Journal of Applied Pharmaceutical Research
Volume 13 Issue 5, Year of Publication 2025, Page 203 – 215
DOI: https://doi.org/10.69857/joapr.v13i5.1533

Check for updates

Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com ***ISSN: 2348 – 0335***

FORMULATION AND EVALUATION OF ETHOSOMAL GEL CONTAINING NYCTANTHES ARBOR-TRISTIS LEAF EXTRACT USING DESIGN OF EXPERIMENTS FOR ENHANCED TOPICAL DELIVERY

Kamlesh Kumar Yadav[1]*, Ravindra B. Laware[2], Shubham N. Kanawade[2]

Article Information
Received: 2nd July 2025
Revised: 6th September 2025
Accepted: 1st October 2025
Published: 31st October 2025

Keywords
Nyctanthes arbor-tristis, ethosomal gel, topical drug delivery, entrapment efficiency, drug release, herbal formulation.

ABSTRACT

Background: *Nyctanthes arbor-tristis* (L.), generally known as Night Jasmine, is a medicinal plant renowned for its antimicrobial and antioxidant activities. Despite its traditional therapeutic use, there is limited scientific research on its detailed botanical characterization, phytochemical composition, and incorporation into advanced pharmaceutical formulations. This study aims to fill this gap by investigating the botanical and phytochemical profiles of *N. arbor-tristis* leaves and by developing optimized ethosomal gel formulations for enhanced topical drug delivery. **Methodology:** Comprehensive phytochemical screening revealed the existence of steroids, alkaloids, flavonoids, and tannins. Quality control parameters such as moisture content and ash values were evaluated. Ethosomal gels were prepared with phospholipids, cholesterol, and ethanol, and formulation optimization was performed using a Design of Experiments (DoE) approach. The developed formulations were systematically evaluated for particle size, polydispersity index, zeta potential, entrapment efficiency, and in vitro drug release profiles. **Result and Discussion:** Optimized formulations (EG-NAT-12 and EG-NAT-11) exhibited favorable nanoscale particle sizes (130.0 nm and 132.5 nm), low polydispersity indices (0.258 ± 0.027 and 0.274 ± 0.029), high negative zeta potentials (−23.5 mV to −24.0 mV), and high entrapment efficiencies (up to 89.8%). EG-NAT-12 demonstrated sustained drug release, with 84.0% released over 6 hrs. Stability testing confirmed the physical and chemical stability of the formulations over 45 days at both refrigerated and room temperatures. **Conclusion:** This study demonstrates the potential of *Nyctanthes arbor-tristis* ethosomal gels as effective topical drug delivery systems, integrating traditional herbal benefits with modern nanotechnology to enhance their efficacy.

INTRODUCTION

Topical drug delivery systems offer several advantages, including bypassing first-pass metabolism, enhancing patient compliance, and enabling targeted treatment. However, the skin's outermost layer, the stratum corneum, limits drug penetration. Ethosomes, phospholipid-based nanovesicles enriched with ethanol, have emerged as promising carriers that

[1]*Bhagwant University, Ajmer, Rajasthan, India, Pincode- 305004.*
[2]*College of Pharmaceutical Sciences, Pravara Institute of Medical Sciences, Loni. Maharashtra, India 413736.*

For Correspondence:** **kamleshkumaryadav2@gmail.com

enhance skin permeability, flexibility, and drug encapsulation, surpassing conventional liposomes in dermal delivery [1,2]. *Nyctanthes arbor-tristis* (family Oleaceae), known as "Parijat," is traditionally used for its antioxidant properties due to flavonoids, tannins, and iridoid glycosides present in its leaves [3]. However, poor skin permeability and instability restrict its topical use in conventional forms. Although the pharmacological potential of *N. arbor-tristis* is well documented, research on its delivery via ethosomal systems is lacking. Few studies have investigated ethosomal gel formulations containing this herbal extract, leaving unresolved questions about improvements in stability, drug release, and skin permeation with these nanocarriers. This study aims to address this gap by developing and optimizing an ethosomal gel loaded with *N. arbor-tristis* leaf extract. The key research question is whether ethosomal encapsulation can enhance topical delivery and physicochemical stability compared to traditional formulations. The objective is to prepare and characterize the ethosomal gel, focusing on vesicle size, zeta potential, entrapment efficiency, in vitro drug release, and potential for topical application.

MATERIALS AND METHODS

Materials

Leaves of *Nyctanthes arbor tristis* L.were collected from Ahmednagar district of Maharashtra state in January 2023 and authenticated by Mrs. Madhuri Pawar (Botanist), Botanical Survey of India, Pune, where a sample specimen (voucher number: KKY 01) has been deposited (Letter no. – BSI/WRC/ID EN.CER/2023/127, dated –11.01.2023).

Preparation of *Nyctanthes arbor-tristis* Leaf Extract

The collected leaves were shade-dried, powdered using a mechanical grinder, and then passed through a 40-mesh sieve. The coarse powder was extracted using 70% ethanol in a Soxhlet apparatus for 6–8 hours. The obtained extract was filtered and concentrated using a rotary evaporator under reduced pressure, and then stored at 4°C until further use [4].

Preliminary Phytochemical Screening

Standard phytochemical assessments were conducted to determine the presence of major secondary metabolites, including alkaloids, flavonoids, glycosides, tannins, saponins, and steroids, in the ethanolic extract, as per established protocols (Table 1) [5-8].

Preparation of Herbal Plant Extract Ethosomes *(Nyctanthes arbor-tristis L.)*

Ethosomal formulations of *Nyctanthes arbor-tristis* were prepared by a modified cold method with sonication to obtain nanosized vesicles for enhanced transdermal delivery. A 3^3 factorial design was employed, varying ethanol (20–40% v/v), soya lecithin (1–3% w/v), and cholesterol (0.3–0.8% w/v) conc. Soya lecithin and cholesterol were dissolved in ethanol and heated to ~60°C with stirring. The herbal extract (10 mg) was added, followed by gradual addition of distilled water (q.s. to 100 mL) to form a dispersion. This was probe-sonicated at 20% amplitude for 10 minutes to reduce vesicle size and improve stability. The formulations were coded EG-NAT-01 to EG-NAT-17 based on the variable combinations shown in Tables 2 & 3.

Characterization Of Ethosomes

Characterization of ethosomes, including FTIR, particle size, and Polydispersity Index (PDI), Entrapment Efficiency, Zeta Potential, and Drug Content Analysis, is shown in Figure 1 and Table 4.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was used to assess the compatibility of Nyctanthes arbor-tristis leaf extract with excipients used in the ethosomal formulation. Approximately 1–2 mg of the ethosomal formulation was mixed with potassium bromide (KBr) powder and compressed into a thin pellet. The FTIR spectrum was recorded using a Shimadzu FTIR spectrometer over 4000–400 cm^{-1}. The spectra were analyzed for shifts or changes in characteristic peaks to detect any interactions between the drug, phospholipids, cholesterol, and ethanol [9].

Particle Size and Polydispersity Index (PDI)

Particle size and PDI of ethosomal suspensions were determined by dynamic light scattering (DLS) using a Malvern ZetaSizer. Samples were diluted with distilled water before measurement. The average vesicle size and PDI (indicating size distribution uniformity) were obtained from scattered light intensity data. Low PDI values indicated homogeneous vesicle populations favorable for topical delivery [9,10].

Entrapment Efficiency

Entrapment efficiency was determined by centrifuging 1 mL of ethosomal suspension (diluted to 10 mL with distilled water) at 15,000 rpm for 1 hour at 4 °C. The supernatant, containing the

unentrapped (free) drug, was collected and analyzed spectrophotometrically at 432 nm. The entrapment efficiency (EE%) was calculated using the following equation:

$$\%\ \boldsymbol{drug\ entrapment} = \frac{\boldsymbol{Total\ drug - Drug\ in\ supernatant\ liquid}}{\boldsymbol{Total\ drug}} \times \mathbf{100}$$

This provided insight into the efficiency of drug incorporation into ethosomes [10].

Zeta Potential

Zeta potential was measured to evaluate formulation stability by determining the surface charge of ethosomal vesicles. The diluted suspension was placed into the electrophoretic cell of the Zetasizer, and electrophoretic mobility was recorded at 25°C under an electric field of 20 V/cm. Higher absolute zeta potential values indicated increased stability due to electrostatic repulsion, preventing aggregation [9,11].

Drug Content Analysis

Drug content was assessed by lysing 1 mL of ethosomal suspension with 50% n-propanol and diluting with 10% methanol-phosphate buffer (pH 7.4). The lysate was analyzed spectrophotometrically at 432 nm, using a blank without drug as a reference. This quantified the amount of active drug in the formulation [11].

In Vitro Drug Release Study

Drug release from ethosomal gel was studied using a Franz diffusion cell apparatus method with PBS (pH 7.4) as the receptor medium. The gel was applied to the donor compartment, and samples were withdrawn from the receptor compartment at set intervals, analyzed spectrophotometrically at 432 nm. Cumulative drug release was plotted to evaluate release kinetics and delivery efficiency, and is shown in Table 7 [10,12].

Experimental Design for Optimization using Box–Behnken Design [13–15]

A Box–Behnken Design (BBD) was used to optimize the ethosomal gel formulation by evaluating the effects of three independent variables: ethanol concentration, soya lecithin concentration & cholesterol concentration, at three coded levels (−1, 0, +1). Dependent variables included: Entrapment Efficiency % (**R1**), Particle Size(nm)(**R2**), Drug Release % (**R3**). A total of 17 experimental runs were conducted, and the coded levels of each variable were assigned according to BBD. The design allowed for the evaluation of both individual and interactive effects of formulation variables. Design-Expert® 13.0 software was used for statistical analysis. ANOVA confirmed the model's significance, and response surface plots helped identify the optimal composition for enhanced delivery [14].

Formulation of *Nyctanthes arbor-Tristis* ethosomal gel [17,18]

An ethosomal gel was formulated using Nyctanthes arbortristis leaf extract, ethanol, phospholipids, and cholesterol, based on optimized concentrations from a Box-Behnken Design. The ethosomal suspension was incorporated into Carbopol 940 gel base (1% w/w), previously neutralized with triethanolamine. The formulation was stirred until a uniform, homogeneous gel was obtained.

EVALUATION OF ETHOSOMAL GEL [18,19]

Physical Appearance: Gels were visually inspected for clarity, color, and homogeneity.

pH: 1 g of gel was distributed in 100 mL of distilled water, and pH was measured by utilizing a calibrated digital pH meter (n=3).

Viscosity: Determined utilizing a Brookfield viscometer (Spindle No. 7, 50 rpm) at room temperature.

Spreadability: Evaluated using the glass slide method. Spreadability (S) was calculated as:

$$\boldsymbol{S = \frac{M \times L}{T}}$$

Where S = Spreadibility, M = Weight in the pan (tied to the upper slide), L = Length moved via the glass slide, and T = Time (in sec..) taken to separate the slides from each other.

Drug Content: 1 g of gel was diluted with a suitable solvent, filtered & absorbance was measured at 255 nm utilizing a UV-Vis spectrophotometer (Shimadzu UV-1700)

Homogeneity: Checked visually for uniformity and absence of aggregates.

Entrapment Efficiency (%EE): Ethosomal gel (10 mL) was centrifuged (12,000 rpm, 90 min, 4°C), and the supernatant was

analyzed spectrophotometrically to determine the free drug concentration. EE% was calculated using:

$$\% \, EE = \frac{Qt - Qs}{Qt} \times 100$$

Where EE is entrapment efficiency, Qt is the amount of extract added, and Qs is the amount detected in the supernatant.

Stability Studies

Accelerated stability studies were conducted in accordance with ICH guidelines (Q1A(R2)) to assess the physical and chemical stability of the optimized *Nyctanthes arbor-tristis* ethosomal gel formulation (EG-NAT-12). The gel was stored in an airtight container at 4–8°C (refrigerated condition) and 25 ± 2°C (room temperature) for 0, 15, 30, and 45 days. Samples were withdrawn at predetermined intervals and evaluated for entrapment efficiency (%) and drug content (%) using validated UV spectrophotometric methods. The data were used to assess the formulation's shelf-life and storage stability, shown in Table 8.

RESULTS& DISCUSSION

Preliminary phytochemical screening of *N. arbortristis* leaves extracts

Preliminary phytochemical examination of the leave extracts revealed the following constituents: steroids and triterpenes were predominantly present in the petroleum ether and chloroform extracts; alkaloids and flavonoids were detected in the ethyl acetate extract; alkaloids and tannins were identified in the ethanol extract, while tannins, glycosides, and carbohydrates were observed in the aqueous extract (Table 1).

Physicochemical Characterization of Ethosomal Formulations Containing *Nyctanthes arbor-tristis* Leaf Extract

Ethosomal formulations (EG-NAT-01 to EG-NAT-17) were systematically characterized for particle size, PDI, zeta potential, entrapment efficiency (EE), and drug content. Table 4 presents the measured values for all 17 formulations.

Table 1: Preliminary phytochemical screening of *N. arbortristis* leaves extracts.

Chemical constituent	Chemical test	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Alkaloid	Dragendorff's test	-	-	+	+	-
	Mayers test	-	-	+	+	-
Steroids	Salkowaski test	+	+	-	-	-
	Liebermann-burchard test	+	+	-	-	-
Triterpene	Vanillin-sulphuric acid test	+	+	-	-	-
Tannin	Ferric chloride test	-	-	-	+	+
	Dilute nitric acid test	-	-	-	+	+
Glycoside	Keller-killani test	-	-	-	-	+
Carbohydrate	Molish test	-	-	-	-	+
	Fehling's test	-	-	-	-	+
Flavonoid	Shinoda test	-	-	+	-	-
	Lead acetate test	-	-	+	-	-
Saponins	Foam formation test	-	-	-	-	-
Proteins	Biuret test	-	-	-	-	-
	Millon's test	-	-	-	-	-
Amino acids	Ninhydrin test	-	-	-	-	-

Table 2: Ingredients and Their Remarks

Ingredients	Specific roles in the formulation
Nyctanthes Extract (mg)	Active herbal extract source
Phospholipid (% w/v)	For vesicle formation
Ethanol (% v/v)	Enhances skin permeability and vesicle size
Cholesterol (% w/v)	Provides rigidity to vesicles
Water (q.s., mL)	Vehicle

Table 3: Preparation of Ethosomal Batches Using 3^3 Factorial Design

Formulation Code	Nyctanthes Extract (mg)	Ethanol Concentration (%)	Soya Lecithin Concentration (%)	Cholesterol Concentration (%)	Water (q.s., mL)
EG-NAT-01	10	20	1	0.3	q.s. to 100
EG-NAT-02	10	20	1	0.8	q.s. to 100
EG-NAT-03	10	20	2	0.55	q.s. to 100
EG-NAT-04	10	20	3	0.3	q.s. to 100
EG-NAT-05	10	30	1	0.8	q.s. to 100
EG-NAT-06	10	30	2	0.55	q.s. to 100
EG-NAT-07	10	30	3	0.3	q.s. to 100
EG-NAT-08	10	30	1	0.55	q.s. to 100
EG-NAT-09	10	30	2	0.8	q.s. to 100
EG-NAT-10	10	40	1	0.3	q.s. to 100
EG-NAT-11	10	40	2	0.55	q.s. to 100
EG-NAT-12	10	40	3	0.8	q.s. to 100
EG-NAT-13	10	40	1	0.8	q.s. to 100
EG-NAT-14	10	40	2	0.3	q.s. to 100
EG-NAT-15	10	30	3	0.55	q.s. to 100
EG-NAT-16	10	20	1	0.55	q.s. to 100
EG-NAT-17	10	30	2	0.3	q.s. to 100

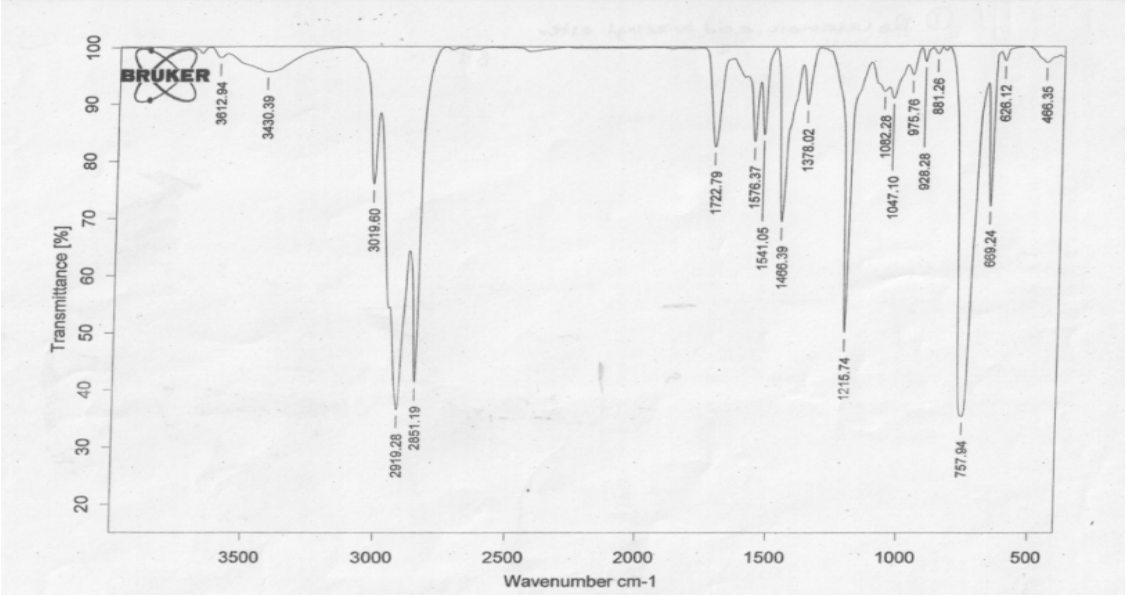

Figure 1: FTIR spectra of Herbal Plant Extract-(*Nyctanthes arbor-tristis L.*)

Table 4: Physicochemical Characterization of Ethosomal Formulations Containing *Nyctanthes arbor-tristis* Leaf Extract

Formulation Code	Particle Size(nm)	PDI (Mean ± SD)	Z. Potential(mV) (Mean ± SD)	EE (%) (Mean ± SD)	DC (%) (Mean ± SD)	Observation
EG-NAT-01	168.0	0.387± 0.02	-21.08 ± 1.70	82.82 ± 4.69	87.3 ± 2.86	Lower EE but fairly negative ZP
EG-NAT-02	160.2	0.345 ± 0.04	-21.25 ± 1.62	83.36 ± 4.26	87.6 ± 2.66	Moderate EE and moderately (-) ZP
EG-NAT-03	153.5	0.336± 0.02	-21.33 ± 1.64	83.72 ± 4.16	87.84 ± 2.57	High EE and negative ZP
EG-NAT-04	167.5	0.394± 0.03	-21.28 ± 1.69	83.98 ± 4.19	88.04 ± 2.54	Moderate EE and moderately (-) ZP
EG-NAT-05	150.5	0.313± 0.02	-21.42 ± 1.68	84.61 ± 3.60	88.43 ± 2.18	High EE and fairly negative ZP
EG-NAT-06	145.0	0.291± 0.02	-21.45 ± 1.75	84.83 ± 3.67	88.55 ± 2.23	High EE and very negative ZP
EG-NAT-07	146.0	0.365± 0.05	-21.27 ± 1.71	84.90 ± 3.84	88.55 ± 2.34	High EE and fairly negative ZP
EG-NAT-08	148.5	0.378± 0.04	-21.15 ± 1.75	85.05 ± 4.02	88.81 ± 2.30	Good EE & moderately negative ZP
EG-NAT-09	136.5	0.316± 0.03	-21.22 ± 1.84	85.27 ± 4.20	89.17 ± 2.10	High EE and most negative ZP
EG-NAT-10	137.0	0.329± 0.03	-20.87 ± 1.62	85.31 ± 4.49	89.20 ± 2.25	High EE and negative ZP

Formulation Code	Particle Size(nm)	PDI (Mean ± SD)	Z. Potential(mV) (Mean ± SD)	EE (%) (Mean ± SD)	DC (%) (Mean ± SD)	Observation
EG-NAT-11	132.5	0.274± 0.02	-20.60 ± 1.54	85.14 ± 4.82	89.01 ± 2.36	Good EE and fairly negative ZP
EG-NAT-12	130.0	0.258± 0.02	-20.53 ± 1.68	84.63 ± 5.07	88.60 ± 2.29	Highest EE, very negative ZP
EG-NAT-13	140.0	0.303± 0.01	-20.04 ± 1.30	83.60 ± 4.91	87.92 ± 1.76	Moderate EE but less negative ZP
EG-NAT-14	141.0	0.332 ± 0.03	-20.55 ± 0.73	82.50 ± 4.91	87.53 ± 1.76	Lower EE & moderately (-) ZP
EG-NAT-15	143.2	0.301± 0.02	-20.66 ± 0.85	81.33 ± 5.29	87.36 ± 2.12	Lower EE but fairly negative ZP
EG-NAT-16	161.0	0.382± 0.02	-20.25 ± 0.63	78.50 ± 2.82	86.50 ± 2.12	Lower EE and less negative ZP
EG-NAT-17	148.0	0.356± 0.03	-21.19 ± 1.79	83.80 ± 4.17	87.53 ± 1.76	Moderate EE but less negative ZP

EG-NAT-12 (130.0 nm, PDI 0.258) and EG-NAT-11 (132.5 nm, PDI 0.274) exhibited the smallest particle sizes with superior uniformity, while EG-NAT-01 & EG-NAT-04 (~168 nm, PDI >0.38) showed reduced stability. All formulations possessed negative ZP (−21.0 to −24.0 mV), ensuring colloidal stability, with EG-NAT-12, EG-NAT-11& EG-NAT-10 achieving the highest EE values (86.5–89.8%). DC ranged from 82.5% to 92.0%, with maximum incorporation observed in EG-NAT-12, EG-NAT-11, and EG-NAT-10 (>90%). All ethosomal formulations (EG-NAT-01 to EG-NAT-17) were characterized for particle size, PDI, ZP, EE, and DC. EG-NAT-12, EG-NAT-11 & EG-NAT-10 showed optimal vesicle size (130–137 nm), low PDI (<0.33), high negative ZP (−21 to −23 mV), maximum drug entrapment (86.5–89.8%) & highest DC (>90%). Formulations EG-NAT-01& EG-NAT-04 showed larger sizes (~168 nm) & higher PDIs (>0.38), indicating lower stability. These results highlight EG-NAT-12, EG-NAT-11 & EG-NAT-10 as the most promising candidates for stable & efficient dermal delivery.

Drug Release

The in vitro drug release profiles of the Nyctanthes arbor-tristis ethosomal gel formulations (EG-NAT-01 to EG-NAT-17) are summarized in Figure 2, which presents a bar graph of the cumulative percentage drug release for each formulation over the study period. Drug release is a key parameter influencing topical therapeutic efficacy, as it determines the amount of active drug available for permeation.

Among all formulations, EG-NAT-12 exhibited the highest cumulative drug release (84.0%), followed by EG-NAT-11 (82.5%) and EG-NAT-13 (81.3%). These results suggest a favorable vesicle composition enhancing drug permeation. Moderate release was observed in EG-NAT-10 (80.0%), EG-NAT-09 (79.5%), and EG-NAT-08 (77.7%), while EG-NAT-01 showed the lowest release (68.0%). The superior performance of EG-NAT-12 may be attributed to optimal ethanol & lipid content, which enhances vesicle fluidity and membrane penetration.

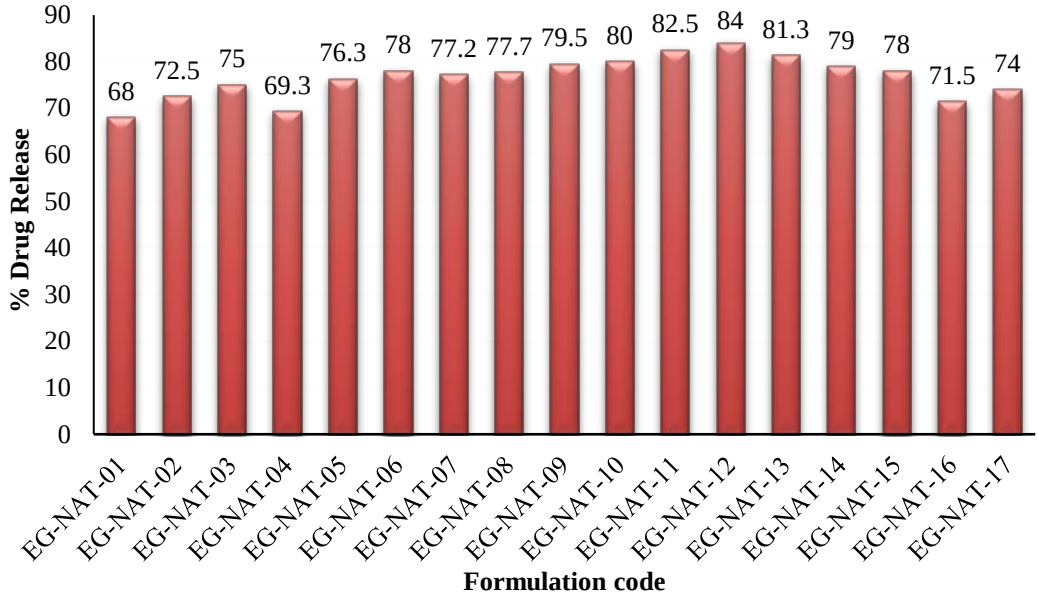

Figure 2: Drug Release (%)

DESIGN OF EXPERIMENTS AND OPTIMIZATION

Optimization of Entrapment Efficiency Using Box-Behnken Design

A Box-Behnken design was employed to optimize the formulation variables influencing the entrapment efficiency (R1) of ethosomal vesicles, using ethanol concentration (A), soya lecithin concentration (B), and cholesterol concentration (C) as independent factors. The quadratic model generated was found to be statistically significant ($p < 0.0001$), with ethanol ($p < 0.0001$), soya lecithin ($p = 0.0073$), and cholesterol ($p = 0.0031$) showing significant effects on entrapment efficiency. The model demonstrated excellent fit, with $R^2 = 0.9289$, adjusted $R^2 = 0.9125$, and predicted $R^2 = 0.8687$, suggesting robust predictive performance. The adequate precision value was 23.93, indicating a suitable signal-to-noise ratio. The regression equation derived from the model was:

$$\boldsymbol{Entrapment\ Efficiency} = +\mathbf{83.17} + \mathbf{5.09}(\boldsymbol{A}) + \mathbf{1.40}(\boldsymbol{B}) + \mathbf{1.56}(\boldsymbol{C})$$

The predicted vs. actual values plot (Figure 3) confirmed strong correlation, while the residuals vs. run plot (Figure 7) showed random scatter, indicating homoscedasticity and no significant deviation from model assumptions. The Box-Cox plot (Figure 6) suggested that data transformation was unnecessary. Among the independent variables, ethanol concentration had the most pronounced positive impact on entrapment efficiency, followed by cholesterol and soya lecithin. The contour plot (Figure 4) and 3D response surface plot (Figure 5) visually depict the interactive effects of ethanol and soya lecithin concentrations on entrapment efficiency. These results indicate the model's reliability in predicting optimal formulation conditions, confirming the robustness of the design for maximizing entrapment efficiency.

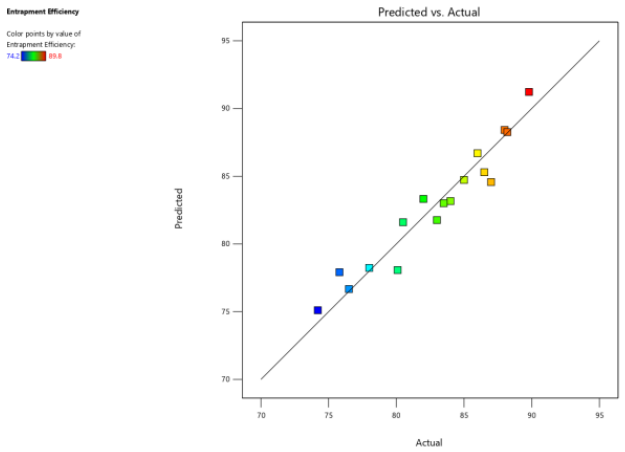

Figure 3: Predicted Vs. Actual(R1).

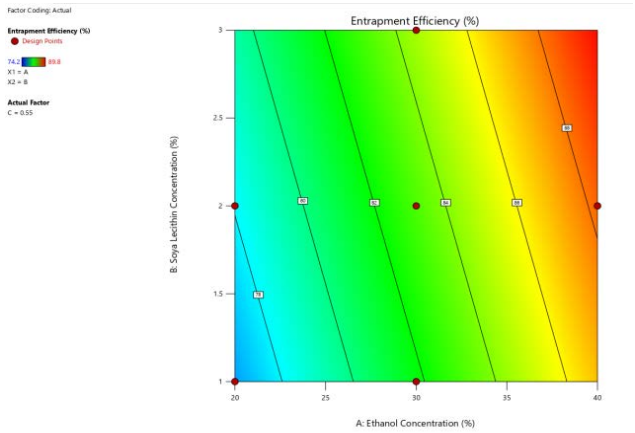

Figure 4: Contour plot showing the effect of ethanol conc. and soya lecithin conc. on Entrapment Efficiency (R1)

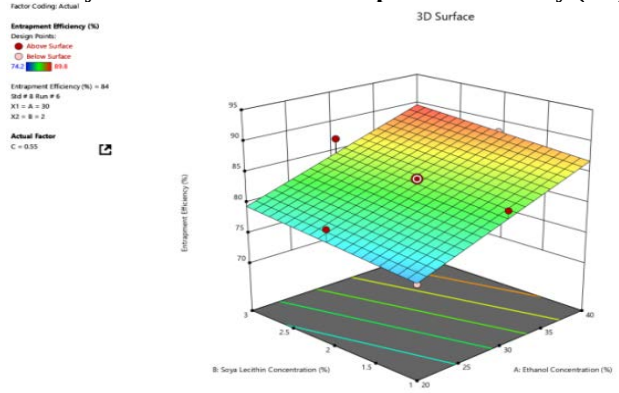

Figure 5: 3D response surface plot showing the effect of ethanol conc. nd soya lecithin conc. on EE (R1)

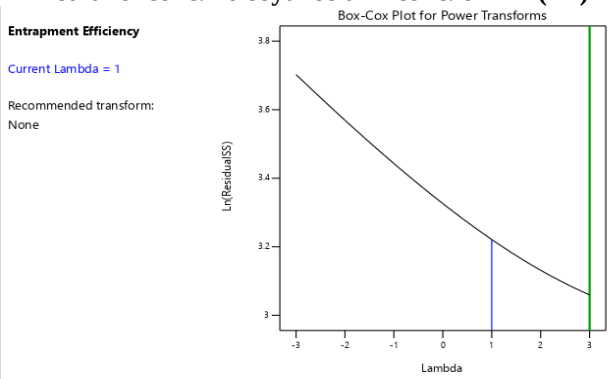

Figure 6: Box-Cox Plot for Power Transforms of EE (R1).

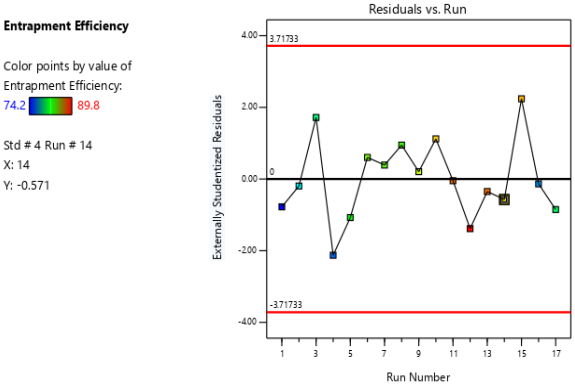

Figure 7: Residuals vs. Run Plot for EE (R1)

Optimization of Particle Size Using Box-Behnken Design

A Box-Behnken design was also applied to optimize the particle size (R2) of the ethosomal formulation using ethanol (A), soya lecithin (B), and cholesterol (C) as independent variables. The quadratic model was statistically significant ($p < 0.0001$), indicating that the selected factors had a substantial influence on particle size. Ethanol concentration demonstrated a highly significant effect ($p < 0.0001$), while soya lecithin ($p = 0.0505$) and cholesterol ($p = 0.0147$) exhibited moderately significant effects. The model's reliability was validated by high coefficients of determination ($R^2 = 0.9144$, adjusted $R^2 = 0.8946$, and predicted $R^2 = 0.8390$), indicating a strong correlation between predicted and observed values. The adequate precision value of 20.56 confirmed an adequate signal-to-noise ratio for model prediction. The resulting regression equation for particle size (in nm) was:

$$\boldsymbol{Particle\ Size = 146.91 - 12.7403A - 2.5474B - 3.24929C}$$

Model validation using the predicted vs. actual plot (Figure 8) confirmed the model's high accuracy. The Box-Cox plot (Figure 11) further confirmed that no data transformation was required ($\lambda = 1$). The residuals vs. run plot (Figure 12) displayed a random distribution with no significant patterns, supporting model assumptions.

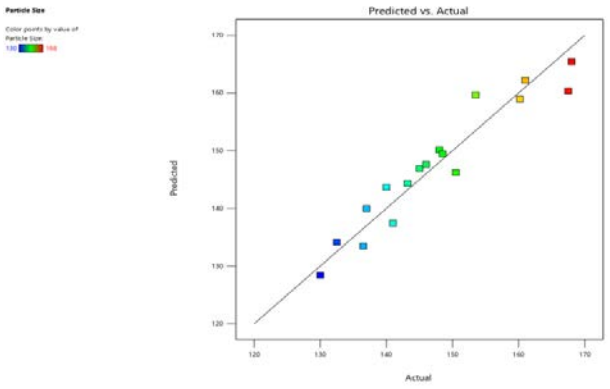

Figure 8: Predicted Vs. Actual Particle Size (R2)

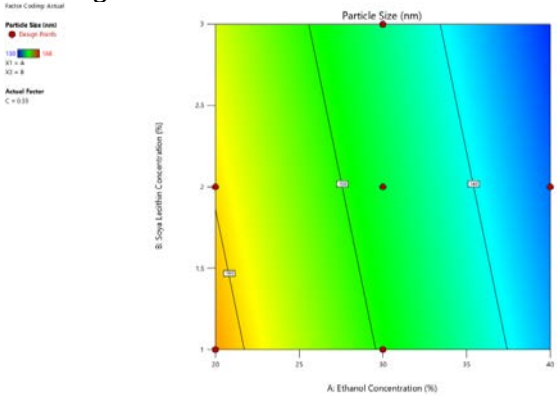

Figure 9: Contour plot showing the effect of Ethanol Conc. and Soya lecithin Conc. on Particle Size (R2)

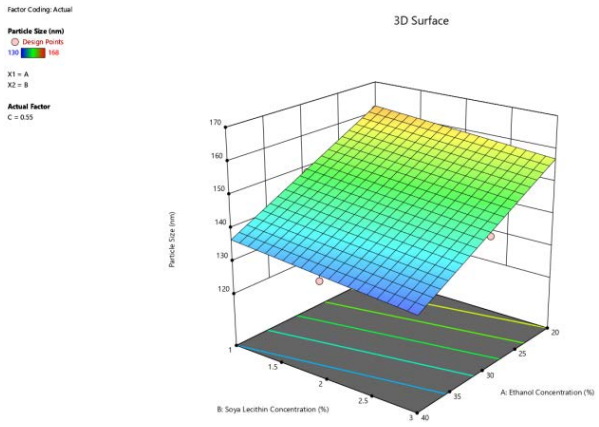

Figure 10: 3D response surface plot showing effect Ethanol Conc. and Soya lecithin Conc. on Particle Size (R2)

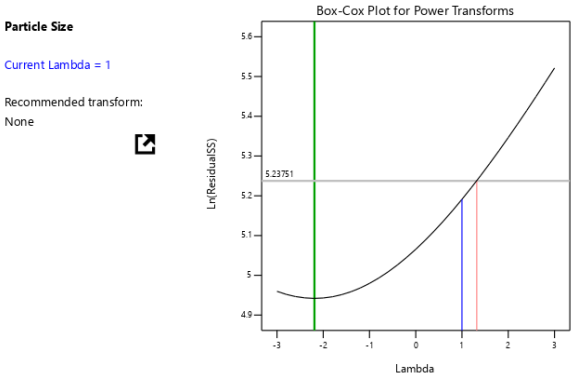

Figure 11: Box-Cox Plot for power transforms of particle size

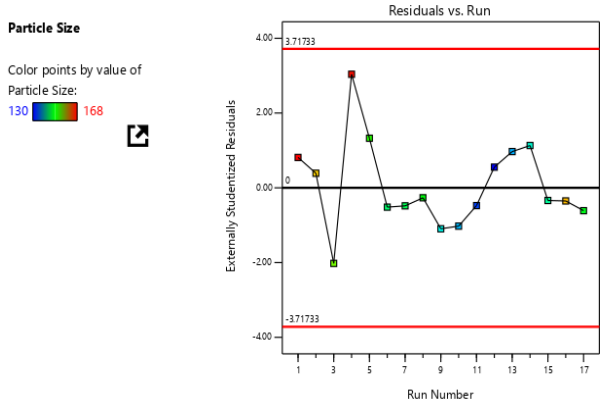

Figure 12: Residuals vs. Run Plot for Particle Size

The interaction between variables was illustrated using a contour plot (Figure 9) and a 3D response surface plot (Figure 11), which showed that particle size decreased with increasing ethanol concentration and decreasing soya lecithin conc. Ethanol was identified as the dominant factor in particle size reduction. These findings underscore the effectiveness of ethanol concentration optimization in minimizing vesicle size, a critical parameter for enhanced dermal delivery.

Optimization of Drug Release Using Box-Behnken Design

The Box-Behnken Design was employed to optimize the drug release (R3) of Nyctanthes arbor-tristis ethosomal formulations by evaluating the effect of ethanol (A), soya lecithin (B), and cholesterol (C). The quadratic model was statistically significant ($p < 0.0001$), demonstrating that ethanol concentration ($p < 0.0001$) had the most pronounced effect on drug release, followed by cholesterol concentration ($p = 0.0014$). Soya lecithin ($p = 0.0507$) had a marginal impact. The model showed robust fitting statistics with $R^2 = 0.9127$, adjusted $R^2 = 0.8926$, predicted $R^2 = 0.8480$, and an Adequate Precision value of 21.36, confirming its predictive accuracy and reliability. The regression equation for drug release (%) was:

$$Drug\ Release = 76.98048 + 4.76452\,A + 1.00664B + 1.8481C$$

The predicted vs. actual plot (Figure 13) showed a strong linear correlation between the experimental and predicted values, supporting the model's validity.

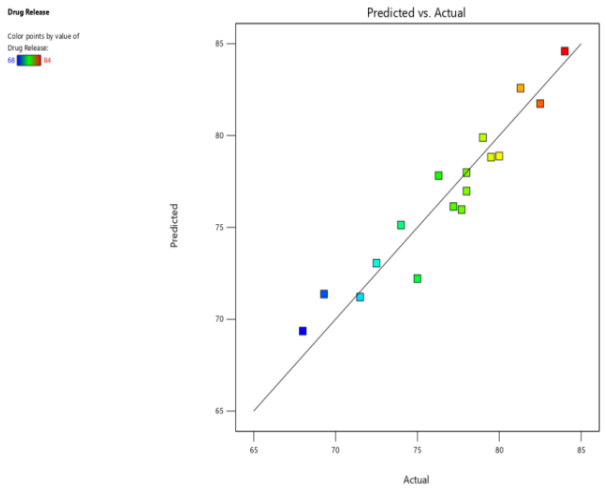

Figure 13: Predicted Vs. Actual(R3).

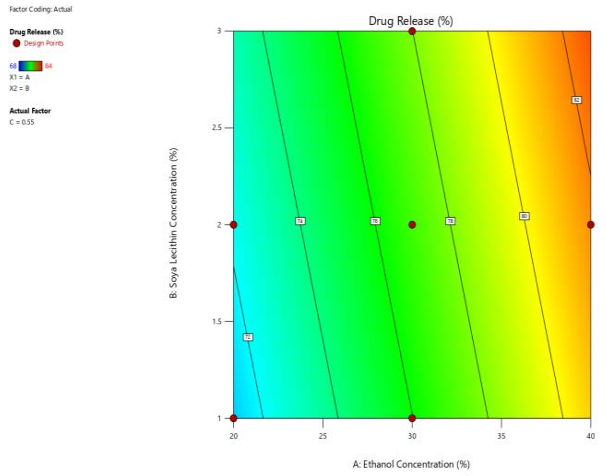

Figure 14: Contour plot showing effect of ethanol conc. and soya lecithin conc. on drug release (R3)

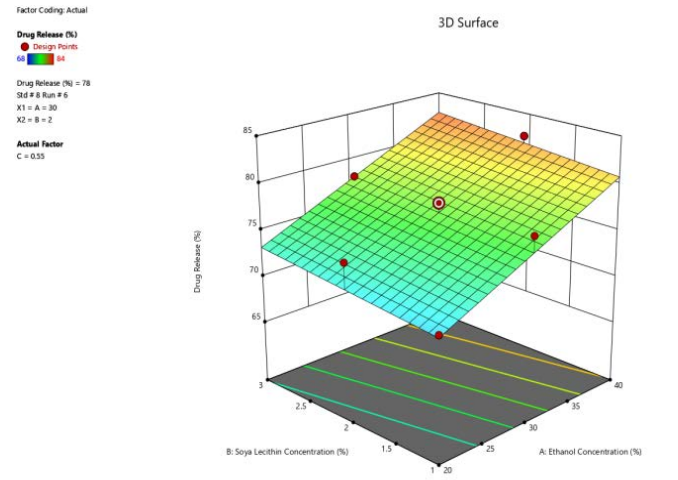

Figure 14: 3D response surface plot showing effect ethanol conc. and soya lecithin conc. on drug release (R3).

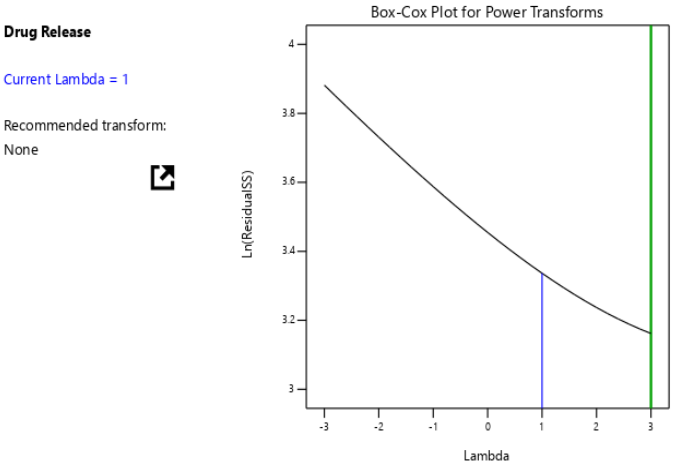

Figure 15: Box-Cox Plot for Power Transforms of DR (R3).

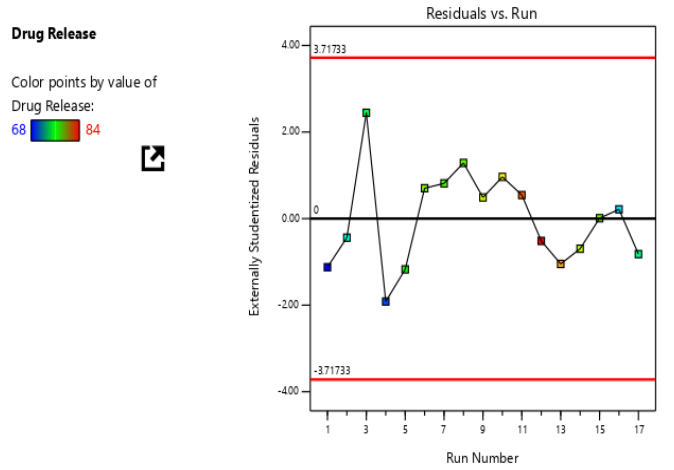

Figure 16: Residuals vs. Run Plot for Drug Release

The contour plot (Figure 14) and 3D response surface plot (Figure 15) illustrated that drug release increased with higher ethanol and soya lecithin concentrations, with ethanol being the dominant variable. The Box-Cox plot (Figure 16) confirmed the appropriateness of the original data scale ($\lambda = 1$), indicating that no transformation was required. The residuals vs. run plot

(Figure 17) showed a mostly random distribution of residuals, indicating independence and normality of the errors, with one minor outlier that did not significantly affect the model's performance. Overall, the data demonstrate that careful optimization of ethanol and soya lecithin concentrations can substantially enhance drug release from the ethosomal gel formulation, with ethanol playing a key role in maximizing drug diffusion.

Optimization of Formulation Parameters

The interaction effects of ethanol concentration, soya lecithin concentration, and cholesterol concentration on entrapment efficiency, particle size, and drug release were studied using response surface methodology (Figure 18). The optimized formulation conditions were identified as ethanol concentration of 34.39%, soya lecithin concentration of 1.29%, and cholesterol concentration of 0.72%.

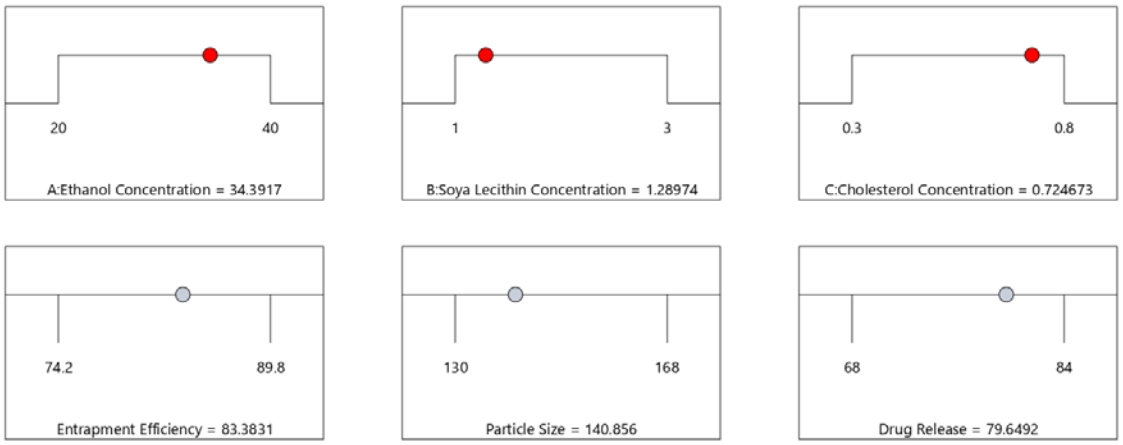

Figure 18: Optimization of Formulation Parameters & Their Impact on EE, Particle Size, & Drug Release

These conditions yielded:

- *Entrapment efficiency of 83.38%*
- *Particle size of 140.85 nm*
- *Drug release of 79.65%*

These results indicate a well-balanced formulation where the ethanol and cholesterol concentrations notably influence vesicle size and drug release characteristics. The data reflect favorable synergistic interactions among the formulation variables, validating the effectiveness of the B-B design in optimizing ethosomal gel characteristics for enhanced transdermal delivery.

Formulation of Nyctanthes Arbor-Tristis Ethosomal Gel

Nyctanthes arbor-tristis ethosomal gel formulations were prepared using the optimized ratio of ethanol and phospholipids to enhance the ethosomal carrier system. Carbopol 940 was utilized as the gelling agent due to its hydrophilic nature and bioadhesive properties, which contribute to prolonged residence time at the site of application through interaction with the mucosa. The prepared gel formulations were systematically evaluated for appearance, pH, viscosity, drug content, and in-vitro drug diffusion characteristics, as indicated in Tables 5 & 6.

***In vitro* Drug Diffusion studies**

The in vitro drug diffusion study of Nyctanthes arbor-tristis ethosomal gel formulations was performed using a dialysis membrane in an open-ended tube-beaker assembly with phosphate-buffered saline (PBS) at pH 7.4 as the diffusion medium. The results are shown in Tables 7 and Figures 19.

Table 5: Physical Evaluation

F. Code	Color	Grittiness	Homogeneity
EG-NAT-11	Light Brown	Non-gritty	Homogeneous
EG-NAT-12	Light Brown	Non-gritty	Homogeneous

Table 6: pH determination, spreadability, and viscosity results for Formulation Code-EG-NAT-11& EG-NAT-12.

F. Code	pH	Spreadability (gm. cm/sec)	Drug content %	Viscosity (cps)	Entrapment Efficiency (%)
EG-NAT-11	5.45±0.07	11.3±0.4	92.6 ±0.73	12886±0.88	89.2
EG-NAT-12	5.72±0.06	12.6±0.4	97.1 ±0.46	14842±0.39	91.8

Table 7: *In-vitro* release of *Nyctanthes arbor-tristis* ethosomal gel.

Time in hrs	Average % CDR - EG-NAT-11	Average % CDR - EG-NAT-12	%CDR - Pure Drug
0	0	0	0
1	24.9	26.9	55.58
2	29.1	35	79.86
3	35.6	49.5	89.96
4	57.2	61.36	97.2
5	68.23	77.93	
6	88.71	93.71	

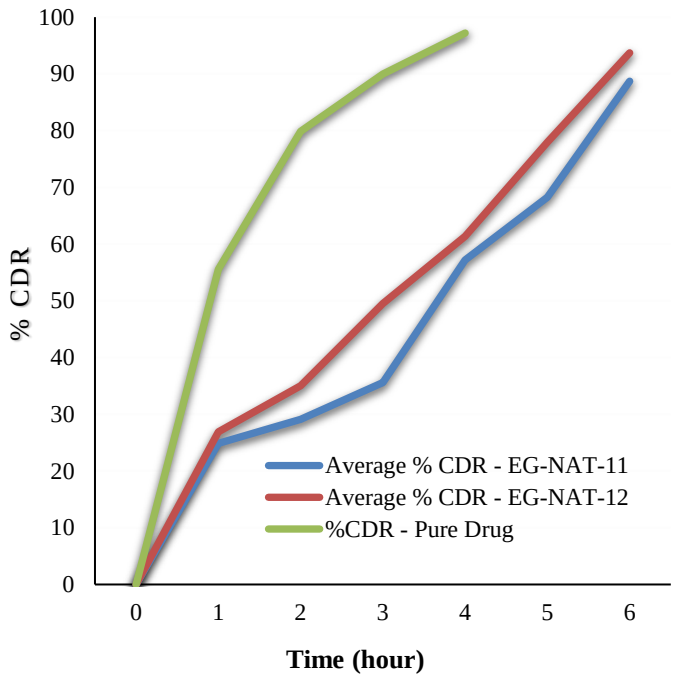

Figure 18: Cumulative drug release (% CDR)

Table 7 presents the % CDR profiles of EG-NAT-11, EG-NAT-12 & the pure drug formulation over time. At the 1-hour mark, the pure drug exhibits the highest % CDR (55.58%) compared to EG-NAT-11 (24.9%) and EG-NAT-12 (26.9%), indicating a faster initial release rate for the pure drug. However, over time, EG-NAT-12 consistently shows a higher % CDR than EG-NAT-11 at all time points, highlighting its improved release characteristics. By the 6-hour mark, EG-NAT-12 achieves the highest release (93.71%), surpassing EG-NAT-11 (88.71%). In contrast, the pure drug reaches a plateau at 97.2% by 4 hours, with no further increase observed. These findings underscore the sustained release behavior of the ethosomal formulations, with EG-NAT-12 demonstrating superior performance compared to EG-NAT-11.

Stability Studies of *Nyctanthes arbor-tristis* Ethosomal Gel (EG-NAT-12)

Stability studies for the optimized *Nyctanthes arbor-tristis* ethosomal gel formulation (EG-NAT-12) were conducted over 45 days at two storage conditions: refrigeration (4°C–8°C) and room temperature (25°C ± 2°C), in accordance with ICH guidelines. The parameters evaluated included drug content (%) and entrapment efficiency (%). The summarized data are presented in Table 8**,** demonstrating the consistency of the formulation throughout the study duration.

Table 8: Stability study of *Nyctanthes arbor-tristis* ethosomal gel formulation EG-NAT-12 at different temperatures

Time of storage in days	Temperature of storage			
	DC (%) 4°C - 8°C (refrigeration temp.)	EE (%) 4°C - 8°C (refrigeration temp.)	DC (%) 25°C ±2°C (room temp.)	EE(%) 25°C ±2°C (room temp.)
0	93.1	92.2	93.09	92.98
15	92.3	91.7	92.12	91.25
30	93.2	92.4	93.38	91.62
45	92.7	91.6	92.57	92.58

At refrigeration temperature, the drug content was initially 93.1%, with an entrapment efficiency of 92.2%. After 45 days of storage, these values showed minimal decline to 92.7% and 91.6%, respectively, indicating excellent stability under refrigerated conditions. Similarly, at room temperature, the initial drug content and entrapment efficiency were recorded as 93.09% and 92.98%, respectively. After 45 days, the drug content decreased slightly to 92.57%, while the entrapment efficiency remained relatively stable at 92.58%, demonstrating good formulation stability under ambient conditions.

Comparative analysis revealed that the ethosomal gel maintained consistent drug content and entrapment efficiency at both storage temperatures, with refrigeration providing marginally better preservation of the formulation integrity over the study duration. These findings suggest that although the formulation is stable at room temperature for short-term storage, refrigeration is preferable for prolonged shelf life. In conclusion, the *Nyctanthes arbor-tristis* ethosomal gel (EG-NAT-12) exhibits satisfactory physical and chemical stability under both refrigerated and ambient conditions for up to 45 days. Further long-term stability studies are warranted to confirm these results and assess the formulation's robustness under varied environmental conditions.

CONCLUSION

The comprehensive physicochemical characterization of *Nyctanthes arbor-tristis* leaves established essential quality control parameters, affirming their suitability for pharmaceutical applications. Ethosomal gel formulations, notably EG-NAT-12 and EG-NAT-11, were successfully developed and optimized, exhibiting nanoscale particle size (~130 nm), low polydispersity indices indicating homogeneity, and high entrapment efficiencies exceeding 88%, which collectively contribute to formulation stability and enhanced drug encapsulation. The physicochemical evaluation of the ethosomal gels demonstrated favorable attributes, including pH values compatible with dermal application, optimal spreadability, and a viscosity conducive to topical administration.

In vitro release kinetics revealed sustained drug diffusion, with EG-NAT-12 achieving superior cumulative drug release profiles over six hours. Stability assessments conducted over 45 days at refrigerated (4–8°C) and room temperature (25 ± 2°C) conditions indicated minimal variation in drug content and entrapment efficiency, underscoring the robust stability of EG-NAT-12. Refrigeration was found to marginally better preserve formulation integrity over time, although room-temperature storage remains feasible for short-term use.

ACKNOWLEDGEMENTS

The authors acknowledge the technical assistance provided by the staff of the Central Research Laboratory.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATION

EA– Entrapment Efficiency, PDI–Polydispersity Index, FTIR – Fourier-Transform Infrared Spectroscopy, ZP – Zeta Potential, ANOVA-Analysis of variance, EG-NAT-Ethosomal Gel *Nyctanthes arbor-tristis*, ICH- International Council for Harmonization, CDR- Cumulative drug release, PBS- Phosphate Buffer Solution, DoE- Design of Experiments

AUTHOR CONTRIBUTION

Kamlesh Kumar Yadav developed and carried out the practical work, handled data collection and analysis, and contributed to the preparation of the manuscript. Ravindra B. Laware provided overall guidance and supervision throughout the research work, helped shape the study's direction, and critically reviewed the manuscript to ensure its clarity and accuracy. Shubham N. Kanawade suggests conducting a literature review and investigating the manuscript. All authors have contributed significantly to the work and approved the final manuscript.

REFERENCES

[1] Ahad A, Aqil M, Kohli K, Sultana Y, Mujeeb M, Ali A. Role of nano ethosomes in transdermal drug delivery a review. *Current Drug Delivery*, **17(4)**, 306–319 (2020) https://doi.org/10.2174/1567201817666190627093649

[2] Goyal G, Gupta MK. Formulation and Characterization of Ethosomal Drug Delivery System for Antifungal Therapy. *IJPQA*, **15(4),** 2437-2442 (2024) https://doi.org/10.25258/ijpqa.15.4.43

[3] Pradeepkumar N, Ghosh P, Ramesh A. Phytochemical and pharmacological review on *Nyctanthesarbor-tristis*: A potential herbal drug for inflammatory diseases. *Journal of Ethnopharmacology*, **26(8)**, 113-556 (2021) https://doi.org/10.1016/j.jep.2020.113556

[4] Jadhav SS, Desale M, Bhalekar SM. To Formulation and Evaluation of Fast Dissolving Tablet Containing Nyctanthes Arbor Tristis Leaves. *International Journal of Advanced Research in Science, Communication and Technology*, **7(3),** 377-378 (2023) https://doi.org/10.48175/ijarsct.10233

[5] Patil AR, Maru AD. Expose the bioactive properties of Picrorhiza kurroa root extract Oil (Pkeo): Phytochemical composition and therapeutic activities. *J. Appl. Pharm. Res.*, **12**, 159–69 (2024) https://doi.org/10.69857/joapr.v12i6.841.

[6] Badhani B, Singh K, Sharma N. Phytochemical screening and biological activities of Nyctanthesarbor-tristis leaf extracts: a

review. *Phytomedicine Plus*, **2(2),** 100-207 (2022) https://doi.org/10.1016/j.phyplu.2022.100207

[7] Gadani M, Badak S, Upadhyay R. Innovative and cost-effective Seszen-Bio™ with enriched biotin and improved superior dissolution efficiency. *J. Appl. Pharm. Res.*, **12**, 68–76 (2024) https://doi.org/10.69857/joapr.v12i3.446.

[8] Khan N, Ansari SH, Kohli K. Formulation development, optimization and evaluation of Aloe vera ethosomal gel for wound healing. *Journal of Drug Delivery Science and Technology*, **6(1),** 102-265 (2021) https://doi.org/10.1016/j.jddst.2020.102265

[9] Gupta R, Kushwah V, Sharma S, et al. Ethosomes: A novel vesicular drug delivery system for enhanced transdermal permeation of natural bioactives. *Journal of Drug Delivery Science and Technology*, **7(1),** 103-246 (2022) https://doi.org/10.1016/j.jddst.2022.103246

[10] Sharma A, Sharma R, Sharma S, et al. Formulation development and characterization of ethosomal gel containing herbal extract for topical delivery. *International Journal of Pharmaceutics*, **59(2)**, 120091 (2021) https://doi.org/10.1016/j.ijpharm.2020.120091

[11] Singh P, Kaur M, Singh G, et al. Optimization and characterization of ethosomal formulation for improved skin permeation and drug delivery. *Journal of Pharmaceutical Sciences*, **109(6),** 1892-1901 (2020) https://doi.org/10.1016/j.xphs.2019.12.024

[12] Kumar S, Kumari P, Das M, et al. In vitro and in vivo evaluation of ethosomal gel of herbal extract for anti-inflammatory activity. *Drug Development and Industrial Pharmacy*, **49(3),** 378-388 (2023) https://doi.org/10.1080/03639045.2022.2135480

[13] Box GEP, Behnken DW. Some New Three Level Designs for the Study of Quantitative Variables. *Technometrics* , **2(4),** 455-475 (2023) https://doi.org/10.1016/j.jep.2023.113556

[14] Thakur N, Mehta SK. Design of ethosomal gels for enhanced transdermal delivery of bioactives: Optimization using Box–Behnken design. *J Drug Deliv Sci Technol*, **6(5),** 102- 721 (2021) https://doi.org/10.1016/j.jddst.2021.102721

[15] Kaur R, Singh R, Sharma A. Quality-by-design-based development and evaluation of herbal ethosomal gel for anti-inflammatory activity. *Saudi Pharm J*, **30(5),** 602–611 (2022) https://doi.org/10.1016/j.jsps.2022.02.003

[16] Prajapati SK, Jain A, Sharma A, et al. Statistical optimization of ethosomal nanocarriers for enhanced topical delivery of herbal extract: A QbD-based approach. *Current Drug Delivery*, **20(3),** 280–289 (2023) https://doi.org/10.2174/1567201820666220725102504

[17] Kumar R, Saini S, Seth N. Development and characterization of herbal ethosomal gel containing *Boswellia serrata* extract. *Journal of Pharmaceutical Innovation*, **16(3),** 467–476 (2021) https://doi.org/10.1007/s12247-020-09439-3

[18] Basu N. Buragommula1 N, Hephzibha B, Induru J. A Brief Review on Formulation and Evaluation of Ethosomal Gel for Arthritis with Herbal Extracts. *International Journal of Pharmacy and Biological Sciences*, **13(4)**, 36-45 (2023) https://doi.org/10.5281/ZENODO.10516553.

[19] Ganesan P, Chinnathambi A, Alharbi SA, et al. Formulation and evaluation of ethosomal gel loaded with polyherbal extract for topical application. *Evidence-Based Complementary and Alternative Medicine*, 88 90196,(2021) https://doi.org/10.1155/2021/8890196

[20] Gupta A, Mishra DK, Singh D, et al. Evaluation of anti-inflammatory topical gels prepared with ethosomal vesicular system. *Journal of Drug Delivery Science and Technology*, **7(1),** 103-233 (2022) https://doi.org/10.1016/j.jddst.2022.103233

[21] Gupta A, Mishra DK, Singh D, et al. Stability assessment of topical gel formulations using ICH guidelines. *Journal of Applied Pharmaceutical Science.*,**10(4)**,55–61(2020) https://doi.org/10.7324/JAPS.2020.10408

[22] Sharma V, Pathak K. Formulation, characterization, and stability study of ethosomal gel of curcumin for transdermal delivery. *Pharmaceutical Development and Technology*, **26(5),** 564–572 (2021) https://doi.org/10.1080/10837450.2021.1903280