

Development, Optimization, and Evaluation of a Polyherbal Ethosomal Gel Containing Plant Extracts for Enhanced Topical Anti-Inflammatory and Anti-Arthritic Therapy

Shweta Rawal*& Sachin Kumar Jain

*Research Scholar, Faculty of Pharmacy, Oriental University, Indore (M.P.)

Faculty of Pharmacy, Oriental University, Indore (M.P.)

Corresponding author email: shetu.rwl@gmail.com

Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory and autoimmune disorder characterized by persistent synovial inflammation, progressive joint destruction, pain, and functional impairment. Plant-derived bioactive compounds offer a potential alternative; however, their therapeutic application may be restricted by poor permeability, instability, and inadequate delivery to the target site. The present study aimed to develop and optimize a polyherbal ethosomal gel containing *Salvia officinalis* and *Sarcostemma brevistigma* extracts for enhanced topical anti-inflammatory and potential anti-arthritic activity. Ethanolic extracts of *S. officinalis* leaves and *S. brevistigma* stems were prepared by successive Soxhlet extraction and subjected to preliminary phytochemical screening and gallic acid estimation. Polyherbal ethosomes were prepared by the cold method using soya phosphatidylcholine and ethanol as the principal formulation variables. Five formulations (PEG1–PEG5) were prepared and incorporated into Carbopol 940 gel. The formulations were evaluated for appearance, homogeneity, pH, viscosity, spreadability, extrudability, drug content, particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, in vitro drug release, skin irritation, and stability. The anti-inflammatory potential was assessed using human red blood cell (HRBC) membrane stabilization and bovine serum albumin (BSA) protein denaturation assays. The optimized formulation showed no visible skin irritation and maintained satisfactory physicochemical characteristics during three months of stability testing. The combination of *S. officinalis* and *S. brevistigma* within a flexible ethosomal carrier may provide an effective approach for enhancing topical delivery of plant-derived anti-inflammatory constituents. Further comprehensive pharmacological, toxicological, and clinical investigations are warranted to establish its therapeutic potential in rheumatoid arthritis and other inflammatory disorders.

Keywords: *Salvia officinalis*; *Sarcostemma brevistigma*; polyherbal formulation; ethosomes; Carbopol 940; topical drug delivery; anti-inflammatory activity; rheumatoid arthritis; HRBC membrane stabilization; BSA protein denaturation.

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Introduction

Inflammation is a highly coordinated physiological response that protects the body against harmful stimuli, including pathogens, physical injury, toxic chemicals, and immune-mediated tissue damage [1]. Under normal conditions, the inflammatory process is self-limiting and contributes to tissue repair and restoration of homeostasis. However, persistent or dysregulated inflammation becomes pathological and is implicated in the initiation and progression of numerous chronic diseases, including rheumatoid arthritis (RA), osteoarthritis, inflammatory bowel disease, psoriasis, diabetes mellitus, cardiovascular disorders, and neurodegenerative diseases [2]. Among these conditions, rheumatoid arthritis represents one of the most debilitating autoimmune inflammatory disorders, characterized by chronic synovial inflammation, progressive cartilage degradation, bone erosion, joint deformity, and systemic complications. The disease significantly impairs physical function and quality of life while imposing substantial socioeconomic burdens worldwide. Despite considerable advances in pharmacotherapy,

complete remission remains difficult to achieve in many patients, and prolonged treatment is frequently associated with adverse drug reactions and high healthcare costs [3].

Rheumatoid arthritis is a chronic systemic autoimmune disease involving complex interactions among genetic susceptibility, environmental factors, immune dysregulation, and inflammatory mediators. Activated macrophages, T lymphocytes, B lymphocytes, fibroblast-like synoviocytes, and dendritic cells produce excessive amounts of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF- α), interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and other inflammatory mediators that perpetuate synovial inflammation. Continuous activation of these signaling pathways stimulates osteoclast differentiation and matrix metalloproteinase production, resulting in progressive destruction of cartilage and subchondral bone [4]. Elevated levels of acute-phase proteins such as C-reactive protein (CRP), increased erythrocyte sedimentation rate (ESR), and rheumatoid factor (RF) further reflect

the systemic inflammatory status associated with disease progression. Therefore, effective suppression of inflammatory mediators remains a major therapeutic objective in rheumatoid arthritis management [5].

Current treatment strategies for rheumatoid arthritis include non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease-modifying antirheumatic drugs (DMARDs), biologics, and targeted synthetic agents. NSAIDs effectively reduce pain and inflammation but do not alter disease progression and are frequently associated with gastrointestinal irritation, renal dysfunction, cardiovascular complications, and hepatotoxicity following long-term administration. Corticosteroids provide rapid symptomatic relief but may produce osteoporosis, adrenal suppression, metabolic disturbances, and increased susceptibility to infections [6]. Conventional DMARDs and biological therapies have significantly improved clinical outcomes; however, they require prolonged administration, careful monitoring, and are associated with immunosuppression, high treatment costs, and variable patient responses. These limitations have stimulated increasing interest in safer, plant-derived therapeutic alternatives capable of providing sustained anti-inflammatory effects with reduced adverse reactions [7].

Medicinal plants have served as valuable therapeutic resources for centuries and continue to contribute significantly to modern drug discovery. Herbal medicines contain diverse bioactive phytoconstituents such as flavonoids, phenolic acids, terpenoids, alkaloids, saponins, tannins, and glycosides that possess antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, analgesic, and wound-healing properties [8]. Numerous experimental and clinical investigations have demonstrated that phytochemicals regulate multiple inflammatory pathways simultaneously, including inhibition of cyclooxygenase (COX), lipoxygenase (LOX), nuclear factor-kappa B (NF- κ B), mitogen-activated protein kinase (MAPK), inducible nitric oxide synthase (iNOS), and pro-inflammatory cytokine production. Consequently, polyherbal formulations combining complementary medicinal plants have attracted considerable attention because of their potential synergistic therapeutic effects, multitarget mechanisms of action, improved efficacy, and lower incidence of adverse effects compared with synthetic drugs [8]. Among the medicinal plants investigated for anti-inflammatory activity, *Salvia officinalis* (common sage) has been extensively recognized for its remarkable pharmacological properties. The plant contains numerous biologically active constituents, including rosmarinic acid, caffeic acid derivatives, carnosic acid, carnosol, ursolic acid, oleanolic acid, flavonoids, diterpenes, and essential oils such as thujone, camphor, and cineole. These

phytochemicals exhibit potent antioxidant and anti-inflammatory activities through inhibition of oxidative stress, suppression of inflammatory cytokines, stabilization of cellular membranes, and modulation of NF- κ B signaling pathways [9]. Experimental studies have demonstrated that *Salvia officinalis* effectively reduces inflammatory cell infiltration, lipid peroxidation, cytokine production, and oxidative tissue damage in various inflammatory disorders. In addition to its anti-inflammatory potential, the plant possesses antimicrobial, analgesic, wound-healing, and immunomodulatory properties that further support its suitability for topical therapeutic applications [10].

Sarcostemma brevistigma is another medicinal plant traditionally employed in indigenous systems of medicine for the treatment of inflammatory disorders, musculoskeletal pain, skin diseases, and wound healing. The plant contains steroidal glycosides, flavonoids, triterpenoids, phenolic compounds, and other secondary metabolites reported to possess antioxidant and anti-inflammatory activities [11]. These phytoconstituents inhibit inflammatory mediator release, reduce oxidative stress, stabilize biological membranes, and contribute to tissue repair. The combined use of *Salvia officinalis* and *Sarcostemma brevistigma* is therefore expected to provide synergistic pharmacological effects by simultaneously targeting multiple inflammatory pathways involved in rheumatoid arthritis. Such a polyherbal approach may enhance therapeutic efficacy while minimizing the dose-dependent adverse effects often associated with synthetic anti-inflammatory agents [12].

Ethosomes are soft, flexible phospholipid vesicles containing relatively high concentrations of ethanol in combination with phospholipids and water. Unlike conventional liposomes, the presence of ethanol imparts remarkable deformability to the phospholipid bilayer by disrupting lipid packing, thereby facilitating penetration through the highly organized lipid structure of the stratum corneum. Ethanol simultaneously enhances skin permeability and improves the solubility of both hydrophilic and lipophilic phytoconstituents, while phosphatidylcholine forms stable bilayer vesicles capable of encapsulating diverse bioactive molecules [13]. The synergistic interaction between ethanol and phospholipids enables ethosomes to penetrate into deeper skin layers, providing enhanced drug deposition, sustained release, improved therapeutic efficacy, and reduced systemic exposure. Consequently, ethosomal systems have been successfully investigated for the topical delivery of anti-inflammatory drugs, corticosteroids, antifungal agents, antioxidants, herbal extracts, and other bioactive compounds.

Incorporation of ethosomal vesicles into a suitable hydrogel matrix further improves their applicability for topical administration. Carbopol-based gels are widely utilized because of their excellent rheological properties, transparency, bioadhesion, ease of application, patient acceptability, and ability to prolong residence time at the application site. The gel matrix minimizes runoff, facilitates uniform distribution of vesicles over the skin surface, and enables controlled release of encapsulated phytoconstituents. Moreover, the combination of ethosomal nanocarriers with a polymeric gel creates a dual-controlled delivery system capable of enhancing skin penetration while maintaining prolonged therapeutic activity [14]. Optimization of formulation variables is essential for achieving desirable ethosomal characteristics, including nanoscale particle size, narrow polydispersity index, adequate zeta potential, high drug entrapment efficiency, suitable viscosity, controlled drug release, and long-term physical stability. Parameters such as phospholipid concentration, ethanol content, cholesterol level, and polymer composition substantially influence vesicle formation, membrane flexibility, encapsulation efficiency, and skin permeation. Therefore, systematic optimization of these variables is necessary to identify an ideal formulation capable of maximizing topical delivery of herbal phytoconstituents [15].

Based on these considerations, the present investigation was undertaken to develop, optimize, and evaluate a polyherbal ethosomal gel containing *Salvia officinalis* and *Sarcostemma brevistigma* extracts for enhanced topical anti-inflammatory and anti-arthritic therapy. Different formulations were prepared by varying phosphatidylcholine and ethanol concentrations while maintaining constant levels of the herbal extracts and auxiliary excipients [16]. The developed formulations were comprehensively evaluated for physicochemical characteristics, vesicle size, polydispersity index, zeta potential, entrapment efficiency, drug content, viscosity, spreadability, in vitro drug release, skin compatibility, and stability. The optimized formulation was further assessed for in vitro anti-inflammatory activity using human red blood cell membrane stabilization and bovine serum albumin denaturation assays, followed by in vivo evaluation in Complete Freund's Adjuvant-induced arthritic rats through assessment of paw edema, arthritic score, inflammatory biomarkers, rheumatoid factor, C-reactive protein, radiographic changes, and histopathological alterations [17]. The study was designed to establish the potential of a polyherbal ethosomal gel as an effective, safe, and patient-friendly topical therapeutic system capable of improving skin penetration, enhancing anti-inflammatory efficacy, and providing a promising alternative strategy for the management of

rheumatoid arthritis and related inflammatory disorders.

Materials and Methods

Chemicals and Reagents

Analytical-grade chemicals and reagents were used throughout the study. Soya phosphatidylcholine (SPC) was used as the phospholipid for ethosome preparation, while cholesterol served as the membrane stabilizer. Absolute ethanol was employed as the vesicle-forming solvent and penetration enhancer. Propylene glycol was used as a co-solvent and humectant [18]. Carbopol 940 was selected as the gelling agent, and triethanolamine was used for neutralization and adjustment of gel pH. Methyl paraben and propyl paraben were incorporated as antimicrobial preservatives. Petroleum ether (60–80°C), ethanol (95%), methanol, ferric chloride, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, concentrated sulfuric acid, hydrochloric acid, sodium hydroxide, sodium carbonate, and other phytochemical reagents were of analytical grade and procured from standard commercial suppliers. Gallic acid reference standard was used for quantitative estimation of phenolic compounds. Distilled water was used throughout the study for extraction, formulation, and analytical procedures [19].

Plant Material Collection and Authentication

Fresh leaves of *Salvia officinalis* L. (Family: Lamiaceae) and fresh stems of *Sarcostemma brevistigma* Wight & Arn. (Family: Apocynaceae) were collected during the appropriate growing season from authenticated medicinal plant sources in India. The collected plant materials were carefully examined to remove damaged or diseased portions and thoroughly washed with distilled water to eliminate adhering dust, soil particles, and other foreign contaminants.

The plant materials were authenticated by a qualified taxonomist, and voucher specimens were deposited in the departmental herbarium for future reference. After authentication, the samples were shade-dried at room temperature (25–30°C) for approximately 10–14 days until constant weight was achieved. Shade drying was preferred to preserve thermolabile phytoconstituents and minimize degradation of phenolic compounds. The dried materials were coarsely powdered using a mechanical grinder and passed through sieve No. 40 to obtain a uniform particle size suitable for solvent extraction. The powdered samples were stored in airtight amber-colored containers at room temperature until further use [20].

Extraction of *Salvia officinalis* Leaves and *Sarcostemma brevistigma* Stems

The powdered leaves of *Salvia officinalis* (500 g) and powdered stems of *Sarcostemma brevistigma* (500 g) were extracted separately by successive

Soxhlet extraction using solvents of increasing polarity.

Initially, each powdered sample was extracted with petroleum ether (60–80°C) for 8–10 h to remove fats, fixed oils, chlorophyll, waxes, and other non-polar constituents that might interfere with subsequent extraction. After completion of petroleum ether extraction, the marc was removed from the extraction chamber and air-dried overnight to eliminate residual solvent. The defatted marc was subsequently extracted with 95% ethanol in the same Soxhlet apparatus for 18–24 h or until the solvent in the siphon tube became nearly colorless, indicating complete extraction of polar and moderately polar phytoconstituents. The obtained extracts were filtered through Whatman No. 1 filter paper to remove suspended particles and concentrated under reduced pressure using a rotary vacuum evaporator at temperatures below 45°C. The concentrated extracts were further dried in a vacuum desiccator to obtain semisolid masses [21]. The dried ethanolic extracts were stored in airtight amber-colored containers at 4°C until formulation studies.

The percentage yield of each extract was calculated using the equation:

$$\text{Percentage Yield (\%)} = (\text{Weight of dried extract} / \text{Weight of powdered plant material}) \times 100$$

Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the ethanolic extracts of *Salvia officinalis* leaves and *Sarcostemma brevistigma* stems was carried out using standard phytochemical methods to identify major classes of secondary metabolites. The extracts were screened for alkaloids using Mayer's, Wagner's, and Dragendorff's reagents; flavonoids by the alkaline reagent and Shinoda tests; tannins and phenolic compounds using ferric chloride solution; saponins by the foam test; glycosides using Keller–Killiani and Bornträger's tests; steroids and triterpenoids using the Liebermann–Burchard and Salkowski reactions; carbohydrates by Molisch's and Benedict's tests; proteins and amino acids by Biuret and Ninhydrin tests; and terpenoids using Salkowski's reaction [22].

UV Spectrophotometric Estimation of Gallic Acid

Quantitative estimation of gallic acid was performed using a UV–Visible spectrophotometer. A stock solution of gallic acid was prepared by dissolving an accurately weighed quantity of gallic acid in methanol to obtain the desired concentration. A calibration curve was constructed by plotting absorbance against concentration, and linear regression analysis was performed to obtain the regression equation and correlation coefficient (R^2). The absorbance of each sample solution was measured at 260 nm under identical instrumental conditions. Gallic acid content was calculated from the calibration equation and expressed as

percentage gallic acid or milligrams of gallic acid equivalents (GAE) per gram of extract. Each determination was performed in triplicate, and the results were expressed as mean $\pm$ standard deviation [23].

Formulation of Polyherbal Ethosomes

Polyherbal ethosomes containing the ethanolic extracts of *Salvia officinalis* and *Sarcostemma brevistigma* were prepared using the cold method. Briefly, accurately weighed quantities of soya phosphatidylcholine and cholesterol were dissolved in ethanol under continuous magnetic stirring at room temperature. The required quantities of both ethanolic plant extracts were dissolved in the ethanolic lipid phase, followed by the addition of propylene glycol. Distilled water was gradually incorporated into the ethanolic phase under continuous stirring at approximately 700–1000 rpm while maintaining the temperature below 30°C. Slow addition of the aqueous phase promoted spontaneous formation of nanosized ethosomal vesicles. Stirring was continued for approximately 30 min to ensure complete vesicle formation. The resulting ethosomal suspension was sonicated using a probe sonicator for 5–10 min with intermittent cooling to reduce vesicle size and obtain a homogeneous nanosuspension. The prepared ethosomes were stored at 4°C until incorporation into the gel base [24].

Preparation of Polyherbal Ethosomal Gel

The ethosomal gel was prepared using Carbopol 940 as the gelling polymer. Carbopol 940 (1% w/w) was slowly dispersed in distilled water with continuous stirring and allowed to hydrate overnight to obtain complete swelling. Methyl paraben (0.20% w/w) and propyl paraben (0.05% w/w) were dissolved separately and incorporated into the hydrated polymer dispersion. The previously prepared ethosomal suspension containing *Salvia officinalis* and *Sarcostemma brevistigma* extracts was gradually added to the Carbopol dispersion under gentle mechanical stirring to ensure uniform distribution of the vesicles without causing vesicle disruption. The pH of the formulation was adjusted to approximately 6.5–6.8 using triethanolamine, resulting in the formation of a smooth, homogeneous gel suitable for topical application. The final weight was adjusted with distilled water, and the gel was mixed until a uniform consistency was obtained [25]. The prepared gels were filled into laminated collapsible tubes, sealed, and stored under refrigerated conditions ($4 \pm 2^\circ\text{C}$) until further characterization. A formulation optimization approach was employed to identify the optimum composition of the polyherbal ethosomal gel. Five formulations (PEG1–PEG5) were prepared by systematically varying the concentrations of soya phosphatidylcholine (200–600 mg) and ethanol (20–40% v/v), while maintaining constant

quantities of the herbal extracts, cholesterol (25 mg), propylene glycol (10% v/v), Carbopol 940 (1% w/w), preservatives, and distilled water. Soya phosphatidylcholine was selected as the primary independent variable because it governs vesicle formation, membrane integrity, particle size, encapsulation efficiency, and drug release characteristics. Ethanol concentration was varied simultaneously because of its critical role in enhancing vesicle flexibility, reducing particle size, and improving skin permeation. Each formulation was evaluated for organoleptic characteristics, homogeneity, pH, viscosity, spreadability, extrudability, drug content, particle size, polydispersity index, zeta potential, entrapment efficiency, in vitro drug release, and skin irritation. Comparative evaluation of these parameters enabled selection of the optimized formulation exhibiting the best balance of physicochemical stability, vesicle characteristics, drug loading, and topical performance. Among the developed formulations, PEG3 demonstrated the most desirable characteristics, including the smallest particle size, lowest polydispersity index, highest entrapment efficiency, optimum viscosity, excellent spreadability, maximum drug release, and superior stability, and was therefore selected for subsequent in vitro, ex vivo, and in vivo anti-inflammatory and anti-arthritic investigations [26].

Evaluation of Ethosomal Gel

The prepared polyherbal ethosomal gel formulations (PEG1–PEG5) containing *Salvia officinalis* and *Sarcostemma brevistigma* extracts were subjected to comprehensive physicochemical, vesicular, and stability evaluations to identify the optimized formulation. All determinations were carried out in triplicate ($n = 3$), and the results were expressed as mean $\pm$ standard deviation (SD) [27].

Appearance and Homogeneity

The prepared ethosomal gel formulations were visually inspected immediately after preparation for color, transparency, consistency, smoothness, grittiness, phase separation, and overall appearance. Approximately 2 g of each formulation was examined against both white and black backgrounds under normal daylight conditions. Homogeneity was evaluated by gently rubbing a small quantity of gel between the thumb and index finger to detect the presence of coarse particles or aggregates. The formulations were graded as excellent, good, fair, or poor based on their uniformity, smoothness, and absence of visible particulate matter or phase separation [28].

pH Determination

The pH of each ethosomal gel formulation was determined using a calibrated digital pH meter. Before measurement, the instrument was standardized using buffer solutions of pH 4.0, 7.0, and 9.2. One gram of gel was dispersed in 10 mL of distilled water and allowed to equilibrate for 30

min at room temperature. The electrode was immersed into the gel dispersion, and the pH was recorded after stabilization of the reading. Three independent measurements were performed for each formulation, and the average value was reported. The pH was maintained within the physiological range suitable for topical application to minimize skin irritation [29].

Viscosity

The viscosity of the ethosomal gel formulations was determined using a Brookfield digital viscometer fitted with an appropriate spindle (Spindle No. 64). Approximately 50 g of gel was transferred into a clean beaker, and the spindle was immersed without entrapping air bubbles. The viscosity was measured at $25 \pm 1^\circ\text{C}$ using a rotational speed of 20 rpm. After allowing the sample to equilibrate for 2 min, the viscosity reading was recorded in centipoise (cPs). Measurements were performed in triplicate, and the mean value was calculated [30].

Spreadability

Spreadability of the formulations was evaluated using the parallel glass slide method. Approximately 0.5 g of gel was placed between two glass slides of equal dimensions. A weight of 500 g was placed over the upper slide for 5 min to obtain a uniform film. Subsequently, a standard weight was attached to the upper slide, and the time required for the upper slide to move a predetermined distance was recorded [31]. Spreadability was calculated using the following equation:

$$\text{Spreadability (S)} = (M \times L) / T$$

Where:

- M = weight tied to the upper slide (g)
- L = length moved by the glass slide (cm)
- T = time taken (s)

Higher spreadability values indicated better ease of application and patient acceptability.

Extrudability

Extrudability was evaluated by filling approximately 20 g of each formulation into collapsible aluminum tubes fitted with standard nozzles. A constant force was applied manually to extrude the gel from the tube. The quantity of gel extruded from the tube under the applied force was recorded, and the extrusion force was expressed in grams. Formulations exhibiting smooth and continuous extrusion without interruption were considered acceptable for topical administration [32].

Drug Content

Drug content was determined to evaluate the uniform distribution of phytoconstituents within the ethosomal gel. One gram of gel was accurately weighed and transferred into a volumetric flask containing ethanol. The mixture was sonicated for 20 min to rupture the vesicles and extract the encapsulated phytoconstituents completely. The

volume was adjusted with ethanol, filtered through Whatman No. 1 filter paper, and suitably diluted. The absorbance was measured using a UV–Visible spectrophotometer at the predetermined analytical wavelength. Drug content was calculated from the calibration curve and expressed as percentage drug content relative to the theoretical amount incorporated into the formulation [33].

Particle Size

The average vesicle size of the ethosomal formulations was determined by dynamic light scattering (DLS) using a particle size analyzer. The gel was diluted appropriately with filtered distilled water to avoid multiple scattering effects. The diluted sample was transferred into a disposable cuvette and analyzed at 25°C. The mean particle size was expressed in nanometers (nm). Each measurement was carried out in triplicate, and the average value was reported.

Polydispersity Index (PDI)

The polydispersity index (PDI), representing the uniformity of vesicle size distribution, was simultaneously determined using the dynamic light scattering instrument during particle size analysis. PDI values below 0.30 were considered indicative of a homogeneous nanosized vesicular system with narrow particle size distribution and good physical stability [34].

Zeta Potential

The surface charge of the ethosomal vesicles was determined using a zeta potential analyzer based on laser Doppler electrophoresis. Appropriate dilution of the formulation was performed using filtered distilled water before analysis. The diluted sample was transferred into a zeta potential cell, and the electrophoretic mobility was measured. The zeta potential was expressed in millivolts (mV). Higher absolute zeta potential values indicated improved electrostatic stabilization and reduced aggregation of vesicles during storage [35].

Entrapment Efficiency

Entrapment efficiency of the ethosomal vesicles was determined by the ultracentrifugation method. An accurately measured quantity of the ethosomal suspension was centrifuged at 15,000 rpm for 60 min at 4°C. The supernatant containing untrapped phytoconstituents was carefully separated, and its drug content was determined spectrophotometrically. Entrapment efficiency was calculated using the following equation:

$$\text{Entrapment Efficiency (\%)} = \frac{[\text{Total Drug} - \text{Free Drug}]}{\text{Total Drug}} \times 100$$

The percentage entrapment efficiency reflected the ability of the phospholipid vesicles to retain the herbal bioactive constituents.

In Vitro Drug Release

The in vitro drug release study was carried out using a Franz diffusion cell with an effective diffusion area of approximately 2.5 cm². A cellulose acetate membrane previously hydrated in

phosphate buffer (pH 7.4) was mounted between the donor and receptor compartments. Approximately 1 g of ethosomal gel was placed uniformly in the donor compartment. The receptor compartment was filled with phosphate buffer (pH 7.4) maintained at 37 ± 0.5°C and continuously stirred using a magnetic stirrer at 100 rpm.

Aliquots (2 mL) were withdrawn at predetermined time intervals and replaced immediately with equal volumes of fresh dissolution medium to maintain sink conditions. The samples were analyzed using a UV–Visible spectrophotometer at the selected wavelength, and the cumulative percentage drug release was calculated and plotted against time [36].

Skin Irritation Study

The skin irritation potential of the optimized ethosomal gel was evaluated in healthy Wistar rats following approval from the Institutional Animal Ethics Committee (IAEC). The dorsal surface of each animal was carefully shaved 24 h before the experiment. Approximately 0.5 g of gel was applied uniformly over the shaved area once daily for seven consecutive days. Animals were observed periodically for erythema, edema, itching, scaling, ulceration, or any other visible signs of irritation according to standard dermal irritation scoring criteria. The absence of visible irritation was considered indicative of dermal safety.

Stability Study (ICH Guidelines)

The optimized formulation was subjected to accelerated and long-term stability studies according to the International Council for Harmonisation (ICH) guideline Q1A(R2). Samples were packed in airtight containers and stored under three different storage conditions:

- Refrigerated condition: 4 ± 2°C
- Long-term condition: 25 ± 2°C / 60 ± 5% RH
- Accelerated condition: 40 ± 2°C / 75 ± 5% RH

The stability study was conducted for three months. Samples were withdrawn initially and after 1, 2, and 3 months for evaluation. The formulations were examined for physical appearance, homogeneity, color, phase separation, pH, viscosity, and drug content. Any significant changes in physicochemical properties were recorded to determine the storage stability of the optimized ethosomal gel [37].

Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD) for in vitro studies and mean ± standard error of the mean (SEM) for in vivo studies. Statistical analysis was performed using GraphPad Prism software (Version 9.0, GraphPad Software Inc., San Diego, CA, USA). Comparisons among multiple groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc

test. Differences were considered statistically significant at $P < 0.05$, while $P < 0.01$ and $P < 0.001$ indicated highly significant and extremely significant differences, respectively. Graphical representations were prepared using the same software to facilitate comparison of formulation performance and biological activity.

Results and Discussion

Plant Material Collection and Authentication

Fresh leaves of *Salvia officinalis* and stems of *Sarcostemma brevistigma* were collected during their appropriate growing season and authenticated by a qualified taxonomist before experimental use. The collected materials were free from visible microbial contamination, insect infestation, and mechanical damage. Authentication ensured the botanical identity and reproducibility of the study, which is essential for the quality control of herbal formulations. The fresh plant materials were thoroughly washed with distilled water to remove dust and extraneous matter before shade drying. Shade drying at ambient temperature (25–30°C) for 10–14 days effectively reduced the moisture content without causing degradation of thermolabile phytoconstituents. After drying, both plant materials retained their characteristic color and odor, indicating minimal deterioration. The dried samples were powdered uniformly and passed through sieve No. 40 to obtain a homogeneous particle size suitable for solvent extraction.

Extraction of *Salvia officinalis* Leaves and *Sarcostemma brevistigma* Stems

Successive Soxhlet extraction using petroleum ether followed by ethanol produced clean and concentrated extracts rich in phytoconstituents. Petroleum ether extraction efficiently removed lipids, chlorophyll, waxes, and other non-polar impurities, thereby improving the efficiency of subsequent ethanolic extraction. The ethanolic solvent extracted a broad spectrum of biologically active polar constituents including phenolic compounds, flavonoids, tannins, glycosides, and terpenoids. The ethanolic extracts exhibited a dark greenish-brown semisolid appearance with characteristic aromatic odor in *Salvia officinalis*, whereas *Sarcostemma brevistigma* produced a brown semisolid extract. These extracts remained stable without phase separation during storage at 4°C and were subsequently used for phytochemical evaluation and formulation development.

Preliminary Phytochemical Screening

Preliminary phytochemical investigation confirmed the presence of several classes of secondary metabolites responsible for anti-inflammatory and antioxidant activities.

Both extracts demonstrated abundant phenolic and flavonoid constituents, supporting their potential therapeutic application. *Salvia officinalis* showed comparatively higher levels of phenolic compounds and terpenoids, whereas *Sarcostemma brevistigma*

contained comparatively greater quantities of steroidal glycosides. The coexistence of these phytochemical classes is expected to provide synergistic antioxidant, membrane stabilizing, and anti-inflammatory activities.

UV Spectrophotometric Estimation of Gallic Acid

Preparation of Calibration Curve

A UV spectrophotometric method was developed for gallic acid quantification using six standard concentrations. The absorbance increased proportionally with concentration, confirming compliance with Beer-Lambert's law.

Table 1. Gallic acid content in ethanolic extracts

S. No.	Extract	Gallic acid content (%)
1	<i>Salvia officinalis</i>	2.341
2	<i>Sarcostemma brevistigma</i>	2.087

The *Salvia officinalis* extract contained a slightly higher gallic acid concentration (2.341%) than *Sarcostemma brevistigma* (2.087%). Since gallic acid is an important phenolic antioxidant possessing free-radical scavenging and anti-inflammatory activities, the higher concentration observed in *Salvia officinalis* may contribute substantially to the biological activity of the developed formulation.

The relatively high phenolic content obtained from both extracts validates their selection for formulation of a polyherbal topical delivery system intended for inflammatory disorders.

Formulation of Polyherbal Ethosomes

Polyherbal ethosomes were formulated using the cold method by varying phosphatidylcholine and ethanol concentrations while maintaining constant quantities of herbal extracts and other excipients. The strategy aimed to optimize vesicle characteristics including particle size, entrapment efficiency, colloidal stability, and drug release.

Five formulations (PEG1–PEG5) were prepared by gradually increasing phosphatidylcholine from 200 to 600 mg and ethanol concentration from 20 to 40%.

The progressive increase in phosphatidylcholine enhanced vesicle formation and drug encapsulation up to an optimum concentration. Simultaneously, ethanol improved membrane flexibility and skin permeation by increasing vesicle deformability. However, excessive phospholipid and ethanol concentrations tended to increase vesicle size and slightly reduce formulation performance because of vesicle aggregation and reduced membrane stability.

This systematic optimization enabled identification of an optimum phospholipid-to-ethanol ratio

suitable for efficient delivery of herbal phytoconstituents through the skin.

Preparation of Polyherbal Ethosomal Gel

The optimized ethosomal dispersions were incorporated into Carbopol 940 gel to improve topical retention and patient compliance. Propylene glycol served as a humectant and permeation enhancer, whereas cholesterol stabilized the phospholipid bilayer. Triethanolamine neutralized Carbopol dispersion to obtain a smooth semisolid gel possessing skin-compatible pH.

The prepared gels exhibited uniform consistency, smooth texture, excellent appearance, and absence of phase separation. Incorporation of ethosomal vesicles into the gel matrix did not adversely influence physical characteristics, indicating excellent compatibility between the vesicular system and polymeric gel network.

The resulting formulation combined the advantages of ethosomal nanovesicles with a bioadhesive hydrogel system capable of prolonged skin residence and controlled release of phytoconstituents.

Evaluation of Polyherbal Ethosomal Gel

The developed polyherbal ethosomal gel formulations (PEG1–PEG5) containing *Salvia officinalis* leaf extract and *Sarcostemma brevistigma* stem extract were evaluated for various physicochemical parameters to identify the optimized formulation. The formulations were prepared by varying soya phosphatidylcholine and ethanol concentrations while maintaining other formulation variables constant. The evaluation parameters included spreadability, extrudability, drug content, particle size, polydispersity index, zeta potential, entrapment efficiency, in vitro drug release, skin irritation potential, and stability behavior.

Table 2. Physicochemical evaluation of polyherbal ethosomal gel formulations

Parameter	PEG 1	PEG 2	PEG3	PEG4	PEG 5
Appearance	Smooth pale green	Smooth pale green	Smooth pale green	Smooth pale green	Smooth pale green
Homogeneity	Good	Good	Excellent	Excellent	Good
pH	6.12 ± 0.05	6.28 ± 0.04	6.42 ± 0.03	6.55 ± 0.04	6.68 ± 0.05
Viscosity (cps)	24850 ± 210	26430 ± 225	28760 ± 240	31120 ± 255	33540 ± 270
Spreadability (g·cm/s)	18.4 ± 0.3	20.1 ± 0.4	22.6 ± 0.5	21.3 ± 0.4	19.8 ± 0.3
Extrudability	145	152	160 ±	168 ±	175

ility (g)	± 5	± 4	5	6	± 5
Drug content (%)	92.8 ± 1.1	95.2 ± 0.9	98.6 ± 0.8	97.4 ± 0.9	95.9 ± 1.0
Particle size (nm)	286.4 ± 8.5	241.7 ± 7.2	198.3 ± 6.4	214.5 ± 6.9	236.8 ± 7.5
PDI	0.321 ± 0.01	0.284 ± 0.02	0.216 ± 0.01	0.238 ± 0.02	0.267 ± 0.02
Zeta potential (mV)	-18.5 ± 0.7	-22.3 ± 0.8	-27.8 ± 0.9	-25.6 ± 0.8	-23.1 ± 0.7
Entrapment efficiency (%)	71.4 ± 1.3	78.9 ± 1.2	86.7 ± 1.0	84.2 ± 1.1	81.6 ± 1.2
Drug release at 8 h (%)	72.5 ± 1.5	81.3 ± 1.4	92.8 ± 1.2	89.6 ± 1.3	85.4 ± 1.4

Appearance and Homogeneity

All prepared formulations exhibited a smooth, uniform, pale green appearance without visible aggregation, phase separation, or particulate matter (Table 1). The pale green coloration was attributed to the incorporation of herbal extracts, whereas the smooth consistency indicated successful dispersion of ethosomal vesicles within the Carbopol 940 gel network. PEG3 and PEG4 demonstrated excellent homogeneity compared with PEG1, PEG2, and PEG5, indicating improved distribution of vesicles and better interaction between phospholipid components and the polymeric gel matrix.

Uniform homogeneity is a critical quality attribute for topical preparations because it ensures consistent distribution of active phytoconstituents during application. The absence of grittiness or phase separation suggested compatibility between herbal extracts, phosphatidylcholine, ethanol, and Carbopol 940.

Spreadability

Spreadability is an important characteristic of topical formulations because it determines ease of application and uniform distribution over the skin surface. The spreadability values of PEG1–PEG5 ranged from 18.4 ± 0.3 to 22.6 ± 0.5 g·cm/s. Among all formulations, PEG3 exhibited the highest spreadability (22.6 ± 0.5 g·cm/s), indicating an optimum balance between viscosity and gel consistency.

The enhanced spreadability of PEG3 may be attributed to its optimized concentration of phosphatidylcholine and ethanol, which provided adequate vesicle flexibility without producing excessive gel resistance. PEG4 and PEG5 showed reduced spreadability due to increased viscosity resulting from higher phospholipid concentration. Although increased viscosity improves retention

time on the skin, excessive viscosity may restrict spreading and patient convenience.

Extrudability

Extrudability represents the ability of a semisolid formulation to be expelled from a container with minimum applied force. The extrudability values increased gradually from 145 ± 5 g for PEG1 to 175 ± 5 g for PEG5. The increase was associated with increasing formulation viscosity.

PEG3 demonstrated acceptable extrudability (160 ± 5 g), providing a suitable balance between ease of dispensing and adequate consistency. The results indicate that all formulations possessed satisfactory handling properties for topical application.

Drug Content

Drug content analysis revealed uniform distribution of herbal phytoconstituents in all formulations, with values ranging from $92.8 \pm 1.1\%$ to $98.6 \pm 0.8\%$. PEG3 exhibited the highest drug content ($98.6 \pm 0.8\%$), indicating efficient incorporation of *Salvia officinalis* and *Sarcostemma brevistigma* extracts into the ethosomal system.

The high drug content of PEG3 may be attributed to optimum phospholipid concentration, which facilitated efficient encapsulation and minimized drug loss during preparation. The results confirmed reproducibility and uniformity of the developed formulations.

Particle Size and Polydispersity Index (PDI)

Particle size significantly influences skin penetration and biological performance of ethosomal systems. The particle size of prepared formulations ranged from 198.3 ± 6.4 to 286.4 ± 8.5 nm. PEG3 showed the smallest vesicle size (198.3 ± 6.4 nm), which is advantageous for enhanced penetration through the stratum corneum. The PDI values ranged from 0.216 ± 0.01 to 0.321 ± 0.01 . PEG3 exhibited the lowest PDI (0.216), indicating narrow particle size distribution and excellent vesicular uniformity. Nanocarriers with PDI values below 0.3 generally exhibit improved physical stability due to reduced aggregation tendency.

Zeta Potential

The zeta potential values ranged between -18.5 ± 0.7 and -27.8 ± 0.9 mV. PEG3 showed the highest negative surface charge (-27.8 ± 0.9 mV), suggesting strong electrostatic repulsion among vesicles and improved colloidal stability.

The negative charge may be attributed to phospholipid molecules present in the ethosomal bilayer. Higher absolute zeta potential values prevent vesicle aggregation and maintain formulation stability during storage.

Entrapment Efficiency

The entrapment efficiency of ethosomal gels ranged from $71.4 \pm 1.3\%$ to $86.7 \pm 1.0\%$. PEG3 demonstrated maximum entrapment efficiency ($86.7 \pm 1.0\%$), indicating effective incorporation of

bioactive constituents within the phospholipid bilayer.

The improved entrapment efficiency of PEG3 may be due to the optimized phosphatidylcholine-to-ethanol ratio, which enhanced membrane flexibility while maintaining vesicle integrity. Excess phospholipid concentration in PEG4 and PEG5 may have increased vesicle rigidity, resulting in slight reduction in encapsulation efficiency.

In Vitro Drug Release Study

The cumulative drug release from PEG formulations was evaluated for 8 h. PEG3 exhibited maximum drug release ($92.8 \pm 1.2\%$), followed by PEG4 ($89.6 \pm 1.3\%$) and PEG5 ($85.4 \pm 1.4\%$). The enhanced release from PEG3 may be attributed to smaller vesicle size, optimized viscosity, and higher entrapment efficiency. The flexible ethosomal vesicles facilitate interaction with skin lipids and promote controlled release of phytoconstituents.

Table 3. In vitro drug release profile

Formulation	Drug release at 8 h (%)
PEG1	72.5 ± 1.5
PEG2	81.3 ± 1.4
PEG3	92.8 ± 1.2
PEG4	89.6 ± 1.3
PEG5	85.4 ± 1.4

Skin Irritation Study

No erythema, edema, redness, or irritation was observed after topical application of PEG formulations. The absence of irritation confirms compatibility of the formulation components with skin tissue.

The skin-compatible pH range (6.12–6.68) and incorporation of biocompatible excipients contributed to the excellent dermal safety profile.

Stability Study (ICH)

PEG3 was selected as the optimized formulation and subjected to stability testing under ICH conditions for three months.

Table 4. Stability study of optimized PEG3 formulation

Condition	Drug content (%) after 3 months	pH	Viscosity (cps)
$4 \pm 2^\circ\text{C}$	98.32 ± 0.49	6.77 ± 0.04	31438 ± 122

25 ±2°C/60% RH	97.58 ±0.52	6.73 ±0.05	30968 ±136
40 ±2°C/75% RH	95.84 ±0.68	6.63 ±0.05	29824 ±151

PEG3 remained physically stable without phase separation or significant changes in appearance. Minor reductions in pH, viscosity, and drug content were observed at accelerated conditions; however, values remained within acceptable limits.

Statistical Analysis

All experimental values were expressed as mean ± SD/SEM (n=3 or n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. The optimized PEG3 formulation showed statistically significant improvement compared with other formulations ($p < 0.001$) for particle size, entrapment efficiency, drug release, HRBC stabilization, and BSA inhibition.

In Vitro Anti-inflammatory Activity

HRBC Membrane Stabilization Assay

The anti-inflammatory activity was evaluated by HRBC membrane stabilization assay. All formulations demonstrated concentration-dependent membrane protection.

Table 5. HRBC membrane stabilization activity

Treatment	Membrane stabilization (%)
PEG1	54.18 ±1.24
PEG2	63.47 ±1.18
PEG3	82.36 ±1.05
PEG4	76.84 ±1.27
PEG5	71.29 ±1.36
Diclofenac sodium	89.74 ±0.92

PEG3 exhibited maximum stabilization activity (82.36%), approaching diclofenac sodium (89.74%). The enhanced activity may be attributed to improved delivery of rosmarinic acid, flavonoids, phenolics, and other bioactive constituents through the ethosomal system.

BSA Protein Denaturation Assay

Table 6. Protein denaturation inhibition activity

Treatment	% Inhibition
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PEG1	63.41 ±1.48
PEG2	71.82 ±1.24
PEG3	86.38 ±1.05
PEG4	82.14 ±1.19
PEG5	77.65 ±1.37
Diclofenac sodium	89.72 ±1.16

PEG3 showed the highest inhibition of protein denaturation (86.38%), confirming its potent anti-inflammatory activity. The activity was comparable with diclofenac sodium and may be attributed to synergistic action of phytoconstituents from both plant extracts.

Based on comprehensive evaluation, PEG3 was selected as the optimized formulation. It exhibited excellent physicochemical properties including particle size of 198.3 nm, PDI 0.216, zeta potential -27.8 mV, entrapment efficiency 86.7%, drug release 92.8% at 8 h, and superior anti-inflammatory activity.

The optimized ethosomal gel demonstrated enhanced vesicular stability, improved topical delivery, and significant membrane stabilization and protein protection effects. The findings suggest that PEG3 represents a promising polyherbal topical system for management of inflammatory disorders such as rheumatoid arthritis and warrants further pharmacological investigation.

Conclusion

The present investigation successfully developed and optimized a polyherbal ethosomal gel containing ethanolic extracts of *Salvia officinalis* leaves and *Sarcostemma brevistigma* stems for topical delivery of anti-inflammatory phytoconstituents. Preliminary phytochemical screening demonstrated the presence of several biologically important secondary metabolites, while gallic acid estimation confirmed the presence of phenolic constituents in both extracts. The use of an ethosomal carrier provided a suitable platform for incorporating the herbal extracts into a nanosized vesicular system, while Carbopol 940 improved the consistency, spreadability, and topical residence of the formulation.

Among the five formulations, PEG3 was identified as the optimized formulation. It exhibited a particle size of 198.3 ± 6.4 nm, PDI of 0.216 ± 0.01 , zeta potential of -27.8 ± 0.9 mV, entrapment efficiency of $86.7 \pm 1.0\%$, drug content of $98.6 \pm 0.8\%$, and $92.8 \pm 1.2\%$ drug release at 8 h. These characteristics indicate the formation of a relatively uniform and physically stable vesicular system with efficient incorporation and release of the herbal phytoconstituents. The formulation also demonstrated satisfactory pH, viscosity,

spreadability, extrudability, and dermal compatibility.

The biological evaluation further supported the potential of the optimized formulation. PEG3 produced $82.36 \pm 1.05\%$ HRBC membrane stabilization and $86.38 \pm 1.05\%$ inhibition of BSA protein denaturation, demonstrating substantial anti-inflammatory activity compared with the other formulations and activity approaching that of diclofenac sodium. The enhanced activity may be associated with the combined effects of phenolic compounds, flavonoids, terpenoids, and other bioactive constituents present in *S. officinalis* and *S. brevistigma*, together with improved topical delivery afforded by the ethosomal system.

Overall, the findings indicate that the optimized polyherbal ethosomal gel represents a promising topical delivery approach for enhancing the therapeutic utilization of *Salvia officinalis* and *Sarcostemma brevistigma* in inflammatory conditions. Nevertheless, the present findings should be considered preliminary with respect to anti-arthritic efficacy. Further studies involving validated ex vivo skin permeation, pharmacokinetic assessment, detailed mechanistic investigations, chronic animal models of arthritis, comprehensive toxicity evaluation, and ultimately well-designed clinical studies are necessary to confirm its safety, efficacy, and therapeutic relevance in rheumatoid arthritis.

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