RESEARCH PAPER

Design, Fabrication and Optimization of Herbal Ethosomal Gel Containing *Carica papaya* and *Bryophyllum pinnatum* Leaf Extracts

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ABSTRACT

Wound healing is a coordinated biological process involving inflammation, cellular proliferation, angiogenesis, extracellular matrix deposition, and tissue remodeling. Delayed wound repair is associated with persistent inflammation, oxidative stress, microbial contamination, and impaired tissue regeneration. The present study describes the development of a combined herbal ethosomal gel containing ethanolic leaf extracts of *Carica papaya* and *Bryophyllum pinnatum*.

The investigation integrates phytochemical screening, spectrophotometric estimation, thin-layer chromatography (TLC), antioxidant evaluation, formulation compatibility testing, factorial optimization, vesicle characterization, gel development, permeation assessment, antimicrobial screening, irritation evaluation, and stability testing. The extracts were prepared by maceration using ethanol, followed by filtration and solvent evaporation. A 3^2 full factorial design comprising nine formulations was used to investigate the effects of lecithin concentration (1.0%, 1.5%, and 2.0%) and ethanol volume (10, 15, and 20 mL) on entrapment efficiency, vesicle size, and zeta potential. Both extracts were incorporated at 1.5 g per batch, while cholesterol was maintained at 0.2%. The selected ethosomal dispersion was subsequently incorporated into a Carbopol 980NF gel base at 0.75% w/w, with meglumine solution used for pH adjustment to 6.0–6.5.

The planned characterization included particle size, polydispersity index, zeta potential, vesicle morphology, extract content, viscosity, spreadability, in vitro release, ex vivo permeation through porcine ear skin, and antimicrobial activity. This formulation-development strategy is intended to investigate whether a combined herbal ethosomal system can provide reproducible phytochemical delivery and suitable topical application characteristics. Numerical experimental outcomes were not included in the available study record; therefore, the optimized formulation's superiority, wound-healing efficacy, and safety cannot yet be established. The work provides a structured basis for the pharmaceutical development and subsequent biological evaluation of a standardized herbal topical preparation.

Keywords: *Carica papaya*; *Bryophyllum pinnatum*; ethosomes; herbal gel; wound healing; phytoconstituents; factorial design; entrapment efficiency; skin permeation; topical drug delivery

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Introduction

Disruption of skin integrity initiates a complex sequence of biological events intended to restore the damaged tissue and re-establish its barrier function. Wound healing involves four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Disturbances in these processes may result in delayed wound closure, chronic ulceration, excessive scar formation, or recurrent tissue breakdown [1]. Persistent oxidative stress, dysregulated inflammatory signaling, impaired circulation, and microbial contamination can further compromise tissue repair. These challenges have stimulated interest in topical preparations capable of maintaining a favorable wound environment and delivering biologically active compounds to the affected site [2].

Although conventional wound-care interventions remain essential, their selection must reflect the wound's cause, severity, infection status, and clinical characteristics. Herbal formulations may be investigated as potential complementary products, but their efficacy and safety must be established experimentally before clinical recommendations can be made [3].

Phenolic compounds and flavonoids have received particular attention because of their ability to interact with reactive chemical species in laboratory assays. Certain plant-derived compounds have also been investigated for their effects on inflammatory mediators, fibroblast activity, collagen synthesis, and epithelial repair. Tannins and other constituents may influence protein interactions or microbial growth under selected experimental conditions. *Carica papaya* is a medicinally investigated plant belonging to the family Caricaceae. Its leaves contain a variety of phytochemicals, and leaf preparations have been examined in different experimental contexts for their potential biological activities. The incorporation of *Bryophyllum pinnatum* leaf extract alongside *C. papaya* extract provides an opportunity to examine a combined botanical formulation [4-6].

A gel provides a semisolid vehicle that can facilitate application, distribution, and retention of an active preparation at the intended site. Carbopol 980NF was selected as the gelling agent in the present work. Its concentration was specified as 0.75% w/w for the final ethosomal gel. The formulation must therefore be evaluated as a complete system rather than assuming that acceptable properties of the initial ethosomal dispersion will automatically be retained after gel incorporation [7-8].

The present work addresses this formulation-development challenge by combining ethanolic extracts of *C. papaya* and *B. pinnatum* in an ethosomal delivery system. The investigation integrates preliminary phytochemical screening, TLC profiling, spectrophotometric measurements, a factorial optimization strategy, and characterization of the resulting gel. Further assessment of release, skin permeation, antimicrobial activity, irritation potential, and stability is intended to provide a broader evaluation of pharmaceutical suitability.

Materials and Methods

Materials

The lecithin and cholesterol was procured from SD Fine-Chem Ltd, India. Polyethylene glycol, Carbopol 980NF, and ethanol were purchased from Merck, India. Ether, potassium dihydrogen phosphate, and sodium hydroxide were procured from CDH Fine Chemical, India. All the other chemicals were of analytical grade. Leaves of *C. papaya* and *B. pinnatum* were collected from Sagar and nearby areas in Madhya Pradesh, India. The plant materials were authenticated by comparison with reference specimens housed in the Department of Botany, Dr. Harisingh Gour University, Sagar.

Preparation of *Carica papaya* leaf extract

Fresh *C. papaya* leaves were washed with distilled water to remove adhering dust and impurities. The leaves were shade-dried at room temperature for 7–10 days until a constant weight was obtained. The dried material was coarsely powdered and stored in an airtight container. A quantity of 500 g of powdered leaves was transferred to a clean conical flask, and 1,000 mL of ethanol was added to immerse the plant material. The mixture was sealed and maintained at room temperature for 48 hours, with occasional shaking or stirring to facilitate extraction. The mixture was filtered through muslin cloth followed by Whatman filter paper. The filtrate was concentrated using a rotary evaporator until a dry extract was obtained. The extract was weighed and stored in a desiccator until further analysis. The extraction yield was calculated from the actual mass of dried extract recovered relative to the initial dry plant mass [9-11].

Preparation of *Bryophyllum pinnatum* leaf extract

Fresh *B. pinnatum* leaves were washed with distilled water and shade-dried at room temperature for 7–10 days. The dried leaves were coarsely powdered and stored in an airtight container. A quantity of 100 g of powdered plant material was transferred to a suitable vessel, and 500 mL of ethanol was added. Following extraction, the mixture was filtered through muslin cloth and Whatman filter paper. The filtrate was concentrated using a rotary evaporator until a dry extract was obtained. The dried extract was weighed to determine extraction yield and stored in a desiccator until further experimental use. The two extraction procedures used different plant-material-to-solvent ratios. Consequently, extraction yields and

phytochemical concentrations were compared using a consistent dry-extract basis rather than the mass of starting plant material alone [12-13].

Preliminary phytochemical screening

The dried extracts were dissolved in ethanol to prepare test solutions for qualitative phytochemical screening. Both extracts were analyzed for alkaloids, flavonoids, tannins saponins, glycosides, steroids and terpenoids. The results were tabulated separately for the two extracts [14-16].

Thin-layer chromatographic characterization

TLC was used for preliminary separation and characterization of extract constituents. Pre-coated silica gel 60 F_{254} aluminum plates were activated at 105°C for 30 minutes. Ethanolic solutions of the extracts were applied to the plates approximately 1–2 cm above the lower edge. For *C. papaya*, chloroform (9:1) was used as the mobile phase. For *B. pinnatum*, the reported mobile phase was ethyl acetate acid:methanol in a ratio of 10:0.5:0.6:0.2 by volume. The plates were developed in a saturated chamber until the solvent front reached approximately 8–10 cm from the origin. After drying, the plates were examined under ultraviolet light at 254 and 365 nm. Anisaldehyde–sulfuric acid was used as a visualization reagent for the *C. papaya* extract, while diphenylboric acid β-aminoethyl ester was specified for the *B. pinnatum* extract [17-20].

Preparation of extract calibration curves

The study record describes preparation of stock solutions by dissolving 100 mg of each dried extract in an ethanol–water mixture (1:1) to obtain a nominal concentration of 1,000 µg/mL. Dilutions were prepared at concentrations of 10, 20, 40, 60, 80, and 100 µg/mL. Absorbance was measured at 415 nm for *C. papaya* and 348 nm for *B. pinnatum*, and calibration plots were constructed by plotting absorbance against nominal extract concentration [21-22].

DPPH radical-scavenging assay

Antioxidant activity was investigated using the DPPH radical-scavenging method. A fresh 0.1 mM DPPH solution was prepared in methanol and protected from light. Different concentrations of each extract were prepared, with the reported range extending from 10 to 100 µg/mL. One milliliter of DPPH solution was mixed with 1 mL of each extract dilution. Ascorbic acid was used as the reference antioxidant. The mixtures were shaken and incubated in darkness at room temperature for 30 minutes. Absorbance was measured at 517 nm against the appropriate blank [23-25].

Preparation of ethosomes

The ethosomes were prepared using a thin-film hydration approach. Lecithin was dissolved in a chloroform mixture in a clean, dry round-bottom flask. The organic solvents were removed under reduced pressure using a rotary evaporator to produce a thin lipid film. The film was hydrated with the herbal extract preparation under continuous rotation at 900 rpm for 10 minutes at 30°C. The resulting dispersion was subjected to sonication to obtain a more uniform vesicular preparation. The study investigated lecithin concentrations of 1.0%, 1.5%, and 2.0%, and ethanol volumes of 10, 15, and 20 mL. Cholesterol was maintained at 0.2%, and the quantity of each extract was specified as 1.5 g per formulation. The final volume was intended to be adjusted to 100 mL [26-27].

Factorial design and optimization

The formulation was optimized using Design-Expert software, version 13.0, according to a (3^2) full factorial design. The independent variables were lecithin concentration (X1) and ethanol volume (X2) (Table 1). Each variable was examined at three levels, resulting in nine experimental runs (Table 2).

Table 1. Independent variables and their experimental levels

Coded level	Lecithin concentration (% w/v)	Ethanol volume (mL)
−1	1.0	10
0	1.5	15
+1	2.0	20

Table 2. Composition of the nine herbal ethosomal formulations

Formulation	Lecithin (% w/v)	Ethanol (mL)
F1	1.0	10
F2	1.5	10
F3	2.0	10
F4	1.0	15
F5	1.5	15
F6	2.0	15
F7	1.0	20
F8	1.5	20
F9	2.0	20

The dependent responses were entrapment efficiency, vesicle size, and zeta potential. The results were intended to be analyzed using analysis of variance, polynomial equations, and response surface plots [28-30].

Characterization of ethosomes

Vesicle size, PDI, and zeta potential

Vesicle size, polydispersity index, and zeta potential were measured using a Malvern Zetasizer Nano ZS

based on dynamic light scattering and electrophoretic mobility measurements [31].

Vesicle morphology

Transmission electron microscopy was used to examine vesicle morphology. A drop of the sample was deposited onto a carbon-coated copper grid, allowed to stand for 15 minutes, negatively stained with 1% aqueous phosphotungstic acid, and air-dried before imaging. Scanning electron microscopy was performed using a JEOL JSM-6360 instrument. Samples were spread on glass slides, dried, sputter-coated with gold, and examined at an accelerating voltage of 20 kV [32-33].

Entrapment efficiency

The reported entrapment-efficiency procedure involved treating 25 mg of ethosomal formulation with 10 mL of Triton X-100, followed by sonication for 20–30 minutes to disrupt the vesicles. The resulting sample was diluted using phosphate-buffered saline at pH 7.4 and analyzed spectrophotometrically at 415 nm for *C. papaya* extract and 348 nm for *B. pinnatum* extract. Total extract content and the quantity of free extract were intended to be determined separately [34-35].

Preparation of ethosomal gel

The selected ethosomal dispersion was incorporated into Carbopol 980NF gel at a concentration of 0.75% w/w. Carbopol 980NF was gradually dispersed into the ethosomal suspension under continuous stirring at 100–200 rpm for approximately 10 minutes. The mixture was allowed to hydrate to form a consistent gel matrix. A 10% w/v aqueous solution of meglumine was then added dropwise to adjust the pH to 6.0–6.5. The formulation was gently mixed until a homogeneous preparation was obtained [36-38].

Evaluation of the ethosomal gel

pH

Approximately 5 g of gel was dispersed in 50 mL of distilled water. The pH was measured at 25°C using a calibrated pH meter. The procedure was reported to be performed in triplicate [39-40].

Extract content

Approximately 1 g of gel was dispersed in 10 mL of methanol and sonicated for 15 minutes. The solution was diluted appropriately and analyzed spectrophotometrically at 415 nm for *C. papaya* and 348 nm for *B. pinnatum* [41-42].

Spreadability

Spreadability was evaluated using the drag-and-slip method. Approximately 2 g of gel was placed between two glass slides, and a known weight was applied to obtain a uniform film. The upper slide was connected to a pulley system, and the time required to travel a distance of 10 cm was recorded [43-44].

Viscosity

Approximately 50 g of gel was transferred to a 100 mL beaker without introducing air bubbles. Viscosity was measured using a Brookfield viscometer with spindle number 64 at 25 ± 1°C. Measurements were intended to be taken at rotational speeds of 10, 20, 50, and 100 rpm. Each measurement was performed in triplicate after stabilization of the reading [45].

***In vitro* release and *ex vivo* skin permeation**

In vitro diffusion was investigated using a vertical Franz diffusion cell. A hydrated dialysis membrane with a molecular-weight cut-off of approximately 12–14 kDa was used as the membrane separating the donor and receptor compartments. The receptor medium consisted of phosphate buffer at pH 6.8 and ethanol in a reported ratio of 2:3. The system was maintained at 32 ± 0.5°C and stirred at 100 rpm. An accurately weighed quantity of gel was introduced into the donor compartment. At predetermined time intervals, samples were withdrawn from the receptor compartment and replaced with an equal volume of fresh medium. The permeated extract-equivalent content was estimated using UV-visible spectrophotometry [46-47].

For *ex vivo* evaluation, porcine ear skin was used as the biological membrane. The skin was cleaned, separated from adhering subcutaneous tissue, hydrated, and mounted between the donor and receptor compartments. The reported diffusion area was 4.9062 cm^2. Cumulative permeation was intended to be expressed as micrograms per square centimeter. Steady-state flux was calculated from the slope of the linear portion of the cumulative permeation-versus-time plot [48].

Antimicrobial activity

Antimicrobial activity was evaluated using the agar well-diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger*, and *Escherichia coli*. The reported procedure involved inoculation of agar medium, preparation of wells, application of the optimized ethosomal gel and control samples, a two-hour prediffusion period under refrigeration, and subsequent incubation. Zones of inhibition were measured to assess antimicrobial activity [49-51].

Statistical analysis

The factorial formulation data should be analyzed using Design-Expert software, version 13.0, or another appropriately documented statistical package.

Results and Discussion

Extraction and phytochemical characterization

Preliminary phytochemical screening of the leaf extracts of *Carica papaya* and *Bryophyllum pinnatum* was performed to identify the presence of major classes of bioactive constituents. The results of phytochemical screening are summarized in Table 3.

Table 3: Phytochemical Screening of Leaf Extracts

Phytochemical Constituents	*Carica papaya*	*Bryophyllum pinnatum*
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Glycosides	+	+
Phenolic compounds	+	+
Terpenoids	-	+
Steroids	+	+

(+): Present, (–): Absent

TLC Profile of *Carica papaya*

The TLC profile of *Carica papaya* extract developed in chloroform (9:1) showed several distinct spots under UV illumination and after derivatization with anisaldehyde–sulfuric acid reagent. The appearance of colored spots (violet, blue, and green) after spraying indicated the presence of terpenoids, steroids, and phenolic compounds (Table 4).

Table 4: TLC Profile of *Carica papaya* Leaf Extract

Spot No.	Rf Value	Observation (UV/Visible)	Probable Phytoconstituent
1	0.21	Faint under UV	Phenolic compounds
2	0.38	Visible at 365 nm	Terpenoids
3	0.56	Strong at 254 nm	Flavonoids
4	0.72	Moderate fluorescence	Steroidal compounds
5	0.84	Weak fluorescence	Non-polar constituents

TLC Profile of *Bryophyllum pinnatum*

The TLC analysis of *Bryophyllum pinnatum* extract using the solvent system ethyl acetate acid:methanol (10:0.5:0.6:0.2) resulted in well-defined spots with good resolution. Visualization under UV light and after spraying with diphenylboric acid β-aminoethyl ester reagent (specific for flavonoids) showed intense fluorescent zones, confirming the abundance of flavonoid compounds (Table 5).

Table 5: TLC Profile of *Bryophyllum pinnatum* Leaf Extract

Spot No.	Rf Value	Observation (UV 365 nm)	Probable Phytoconstituent
1	0.18	Strong	Flavonoid glycosides
2	0.34	Intense	Flavonoids
3	0.49	Moderate	Phenolic compounds
4	0.63	Moderate	Coumarins
5	0.79	Weak	Less polar flavonoids

The presence of terpenoids and steroids in *Carica papaya* may contribute to enhanced anti-inflammatory and antimicrobial effects, while the high abundance of flavonoids in *Bryophyllum pinnatum* suggests superior antioxidant capacity. The diversity of compounds observed in TLC profiles indicates the possibility of synergistic interactions when both extracts are combined.

Calibration Curve of *Carica papaya* and *Bryophyllum pinnatum* Leaf Extracts

The absorbance of the prepared standard solutions was measured at 415 nm for *Carica papaya* and 348 nm for *Bryophyllum pinnatum*, corresponding to their respective absorption maxima. The absorbance values recorded for different concentrations (10–100 µg/mL) demonstrated a proportional increase with concentration for both extracts, indicating good linearity within the selected range (Figure 1 and 2).

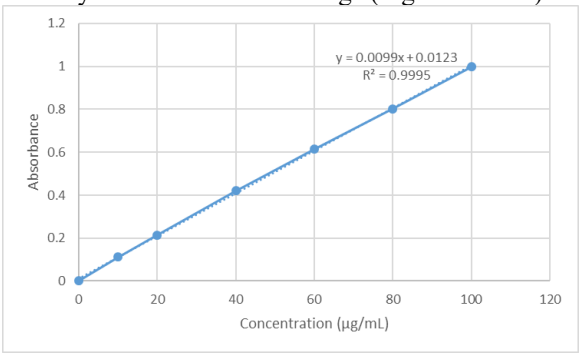


Figure 1: Calibration curve for *Carica papaya* Extract (λmax = 415 nm)

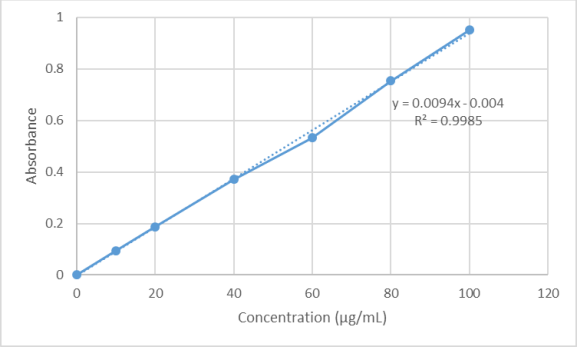


Figure 2: Calibration curve for *Bryophyllum pinnatum* Extract (λmax = 348 nm)

Antioxidant Activity of *Carica papaya* and *Bryophyllum pinnatum* Leaf Extracts by DPPH Free Radical Scavenging Assay

Both extracts exhibited concentration-dependent free radical scavenging activity, indicating their ability to donate hydrogen atoms or electrons to neutralize DPPH radicals. The percentage inhibition increased with increasing concentration (10-100 µg/mL), demonstrating dose-dependent antioxidant potential (Figure 3).

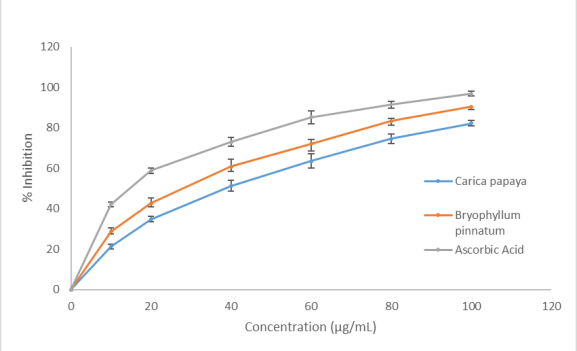


Figure 3: Comparison of Antioxidant Activity of Extracts and Standard

The IC_{50} values, representing the concentration required to inhibit 50% of DPPH radicals, were calculated from the plotted graphs. The lower IC_{50} value of *Bryophyllum pinnatum* (29.45 µg/mL) compared to *Carica papaya* (38.72 µg/mL) further confirms its stronger free radical scavenging efficiency. Nevertheless, both extracts showed appreciable activity, although lower than the standard antioxidant, ascorbic acid.

Compatibility Studies of Prepared Extract With Excipients

The FTIR spectra of both extracts exhibited prominent characteristic peaks corresponding to functional groups associated with phytoconstituents such as phenolics, flavonoids, and terpenoids. The slight broadening and marginal shifting of the O–H stretching band suggest possible intermolecular hydrogen bonding interactions, particularly with excipients such as propylene glycol and carbopol, which contain hydroxyl and carboxyl groups. These interactions are considered beneficial as they may enhance formulation stability and drug dispersion.

Optimization of Ethosomes of *Carica papaya* and *Bryophyllum pinnatum* Extracts

Effect of variables on entrapment efficiency

Data was analyzed to fit full second-order quadratic or cubic polynomial equation(s) with added interaction terms to correlate the various studied responses with the examined variables. As depicted by 3D response surface plot (Figure 4), percent entrapment efficiency of drug is positively correlated with X_1 i.e. lecithin concentration. The quadratic equation indicated that on increasing X_1 from low to intermediate levels, % EE increases drastically while, increasing beyond it up to higher levels, extent of increase in % EE was found to be less.

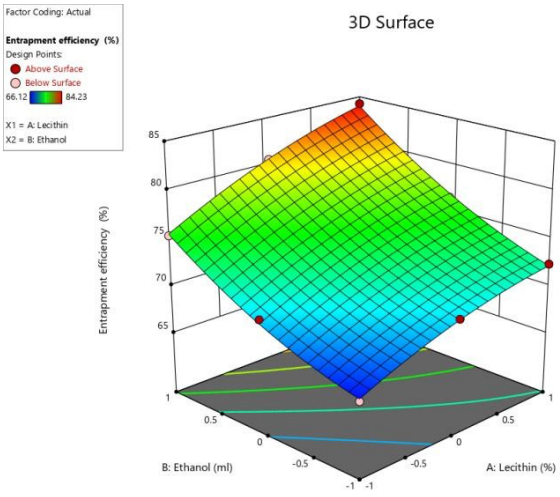


Figure 4: 3D Response surface plots for evaluating influence of lecithin (X1) and ethanol (X2) on Entrapment efficiency (Y1).

Effect of variables on vesicle size

As depicted in 3D-plots (Figure 5), it is indicated that at lower levels of lipid, increase in the levels of ethanol concentration showed negative influence on vesicle size. On the contrary, increasing the levels of lipid, at constant ethanol concentration, increase in vesicle size was observed (positive influence). Thus, lowest level of lipid while highest level of ethanol concentration, resulted in minimum vesicle size. This can be attributed due to steric stabilization mechanism and edge activation due to high ethanol concentration which resulted in low vesicle size.

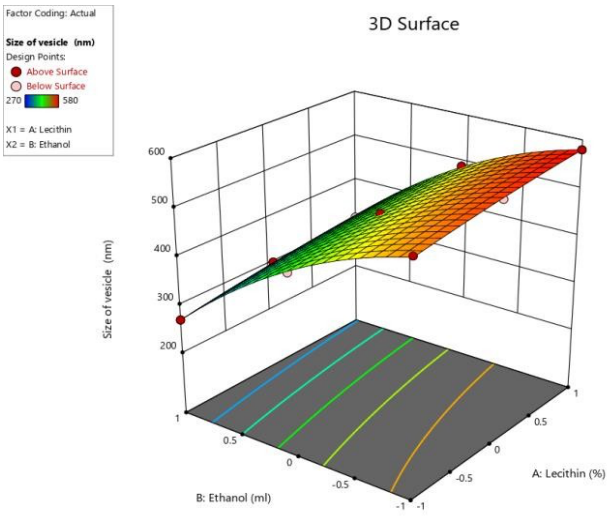


Figure 5: 3D Response surface plots for evaluating influence of lecithin (X1) and ethanol (X2) on Vesicle size (Y2).

Effect of variables on zeta potential

The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential, then they will tend to repel each other and there will be no tendency for the particles to come together. However, if the particles have low zeta potential values, then there will be no force to prevent the particles to come together and flocculate.

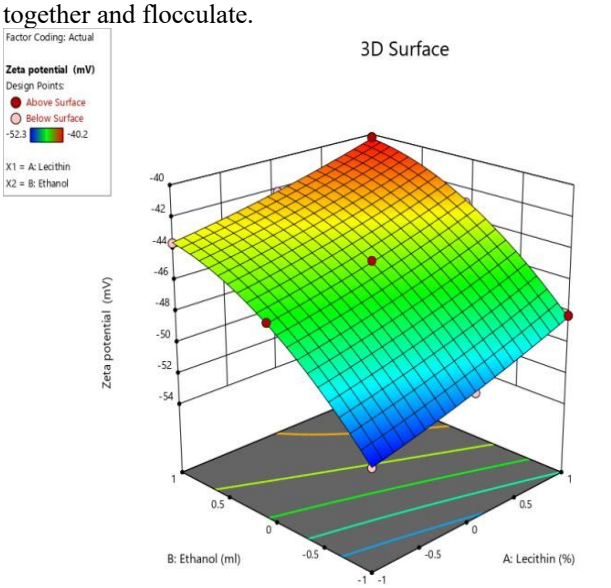


Figure 6: 3D Response surface plots for evaluating influence of lecithin (X1) and ethanol (X2) on Zeta potential (Y3).

Although F7 showed the smallest vesicle size (170 nm), its entrapment efficiency was comparatively lower than F9. Therefore, considering the balance between all critical parameters, F9 was identified as the optimized formulation.

Characterization of *Carica papaya* and *Bryophyllum pinnatum* Extract-Loaded Ethosomes

Vesicle Size, Particle Size Distribution Index, and Zeta Potential

The evaluation revealed a uniform size distribution of vesicles with a particle size of 189.3 ± 2.55 nm. The particle size distribution index (PDI) and zeta potential values of the optimized ethosomal formulations. The PDI value of the prepared vesicle was 0.6436 ± 0.03. The zeta potential of the ethosomal formulation was observed to -21.79 ± 0.75 mV. This value reflect a high degree of surface charge, which is indicative of good electrostatic stabilization.

Vesicle Morphology

The particle size observed in TEM analysis was found to be in the nanometric range (~200nm), consistent with particle size analysis data, indicating efficient vesicle formation. The presence of distinct lipid bilayers confirms the characteristic structure of ethosomes, which is essential for enhanced skin permeation.

SEM micrographs of the ethosomal formulations revealed that the vesicles were predominantly spherical to slightly irregular in shape with a relatively smooth surface morphology. The particles appeared to be well-dispersed with minimal aggregation, indicating good formulation stability. The absence of large clusters or crystalline structures suggests that the extracts were successfully incorporated within the lipid matrix without phase separation.

Entrapment Efficiency (%EE)

The calculated %EE value for optimized formulation was found to be 84.23±2.15%, indicating effective incorporation of the herbal extracts within the vesicular system. The high %EE values obtained in this study confirm the suitability of ethosomal systems for encapsulating phytoconstituents of *Carica papaya* and *Bryophyllum pinnatum*.

Evaluation of Prepared Ethosomal Gel

The pH of the formulated ethosomal gel was found to be 6.8, which is close to the physiological pH of the skin. This indicates that the formulation is suitable for topical application and is unlikely to cause irritation or discomfort upon administration.

The drug content of the formulation was determined to be 90.95% for *Carica papaya*, and 88.65% for *Bryophyllum pinnatum* extract indicating uniform distribution of the encapsulated extracts within the gel matrix. High extract content ensures dose uniformity and effective therapeutic performance.

The spreadability value was found to be 33.65 g·cm/sec, suggesting that the gel can be easily spread

over the skin with minimal effort. Good spreadability is an important parameter for patient compliance, as it ensures uniform application and better coverage of the affected area.

The viscosity of the gel was measured to be 39435 cP, indicating a moderately high consistency. This level of viscosity is desirable for topical formulations as it ensures adequate retention at the site of application without being too stiff, thereby facilitating ease of handling and application.

In Vitro and _Ex Vivo_ Diffusion Studies Ethosomal Gel

The optimized ethosomal gel demonstrated a time-dependent and sustained release of both herbal extracts. The cumulative percentage release increased progressively from the initial sampling point and reached 91.26 ± 1.86% for *Carica papaya* and 94.18 ± 1.93% for *Bryophyllum pinnatum* at 24 h (Figure 7).

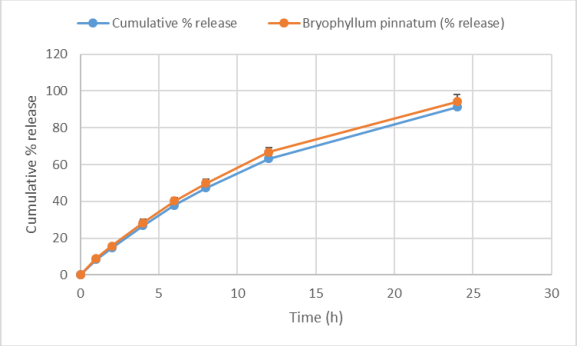


Figure 7: _In vitro_ cumulative % release of _Carica papaya_ and _Bryophyllum pinnatum_ extracts

The *ex vivo* study demonstrated a continuous increase in cumulative permeation over 24 h, with 63.28 ± 1.62% of *Carica papaya* extract and 67.42 ± 1.71% of *Bryophyllum pinnatum* extract permeating across porcine skin by the end of the study. The lower cumulative permeation compared with the in vitro dialysis membrane study confirms the additional diffusional resistance provided by the biological skin barrier.

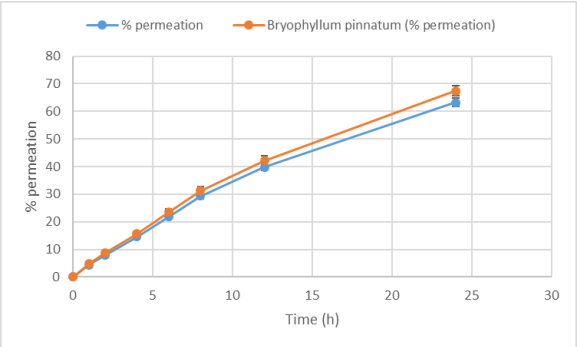


Figure 8: _Ex vivo_ cumulative percentage permeation of _Carica papaya_ and _Bryophyllum pinnatum_ extracts

Antimicrobial activity of prepared gel

The optimized ethosomal gel exhibited distinct antimicrobial activity against all four tested microorganisms, as demonstrated by the formation of clear inhibition zones surrounding the formulation wells. The highest zone of inhibition was observed against *S. aureus* (18.6 ± 0.6 mm), followed by *B. subtilis* (16.8 ± 0.5 mm), *E. coli* (14.2 ± 0.4 mm), and *A. niger* (12.7 ± 0.5 mm). In contrast, DMSO used as the vehicle control did not produce any measurable inhibition zone, indicating that the observed antimicrobial activity was attributable primarily to the plant extracts and/or their incorporation into the ethosomal system.

Conclusion

The study describes the design and development of an ethosomal gel containing ethanolic leaf extracts of *Carica papaya* and *Bryophyllum pinnatum* for investigation as a potential topical wound-care formulation. The documented procedures include plant authentication, ethanolic extraction, qualitative and quantitative phytochemical analysis, TLC characterization, DPPH antioxidant testing, FTIR compatibility assessment, and preparation of nine ethosomal formulations using a (3^2) full factorial design.

The formulation variables comprised lecithin concentrations of 1.0%, 1.5%, and 2.0%, and ethanol volumes of 10, 15, and 20 mL. Each batch was specified to contain 1.5 g of each plant extract and 0.2% cholesterol. The selected ethosomal dispersion was intended to be incorporated into Carbopol 980NF gel at 0.75% w/w, with meglumine solution used to adjust the pH to 6.0–6.5. The planned evaluation program encompasses vesicle size, PDI, zeta potential, entrapment efficiency, morphology, extract content, viscosity, spreadability, *in vitro* release, *ex vivo* skin permeation, and antimicrobial activity testing.

The principal contribution of the work is the integration of combined herbal extracts with a vesicular topical delivery platform and a structured formulation-optimization strategy. However, the numerical results required to establish the optimized formulation and demonstrate its pharmaceutical performance were not included in the available experimental record. Accordingly, superiority of any formulation, enhanced skin permeation, antimicrobial efficacy, irritation safety, and wound-healing effectiveness remain unconfirmed.

Declarations

Funding: Not applicable.

Clinical trial number: Not applicable.

Ethics and Consent to Participate declarations: Not applicable

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