



DEVELOPMENT AND EVALUATION OF HERBAL PLANT LOADED ETHOSOMAL FORMULATIONS FOR TOPICAL DELIVERY

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ABSTRACT

Polycystic Ovarian Syndrome (PCOS) is a complex endocrine disorder commonly affecting women of reproductive age. In India, nearly two out of every ten women are affected by PCOS. The condition is characterized by polycystic ovarian morphology, ovulatory dysfunction, and hyperandrogenism. PCOS is considered one of the major causes of female infertility and is associated with several complications such as metabolic disorders, irregular menstruation, hypertension, insulin resistance, and elevated androgen levels including testosterone. Women with PCOS may also experience multiple sub-follicular cysts in the ovaries. Additionally, vitamin deficiencies in PCOS patients may increase the risk of severe COVID-19 infection, especially due to reduced sun exposure during quarantine periods. Hence, increasing public awareness regarding PCOS is essential. Although PCOS is a manageable disorder, natural remedies including herbs and seeds have gained importance because of their safety and effectiveness. Development of polyherbal formulations may help reduce the cost, duration, and side effects associated with conventional therapies. Furthermore, lifestyle modifications such as a balanced diet, regular exercise, yoga, and healthy habits play a significant role in controlling PCOS. Therefore, this review highlights the potential use of natural remedies in the management of PCOS.

KEYWORDS: PCOS; hyperandrogenism; herbs; seeds; yoga asanas.

1. INTRODUCTION

Topical drug delivery systems (TDDS) represent a cornerstone in modern pharmaceutical science, providing a direct and localized method for administering therapeutic agents. The skin, being the largest and most accessible organ of the body, offers an attractive route for drug delivery. Historically, TDDS have been utilized for a variety of applications, ranging from cosmetic formulations to treatments for localized skin conditions like eczema, psoriasis, and acne, as well as systemic conditions via transdermal patches (Liu *et al.*, 2025). One of the primary advantages of TDDS lies in its ability to bypass the gastrointestinal tract and hepatic first-pass metabolism, which can degrade drugs and reduce bioavailability. Moreover, this route allows for the direct

delivery of therapeutic agents to the site of action, thereby minimizing systemic exposure and reducing the likelihood of side effects. These systems also offer a non-invasive, patient-friendly approach, which enhances compliance, particularly in populations where oral administration may be challenging, such as pediatrics and geriatrics (Xu *et al.*, 2025).

The QbD approach accentuates the comprehension of different components of the formulation for improved command over wanted yield. Design of experiments (DoE) and multivariate statistical data analysis are essential elements of QbD, recognized by the recent International Conference of Harmonization Q8 guideline (Gurba-Bryśkiewicz *et al.*, 2023). These tools facilitate

the simultaneous variation of all the formulation variables, allowing quantification and prioritization of the consequences made by these variables in the defined design space, together with any possible interaction between them. Using QbD approach, the effect of formulation variables on vesicle size and the percentage entrapment efficiency of ethosomal formulations was studied. Based on the data gained by multivariate analysis, the idea of “desirability function” was applied to attain optimum formulation conditions within the defined design space (Arora and Nanda, 2019).

Ethosomes are non-invasive delivery carriers that enable drugs to reach the deep skin layers and /or the systemic circulation. Plant drugs are considered safe of their natural origin. Even after exhibiting the promising therapeutic effects, most of the phytoconstituents fail to achieve bioavailability because of poor absorption (Chauhan *et al.*, 2022). Large molecular sizes and low lipid solubilities are the prominent factors causing poor absorption of phytoconstituents resulting in reduced bioavailability. Incorporation of these plant actives or extracts into vesicular carriers vastly improves their absorption and consequently bioavailability. Indirectly ethosomes has become an area of research in herbal formulations because of their enhanced skin permeation and improved entrapment efficiency (Huanbutta *et al.*, 2022).

The ethosomal drug delivery system involves a synergistic interaction between ethanol, vesicles, and skin lipids, enhancing the delivery of active ingredients. Ethanol interacts with lipid molecules in the stratum corneum, making it more permeable (Paiva-Santos *et al.*, 2021). The "Ethosome effect" allows ethosomes to penetrate and fuse with skin lipids, creating new pathways for drug penetration into deeper layers. Ethanol contributes to the soft and flexible characteristics of ethosomes, allowing them to penetrate more easily. The drug released from ethosomes can reach deeper layers of the skin and potentially be absorbed into systemic circulation. This mechanism provides a promising approach for improving transdermal drug delivery (Almuqbil and Aldhubiab, 2025).

Butterfly pea, or *Clitoria ternatea* (Fabaceae), is a perennial herb that is widely used in traditional medicine to treat conditions related to oxidative stress, fever, and inflammation. It has recently drawn a lot of attention because of its potential use in modern health and agriculture, as well as its capacity to offer antioxidants and natural food coloring (Oguis *et al.*, 2019). Flavonoids (quercetin, kaempferol, and rutin), phenolic acids (gallic and chlorogenic acid), and anthocyanins are among its phytochemical constituents, which have strong antiinflammatory and antioxidant properties. These substances serve as metal chelators, enzyme modulators, and free radical scavengers. Its therapeutic value has been validated by prior research showing that *C. ternatea* extracts have a strong antioxidant capacity that is

comparable to that of conventional antioxidants (Vidana Gamage *et al.*, 2021).

In the present study, a QbD-based strategy was employed for the preparation, development, optimization, and evaluation of herbal plant-loaded ethosomal formulations intended for topical delivery. The formulation and process variables were systematically investigated to identify their influence on the selected CQAs.

2. MATERIALS AND METHODS

2.1 Chemicals

Ethanol and Ascorbic Acid were obtained from Merck, India. HiMedia provided the Carboxymethylcellulose (CMC). JoinHub Pharma provided the Normal saline while Niksan Pharmaceuticals supplied Donepezil. Sisco Research Laboratories were obtained from DPPH (2,2-Diphenyl-1-picrylhydrazyl) and Clorofiltind provided the 95% Alcohol. SRF Limited obtained from Chloroform. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Ams Fine Chemical, Pandora Industries, Suvchem, Manas Petro Chem and Vizag chemical.

2.2 Procurement of Material

Fresh aerial parts of *Clitoria ternatea* were collected directly from its natural habitat where it grows abundantly and authenticated by a qualified botanist. To prevent contamination and deterioration, dried plant was stored in airtight glass containers in a dry, cool environment. The plant material was washed, air-dried in shade at room temperature, and pulverized to a coarse powder.

2.3 Preparation of Extract: Drying, Powdering, and Extraction of Plant Material

The dried and coarsely powdered material of *Clitoria ternatea* is subjected to extraction using the Soxhlet apparatus, a widely used method for efficient extraction of bioactive compounds. The continuous circulation of the solvent facilitates the efficient extraction of phytoconstituents. The process is carried out for 6 to 8 hours or until the solvent in the siphon tube appears clear, indicating the exhaustion of plant material. After completion, the extract is filtered to eliminate any solid impurities and concentrated using a rotary evaporator under reduced pressure to remove excess solvent. The semi-solid crude extract is then dried and stored in an airtight container in a desiccator at low temperature for further pharmacological and phytochemical investigations. This method ensures maximum yield and purity of bioactive substances for neuroprotective evaluation (Deorankar *et al.*, 2020).

The percentage yield of the *Clitoria ternatea* extract is determined using the following formula.

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

The prepared extracts were labeled and kept in container until further usage after being examined for organoleptic characteristics (percentage yield, color, and odor).

2.4 Formulation of extract Loaded Ethosomes via Cold method

Clitoria ternatea extract-loaded ethosomes were prepared by the cold method. Soya lecithin and cholesterol were dissolved in ethanol under continuous stirring at room temperature, followed by the addition of *Clitoria ternatea* extract and propylene glycol. Distilled water heated to 30–40°C was added slowly with continuous stirring, and the dispersion was stirred for 1–3 h according to the formulation design. The resulting

formulation was equilibrated and sonicated for 5–10 min to obtain a uniform nanosized vesicular dispersion. The prepared ethosomes were stored in airtight amber-colored containers at $4 \pm 2^\circ\text{C}$ until further evaluation. The formation of ethosomes was facilitated by the combined action of phospholipids and ethanol, which provide flexibility and deformability to the vesicles. Cholesterol improved membrane stability, while propylene glycol acted as a co-solvent and permeation enhancer. Encapsulation of *Clitoria ternatea* phytoconstituents was expected to enhance their stability and topical skin delivery (Morya *et al.*, 2025).

Table 1: Composition of Ethosomes Formulation.

Soya lecithin (%)	Cholesterol (%)	Stirring time (hrs)	Ethanol (%)	<i>Clitoria ternatea</i> extract (%)	Distilled Water (ml)	Propylene Glycol (ml)
3	1	2	20.0	2.0	10.0	5.0
2	1	3	20.0	2.0	10.0	5.0
3	0.65	3	20.0	2.0	10.0	5.0
2	0.3	1	20.0	2.0	10.0	5.0
3	0.65	1	20.0	2.0	10.0	5.0
2	1	1	20.0	2.0	10.0	5.0
2	0.3	3	20.0	2.0	10.0	5.0
1	1	2	20.0	2.0	10.0	5.0
1	0.3	2	20.0	2.0	10.0	5.0
3	0.3	2	20.0	2.0	10.0	5.0
1	0.65	1	20.0	2.0	10.0	5.0
1	0.65	3	20.0	2.0	10.0	5.0

2.5 Characterization parameter of *Clitoria ternatea* loaded Ethosomes

2.5.1 Physical Appearance

The ethosomal formulations (ES1–ES12) were visually inspected to evaluate their color, appearance, clarity, and homogeneity. The formulations were examined for the presence of uniform dispersion, turbidity, phase separation, sedimentation, or visible aggregates (Dadwal *et al.*, 2023).

2.5.2 Particle Size Analysis

The size of vesicular carriers plays an important role in determining the performance of topical and transdermal drug delivery systems. For the extract-loaded ethosomal formulation, the average particle size and size distribution were evaluated using dynamic light scattering (DLS) technique with a Zetasizer Nano ZS (Malvern Instruments, UK) (Qureshi *et al.*, 2023).

2.5.3 Surface Charge Evaluation

The surface charge of herbal plant extract-loaded ethosomes was determined by zeta potential analysis to evaluate the colloidal stability and electrostatic characteristics of the vesicular system. The analysis was carried out using a Zetasizer Nano ZS (Malvern Instruments, UK) based on the principle of electrophoretic light scattering, which measures the mobility of charged vesicles under an applied electric field (Kharwade *et al.*, 2023).

2.5.4 Morphological Examination Using Scanning Electron Microscopy

The surface morphology and structural characteristics of ethosomal formulation were evaluated using Scanning Electron Microscopy (SEM). A small quantity of the lyophilized ethosomal powder was carefully mounted onto a metallic SEM stub using double-sided carbon adhesive tape. The samples were then coated with a thin gold layer under vacuum conditions to improve electrical conductivity and minimize charging effects during imaging (Mohamed, *et al.*, 2022).

2.6 Method of Ethosomal Cream Formulation

The optimized *Clitoria ternatea*-loaded ethosomal suspension was incorporated into a cream base prepared by the fusion method. Stearic acid, cetyl alcohol, and other oil-soluble ingredients were melted at $70 \pm 2^\circ\text{C}$, while the aqueous phase was heated separately to the same temperature. Both phases were mixed with continuous stirring to form a smooth cream base. After cooling to 35–40°C, the ethosomal suspension was gently incorporated and stirred until a homogeneous ethosomal cream was obtained. The formulation was stored in airtight containers at $4 \pm 2^\circ\text{C}$ until further evaluation. Incorporation below 40°C helped preserve vesicle integrity, while gentle mixing ensured uniform distribution of ethosomes. The developed cream was expected to improve skin retention and topical delivery of *Clitoria ternatea* phytoconstituents (Verma *et al.*, 2010).

Table 2: Composition of cream formulation.

Ingredients	Quantity (% w/w)	Function
<i>Clitoria ternatea</i> Loaded Ethosomal Suspension	2.0	Active ingredient and vesicular carrier
Stearic Acid	12.0	Emulsifying agent and consistency enhancer
Cetyl Alcohol	3.0	Stiffening agent and emollient
Liquid Paraffin	5.0	Emollient and oil phase component
Propylene Glycol	5.0	Humectant and penetration enhancer
Triethanolamine	1.5	Neutralizing and emulsifying agent
Methyl Paraben	0.18	Preservative
Propyl Paraben	0.02	Preservative
Distilled Water	q.s. to 100	Vehicle

2.7 Evaluation of *Clitoria ternatea* Loaded Ethosomal Cream

2.7.1 Organoleptic Evaluation

The prepared ethosomal cream was visually inspected for color, odor, appearance, texture, consistency, and phase separation. Organoleptic evaluation is important for assessing patient acceptability and overall product quality (Thomas *et al.*, 2019).

2.7.2 pH Determination

The pH of the ethosomal cream was determined using a calibrated digital pH meter (Sharma *et al.*, 2023).

2.7.3 Homogeneity

Homogeneity was evaluated by visual inspection after the cream had been packed in the container. The cream was examined for the presence of lumps, aggregates, phase separation, and uniformity of distribution. A homogeneous formulation should exhibit smooth consistency and uniform distribution of the active ingredient throughout the cream base (Verma and Pathak 2010).

2.7.4 Viscosity Measurement

The viscosity of the ethosomal cream was measured using a Brookfield Viscometer fitted with a suitable spindle at room temperature (25 ± 2°C). Approximately 50 g of cream was transferred into the sample container and viscosity readings were recorded at different rotational speeds. Viscosity is a critical parameter affecting release, spreadability, stability, and patient compliance. Increased viscosity generally decreases drug diffusion and permeation through the skin (Binder *et al.*, 2019).

2.7.5 Spreadability

Spreadability was determined using the parallel-slide method. Approximately 3–5 g of cream was placed between two glass slides. A known weight was placed on the upper slide to obtain a uniform film, and an additional weight was attached to move the upper slide. The time required for the upper slide to move a specified distance was recorded. Spreadability was calculated using the following equation.

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

Where

- S = Spreadability (g·cm/sec)
- M = Weight tied to upper slide (g)
- L = Length moved by the slide (cm)
- T = Time taken to separate the slides (sec)

Higher spreadability values indicate better ease of application and improved patient compliance. Spreadability is considered an important quality attribute for semisolid formulations (Barghouthy *et al.*, 2025).

2.7.6 Extrudability

Extrudability was determined by filling the cream into collapsible aluminum tubes. The tube was pressed with a known weight and the amount of cream extruded from the tube was measured. Extrudability reflects the force required to expel the cream from the container and is an important parameter for consumer convenience. Formulations exhibiting easy extrusion with minimal force are considered acceptable (Phad *et al.*, 2018).

2.8 Antibacterial Activity of herbal cream formulation

Nutrient agar medium was prepared by dissolving the required quantity of agar powder in distilled water, followed by sterilization in an autoclave at 121°C for 15 min. The sterilized medium was poured into sterile Petri plates and allowed to solidify. Different concentrations of the herbal cream formulation were prepared, including placebo cream as the negative control, extract mixture (1 mg/mL), and extract-loaded herbal cream formulations at 0.5 and 1.0 mg/mL. The prepared samples were carefully introduced into wells in the agar plates under aseptic conditions using a micropipette. The inoculated plates were incubated at 37°C for 24 h to allow bacterial growth and diffusion of the test formulations. After incubation, the plates were examined for clear zones surrounding the wells, and the diameter of each zone of inhibition was measured in millimetres using a ruler or vernier caliper. The antibacterial activity was evaluated based on the diameter of the inhibition zones, with larger zones indicating greater antibacterial activity of the herbal cream formulation (Seth and Ramesh, 2026).

3. RESULTS AND DISCUSSION

3.1 Percentage Yield

Table 3: Percentage Yield of plant material.

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
<i>Clitoria ternatea</i>	Ethanol	350	26.8	7.65 %

3.2 Qualitative Phytochemical estimation.

Table 4: Phytochemical test of *Clitoria ternatea* extract of Methanolic extract.

Experiment	Presence or absence of phytochemical test Methanolic extract
Alkaloids	
Dragendorff's test	Present
Mayer's reagent test	Present
Wagner's reagent test	Present
Hager's reagent test	Present
Glycosides	
Borntrager test	Present
Legal's test	Present
Killer-Killiani test	Present
Carbohydrates	
Molish's test	Absent
Fehling's test	Absent
Benedict's test	Absent
Barfoed's test	Absent
Proteins and Amino Acids	
Biuret test	Absent
Ninhydrin test	Absent
Flavonoids	
Alkaline reagent test	Present
Lead Acetate test	Present
Tannin and Phenolic Compounds	
Ferric Chloride test	Present
Saponin	
Foam test	Present
Test for Triterpenoids and Steroids	
Salkowski's test	Absent
Libermann-Burchard's test	Absent

3.3 Optimization of Ethosomes formulations by the DoE approach

3.3.1 Build Information

Table 5: Build information of DOE software.

File Version	12.0.1.0		
Study Type	Response Surface	Subtype	Randomized
Design Type	Box-Behnken	Runs	12
Design Model	Quadratic	Blocks	No Blocks

3.3.2 Formulation trials as per Box–Behnken design.

Table 6: Formulation trials as per Box–Behnken design.

Factor 1 Soya lecithin (%)	Factor 2 Cholesterol (%)	Factor 3 Stirring time (hrs)	Ethanol (%)	<i>Clitoria ternatea</i> extract (%)	Distilled Water (ml)	Propylene Glycol (ml)	Response 1 Particle size	Response 2 Zeta potential
3.0	1	2.0	20.0	2.0	10.0	5.0	256.35	-31.04
2.0	1	3.0	20.0	2.0	10.0	5.0	183.26	-33.62
3.0	0.65	3.0	20.0	2.0	10.0	5.0	163.22	-33.11
2.0	0.3	1.0	20.0	2.0	10.0	5.0	288.47	-30.52
3.0	0.65	1.0	20.0	2.0	10.0	5.0	360.51	-30.22
2.0	1	1.0	20.0	2.0	10.0	5.0	401.25	-29.82
2.0	0.3	3.0	20.0	2.0	10.0	5.0	145.28	-34.86

1.0	1	2.0	20.0	2.0	10.0	5.0	271.36	-30.15
1.0	0.3	2.0	20.0	2.0	10.0	5.0	230.55	-32.53
3.0	0.3	2.0	20.0	2.0	10.0	5.0	212.38	-32.77
1.0	0.65	1.0	20.0	2.0	10.0	5.0	362.05	-29.25
1.0	0.65	3.0	20.0	2.0	10.0	5.0	170.66	-33.56

3.3.3 Limits of Variables (Constraints)

Table 7: Variables operating range for Ethosome formulation.

Name	Goal	Lower Limit	Upper Limit	Importance
A:Soya Lecithin	is in range	1	3	3
B:Cholesterol Concentration	is in range	0.3	1	3
C:Stirring time	is in range	1	3	3
Particle Size	none	145.28	401.25	3
Zeta potential	none	-34.86	-29.25	3

3.3.4 Fit Summary

Response 1: Particle size

Table 8: Response 1: Particle size (Fit Summary).

Source	Sequential p-value	Adjusted R ²	Predicted R ²	
Linear	< 0.0001	0.9480	0.9149	Suggested
2FI	0.3367	0.9553	0.8829	
Quadratic	0.2871	0.9676	0.8585	Aliased

3.4 Effect of formulation variables on particle size (ANOVA for Linear model).

3.4.1 Response 1: Particle size.

Table 9: Response 1: Particle size (ANOVA for Linear model).

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	77443.32	25814.44	67.80	< 0.0001	significant
A-Soya Lecithin	222.18	222.18	0.5836	0.4668	
B-Cholesterol Concentration	6934.89	6934.89	18.22	0.0027	
C-Stirring time	70286.25	70286.25	184.61	< 0.0001	
Residual	3045.78	380.72			
Cor Total	80489.11				

3.4.2 Coefficients Coded Factors

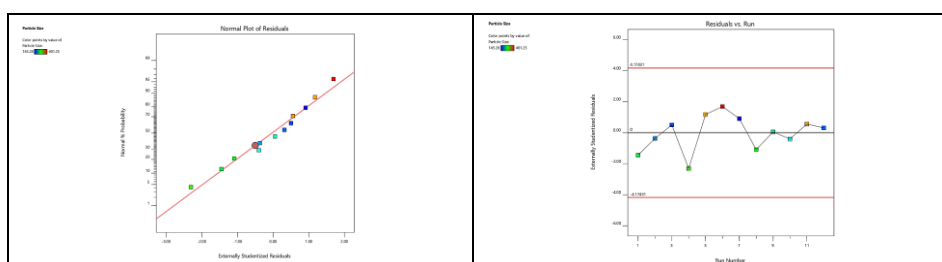
Table 10: Coefficients in Terms of Coded Factors.

Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High	VIF
Intercept	253.78	5.63	240.79	266.77	
A-Soya Lecithin	-5.27	6.90	-21.18	10.64	1.0000
B-Cholesterol Concentration	29.44	6.90	13.53	45.35	1.0000
C-Stirring time	-93.73	6.90	-109.64	-77.82	1.0000

3.3.3 Final Equation in Terms of Coded Factors

Particle size (R₁) = +253.78 -5.27 AX₁ +29.44 BX₂ - 93.73 CX₃

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1.

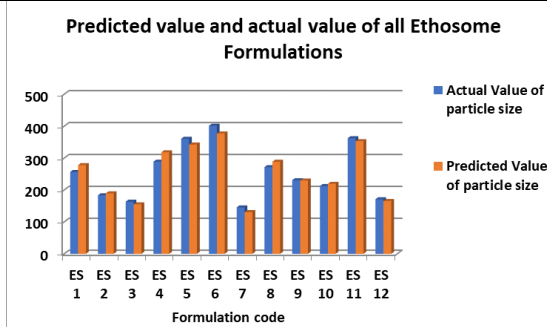


Graph 1: Diagnostic Residual Plots (Normal Plot and Residuals vs. Run) for Particle Size Response.

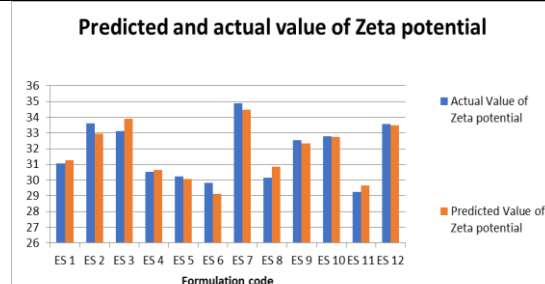
3.4.4 Predicted value and actual value of all Formulations.

Table 11: Predicted value and actual value of Particle size and Zeta potential of all Ethosome Formulations.

Formulations	Actual Value of particle size	Predicted Value of particle size	Actual Value of Zeta potential	Predicted Value of Zeta potential
ES 1	256.35	277.95	-31.04	-31.24
ES 2	183.26	189.49	-33.62	-32.95
ES 3	163.22	154.78	-33.11	-33.91
ES 4	288.47	318.07	-30.52	-30.63
ES 5	360.51	342.24	-30.22	-30.08
ES 6	401.25	376.95	-29.82	-29.11
ES 7	145.28	130.60	-34.86	-34.46
ES 8	271.36	288.49	-30.15	-30.83
ES 9	230.55	229.61	-32.53	-32.34
ES 10	212.38	219.07	-32.77	-32.75
ES 11	362.05	352.78	-29.25	-29.66
ES 12	170.66	165.32	-33.56	-33.50



Graph 2: Comparison of Actual and Predicted Values for All Ethosome Formulations.



Graph 3: Graphical Correlation between Actual and Predicted Zeta potential.



Graph 4: Response Surface Plot Illustrating the Combined Effect of Formulation Variables on Ethosome Characteristics.

3.4.5 Effect of formulation variables on Zeta potential.

Table 12: Response 2: Zeta potential (Fit Summary).

Source	Sequential p-value	Adjusted R ²	Predicted R ²	
Linear	< 0.0001	0.9070	0.8479	Suggested
2FI	0.6267	0.8920	0.7174	
Quadratic	0.2651	0.9258	0.6760	Aliased

3.5 ANOVA for linear model

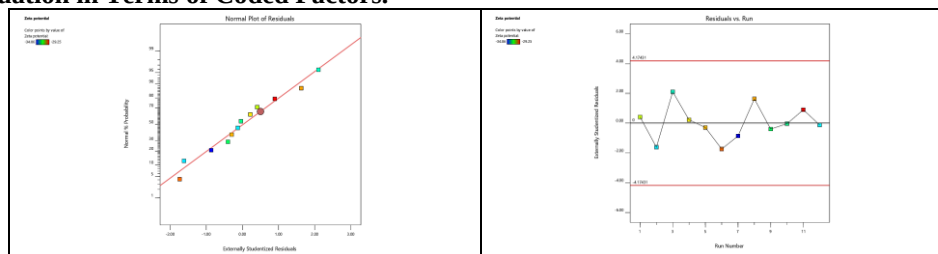
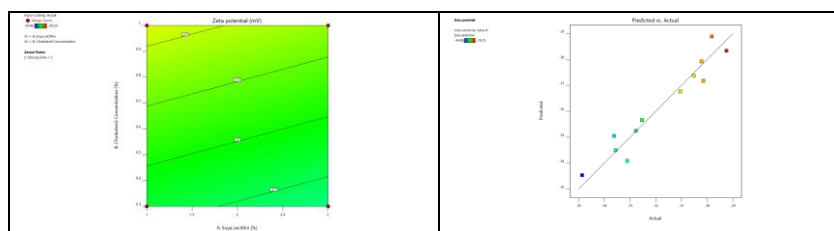
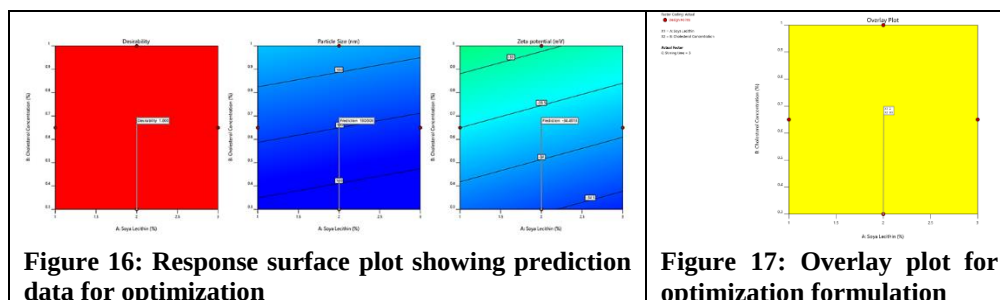
3.5.1 Response 2: EE (ANOVA Linear)

Table 13: Response 2: Zeta potential.

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	34.33	11.44	36.78	< 0.0001	significant
A-Soya Lecithin	0.3403	0.3403	1.09	0.3262	
B-Cholesterol Concentration	4.58	4.58	14.70	0.0050	
C-Stiring time	29.41	29.41	94.53	< 0.0001	
Residual	2.49	0.3112			
Cor Total	36.82				

Table 14: Coefficients in Terms of Coded Factors.

Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High	VIF
Intercept	-31.79	0.1610	-32.16	-31.42	
A-Soya Lecithin	-0.2062	0.1972	-0.6610	0.2485	1.0000
B-Cholesterol Concentration	0.7563	0.1972	0.3015	1.21	1.0000
C-Stirring time	-1.92	0.1972	-2.37	-1.46	1.0000

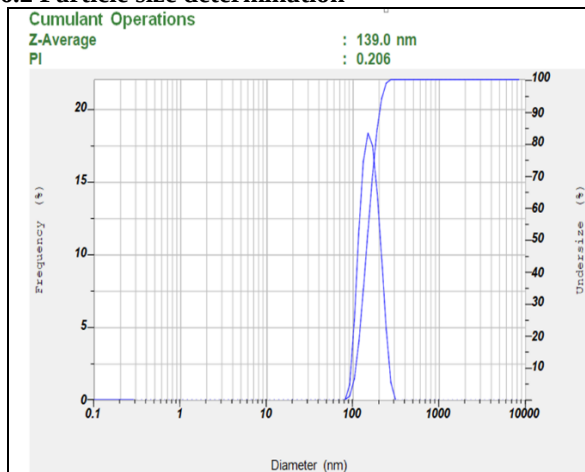
3.5.2 Final Equation in Terms of Coded Factors.**Graph 5: Graphical representation of Residuals vs. run, Normal plot of Ethosome formulation on Zeta potential.****Graph 6: Two-dimensional contour plots for the effect of Phospholipid and cholesterol concentration on Zeta potential and predicted vs. actual.****Figure 16: Response surface plot showing prediction data for optimization****Figure 17: Overlay plot for optimization formulation****3.5.3 Optimized formula of Ethosome formulation.****Table 15: Optimized formula of Ethosome formulation.**

Soya Lecithin	Cholesterol Concentration	Stirring time	Particle Size	Zeta potential	Desirability	
3.000	1.000	2.000	277.951	-31.238	1.000	
2.000	0.300	3.000	130.603	-34.461	1.000	Selected
2.590	0.896	2.347	238.819	-32.043	1.000	

3.6 Characterization of optimized Ethosome formulation.**3.6.1 Physical Appearance.****Table 16: Physical Appearance of extract loaded Ethosome.**

Parameter	Observation
Color	White to off-white
Odor	Odorless
Appearance	Clear and homogeneous
State	Liquid

3.6.2 Particle size determination



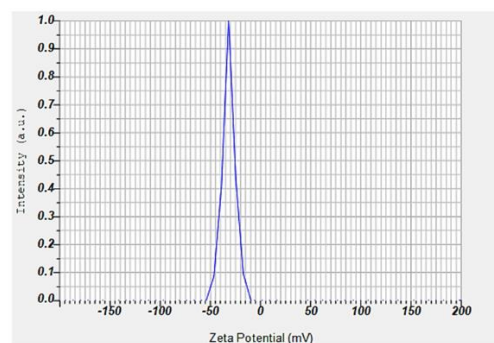
Graph 7: Particle size of optimized formulation

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-31.5 mV	-0.000244 cm ² /Vs
2	---	---
3	---	---

Zeta Potential (Mean) : -31.5 mV

Electrophoretic Mobility Mean : -0.000244 cm²/Vs



Graph 8: Zeta potential of optimized formulation

Table 17: Particle size of optimized formulation.

Formulation	Particle size (Predicted value)	Particle size (Actual value)	Zeta potential (Predicted value)	Zeta potential (Actual value)
Ethosome	130.0 nm	139.0 nm	-34.46 mV	-31.5 mV

3.6.3 SEM analysis of Optimized formulation

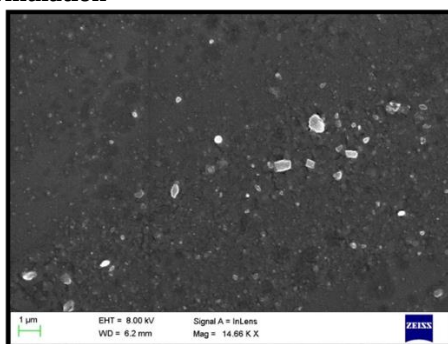


Figure 1: Scanning electron microscope (SEM).

3.7 Evaluation parameter of extract loaded Ethosome Cream formulation.

3.7.1 Physical appearance of ethosomal cream formulation.

Table 18: Physical appearance.

Parameter	Observation
Colour	White to off-white
Odour	Odourless
Appearance	Smooth and uniform Cream
Homogeneity	Homogeneous
Texture	Soft, non-gritty, and smooth
Phase Separation	Absent

3.7.2 Viscosity (cps), pH and Spreadability Cream formulation.

Table 19: Viscosity (cps), pH and Spreadability of extract loaded Ethosome Cream formulation.

Formulation	Viscosity (cps)	pH	Spreadability (g·cm/sec)	Extrudability
Ethosomal Cream formulation	4089 ± 0.47 cps	6.3	11.60 g·cm/sec	Good

3.8 Results of antimicrobial activity of Herbal cream formulation.

3.8.1 Antimicrobial activity of ethosomal cream formulation.

Table 20: Antimicrobial activity of cream formulation against *E.coli*.

Formulation	<i>E. coli</i> (Zone of Inhibition, mm)
Placebo cream (Control)	0
Plant Extracts (1 mg/mL)	12± 0.6
Ethosomal cream formulation (0.5 mg/mL)	14 ± 0.8
Ethosomal cream formulation (1.0 mg/mL)	18± 0.5

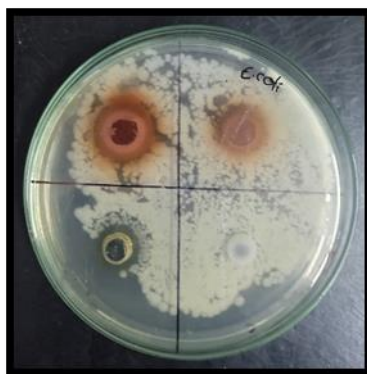
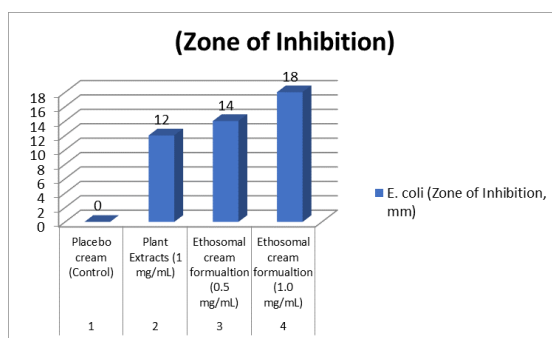


Figure 2: Photograph showing zone of inhibition of plants extract loaded formulation against *A: Escherichia coli*.



Graph 9: Graphical representation of Zone of inhibition of extract loaded Cream formulation.

DISCUSSION

The study demonstrated the successful development of *Clitoria ternatea* extract-loaded ethosomal cream using a QbD-based optimization approach. The optimized ethosomes showed a nanosized particle diameter of 139.0 nm and a zeta potential of -31.5 mV, indicating suitable vesicular characteristics and good physical stability. SEM analysis confirmed the formation of smooth, nearly spherical vesicles without significant aggregation.

The incorporation of optimized ethosomes into the cream base produced a homogeneous formulation with acceptable topical properties. The pH of 6.3 suggested good skin compatibility, while the viscosity and spreadability values indicated suitable consistency and ease of application. The ethosomal cream also showed greater antibacterial activity against *E. coli* than the crude extract, with the 1.0 mg/mL formulation producing the highest inhibition zone of 18 ± 0.5 mm. This enhanced activity may be associated with improved delivery of the plant bioactive constituents through the

ethosomal vesicular system. Overall, the results support the potential of *Clitoria ternatea* ethosomal cream as a promising herbal topical delivery system.

4. CONCLUSION

The present study successfully formulated and optimized a *Clitoria ternatea* loaded ethosomal cream using a systematic Design of Experiments approach based on the Box–Behnken design. The optimized ethosomal formulation exhibited desirable characteristics, including nanosized vesicles, suitable zeta potential, uniform morphology, and good physical stability. The successful incorporation of the optimized ethosomes into a cream base resulted in a formulation with acceptable organoleptic properties, suitable pH, optimum viscosity, good spreadability, and excellent patient applicability. Furthermore, the formulation showed enhanced antimicrobial activity against *Escherichia coli* when compared with the crude plant extract, highlighting the beneficial role of ethosomal vesicles in improving the delivery and biological efficacy of herbal constituents. Therefore, it can be concluded that the *Clitoria ternatea*

loaded ethosomal cream represents a promising novel topical drug delivery system with improved stability, sustained release characteristics, and significant antimicrobial activity. The findings of this research suggest that the developed formulation may have potential applications in the management of microbial skin infections and other dermatological disorders. However, further studies including long-term stability studies, skin permeation studies, toxicity evaluation, and clinical investigations are required to establish its safety, efficacy, and therapeutic potential for commercial pharmaceutical applications.

REFERENCES

1. Liu, S., Deng, T., Cheng, H., Lu, J., & Wu, J. (2025). Advances in transdermal drug delivery systems and clinical applications in inflammatory skin diseases. *Pharmaceutics*, 17(6): 746.
2. Xu, F., Qiu, Z., Zhang, M., Ren, Y., Kong, L., Liu, Y., & Wang, P. (2025). Transdermal drug delivery systems: a comprehensive review of mechanisms, technologies, and clinical applications. *Pharmaceutical Research*, 42(12): 2429-2442.
3. Gurba-Bryśkiewicz, L., Maruszak, W., Smuga, D. A., Dubiel, K., & Wieczorek, M. (2023). Quality by design (QbD) and design of experiments (DOE) as a strategy for tuning lipid nanoparticle formulations for RNA delivery. *Biomedicines*, 11(10): 2
4. Arora, D., & Nanda, S. (2019). Quality by design driven development of resveratrol loaded ethosomal hydrogel for improved dermatological benefits via enhanced skin permeation and retention. *International journal of pharmaceutics*, 567: 118448.
5. Chauhan, N., Vasava, P., Khan, S. L., Siddiqui, F. A., Islam, F., Chopra, H., & Emran, T. B. (2022). Ethosomes: A novel drug carrier. *Annals of Medicine and Surgery*, 82: 104595.
6. Huanbutta, K., Rattanachitthawat, N., Luangpraditkun, K., Sriamornsak, P., Puri, V., Singh, I., & Sangnim, T. (2022). Development and evaluation of ethosomes loaded with zingiber zerumbet linn rhizome extract for antifungal skin infection in deep layer skin. *Pharmaceutics*, 14(12): 2765.
7. Paiva-Santos, A. C., Silva, A. L., Guerra, C., Peixoto, D., Pereira-Silva, M., Zeinali, M., ... & Veiga, F. (2021). Ethosomes as Nanocarriers for the Development of Skin Delivery Formulations: Paiva-Santos et al. *Pharmaceutical research*, 38(6): 947-970.
8. Almuqbil, R. M., & Aldhubiab, B. (2025). Ethosome-based transdermal drug delivery: its structural components, preparation techniques, and therapeutic applications across metabolic, chronic, and oncological conditions. *Pharmaceutics*, 17(5): 583.
9. Oguis, G. K., Gilding, E. K., Jackson, M. A., & Craik, D. J. (2019). Butterfly pea (*Clitoria ternatea*), a cyclotide-bearing plant with applications in agriculture and medicine.
10. Vidana Gamage, G. C., Lim, Y. Y., & Choo, W. S. (2021). Anthocyanins from *Clitoria ternatea* flower: Biosynthesis, extraction, stability, antioxidant activity, and applications. *Frontiers in Plant Science*, 12: 792303.
11. Deorankar, P., Gangiwale, R., Chintamani, R., & Singh, R. P. (2020). Evaluation of ethanolic and aqueous extract of *Clitoria ternatea* for antimicrobial activity. *Indian Journal of Natural Products and Resources (IJNPR)[Formerly Natural Product Radiance (NPR)]*, 11(3): 194-198.
12. Morya, N., Hirave, K., Banerjee, D., Singh, A., & Saharan, V. A. (2025). Herbal Extracts and Phytoconstituents-Loaded Transfersomes, Ethosomes, and Transdermal Patches in Drug Delivery. *Formulating Pharma-, Nutra-, and Cosmeceutical Products from Herbal Substances: Dosage Forms and Delivery Systems*, 453-494.
13. Dadwal, N., Amisha, Singh, D., & Singh, A. (2023). Quality-by-design approach for investigating the efficacy of tacrolimus and hyaluronic acid-loaded ethosomal gel in dermal management of psoriasis: In Vitro, Ex Vivo, and In Vivo Evaluation. *AAPS PharmSciTech*, 24(8): 220.
14. Qureshi MI, Jamil QA, Usman F, Wani TA, Farooq M, Shah HS, Ahmad H, Khalil R, Sajjad M, Zargar S, Kausar S. Tioconazole-loaded transethosomal Cream using box-behnken design for topical applications: in vitro, in vivo, and molecular docking approaches. *Creams*, 2023 Sep 21; 9(9): 767.
15. Kharwade R, Hatwar N, Mahajan N. Herbal Nano Therapy: Mechanism of Action. In *Herbal Nanotherapy for Diabetes 2025* Oct 22 (pp. 112-143). Bentham Science Publishers.
16. Mohamed, J. M. M., Khan, B. A., Rajendran, V., El-Sherbiny, M., Othman, G., Hussamuldin, A. B. A., & Al-Serwi, R. H. (2022). Polymeric ethosomal gel loaded with nimodipine: Optimisation, pharmacokinetic and histopathological analysis. *Saudi pharmaceutical journal*, 30(11): 1603-1611.
17. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: An overview. *Journal of Advanced Pharmaceutical Technology & Research*, 2010; 1(3): 274-282.
18. Thomas AP, Nair AB, Shah J, Jacob S, Al-Dhubiab BE, Sreeharsha N. Formulation and evaluation of ethosomal Cream of tazarotene for topical delivery. *Asian Journal of Pharmaceutics*, 2019; 13(4): S1080-S1088.
19. Sharma, T., Thakur, S., Kaur, M., Singh, A., & Jain, S. K. (2023). Novel Hyaluronic Acid ethosomes based gel formulation for topical use with reduced toxicity, better skin permeation, deposition, and improved pharmacodynamics. *Journal of liposome research*, 33(2): 129-143.
20. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: An overview. *Journal of*

- Advanced Pharmaceutical Technology & Research, 2010; 1(3): 274–282.
21. Binder L, Mazál J, Petz R, Klang V, Valenta C. Topical dosage form development and evaluation. In: Dermatological and Transdermal Formulations. Bethesda (MD): National Center for Biotechnology Information (US), 2019.
 22. Al-Barghouthy EY, Abu-Diak OA, Al-Horani RA, Bustanji Y. Comparative evaluation of spreadability measurement methods for topical semisolid formulations. *Creams*, 2025; 11(12): 1006.
 23. Phad, A. R., Dilip, N. T., & Ganapathy, R. S. (2018). Emulgel: A comprehensive review for topical delivery of hydrophobic drugs. *Asian journal of pharmaceuticals*, 12(2): S382-S393.
 24. Seth, S., & Ramesh, R. (2026). Comparison of nanosurface evaluation and antimicrobial efficacy of calcium hydroxide-coated stainless steel and Zirconia crowns in pediatric dentistry: An atomic force microscopic study. *The Journal of Contemporary Dental Practice*, 27(1): 37-43.