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FORMULATION AND DEVELOPMENT OF NIOSOMAL GEL CONTAINING NEOLAMARCKIA CADAMBA LEAVES EXTRACT FOR ENHANCING ITS EFFICACY

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ABSTRACT

The study aimed to formulate and evaluate a niosomal gel incorporating *Neolamarckia cadamba* leaves extract for its potential antimicrobial activity. Niosomes, as vesicular systems, enhance the bioavailability and stability of phytochemicals. *Neolamarckia cadamba*, known for its rich array of bioactive compounds, presents promising antimicrobial properties. This research encapsulated the methanolic extract of *Neolamarckia 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 83.12 nm to 417.67 nm for all formulations, and a zeta potential of average from -28.9 mV, indicating good stability. These niosomes were then incorporated into a carbopol gel matrix to enhance the formulation's applicability. 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 enhanced activity is attributed to the improved penetration and sustained release properties of the niosomal gel formulation. In conclusion, the study successfully formulated a niosomal gel

of *Neolamarckia cadamba* leaves extract with promising antimicrobial efficacy. 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, *Neolamarckia cadamba*, Leaves extract, antimicrobial activity, phytoconstituents.

1. INTRODUCTION

Drug delivery is the administration of pharmaceutical substances to treat diseases, particularly in amide and macromolecule medicine. Various technologies are being developed for nasal and respiratory organs, including cyclodextrins, liposomes, proliposomes, microspheres, gels, and prodrugs. Perishable polymer nanoparticles, which meet aerosol transfer, stability, biocompatibility, targeting specific sites or cell populations, preset drug release, and appropriate degradation intervals, are promising alternatives to parenteral medication administration **(Tiwari et al. 2012).** Niosomes are vesicles with a nanometric size composed of cholesterol and non-ionic surfactant. Because of the structural elements of niosomes, they are more stable than liposomes. Because they contain a non-ionic surfactant, they are therefore less hazardous and more stable than liposomes. Niosomes have particle sizes that vary from 20 to 100 nm **(Chandu et al., 2012).** Niosomes are spherical structures consisting of tiny lamellar structures, formed by combining nonionic surfactants with or without cholesterol and a charge inducer. These surfactants include alkyl ethers, alkyl glyceryl ethers, sorbitan fatty acid esters, and polyoxyethylene fatty acid esters. Cholesterol addition reduces leaky niosomes by keeping the bilayer tight. Charge inducers, such as cetylpyridinium chloride and stearylamine, give the vesicles a charge and enlarge them, improving drug entrapment effectiveness. Positive and negative charge inducers stabilize the vesicles **(Gandhi and Sen 2012).**

Plants continue to be important sources of bioactive chemicals for human health. Plants have several secondary metabolites with diverse biological functions **(Yousuf et al., 2014).** It is an excellent source of natural antioxidants for treating aging and diseases caused by radicals, including cancer **(Alekhya et al., 2013).** *Neolamarckia cadamba* is a medicinal herb that the Indians have historically employed. It has been used in Indian medical literature to treat fever, anemia, diabetes, uterine and liver problems, menorrhagia, blood and skin illnesses,

diarrhoea, colitis, stomatitis, dysentery, and increase sperm quality **(Kakkar et al. 1992).** Various portions of this plant have historically been used to treat a variety of ailments **(Duke JA 2007).** Bioactivity investigations on this plant indicated antibacterial, oxidative, and wound healing characteristics, as well as antimalarial, antihepatotoxic, hepatoprotective, analgesic, anti-inflammatory, antipyretic, anthelmintic, diuretic, laxative, and antidiabetic activities **(Umachigi et al 2007, Acharyya et al., 2010)**. This plant's leaves and bark preparations shown antifungal efficacy against Aspagillus fumigates and Candila albicans **(Patel et al., 2012).** The tribes of Orissa's Ganjam district drink root paste suspended in water for its antibacterial and anthelmintic properties. Phytochemistry techniques have been used to identify various phytochemical components in *N. cadamba. N. cadamba* leaf extracts contain secondary metabolites such as glycosides, alkaloids, tannins, phenols, steroids, and flavonoids **(Usmanet al., 2012, Madhuet al., 2012)**. The bark includes several alkaloids, saponins, glycosides, triterpenoids, cadambagic acid, quinovic acid, and β-sitosterol **(Kumar et al 2010)**. Alkaloids, steroids, and flavonoids are effective antiepileptics in diverse seizure models **(Hegde et al., 2009).** Saponins can alter neurotransmitter levels and have anti-convulsant properties. N. cadamba bark powder contains saponins, proteins, terpenes, carbohydrates, and alkaloids, according to qualitative chemical testing. A phytochemical analysis of N. cadamba's methanolic extract revealed the presence of flavonoids, alkaloids, carbohydrates, proteins, and glycoside chemicals **(Himanshu et al 2010).** *N. cadamba* flowers produce essential oils containing linalool, geraniol, geranylacetate, linalyl acetate, α-selinene, 2-nonanol, βphellandrene, α-bergamottin, p-cymol, curcumene, terpinolene, camphene, and myrcene **(Wealth of India 2006).** N. cadamba seeds contain the water-soluble polysaccharides D-xylose, D-mannose, and Dglucose in a 1:3:5 molar ratios **(Chandra O, Gupta 1980).**

2. MATERIAL AND METHOD

2.1 Plant collection

Neolamarckia 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 *Neolamarckia cadamba*.

2.2 Extraction

Plant material was extracted for the current investigation utilizing the Soxhlet apparatus and a continuous hot percolation process. *Neolamarckia 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:

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

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use **(Baidya et al., 2002).**

2.3 Formulation of niosomes

Niosomes were formulated using the thin film hydration method as given in Table 4. An Definite weights of the nonionic surfactant Span 60 and cholesterol, at different concentration (50 to 250), were dissolved in 20 mL chloroform in a rounded bottom flask. Then the following mixture was subjected to thin film hydration at a temperature of 50°C until the thin film was found. The film was hydrated with 10ml phosphate buffer of pH 5.5 containing *Neolamarckia cadamba* leaves extract for 3 hr with gentle shaking (**Hegdekar et al., 2023, Sangkana *et al.*, 2024)**.

Table 1: Composition of noisome formulation.

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

2.4 Characterization of niosomes

2.4.1 Particle size

The particle size is one of the most important parameter for the characterization of niosome. The size of niosomes was measured using Malvern Zeta sizer (Malvern Instruments). The dispersions were diluted with Millipore filtered water to an appropriate scattering intensity at 25°C and sample was placed in disposable sizing cuvette. The size data is documented in Table **(Singh and Vingkar 2008).**

2.4.2 Zeta potential

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge. In the present work, the niosome was diluted 10 times with distilled water and analyzed by Zetasizer Malvern instruments. Formulation was sonicated for 5-15 minutes before zeta potential measurements. The zeta potential data is documented in Table **(Đorđević *et al.*, 2015).**

2.4.3 Scanning Electron Microscopic (SEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the extract loaded niosomes were coated with a thin layer (2–20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pre-treated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography **(Anwer *et al*., 2019).**

2.5 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 (**Abbas *et al*., 2019, Silpa *et al*., 2021**).

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

2.6 Characterization of niosome loaded gel

2.6.1 Physical appearance

The prepared Gel formulation was evaluated for appearance, Color, Odor, and homogeneity by visual observation **(Kumar and Eswaraiah 2023).**

2.6.2 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 (**McGlynn, W. 2003).**

2.6.3 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 **(Monica and Gautami 2014).**

2.6.4 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 **(Sandeep, D. S. 2020).**

2.7 Anti-microbial activity

2.7.1 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.

2.7.2 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 **(Mohammadi *et al.*, 2012)**.

3. RESULTS AND DISCUSSION

3.1 Percentage yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Neolamarckia cadamba* is shown in Table: 3

Table 3: Percentage Yield of crude extracts of *Neolamarckia cadamba* extract.

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

3.2 Characterization of niosomes formulations

3.2.1 Particle size determination

Figure 1: Particle size (F1), (F2), (F3), (F4), (F5).

Table 4: Particle size.

S. No	Formulation code	Particle size (nm)	Polydispersity index
1.	NS 1	331.21 nm	0.212
2.	NS 2	83.12 nm	0.241
3.	NS 3	213.79 nm	0.241
4.	NS 4	187.26 nm	0.321
5.	NS 5	417.67 nm	0.241

The particle size is one of the most important parameter for the characterization of niosomes. The average particle size of the prepared drug loaded niosomes was measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of drug loaded niosomes was found to be range 511.6 nm to 980.6 nm.

3.2.2 Zeta potential

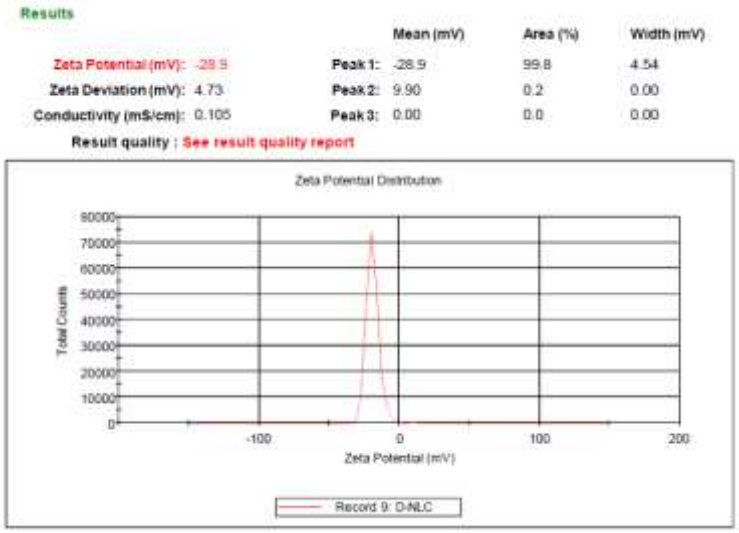

Figure 2: Zeta potential (F2).

Table 5: Zeta potential.

S. No	Formulation Code	Zeta potential
1.	NS 2	-28.9 mV

Zeta potential analysis is carried out to find the surface charge of the particles to know its stability during storage. The magnitude of zeta potential is predictive of the colloidal stability. If the particles in niosome have a large positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating for niosome. Zeta potential of best formulation was found to be -28.9 mV with peak area of 100% intensity. These values indicate that the formulated niosomes are stable.

3.2.3 Scanning electron microscope (SEM) of F2 Formulation (Optimized)

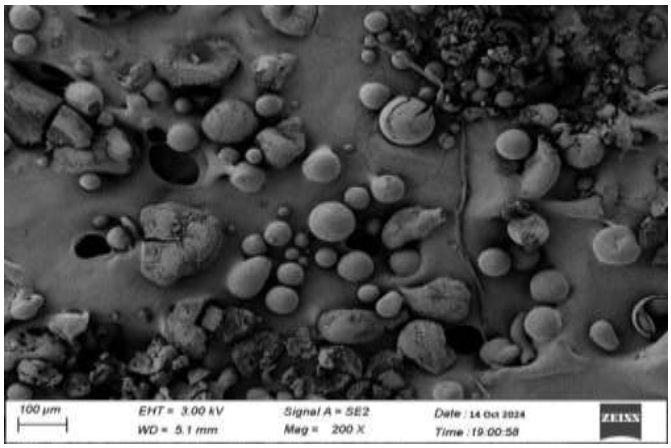

Figure 3: Scanning electron microscope.

SEM analysis was performed to determine their microscopic characters (shape & morphology) of prepared niosomes. Niosomes were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy. Scanning electron micrograph of the prepared niosomes at 200.00 k x magnification showed that the niosomes were smooth surface morphology and spherical shape. The porous nature of niosomes was clearly observed in the SEM images.

3.3 Characterization of niosomal gel formulation

3.3.1 Physical appearance

Table 6: Physical appearance.

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

An evaluation of the gel, including color, odor, appearance and homogeneity, was conducted. Gel was discovered to have a white color to it when tested. Gel does not have a distinctive odor and has a semisolid appearance, according to research conducted on it. Gel exhibited the same color, odor, and Appearance as the I.P. requirements for these characteristics.

3.3.2 Viscosity, pH, Spreadability of gel

Table 7: Viscosity, pH, Spreadability

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

The viscosity was measured by the Brookfield viscometer spindle no. 61 at 100rpm. The result was shown in the table 8. The viscosity of Gel was found to be 5989 ±0.54 centipoise respectively. The pH of the gel formulation was found to be 6.7, which lies in the normal pH range of the skin and would not produce any skin irritation. There was no significant change in pH values as a function of time. The physicochemical properties of prepared gel formulation were in good agreement. One of the essential criteria for a Gel is that it should possess good spreadability. Spreadability depends on the viscosity of the formulation and physical characteristics of the polymers used in the formulation. A more viscous formulation would have poor spreadability. Spreadability is a term expressed to denote the extent of area on which the gel readily spreads on application to the skin. The therapeutic efficacy of a formulation also depends upon its spreading value. The spreadability of Gel formulation is found to be 10.59g.cm/s

3.4 Anti-microbial activity

3.4.1 Results of antimicrobial activity of Niosomal gel formulation against *E.coli*

Table 8: Antimicrobial activity of Niosomal against *E.coli.*

S No.	Sample name	Zone of Inhibition (mm)
1	Extract	9 mm
2	Niosomal gel (1.0 mg/ml)	12mm

Figure 4: Antimicrobial activity against *E. coli.*

Table demonstrates the antimicrobial activity of different samples against *E. coli*, as indicated by the zone of inhibition measured in millimeters (mm). The extract alone exhibited a modest inhibition zone of 6 mm. In contrast, the niosomal gel formulations showed enhanced antimicrobial activity. Specifically, the niosomal gel at a concentration of 1.0 mg/ml produced an inhibition zone of 12 mm, that niosomal gel encapsulation significantly enhances the antimicrobial efficacy of the extract against *E. coli*.

4. CONCLUSION

The formulation of niosomal gels was carried out using a thin film hydration method, with cholesterol and surfactant (Span 60) being suitable for the preparation. The niosomal gels showed smooth surface morphology and spherical shape, with a porous nature. The particle size and zeta potential were determined using the Malvern Zeta sizer, with the average particle size ranging from 83.12 nm to 417.67 nm. The niosomal gel's viscosity was 5989±0.54cps, with a ph of 6.7 and spreadability of 10.59, indicating high release and permeability. The study highlights the potential of niosomal gel formulations to enhance bioavailability and the potential of the noisome-based drug delivery approach to improve the therapeutic efficacy of phytochemicals by altering their physicochemical and release properties.

REFERENCES

1. Tiwari G, Tiwari R, Sriwastawa B, Bhati L, Pandey S, Pandey P, Bannerjee SK. Drug delivery systems: An updated review. International journal of pharmaceutical investigation, 2012; 2(1): 2.
2. Chandu VP, Arunachalam A, Jeganath S, Yamini K, Tharangini K, Chaitanya G. Niosomes: a novel drug delivery system. International journal of novel trends in pharmaceutical sciences, 2012; 2(1): 25-31.
3. Gandhi A, Sen SO, Paul A. Current trends in niosome as vesicular drug delivery system. Asian Journal of Pharmacy and Life Science ISSN, 2012; 2231: 4423.
4. Yousuf S, Bachheti RK, Joshi A, Mathur A. Evaluation of antioxidant potential and phytochemicals of Morina longifolia. Int J Pharm Pharm Sci, 2014; 6(6): 208-12.
5. Alekhya V, Deepan T, Shaktiprasanna S, Dhanaraju MD. Preliminary phytochemical screening and evaluation of in vitro antioxidant activity of Anthocephalous cadamba by using solvent extracts. Eur J Biol Sci, 2013; 5(1): 34-7.
6. Duke JA. Phytochemical and Ethnobotanical Databases. (Http: //www. ars-grin. gov/duke/chem-activities. html), 2007.
7. Umachigi SP, Kumar GS, Jayaveera KN, Kumar KDV, Kumar ACK, Dhanpal R. Antimicrobial, wound healing and antioxidant activities of Anthocephalus cadamba. Afr J Tradit Complementary Altern Med, 2007; 4(4): 481-7.
8. Acharyya S, Dash DK, Mondal S, Dash SK. Studies on glucose lowering efficacy the Anthocephalus cadamba (Roxb.) Miq roots. Int J Pharm Bio Sci, 2010; 2(1): 1-9.

9. Patel DA, Patel YK, Shah PB. Pharmacognostical study of Neolamarckia cadamba (Roxb.) Bosser bark. Int Res J Pharm, 2012; 3(6): 120-21.
10. Usman MRM, Purushottam SR, Abullais MD, Usman MD. Evaluation of antipyretic activity of Anthocephalus cadamba Roxb. leaves extracts. Res J Pharm Biol Chem Sci, 2012; 3(1): 825-34.
11. Madhu C, Sharma U, Kumar N, Singh B, Satwinderjeet K. Antioxidant activity and identification of bioactive compounds from leaves of Anthocephalus cadambaby Asian Pacific J Tropical Medicine, 2012; 5(12): 977-85.
12. Hegde K, Thakker SP, Joshi AP, Shastry CS, Chandrashekhar KS. Anticonvulsant activity of Carissa carandas Linn. root extract in experimental mice. Tropical J Pharm Res, 2009; 8: 117–25.
13. Himanshu GSK, Nandanwar JR, Vinod KS. Phytochemical screening on the stem bark of Anthocephalus cadamba (Roxb.) Miq. Int J Pharm Sci Res, 2010; 1(7): 108-15.
14. The Wealth of India. A dictionary of Indian raw materials and industrial products. New Delhi: NISCAIR press publishers, 2006.
15. Chandra O, Gupta D. A complex polysaccharide from the seeds of Anthocephalus indicus. Carbohydrate Res, 1980; 83: 85-92.
16. Hegdekar NY, Priya S, Shetty SS, Jyothi D. Formulation and Evaluation of Niosomal Gel Loaded with *Asparagus racemosus* Extract for Anti-inflammatory Activity. Indian J of Pharmaceutical Education and Research, 2023; 1, 57(1s): s63-74.
17. Sangkana, S., Eawsakul, K., Ongtanasup, T., Mitsuwan, W., Chimplee, S., Paul, A. K., & Nissapatorn, V. Preparation and evaluation of a niosomal delivery system containing *G. magostana* extract and study of its anti-Acanthamoeba activity. Nanoscale Advances, 2024.
18. Singh KK, Vingkar SK. Formulation, antimalarial activity and biodistribution of oral lipid nanoemulsion of primaquine. International Journal of Pharmaceutics, 2008; 22, 347(1-2): 136-43.
19. Đorđević SM, Cekić ND, Savić MM, Isailović TM, Ranđelović DV, Marković BD, Savić SR, Stamenić TT, Daniels R, Savić SD. Parenteral nanoemulsions as promising carriers for brain delivery of risperidone: Design, characterization and in vivo pharmacokinetic evaluation. International journal of pharmaceutics, 2015; 30, 493(1-2): 40-54.
20. Anwer MK, Mohammad M, Ezzeldin E, Fatima F, Alalaiwe A, Iqbal M. Preparation of sustained release apremilast-loaded PLGA nanoparticles: *in vitro* characterization and *in*

vivo pharmacokinetic study in rats. International journal of nanomedicine, 2019; 1: 1587-95.

21. Abbas N, Parveen K, Hussain A, Latif S, uz Zaman S, Shah PA, Ahsan M. Nanosponge-based hydrogel preparation of fluconazole for improved topical delivery. Tropical Journal of Pharmaceutical Research, 2019; 11, 18(2): 215-22.
22. Silpa GS, Mathan S, Dharan SS. Formulation and Evaluation of Nanosponges Loaded Hydrogel Using Different Polymers Containing Selected Antifungal Drug. Journal of Pharmaceutical Sciences and Research, 2021; 1, 13(2): 101-11.
23. Islam M. Topical Drug Delivery of Polyherbal Drugs Entrapped Gel with Design and Validation.
24. McGlynn W. Choosing and using a pH meter for food products. Oklahoma Cooperative Extension Service, 2003.
25. Monica AS, Gautami J. Design and evaluation of topical hydrogel formulation of diclofenac sodium for improved therapy. International Journal of Pharmaceutical Sciences and Research, 2014; 1, 5(5): 1973.
26. Sandeep DS. Development, characterization, and *in vitro* evaluation of aceclofenac emulgel. Asian Journal of Pharmaceutics (AJP), 2020; 22: 14(03).
27. Giri MA, Bhalke RD. Formulation and evaluation of topical anti-inflammatory
28. Murthy SN, Hiremath SR. Physical and chemical permeation enhancers in transdermal delivery of terbutaline sulphate. AAPS Pharm Sci Tech, 2001; 2(1): Technical-Note.