ORIGINAL ARTICLE

Development, Optimization, and *In vitro* Evaluation of Ethosomal Gel of *Dalbergia sissoo* Leaves Extract

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Abstract

Aim: The present study aimed to develop an ethosomal gel loaded with *Dalbergia sissoo* leaves extract for transdermal application. **Materials and Methods:** *D. sissoo* extract was subjected to phytochemical analysis, flavonoid content, total phenolic content, and Fourier-Transform Infrared Spectroscopy analysis. The ethosomes containing *D. sissoo* extract were formulated and characterized based on morphology, particle size, surface charge, and encapsulation efficiency. Subsequently, the optimized ethosomes were incorporated into Carbopol gel and then evaluated for organoleptic properties, pH, viscosity, and skin permeation. **Results and Discussion:** *Dalbergia* extract incorporated in the ethosomes showed spherical shape, vesicle size (143 nm), −14.9 mV surface charge, and good entrapment efficiency (84.4%). The optimized formulation was further incorporated into Carbopol gel and showed optimal pH (6.2), good spreadability, high release percentage (85.92%), as well as stability over 60 days. **Conclusion:** The findings revealed that the ethosomal gel loaded with *D. sissoo* leaves extract was found to be a potential strategy for effective delivery.

Key words: *Dalbergia sissoo* L. extract, ethosomes, gel, herbal, release, transdermal delivery

INTRODUCTION

Over the past decade, there has been a significant global surge in the utilization of herbal products, including dietary ingredients, supplements, and herbal formulations. The main benefits of herbal preparations are that they have lesser adverse effects. Yet, the utilization of herbal preparations has challenges including low aqueous solubility and poor oral bioavailability. These challenges require concise refinement to enhance their effectiveness.[1]

Dalbergia sissoo Roxb. ex DC is a significant medicinal plant that is indigenous to tropical and subtropical regions belonging to the family of Fabaceae of genus *Dalbergia* often known to be Sheesham.[2] According to an extensive review of the literature, this plant's phytoconstituents include flavonoids, glycosides, polysaccharides, or gums. Pharmacological database studies have found significant effects against inflammation, diarrhea, antimicrobial resistance, anticancer, antioxidants, antidiabetic, gastroprotective, osteogenic, bacterial, anthelmintic, cardioprotective, neuroprotective, and reproductive.[3] An issue with herbal medications is their potential therapeutic effects. The size of the particles might be the cause of these. The larger size of bioactive substances makes them more difficult to pass across lipid membranes, and in certain circumstances, their solubility raises additional concerns.[4] These factors eventually impact the drug's bioavailability. Due to these factors, extracts are seldom used clinically to treat illnesses. The conventional formulations of the plant-based extract are transformed into nanoformulation with the aid of nanotechnology, providing a number of advantages including improved solubility, bioavailability, permeability, stability, and, eventually, therapeutic outcomes.[5]

Further, trans-or intradermal administration of drugs circumvents multiple constraints that might arise in systemic applications, such as drug metabolism first-pass occurring in the lungs, liver, or gastrointestinal tract before reaching

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the intended target.[6] Transdermal delivery offers various advantages as it delivers the drug through the skin at a specified and controlled rate by passive diffusion, which optimizes systemic effect. The skin is the biggest and most accessible organ; yet, it also acts as an extremely selective barrier that prevents large molecules and hydrophilic substances from passing through.[7] To address this challenge, drugs must be enclosed in nanovectors that are "skin-friendly," often utilizing lipid-based vesicular systems.[8] Ethosomes have been described as the most recent, innovative non-invasive, passive carriers for lipid-based systems, with enticing characteristics.[9] Fundamentally, they are soft, flexible, and phospholipid vesicles that have been altered for better drug delivery. It is mainly composed of water, a high concentration of ethanol, multiple, and concentric layer of phospholipid.[10] Consequently, in contrast to the liposomal drug delivery methods, it enhances the penetration through the stratum corneum barrier, directing the penetration in deeply embedded skin layers. In addition to the effect of ethanol on the stratum corneum, the permeability and deformability of vesicles also contribute. Both the fluidity of ethanol and the lipid molecules in the polar head region contribute to the enhanced permeability across the membrane. Thus, due to these distinctive features, ethosomes are regarded to be a superior carrier for the transdermal delivery of drugs.[11]

Quality by design is a robust and cost-effective method for optimizing the connection between independent and dependent variables in pharmaceutical nanoformulation development.[12] One-way assessment of variance (ANOVA) statistics is a useful tool for this purpose. Using this approach, designing the studies and interpreting the variable effects becomes easy.[13] This study aimed to enhance the release of drug by the incorporation of the extract into ethosomal gel. Comparing the study's outcome to the various experimental dose types revealed that it was superior. Ultimately, this composition has improved therapeutic outcomes.

The present study intended to develop and characterize *D. sissoo* leaves extract loaded ethosomal gel and to determine the best optimum formula for testing its anti-osteoporotic activity.

MATERIALS AND METHODS

The *D. sissoo* leaves extract was obtained from vital herbs, New Delhi. Chemicals such as ethanol, soya lecithin, iso-propylene alcohol, methanol, Carbopol 934 K, and propylene glycol were obtained from Sigma-Aldrich. Throughout the experiment, only analytical-grade chemicals and double-distilled water were utilized. The functional groups of *D. sissoo* extract-loaded with ethosomes were analyzed using Fourier-Transform Infrared Spectroscopy (FTIR). The size and surface charge of ethosome vesicles loaded with *D. sissoo* leaves extract was determined by DLS and transmission electron microscopy spectroscopy (TEM) and entrapment efficiency by ultracentrifugation.

Analysis of the extract

Activity of 2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging

The free radical scavenging activity of the extract was assessed through the use of DPPH.[14] The ascorbic acid was employed as the standard and the formula was used to calculate the percentage inhibition

$$\text{Inhibition } (\%) = \frac{\text{Absorbance of the test solution}}{\text{Absorbance of the control}} \times 100$$

where,

Absorbance of the test solution = activity in the presence of test compound

Absorbance of the control = total activity without inhibitor

Phytochemical screening

The phytochemicals (alkaloids, flavonoids, tannins, glycosides, saponins, carbohydrates, and proteins) included in the extract were identified by a phytochemical examination. Briefly, Mayer's reagent, Dragondroff's reagent, Wagner's reagent, and Hager's reagent were used to identify the alkaloids. Lead acetate test was used to identify flavonoids, ferric chloride test was used to identify tannins, Salkowski's test for diterpenes, froth test for saponins, and Libermann Burchard's test for photosterols.[15] Ferric chloride test for phenols, Molisch and Benedict's test for carbohydrates and reducing sugars, ninhydrin test for proteins and amino acids.[16]

Total phenolic and flavonoid content

The total phenolic contents were calculated using the Folin Ciocalteus technique, and the findings were reported as milligrams of GAE/g, or gallic acid equivalent per gram of extract. Likewise, the total flavonoid content (TFC) was determined by the aluminium chloride colorimetric technique. Quercetin in milligrams equivalent per gram of extract (mg QE/g extract) was used to characterize the TFC findings.[17]

FTIR analysis

The extract's active functional groups have been identified using FTIR.

Formulation of ethosomes incorporated with D. sissoo leaves extract

The ethosomes loaded with extract were formulated with the consideration of the approach mentioned by Touitou *et al*.[18] As listed in Table 1, all necessary ingredients were weighed in different concentrations to prepare the ethosomal formulation. First, the extract and soy lecithin were mixed

Table 1: Composition and responses in the CCD for ethosomal suspension when two independent variables are optimized using Design Expert software

Runs	Independent variables	The amount of ethanol (ml)	Responses	Entrapment efficiency
	The amount of soy lecithin (mg)		Particle size	
1	250	25	130.6	78.62
2	250	25	133.4	80.83
3	250	30	138.9	82.64
4	250	25	142.6	81.6
5	250	40 (High)	143.4	84.6
6	100 (Low)	40	113.2	72.45
7	250	25	151.5	81.24
8	462.13	25	180.3	75.46
9	400 (High)	46.21	175.7	76.2
10	250	25	155.3	79.45
11	100	10	117.8	67.76
12	200	25	106.9	78.24
13	400	10 (Low)	178.1	65.35

using ethanol and propylene glycol. The resultant mixture was then heated up to 30°C on a water bath. Furthermore, distilled water was added to the solution in a fine stream. In a sealed jar, the solution was continuously stirred at a speed of 700 revolutions per minute using a magnetic stirrer (Remi Equipment, located in Mumbai). Following that, the resultant solution was then maintained at 4°C. Then, sonication was performed 3 times using a probe sonicator, for a total of around 5 min each, with a 5-min interval between each.[19] Each formulation was examined visually for homogeneity with no precipitation or phase separation. Then, the most appropriate formulation was, further, subjected to detailed analysis.[20] Thirteen ethosomal formulations with varying concentrations have been designed using the aforementioned techniques, and they were then utilized for a number of evaluation criteria.

Experimental design

The experimental design was statistically evaluated using Design Expert software (Demo version). The central composite design (CCD) was the most widely used response surface design. The amount of soy lecithin and ethanol have been selected in the form of independent variables having two levels as indicated in Table 1. The development process and composition of ethosome were significantly impacted by formulation optimization. The impacts of independent factors, that is, ethanol (X2) and lecithin (X1), on the dependent variables, that is, entrapment efficiency (%) and particle size (nm), were investigated using the 2^2 designs for ethosomal optimization. The independent variables were optimized to maximize entrapment efficiency. Table 1 provides the ethosomal formulation composition and responses in CCD. ANOVA was used to assess the significant model.

Characterization of ethosomes

Vesicle size

The size of the vesicle is measured using a dynamic light scattering (DLS) approach employing Malvern Zetasizer (Nano ZS, Malvern, UK). All formulations were diluted with distilled water and shaken together before measurements to eradicate multiple scattering phenomena and increase the intensity of the scattering. Following the placement of the samples in glass cuvettes, the particle size was determined. For each formulation, three replicates were conducted and the results are shown as mean ± SD.[20]

Zeta potential analysis

Based on electrophoretic mobility, we calculated the ZP using computerized Malvern Zetasizer (instrument at GLA University, Mathura, India). To ensure the stability of the ethosomal suspension, the particle charge is an important component.[21]

FTIR analysis

The ethosomes were analyzed using FTIR analysis as mentioned above.

Morphology of ethosomes loaded extract using TEM

The formulation's morphology was assessed using spectroscopyTEM. TEM (Jamia Hamdard) was employed for the assessment of internal composition, morphology, and crystallization of the sample. To carry out the experiment, a 9000× and 200 kV magnifier was used. With the help

of deionized water, 20 µL of the formulation has been diluted 10 times in a nutshell. Staining took place with the 2% w/v phosphotungstic acid for at least 30 s. In the end, the specimens were put on a copper-laminated grid and then allowed to dry. Two grids were set up and regularly monitored for every specimen.[22]

Determination of entrapment efficiency

EE refers to the total content of the drug encapsulated in vesicles within formulations. The unentrapped drug was, then, separated using a cooling centrifuge operating at 16,000 rpm and 4°C (Remi Laboratories, Mumbai, India).[23] Distilled water was used to dilute the supernatants (10 mL, 3 min).

An Eppendorf tube containing a small amount of the formulation was first filled, and it was centrifuged for 15 min at a temperature of 4°C and a speed of 14,000 rpm. The procedure was repeated until a cloudless sort of supernatant was produced using the Remi ultracentrifuge, which has a TLA-45 rotor. For the final result, a cloudless supernatant was obtained, and a UV/visible spectrophotometer was, then, used to detect drug content at a wavelength of 280 nm. Further, the following formula was employed to calculate the quantity of medication entangled in the colloidal system. Three approximations were made for every sample.[24]

$$EE\% = \frac{\left(\text{Amount of the incorporated drug} - \text{Amount of free drug present in the supernatant}\right)}{\text{Amount of drug incorporated}} \times 100$$

Statistical analysis

Polynomial models are generated through multiple linear regression analysis for all the response components, including quadratics and interactions. Equations have been developed for analysis of the influence of each variable on the formulation properties, and the interaction coefficient has been calculated. To validate the model, an analysis of variance was performed. Three-dimensional response graphs were created using Design Expert software.

Characterization of gel

Evaluation of the pH, viscosity, spreadability, and extrudability

Utilizing a digital pH meter the composition was ascertained. After completely submerging the glass electrode in ethosomal gel, the results were presented and recorded. The viscosity of the formulation was estimated by immersing a Brookfield viscometer (model LVDV-II Pro) spindle of S 96 into a beaker which contains ethosomal gel at 10, 15, and 20 rpm for different intervals at upper, middle, and bottom case.[25]

To determine spreadability, 1 g of the ethosomal gel was precisely weighed and then placed between two 8-cm long glass slides. The weight at which the glass slide moved out of place was measured using different weights fastened to the pulley, and the duration required to move the top slide and for the gel that extend farther to the bottom slide was noted. After taking the measurement 3 times, the spreadability readings were calculated using the following formula:

S = M * L/T, where S stands for spreadability of the ethosomal gel, M is the weight tide to the upper slide (g), L is the distance moved by this slide (cm), and T is the time taken by the upper slide to further move downward.

Extrudability ensued after placing 20 g of the formulated ethosomal gel into a collapsible tube, with subsequent application of pressure. A clamp had been fastened to the tube to prevent content from flowing backward. When the tube has been finally opened, the quantity of the gel that continued to extrude until the pressure persisted was measured and noted.[26]

In vitro drug release

A modified Franz diffusion cell with an egg membrane was utilized to conduct the *in vitro* permeation investigation study. A pH 7.4 phosphate buffer saline was used for this study. On the top side of the skin in the donor compartment, formulation – which is equivalent to 2.5 mg of drug – was placed. The assembly's temperature was kept constant at 37 ± 2°. Every hour, samples were taken out of the receptor media using the sampling tube, and to create sink conditions, the same volume of new receptor media was added at the same time. The solutions were measured at 280 nm using a UV/visible spectrophotometer (Shimadzu, 1700, Japan) against a suitable blank. All formulations underwent a similar process, and at the end, a graph was plotted between the percentage of cumulative drug release and the passage of time. The experiment was run 3 times for each ethosomal formulation, and the results were presented as mean ± SD.

RESULTS

Free radical scavenging activity by DPPH

In this study, *D. sissoo* leaves extract's capacity to scavenge free radicals was determined using the DPPH technique.

It was measured using the DPPH method. The plant demonstrated 78.84% antioxidant activity in comparison to ascorbic acid, which is used as standard.

Analysis of the phytochemical activity

The leaves extract of *D. sissoo* was put through a variety of phytochemical assays to determine whether

secondary metabolites were present, as indicated in Table 2. The results demonstrated presence of the various phytochemicals including tannins, flavonoids, saponins, terpenoids, glycosides, reducing sugars, proteins, and amino acids.

Total phenolic and flavonoid content

The total phenolic content value of *D. sissoo* leaves extract was found to be 40.39 mg QE/g and total phenolic content was 20.85 ± 1.34 mg GAE/g.

FTIR analysis

FTIR spectrophotometer was used for the identification of the functional groups found in the plant extract and herbal ethosomal system. Table 3 provides the spectrum interpretation for each sample. The main peaks in the ethosomal system were nearly identical to those observed in pure ethanolic extracts, suggesting that the herbal extract and the other excipients in the distributed system did not significantly interact. Figure 1 displays the FTIR spectra of the extracting antenna sections in *D. sissoo* leaves extract. The peak which emerged at 3338 cm^{-1} shows the existence of phenols and alcohols in the extract. The peak at 1647 cm^{-1} demonstrates the existence of an alkene group, the peak at 2920 cm^{-1} shows C-H (stretching), and at 1347 cm^{-1} shows C-H (bending) in the sample.

Table 2: Phytochemical screening of *Dalbergia sissoo* leaves extract

S. No.	Secondary metabolites	Results
1.	Tannins	+
2.	Flavonoids	+
3.	Saponins	+
4.	Terpenoids	+
5.	Reducing sugars	+
6.	Carbohydrates	+
7.	Glycosides	+
8.	Anthraquinones	-
9.	Proteins and amino acids	+

+ denotes the existence of secondary metabolites in the extract and – denotes the lack of secondary metabolite in the extract

Visualization of ethosomal vesicles

Determination of particle size, zeta potential, and polydispersity index

Based on DLS, the particle size has been estimated, and Table 2 presents the results for both particle size and PDI. The particle size of the prepared ethosomal formulation was found to be 113.2–180.3 nm and PDI was found to be in the range of 0.324–0.613. With an optimal particle size and PDI of 143.4 nm and 0.447, respectively, formulation F5 exhibits the highest entrapment efficiency when compared to the remaining formulations as represented in Figure 3. On the other hand, formulation F12 shows the highest values for PDI and particle size, which are 180.8 and 0.613, respectively. The vesicle's size is particularly important when it comes to topical delivery of drug. The study revealed that there is an abrupt increase in the formulation's particle size when the concentrations of soy lecithin are raised over 300 mg. Thus, it can be inferred from the previous statement that changing the ratio of lipid to ethanol affects the formulation's physical characteristics in one way or another. Furthermore, the rate at which the formulation penetrates the skin can be influenced by the size of the particles and the quantity of ethanol. The smaller the size of the vesicle, the more effectively it will penetrate into underlying layers of skin. Based on the aforementioned results, formulation F5 demonstrated an optimal particle size of about 143.4 nm, indicating great suitability for topical administration.

The stability of the ethosomal formulation is assessed using the zeta potential.

Table 4 lists the zeta potential values, which ranged from –11.6 to –35.6 mV. Zeta potential has a maximum value represented by F6, which is –35.6 mV, and a minimum value represented by F8, which is about –24.4 mV. Zeta potential values did not significantly alter for the formulations F1, F2, and F3 that are –18.6, −17.9, and –18.5. Figure 4 represents the zeta potential of optimized formulation.

Experimental design and optimization of ethosomal formulation

Phospholipids and ethanol have been selected as independent variables. The particle size and entrapment efficiency were examined as a function of the dependent variables. The CCD used such factors as two levels. The table presents that these formulations were prepared in 13 batches with their summaries of the response.

Table 3: Plant extract spectral interpretation using FTIR spectra

S. No	Plant-based extract	Peak values	Functional groups	Potential metabolites
	Dalbergia sissoo	3338	O-H (alcohol)	Flavonoids, tannins, saponins, terpenoids, glycosides, reducing sugars, proteins, and amino acids.
		2920	C-H (Stretching)	
		1347	C-H (Bending)	
		1647	C=C	
		1039	C-C (stretching)	

Effect of variables on particle size

Data have been processed to fit full second-order cubic as well as quadratic polynomial equations with additional interaction factors to establish a correlation between different studied responses and the examined variables. The plots in Figure 2a depicted that an optimum level of lecithin and an increased level of ethanol demonstrated a positive influence on the particle size. As the amount of soy lecithin increases, at a constant concentration of ethanol, an increase in the particle size was observed.

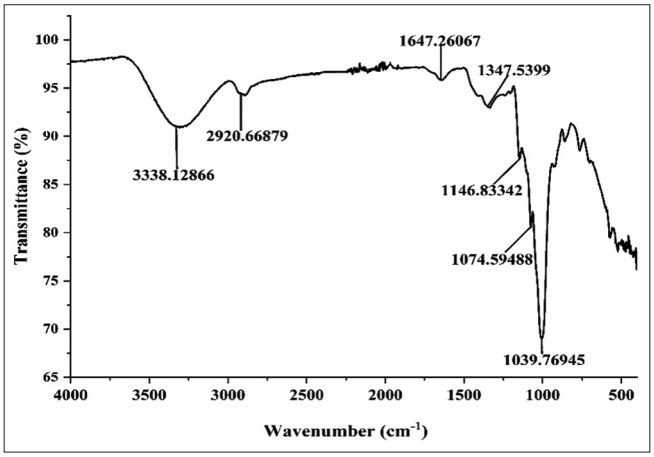

Figure 1: FTIR spectrum of *D. sissoo* leaves extract

The quadratic equation generated by the software is:

Particle size=+143.67+28.32×A+0.13×B

The ANOVA results demonstrated p <0.0001, which indicates that model terms are significant. The model F-value of 55.25 implies that the model is significant. The value of the predicted determination coefficient (R2) was 0.9170 and adjusted R2 was 0.9004. The signal-to-noise ratio of 21.877 indicates an adequate signal.

Effect of the variables on entrapment efficiency

The drug's entrapment efficiency was directly correlated with the concentration of phospholipid and ethanol which has been shown in 3-D plot [Figure 2b]. Equation (2) shows the final mathematical model for entrapment efficiency, based on coded parameters calculated by Design Expert software.

Entrapment efficiency =+80.45+1.35×A+5.24×B+1.85×A×B−4.04×A^2−4.93×B^2

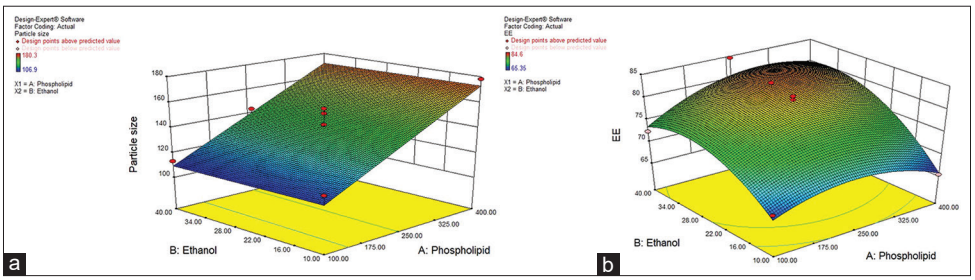

Figure 2: Three-dimensional response surface graph indicating the impact of independent variables (phospholipid and ethanol) on these (a) particle size (nm) and (b) entrapment efficiency (%w/w)

Table 4: Entrapment efficiency, particle size, zeta potential, and PDI of prepared ethosomal formulation

Formulation code	Entrapment efficiency	Particle size	Polydispersity index	Zeta potential
F1	78.62	130.6	0.482	-18.6
F2	80.83	133.4	0.324	-17.9
F3	82.64	138.9	0.525	-18.5
F4	81.6	142.6	0.542	-13.6
F5	84.6	143.4	0.447	-13.9
F6	72.45	113.2	0.336	-35.6
F7	81.24	151.5	0.403	-14.9
F8	75.46	180.3	0.511	-24.4
F9	76.2	175.7	0.459	-18.9
F10	79.45	155.3	0.342	-13.7
F11	67.76	117.8	0.497	-15.4
F12	78.24	106.9	0.613	-14.6
F13	65.35	178.1	0.432	-15.8

Table 5 shows that both linear as well as quadratic models have significant p-values for entrapment efficiency. The ANOVA results demonstrated *P* value having <0.05, indicating that model terms are significant. The F-value of 15.75 implies the model is significant. The signal-to-noise ratio of 11.048 indicates an adequate signal.

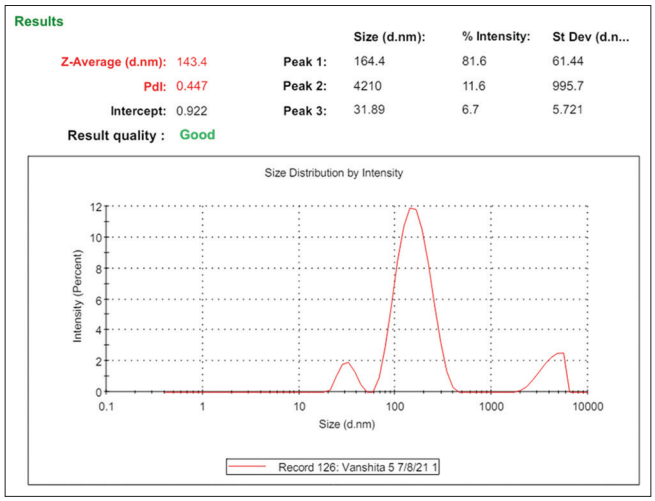

Figure 3: Size of optimized formulation

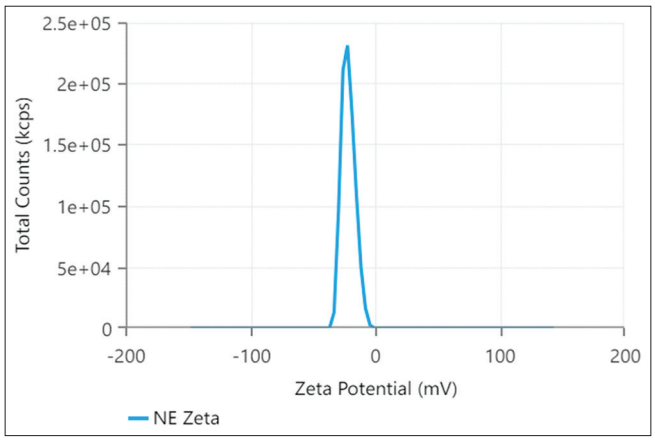

Figure 4: Zeta potential of optimized formulation

Desirability function

The present study demonstrated particle size in the range of 106.9–180.3 nm. The ethosomal formulation with a desirability of 1.00 was selected as the optimized formulation. The optimized formulation had phospholipid (250 mg) and ethanol (40 mL). Table 6 compares the experimental values of particle size and entrapment efficiency of optimized ethosomal formulation with predicted values, and calculating the percentage bias.

Entrapment efficiency

The ethosomal dispersion's entrapment effectiveness directly affects the delivery potential. Table 4 displays the results of the determination of each formulation's entrapment efficiency. The formulation developed with 300 mg soy lecithin and 30 mL ethanol demonstrated maximum entrapment efficiency, that is, 84.6%. This could be due to the increased ethanol content, which makes the extract more soluble in ethosomes.

Characterization of optimized ethosomal formulation

Morphology through transmission electron microscopy

TEM was utilized to represent the surface morphology of the optimized and prepared F5 accumulation in Figure 5. TEM images reveal information on the spherical shape of the particles in the formulation in addition to surface morphology. Images acquired using TEM demonstrated that the ethosomes had a spherical form, were smooth, free from crystallinity, and were within the nanometre range. The average particle size was 59.91 ± 43.75, which was calculated using Image J software. While, the mean particle size measured by DLS (143.4 nm) was substantially greater than TEM, which may be due to the fact that DLS assessed the hydrodynamic diameter of the particle's core as well

Table 5: Model fit summary for responses

S. No	Source	Particle size		Entrapment efficiency	
		F-value	*P*-value	F-value	*P*-value
1	Linear versus Mean	55.25	<0.0001	1.83	0.2105
2	2FI versus Linear	0.042	0.8424	0.028	0.8748
3	Quadratic versus 2F1	0.091	0.09143	27.81	0.0005
4	Cubic versus Quadratic	0.27	0.8457	5.63	0.0642

Table 6: Comparison of experimental and predicted responses of optimized formulation

Responses	Predicted values	Experimental values	Bias (%)[a]
Particle size (nm)	140.505	143.4	–2.06
Entrapment efficiency (%)	80.76	84.6	–4.53

[a]Percent bias= (Predicted value- Experimental value)/Predicted value×100

as the solvent layer attached to the particles. TEM sample preparation comprises drying, dehydration, staining, and electron irradiation of ethosomes, resulting in shrinkage of the particles, as described in prior publications.[27] Previous investigations reported similar variances in particle size.[28]

Ethosomal gel characterization

The optimized ethosomal gel loaded with ethosomes containing *D. sissoo*leaves extract was examined for organoleptic properties (phase separation, odor, and color,), pH, and viscosity.

Organoleptic evaluation

Homogeneity and organoleptic properties did not change at various temperatures over the stability investigations' allotted duration. At a higher temperature, the light brown color of all the formulations changed to a dark brown color.

FTIR spectroscopy

The FTIR spectra of extract, soy lecithin, ethanol, physical mixture, cholesterol, ethosomes, and gel are shown in Figure 6. The FTIR spectra of the extract [Figure 1] revealed absorption bands of O-H (alcohol) stretching vibrations at 3338 cm^{-1}, C-H stretching vibrations at 2920 cm^{-1}, C-H bending vibrations at 1347 cm^{-1}, and C=C stretching vibrations at 1647 cm^{-1} which were observed in relation with the previously reported values. The soy lecithin spectrum showed peaks at 3409 cm^{-1} (OH stretching), C=O stretching at 1698 cm^{-1}, and C-O stretching at 1062 cm^{-1}. Ethanol showed peaks at OH stretching at 3391 cm^{-1}, CH stretching at 2961 cm^{-1}, and CO stretching at 1055 cm^{-1}. Carbopol showed peaks at 3422 cm^{-1}, CH stretching at 2932.8 cm^{-1}, and C=O stretching at 1724.2 cm^{-1}. Cholesterol showed C-H stretching at 2936 cm^{-1}, C-H bending at 1464 cm^{-1}, and CO stretching at 1056 cm^{-1}. The physical mixture's spectrum revealed all the distinct peaks of extract, lipid, ethanol, and Carbopol and hence indicating no interaction. The gel containing optimized ethosomal formulation confirmed good solubilization of extract in both lipid and ethanol as indicated by the reduced intensity and the broadening of the peaks, thus demonstrating entrapment of the extract in the formulation.

Viscosity, pH, spreadability, and extrudability

The viscosity and the pH of all ethosomal formulations ranging from 5435–8954 cps to 6.5–5.7, Table 7 shows the results for all formulations including excellent values for spreadability and extrudability. The results indicate that the formulation is appropriate for topical use. The formulation EG3 had optimal pH, viscosity, and spreadability, indicating superior gelling properties for topical application.

In vitro release studies

The *in vitro* release profiles of ethosomal gel with different concentrations of Carbopol are shown in Figure 7. The ethosomal gel with a concentration of 1% Carbopol showed a maximum release of 85.19% after 24 h.

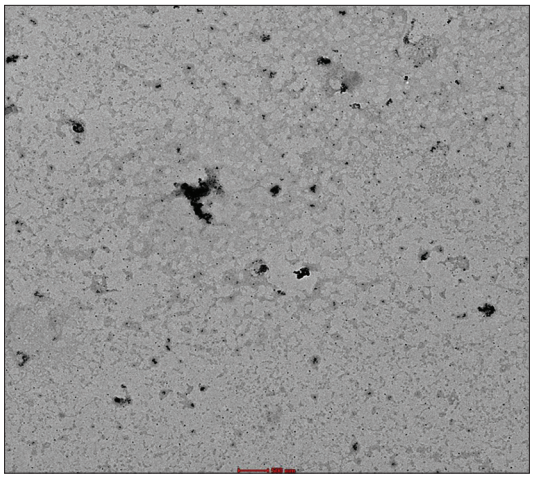

Figure 5: Transmission electron microscopy image of optimized ethosomal formulation

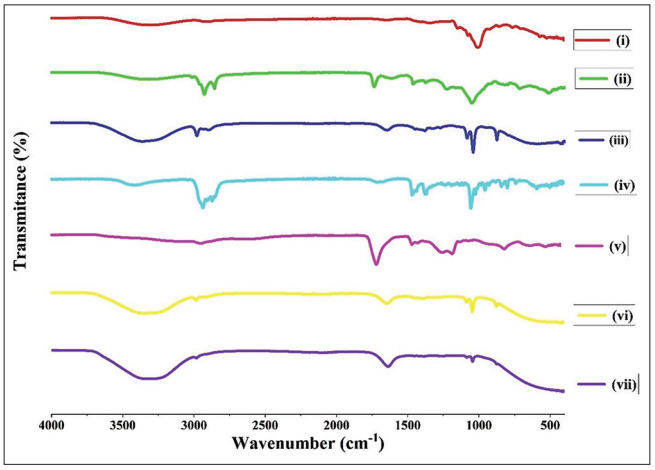

Figure 6: FTIR spectra of (i) extract, (ii) soy lecithin, (iii) ethanol, (iv) cholesterol, (v) Carbopol, (vi) physical mixture, and (vii) ethosomal gel

Stability studies

Stability tests were used to assess the ethosomal system's ability to adhere to the drug. Table 8 shows the stability of selected ethosomal gel using different parameters including color, odor, and phase separation. Figure 8 depicts pH of ethosomal gel at different temperatures. As temperature rises, molecular vibrations and ionization accelerate, leading to a decrease in pH. It is possible that at higher temperature, there is less polymer entanglement. However, the shift has not been drastic. Figure 9 depicted viscosity of ethosomal gel at different temperatures. Stability studies show that viscosity reduces as temperature increases. The study concludes that Carbopol-based ethosomal gel is thermally stable.

Table 7: pH, viscosity, spreadability, and extrudability of prepared ethosomal gel containing different concentrations of Carbopol

Formulation	pH	Viscosity	Spreadability	Extrudability
EG1	6.4	5435	7.12 ± 0.3	+++
EG2	5.7	5867	6.84 ± 0.2	++
EG3	6.2	7128	6.83 ± 0.4	+++
EG4	6.5	8120	5.56 ± 0.6	++
EG5	5.8	8954	4.33 ± 0.8	+

Table 8: Evaluation of organoleptic stability of ethosomal gel at different temperatures

Parameter	Temp	Initial	7 days	15 days	30 days	60 days	90 days
Color	4	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown
	25	Light brown	Light brown	Light brown	Light brown	Light brown	Brown
	40	Light brown	Light brown	Brown	Brown	Brown	Dark brown
Odor	4	-	-	-	-	-	-
	25	-	-	-	-	-	-
	40	-	-	-	-	-	-
Phase separation	4	-	-	-	-	-	-
-	25	-	-	-	-	-	-
	40	-	-	-	-	-	-

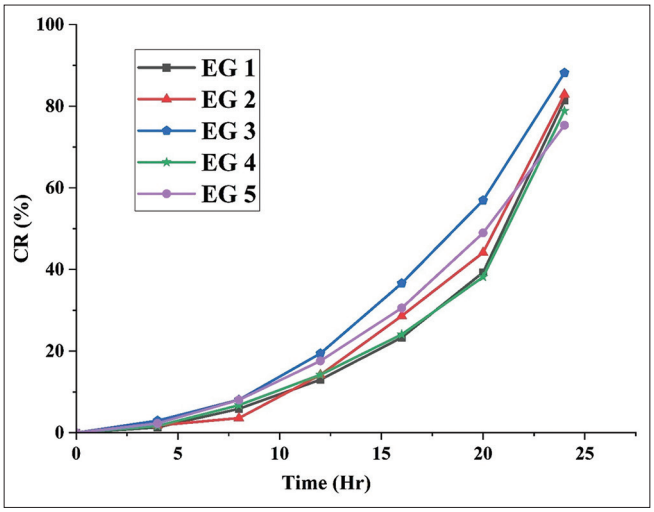

Figure 7: *In vitro* release profile of ethosomal gel with different concentrations of Carbopol

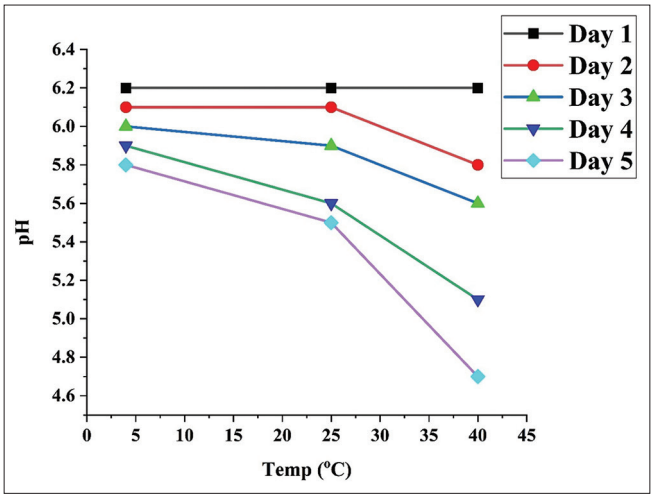

Figure 8: Effect of pH of ethosomal gel at different temperatures

DISCUSSION

Herbal ethosomal gel was formulated by incorporating *D. sissoo* leaves extract. To demonstrate the formulation's efficacy and suitability, it underwent a series of characterizations. The FTIR studies of the extract revealed the presence of important groups including flavonoids, phenols, tannins, and saponins.

Vesicles smaller than 300 nm can penetrate deeper layers of skin. In the analysis of the particle size and polydispersity index, it has been observed that the amount of soy lecithin is directly correlated with the vesicle size. An increase in soy lecithin concentration beyond 300 mg is associated with an abrupt increment in the particle size and narrow distribution of PDI. Propylene may allow penetration into deeper layers of the skin. Therefore, it can be inferred that alterations in the ratio of lipid to ethanol, either increased or decreased, significantly affect the physical characteristics of ethosomes. Particle size and the concentration of ethanol have an impact on the skin's permeability since only smaller particles can pass through it, whereas ethanol concentration typically has an impact on a drug's permeability. With the foregoing considerations, we may conclude that F5 is entirely

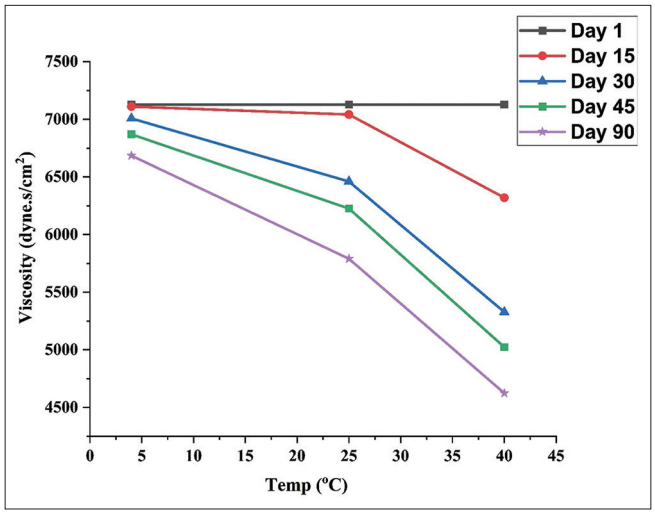

Figure 9: Effect of viscosity of ethosomal gel at different temperatures

appropriate for skin administration. Further, in the case of zeta potential, research revealed that particles will encounter repulsion among themselves and resist agglomeration if the suspension contains a high amount of cationic and anionic charge in the system (i.e., a high value of both positive and negative zeta potential). Consequently, increased surface charge improves the stability of ethosomes. The aforementioned statement indicates that F5, with its greatest zeta potential value, has the best level of stability compared to the remaining formulations. PDI values above 0.7 suggest the existence of bigger particles in ethosomal dispersions and a heterogeneous system. The present system has a homogeneous and narrow particle size distribution, as shown by the majority of the values. The stability studies confirmed that the prepared ethosomal gel was thermally stable.

CONCLUSION

The development of an appropriate carrier system for dermal delivery of the plant extract was highly difficult due to the hydrophilic nature, leading to limited skin liberation. Due to the substantial amount of ethanol in their composition, ethosomes have been found to provide both continuous skin permeability and enhanced medication permeability through lipid vesicles. A stable gel containing ethosomes of nanometre range loaded with *D. sissoo* leaves extract increased the penetration to the skin and could be a potential product for researchers.

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CONSENT FOR PUBLICATION

None.

AUTHORS' CONTRIBUTIONS

All authors have an equal contribution.

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