2014).

Viscosity

The viscosity of the gel formulations was determined using Brookfield viscometer with spindle no. 61 at 100 rpm at the temperature of 25^0C (Sandeep; 2020).

Spreadability

When applied or rubbed on the skin's surface, an optimal topical gel should have a high enough spreading coefficient. One gram of the mixture was applied to a glass slide in order to assess this. To ensure that the gel is sandwiched between the two glass slides and spreads at a specific distance, another glass slide of the same length was positioned above it. A mass of 50 mg was then placed on the glass slide. The distance that the gel took to travel from its site was measured and recorded. The following formula was used to calculate spreadability.

S= M*L/T

Where, S-Spreadability, g.cm/s M-Weight put on the upper glass L-Length of glass slide T-Time for spreading gel in sec (Giri *et al.,* 2019).

Skin irritation test

Wistar rats of both sexes, weighing 150–200 g on average, with their skin intact, were used. The rat was dehaired two to three days before to the experiment. The rat's skin was carefully shaved before the gel was administered. The animals received daily treatment for two to three days. During that time, any undesired skin changes such as color changes or morphological changes were monitored for twenty-four hours, and any erythema or edema on the treated skin were investigated (Murthy *et al.,* 2001).

Anti-microbial activity

Preparation of Nutrient Agar Media

One liter of distilled water was used to dissolve 28 grams of nutritional media. The media's pH was measured prior to sterilization. The medium was autoclaved for 15 minutes at 121 degrees Celsius and 15 pounds of pressure. After adding nutritional media to plates, they were put in a laminar air flow until the agar solidified.

Well Diffusion Assay

The *E. coli* bacterial suspension was maintained in the shaker and standardized to 108 CFU/ml of bacteria. Next, using a micropipette, 100µl of the inoculums (containing 108 CFU/ml) from the broth were collected and transferred to a fresh, sterile, solidified Agar Media Plate. Using a sterile spreader, the inoculums were applied to the whole surface of the sterile agar to inoculate the agar plate. Using a sterile cork-borer, three 6 mm wells were drilled into the inoculation material. Next, the wells were created for the inoculation of the Gel solution containing the noisome (1 mg/ml) and 0.5 mg/ml). The sample was loaded in 100 µl. It was incubated for 18 to 24 hours after being allowed to diffuse for roughly 30 minutes at room temperature. Following incubation, plates were checked to see if a clear zone formed around the well, indicating that the chemicals under test had antimicrobial activity. A measurement of the zone of inhibition (ZOI) in millimeters was made.

Zones were measured with a ruler, held on the back of the inverted Petri plate, to the closest millimeter. A few inches above a black, non-reflective background was where the Petri plate was held. The diameters of the well and the zone of complete inhibition (as determined by unaided eye) were measured.

RESULTS AND DISCUSSION

The formulation and evaluation of niosomal gel containing *Neolamarekia cadamba* leaves extract demonstrated promising results in various analyses, including yield, phytochemical testing, particle size, zeta potential, and antimicrobial activity. The extraction process yielded 0.59% with petroleum ether and 2.29% with methanol (Table 3).

The higher yield with methanol suggests it is a more effective solvent for extracting bioactive compounds from Neolamarekia cadamba leaves.Phytochemical analysis revealed that the methanolic extract contained alkaloids, glycosides, carbohydrates, flavonoids, tannins, and phenolic compounds, whereas the petroleum ether extract mainly showed the presence of saponins, triterpenoids, and steroids (Table 4).

This indicates that methanol is more efficient at extracting a wider range of phytochemicals from the plant.The particle size of niosomal formulations ranged from 511.6 nm to 980.6 nm, with NS 5 having the smallest particle size (Table 5). The polydispersity index indicated varying degrees of uniformity across formulations, with NS 2 exhibiting the highest homogeneity.

Zeta potential values ranged from -0.4 mV to -4.4 mV (Table 6), suggesting moderate stability of the niosomal formulations. The physical appearance of the optimized niosomal gel was brown, odorless, semisolid, and homogeneous (Table 7).

The viscosity of the gel was 5989 ± 0.54 cps, indicating a suitable consistency for topical application. The pH was 6.7, which is close to the skin's natural pH, and the spreadability was 10.59 g.cm/s, suggesting good applicability (Table 8).

The niosomal gel exhibited significant antimicrobial activity against E. coli, with zones of inhibition measuring 11 mm and 14 mm for 0.5 mg/ml and 1 mg/ml concentrations, respectively. In comparison, the extract alone showed a smaller inhibition zone of 6 mm (Table 9). This demonstrates the enhanced antimicrobial efficacy of the niosomal gel formulation.

Table 1: Composition of noisome formulation

S. No.	F. Code	Span 60 (mg)	Chloroform (ml)	Cholesterol (mg)	Phosphate buffer (pH 5.5)	Extract (mg)	Temp. (°C)
1	F1	100	10	300	10	200	50°C
2	F2	150	10	250	10	200	50°C
3	F3	200	10	200	10	200	50°C
4	F4	250	10	150	10	200	50°C
5	F5	300	10	100	10	200	50°C

Table 2: Composition of gel formulation

S. No.	Excipients	Quantity (gm)
1.	Carbopol 934	1.00 gm
2.	Carboxymethyl cellulose	1.00 gm
3.	Propylene glycol	0.5 ml
4.	Methyl paraben	0.2 ml
5.	Niosomes	10 ml
6.	Triethanolamine	q.s
7.	Water	100 ml

Table 3: Results of percentage yield of crude extracts of *Neolamarekia cadamba* extract

S. No	Plant name	Solvent	Theoretical weight	Yield (gm)	% yield
1	*Neolamarekia cadamba*	Pet ether	297	1.76	0.59%
2		Methanol	288.29	6.61	2.29%

Table 4: Results of phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	**Glycoside**		
2.1	Borntrager test	Absent	Present
2.2	Legal's test	Absent	Present
2.3	Killer-Killiani test	Absent	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Present
3.2	Fehling's test	Absent	Present
3.3	Benedict's test	Absent	Present
3.4	Barfoed's test	Absent	Present

4.	**Proteins and Amino Acids**		
4.1	Biuret test	Absent	Absent
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Present
5.2	Lead Acetate test	Absent	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Present	Absent
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Present	Absent
8.2	Libbermann-Burchard's test	Present	Absent

Table 5: Results of Particle size of niosomes formulation NS 1 to NS 5

S. No	Formulation code	Particle size (nm)	Polydispersity index
1.	NS 1	859.7 nm	287.4 %
2.	NS 2	932.4 nm	41.7 %
3.	NS 3	980.6 nm	93.6 %
4.	NS 4	817.9 nm	141.4 %
5.	NS 5	511.6 nm	122.9 %

Table 6: Results of Zeta potential niosomes formulation NS 1 to NS 5

S. No	Formulation Code	Zeta potential
1.	NS 1	-0.4 mV
2.	NS 2	-3.3 mV
3.	NS 3	-4.3 mV
4.	NS 4	-1.5 mV
5.	NS 5	-4.4 mV

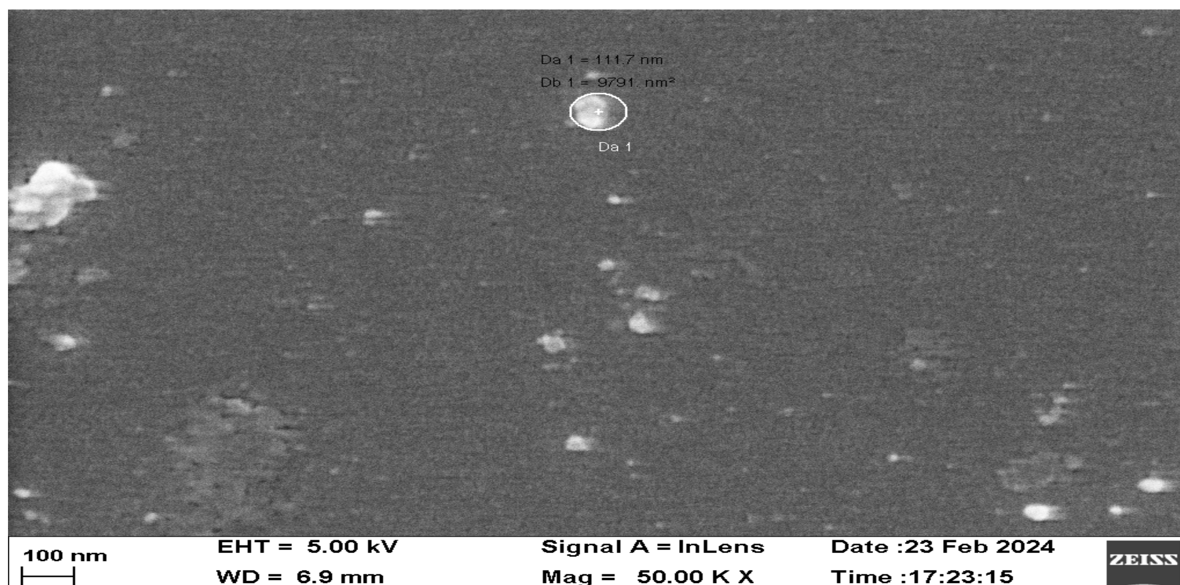

Figure 1: Scanning electron microscope image of optimized formulation F5

Table 7: Physical appearance

S. No	Parameter	Result
1.	Colour	Brown colour
2.	Odour	Odourless
3.	Appearance	Semisolid
4.	Homogeneity	Homogeneous

Table 8: Results of Viscosity, pH and Spreadability

S. No.	Formulation	Viscosity (cps)	pH	Spreadability (g.cm/s)
1.	Gel	5989±0.54	6.7	10.59

Table 9: Antimicrobial activity of Niosomal against *E.coli*

S. No.	Sample name	Zone of Inhibition (mm)
1	Extract	6 mm
2	Niosomal gel (0.5 mg/ml)	11mm
3	Niosomal gel (1mg/ml)	14 mm

Figure 2: Antimicrobial activity against *E. coli*

CONCLUSION

The study successfully formulated and evaluated a niosomal gel containing Neolamarekia cadamba leaves extract. The niosomal gel exhibited good physical and chemical properties, enhanced stability, and significant antimicrobial activity. These findings suggest that the niosomal gel could be a promising topical formulation for antimicrobial applications.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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