

Quality by Design-Based Development and Optimization of a *Parmotrema perlatum* Herbal Ointment: Analytical Characterization, In Vitro Release, and Pharmaceutical Evaluation

Ms. Ruksar Ibrahim Shaikh^{1*}, Dr. Allabaksha Mahboob Shaikh²

^{1*}Research scholar, MCE Society's Dr. P. A. Inamdar University, Allana College of Pharmacy, Azam Campus, Camp, Pune, Maharashtra. Email: ruksaritis@gmail.com
ORCID ID: 0009-0005-2257-064X

² Professor and HOD (Department of Pharmacology), MCE Society's Dr. P. A. Inamdar University, Allana College of Pharmacy, Azam Campus, Camp, Pune, Maharashtra Email: amaanshaikh.shaikh@gmail.com

Abstract

Parmotrema perlatum (Black Stone Flower) is a medicinal lichen known for its rich phytochemical composition and diverse pharmacological activities, including anti-inflammatory and antioxidant effects. Despite its therapeutic potential, limited research has focused on the development of standardized topical formulations utilizing this natural bioresource. The present study was undertaken to develop, optimize, and evaluate a *Parmotrema perlatum*-based herbal ointment using a Quality by Design (QbD) approach to ensure formulation robustness, reproducibility, and desirable pharmaceutical attributes.

The ethanolic extract of *Parmotrema perlatum* was prepared and subjected to phytochemical and analytical characterization. Preliminary phytochemical screening confirmed the presence of flavonoids, phenolic compounds, tannins, terpenoids, glycosides, and alkaloids. The extract was further characterized using UV-visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, and High-Performance Thin Layer Chromatography (HPTLC), which established its chemical profile and suitability for formulation development. A systematic QbD framework incorporating risk assessment, Design of Experiments (DoE), factorial design, and Response Surface Methodology (RSM) was employed to optimize formulation variables and identify critical quality attributes affecting product performance.

Several ointment batches were prepared and evaluated to obtain an optimized formulation. The selected formulation was assessed for physicochemical parameters including organoleptic properties, color and appearance, homogeneity, consistency, pH, spreadability, extrudability, washability, viscosity, and stability. The optimized ointment exhibited smooth texture, uniform consistency, acceptable pH, satisfactory spreadability, good extrudability, and desirable rheological behavior. Stability studies demonstrated the absence of significant physical changes during the storage period, indicating good formulation stability. In vitro drug release studies performed using Franz diffusion cell apparatus revealed sustained release of phytoconstituents from the ointment matrix, suggesting prolonged availability of active constituents at the site of application.

The findings of the study demonstrate that the QbD-based approach successfully facilitated the development of a stable and pharmaceutically acceptable *Parmotrema perlatum* herbal ointment with favorable physicochemical characteristics and controlled release behavior. The developed formulation represents a promising topical drug delivery system and provides a scientific foundation for further pharmacological evaluation and future clinical investigations.

Keywords: *Parmotrema perlatum*, Herbal ointment, Quality by Design, Formulation optimization, Topical drug delivery, FTIR, HPTLC, In vitro drug release, Pharmaceutical evaluation, Response Surface Methodology.

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INTRODUCTION

Topical drug delivery systems have gained considerable attention in recent years owing to their ability to deliver therapeutic agents directly to the site of action while minimizing systemic adverse effects. Among the various topical dosage forms, ointments remain one of the most widely used formulations because of their ability to enhance skin hydration, prolong drug residence time, and improve local therapeutic efficacy. The growing demand for safer and more effective treatments for inflammatory skin disorders has stimulated interest

in the development of herbal-based topical formulations containing bioactive natural products. Natural products have long served as valuable sources of therapeutic agents owing to their diverse chemical composition and pharmacological activities. Herbal medicines are increasingly being explored for the management of chronic inflammatory conditions because of their relatively favorable safety profiles and multitarget mechanisms of action. Among these natural resources, lichens represent a unique group of

symbiotic organisms known to produce a wide variety of secondary metabolites with significant biological activities. Several lichen-derived compounds have been reported to exhibit antimicrobial, antioxidant, anti-inflammatory, anticancer, and wound-healing properties, making them attractive candidates for pharmaceutical applications.

Parmotrema perlatum commonly known as Black Stone Flower, is a medicinal lichen widely distributed in tropical and subtropical regions. Traditionally, it has been used as a flavoring agent and in various indigenous medicinal systems for the treatment of inflammatory and infectious conditions. Phytochemical investigations of *P. perlatum* have revealed the presence of several bioactive constituents, including phenolic compounds, flavonoids, tannins, terpenoids, and characteristic lichen metabolites such as atranorin and other depsides and depsidones. These constituents have been associated with potent antioxidant and anti-inflammatory activities, suggesting the therapeutic potential of *P. perlatum* for the management of inflammatory skin disorders. Despite its promising pharmacological profile, the pharmaceutical development of *P. perlatum*-based topical formulations remains largely unexplored.

Successful development of herbal topical formulations requires not only incorporation of the active extract into a suitable base but also systematic optimization of formulation variables to ensure product quality, stability, and reproducibility. Conventional formulation development approaches often rely on trial-and-error experimentation, which may be time-consuming and may fail to adequately identify the influence of critical formulation variables. In recent years, the Quality by Design (QbD) paradigm has emerged as a scientifically sound and systematic approach for pharmaceutical product development. QbD emphasizes predefined quality objectives, risk assessment, understanding of formulation and process variables, and establishment of design space to achieve consistent product performance.

The application of QbD principles in semisolid dosage forms enables identification of critical material attributes (CMAs) and critical process parameters (CPPs) that influence critical quality attributes (CQAs) such as viscosity, spreadability, homogeneity, extrudability, stability, and drug release characteristics. Furthermore, statistical tools such as Design of Experiments (DoE) and Response Surface Methodology (RSM) facilitate efficient optimization of formulation variables while minimizing experimental workload. The integration of QbD principles into herbal formulation development can significantly improve product robustness, reproducibility, and overall quality.

In addition to formulation optimization, comprehensive analytical characterization of herbal

extracts is essential to ensure standardization and quality control. Techniques such as UV-visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, and High-Performance Thin Layer Chromatography (HPTLC) provide valuable information regarding phytochemical composition, functional groups, and chemical fingerprints of herbal materials. These analytical tools contribute to the establishment of reproducible and scientifically validated herbal formulations.

Considering the therapeutic potential of *Parmotrema perlatum* and the need for standardized herbal topical products, the present study was undertaken to develop and optimize a *Parmotrema perlatum*-loaded herbal ointment using a Quality by Design approach. The extract was subjected to phytochemical and analytical characterization, followed by systematic formulation development and optimization. The optimized ointment was evaluated for physicochemical properties, stability, and in vitro drug release behavior. The study aims to provide a scientific basis for the development of a stable, effective, and pharmaceutically acceptable topical delivery system based on *Parmotrema perlatum*, thereby contributing to the growing field of herbal drug delivery and formulation science.

1. MATERIALS AND METHODS

Materials

Plant Material

The dried thalli of *P. perlatum* were procured from a certified herbal supplier and authenticated by a qualified taxonomist in the Department of Botany. A voucher specimen was prepared and deposited for future reference.

The collected material was carefully examined to remove extraneous matter such as dust, soil particles, and foreign plant materials. The cleaned lichen material was washed with distilled water, shade-dried at room temperature, and pulverized using a mechanical grinder to obtain a coarse powder. The powdered material was passed through a suitable sieve to achieve uniform particle size and stored in airtight containers protected from light, moisture, and environmental contamination until further use.

Chemicals and Reagents

All chemicals, solvents, and reagents used in the study were of analytical reagent (AR) grade and were utilized without further purification.

Ethanol was employed as the extraction solvent for preparation of the *Parmotrema perlatum* extract. Methanol, chloroform, acetone, and distilled water were used during solubility studies and analytical investigations. Petroleum jelly, hard paraffin, and cetostearyl alcohol were used as major components of the ointment base during formulation development.

Phosphate buffer solutions were prepared and used during pH determination and in vitro drug release studies. Reagents required for phytochemical

screening, including Dragendorff's reagent, Mayer's reagent, Wagner's reagent, ferric chloride solution, sodium hydroxide solution, concentrated sulfuric acid, glacial acetic acid, and other analytical reagents, were employed for qualitative identification of phytoconstituents.

For analytical characterization, HPTLC-grade solvents and reagents were used for chromatographic fingerprinting studies, while spectroscopic-grade chemicals were utilized during UV-visible and FTIR analyses.

All reagents were freshly prepared wherever required, and solutions were stored under appropriate laboratory conditions to maintain their stability and analytical reliability throughout the study.

Instruments and Equipment

The experimental work was carried out using standard laboratory instruments and analytical equipment. The extraction of *Parmotrema perlatum* was performed using a Soxhlet extraction apparatus, while concentration of the extract was carried out using a rotary vacuum evaporator.

A UV-Visible spectrophotometer was used for spectroscopic analysis and determination of absorption characteristics of the extract. Fourier Transform Infrared (FTIR) spectroscopy was employed for identification of functional groups and characterization of phytoconstituents. High-Performance Thin Layer Chromatography (HPTLC) equipment was utilized for chromatographic fingerprinting and extract standardization.

A calibrated digital pH meter was used for determination of pH of the formulated ointments. Viscosity measurements were performed using a Brookfield rotational viscometer. Analytical weighing balance with appropriate sensitivity was employed for accurate weighing of plant material, chemicals, and formulation components.

The spreadability apparatus and extrudability testing setup were used for evaluation of semisolid formulations. Stability studies were conducted using a stability chamber maintained under controlled temperature and humidity conditions. In vitro drug release studies were carried out using a Franz diffusion cell apparatus coupled with a magnetic stirrer and thermostatically controlled water bath.

Other commonly used laboratory equipment included a hot plate, magnetic stirrer, mechanical grinder, sieve set, laboratory glassware, micropipettes, centrifuge, water bath, desiccator, and refrigerator.

All instruments were calibrated periodically according to standard laboratory procedures to ensure accuracy, precision, and reproducibility of the experimental results.

2. Preparation and Standardization of Extract Collection and Authentication of *Parmotrema perlatum*

The collected material was carefully inspected for the presence of foreign matter, dust particles, and other contaminants. Morphological characteristics including color, texture, and thallus morphology were examined and compared with standard taxonomic descriptions to confirm authenticity. The authenticated material was stored in clean polyethylene bags under dry conditions until further processing.

Processing of Plant Material

The authenticated *Parmotrema perlatum* thalli were washed thoroughly with distilled water to remove adhering dust and impurities. The cleaned material was shade-dried at room temperature ($25 \pm 2^\circ\text{C}$) for 10 days to prevent degradation of thermolabile phytoconstituents.

After complete drying, the material was pulverized using a mechanical grinder to obtain a coarse powder. The powdered material was passed through sieve No. 40 to achieve uniform particle size distribution. The sieved powder was collected and stored in airtight amber-colored containers protected from moisture, light, and environmental contamination until extraction.

The total quantity of dried powdered material obtained after processing was approximately 1.2 kg and was used for subsequent extraction studies.

Solubility Studies

Preliminary solubility studies were performed to identify the most suitable solvent for extraction of bioactive constituents from *Parmotrema perlatum*. Five different solvents with varying polarities were selected, namely distilled water, methanol, ethanol, acetone, and chloroform.

One gram of powdered *Parmotrema perlatum* was transferred separately into test tubes containing 10 mL of each solvent. The mixtures were vortexed for 5 min and allowed to stand at room temperature for 24 h with intermittent shaking. The degree of dissolution was assessed visually based on clarity, sedimentation, and extractive behavior.

Among the solvents evaluated, ethanol exhibited maximum solubilization of phytoconstituents and produced a dark brown extract with minimal insoluble residue. Methanol showed moderate solubility, whereas distilled water, acetone, and chloroform demonstrated comparatively lower extraction efficiency. Based on these observations, ethanol was selected as the extraction solvent for subsequent studies.

Preparation of Ethanolic Extract

The ethanolic extract of *Parmotrema perlatum* was prepared using the Soxhlet extraction technique. Approximately 500 g of powdered lichen material was packed in a cellulose extraction thimble and placed in a Soxhlet extractor. Extraction was carried out using 2.5 L of 95% ethanol as solvent. The extraction process was continued for 18 h at a controlled temperature of 60–70°C until the siphon

tube solvent became colorless, indicating exhaustive extraction.

The obtained extract was filtered through Whatman No. 1 filter paper to remove particulate matter. The filtrate was concentrated under reduced pressure using a rotary vacuum evaporator maintained at 45°C. The concentrated extract was further dried in a vacuum desiccator until a constant weight was obtained.

A dark brown semisolid extract with characteristic aromatic odor was obtained and stored in airtight amber-colored containers at 4°C for further phytochemical, analytical, and formulation studies.

Percentage Yield Determination

The extraction efficiency was determined by calculating the percentage yield of the dried ethanolic extract.

The weight of the dried extract obtained after solvent evaporation was recorded accurately using an analytical balance. Percentage yield was calculated using the following equation:

$$\text{Percentage Yield (\%)} = (\text{Weight of Dried Extract} / \text{Weight of Powdered Plant Material}) \times 100$$

For the present study, 500 g of powdered *Parmotrema perlatum* yielded 82.5 g of dried ethanolic extract.

Therefore:

$$\text{Percentage Yield (\%)} = (82.5 / 500) \times 100$$

$$\text{Percentage Yield (\%)} = 16.5\%$$

The percentage yield obtained indicated good extraction efficiency of ethanol for recovery of phytoconstituents from *Parmotrema perlatum*. The extract was subsequently utilized for phytochemical screening, analytical characterization, formulation development, and in vitro evaluation studies.

3. Phytochemical and Analytical Characterization

Preliminary Phytochemical Screening

The ethanolic extract of *Parmotrema perlatum* was subjected to preliminary qualitative phytochemical screening to identify the major classes of secondary metabolites present in the extract. Standard phytochemical tests were performed for the detection of alkaloids, flavonoids, phenolic compounds, tannins, glycosides, terpenoids, saponins, and steroids.

For alkaloid detection, Mayer's, Wagner's, and Dragendorff's tests were employed. Flavonoids were evaluated using the alkaline reagent test and Shinoda test. Ferric chloride test was performed for the detection of phenolic compounds and tannins. Glycosides were identified using Keller–Killiani test, while terpenoids were detected by Salkowski reaction. Foam test was used for the detection of saponins and Liebermann–Burchard test was performed for identification of steroids.

All tests were carried out in triplicate, and observations were recorded based on the appearance of characteristic color changes or precipitates according to standard phytochemical procedures.

UV–Visible Spectroscopic Analysis

UV–Visible spectroscopic analysis of the ethanolic extract was performed using a UV–Visible spectrophotometer to establish its absorption characteristics.

A stock solution of the extract was prepared by dissolving an accurately weighed quantity of dried extract in ethanol. The solution was filtered through a 0.45 µm membrane filter prior to analysis.

The absorbance spectrum was recorded over the wavelength range of 200–800 nm using ethanol as the blank. Quartz cuvettes with a path length of 1 cm were used throughout the analysis. The scan was performed at room temperature, and the absorption spectrum was recorded for further characterization and selection of analytical wavelength for subsequent studies.

FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy was carried out to identify the functional groups present in the ethanolic extract of *Parmotrema perlatum*.

The dried extract was mixed with spectroscopic-grade potassium bromide (KBr) and compressed into pellets using a hydraulic press. The prepared pellets were scanned using an FTIR spectrophotometer over the spectral range of 4000–400 cm⁻¹ at appropriate resolution settings.

The obtained spectra were recorded and used for identification of characteristic functional groups present in the extract. The spectral data were subsequently analyzed by comparing the absorption bands with standard reference values reported in the literature.

HPTLC Fingerprinting

High-Performance Thin Layer Chromatography (HPTLC) fingerprinting was performed to establish the chromatographic profile of the ethanolic extract and to facilitate its standardization.

The extract solution was prepared in methanol and filtered through a membrane filter before application. Chromatographic separation was carried out using precoated silica gel 60 F254 aluminum plates. Sample application was performed using an automated sample applicator.

The plates were developed in a pre-saturated twin-trough chromatographic chamber using a suitable mobile phase system optimized for separation of phytoconstituents. After development, the plates were dried and scanned using an HPTLC scanner at selected wavelengths.

Densitometric analysis was carried out using chromatographic software, and the fingerprint profile of the extract was recorded for quality control and standardization purposes.

HPLC Analysis

High-Performance Liquid Chromatography (HPLC) analysis was carried out for quantitative estimation and chromatographic characterization of selected phytoconstituents present in the ethanolic extract.

The extract solution was prepared in HPLC-grade methanol, filtered through a 0.22 µm membrane filter, and injected into the HPLC system. Separation was achieved using a reverse-phase C18 column maintained at controlled temperature conditions. The mobile phase consisted of suitable solvent systems delivered at a constant flow rate. Detection was carried out using a UV detector at an appropriate wavelength selected based on the absorption characteristics of the extract.

Chromatographic data including retention times and peak characteristics were recorded and used for standardization and quantitative evaluation of the extract.

4. Quality by Design-Based Formulation Development

A Quality by Design (QbD) approach was adopted for the systematic development and optimization of the *Parmotrema perlatum* herbal ointment.

Quality Target Product Profile (QTPP)

The Quality Target Product Profile was established prior to formulation development to define the desired characteristics of the final product. The target product was designed as a semisolid herbal ointment intended for topical application in inflammatory skin disorders.

The predefined quality objectives included:

- Smooth semisolid appearance
- Uniform distribution of extract
- Acceptable skin-compatible pH (5.5–7.0)
- Good spreadability
- Adequate extrudability
- Suitable viscosity for topical retention
- Physical stability during storage
- Sustained release of phytoconstituents
- Patient acceptability and ease of application

The QTPP served as the foundation for identification of critical quality attributes and formulation optimization.

Identification of Critical Quality Attributes (CQAs)

Based on the intended use and QTPP, critical quality attributes were identified as formulation characteristics that could significantly influence product quality, performance, and patient acceptability.

The following CQAs were selected:

- pH
- Viscosity
- Spreadability
- Extrudability
- Homogeneity
- Consistency
- Physical stability
- Drug release profile

These attributes were monitored throughout formulation development and optimization. Acceptance criteria were established based on

pharmaceutically acceptable limits and literature reports for topical semisolid dosage forms.

Risk Assessment

A preliminary risk assessment was performed to identify formulation variables that could potentially affect the critical quality attributes of the ointment.

An Ishikawa (fishbone) diagram was constructed to evaluate possible sources of variability associated with:

- Raw materials
- Extract concentration
- Base composition
- Manufacturing process
- Environmental conditions
- Analytical procedures

Subsequently, a Failure Mode and Effects Analysis (FMEA) approach was adopted to rank the formulation variables according to their potential impact on product quality.

Risk Priority Numbers (RPNs) were assigned based on:

- Severity (S)
- Occurrence (O)
- Detectability (D)

Each parameter was scored on a scale of 1–10, and the RPN was calculated using:

$$\text{RPN} = \text{S} \times \text{O} \times \text{D}$$

Variables exhibiting higher RPN values were considered critical and selected for further optimization through Design of Experiments.

Selection of Critical Material Attributes (CMAs)

Based on risk assessment outcomes and preliminary formulation trials, the following formulation variables were identified as Critical Material Attributes (CMAs):

Independent Variables

X₁ = Concentration of *Parmotrema perlatum* extract (% w/w)

X₂ = Concentration of cetostearyl alcohol (% w/w)

X₃ = Concentration of hard paraffin (% w/w)

The concentration ranges selected for optimization were:

- Extract concentration: 2–10% w/w
- Cetostearyl alcohol: 3–8% w/w
- Hard paraffin: 2–6% w/w

These variables were selected because preliminary trials demonstrated their significant influence on spreadability, viscosity, consistency, and drug release characteristics of the formulation.

Design of Experiments (DoE)

A systematic Design of Experiments approach was employed to evaluate the influence of formulation variables on the quality attributes of the ointment.

Initially, several trial batches were prepared using a conventional trial-and-error approach. Based on the observations obtained, a factorial experimental design was selected for further optimization.

A three-factor, three-level design was employed to investigate the effects of:

- Extract concentration (X_1)
- Cetostearyl alcohol concentration (X_2)
- Hard paraffin concentration (X_3)

on the selected response variables.

The dependent responses evaluated included:

Y_1 = Spreadability

Y_2 = Viscosity

Y_3 = Extrudability

Y_4 = In vitro drug release

Experimental runs were generated using Design-Expert® software. Each formulation batch was prepared according to the experimental design matrix and evaluated under identical conditions.

The generated experimental data were subjected to analysis of variance (ANOVA) to assess the significance of formulation variables and their interactions.

Response Surface Methodology (RSM)

Response Surface Methodology was employed to establish mathematical relationships between formulation variables and response parameters.

The experimental data generated from the DoE study were fitted into a second-order polynomial model represented by:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

where:

Y = response variable

β_0 = intercept

β_1 – β_3 = regression coefficients

X_1 – X_3 = formulation variables

Three-dimensional response surface plots and contour plots were generated using Design-Expert® software to visualize the effect of formulation variables on critical quality attributes.

The generated models were used to predict the optimum formulation region and evaluate variable interactions.

Optimization Strategy

Numerical optimization was performed using the desirability function approach available in Design-Expert® software.

The optimization criteria were defined as follows:

- Maximum spreadability
- Optimum viscosity
- Maximum extrudability
- Acceptable pH
- Maximum drug release
- Good physical stability

The software generated optimized formulation compositions based on the predefined constraints.

The optimized formulation was subsequently selected for detailed physicochemical evaluation, stability studies, and in vitro release investigations.

5. Formulation of Herbal Ointment

Composition of Ointment Formulation

Initially, nine trial batches (F1–F9) were prepared by varying the concentrations of extract, hard paraffin, and cetostearyl alcohol while maintaining the quantity of white soft paraffin as the base

component. The concentration of *Parmotrema perlatum* extract was varied between 2–10% w/w, cetostearyl alcohol between 3–8% w/w, and hard paraffin between 2–6% w/w.

Based on preliminary evaluation and QbD optimization studies, an optimized formulation was selected for further investigations.

The composition of the optimized formulation prepared per 100 g batch is presented below:

Table 1. Composition of the optimized formulation

Ingredient	Quantity (% w/w)
<i>Parmotrema perlatum</i> extract	5.0
Cetostearyl alcohol	5.0
Hard paraffin	3.0
White soft paraffin	q.s. to 100 g

The selected formulation composition was found suitable for obtaining the desired semisolid consistency, spreadability, viscosity, and stability required for topical application.

Preparation of Ointment Base

The ointment base was prepared by the fusion method using white soft paraffin, hard paraffin, and cetostearyl alcohol.

Accurately weighed quantities of hard paraffin and cetostearyl alcohol were transferred into a stainless-steel beaker and heated on a thermostatically controlled water bath maintained at $70 \pm 2^\circ\text{C}$. After complete melting of these ingredients, white soft paraffin was added gradually to the molten mixture with continuous stirring.

The entire system was maintained at the same temperature until complete liquefaction and formation of a clear homogeneous mixture. The molten base was stirred continuously using a mechanical stirrer operating at approximately 300 rpm to ensure uniform mixing of all components.

After complete homogenization, the molten ointment base was allowed to cool gradually to approximately 40 – 45°C while continuous stirring was maintained to prevent crystallization and phase separation. The prepared base was then subjected to visual examination to confirm homogeneity and absence of particulate matter prior to incorporation of the herbal extract.

Incorporation of Herbal Extract

The dried ethanolic extract of *Parmotrema perlatum* was incorporated into the prepared ointment base using the geometric dilution method.

The required quantity of extract was accurately weighed and triturated in a porcelain mortar to obtain a fine and uniform consistency. A small quantity of molten ointment base maintained at

approximately 40°C was gradually added to the extract and mixed thoroughly to form a smooth paste.

Subsequently, increasing quantities of ointment base were incorporated in a stepwise manner with continuous trituration to ensure uniform distribution of the extract throughout the formulation. The process was continued until the entire quantity of ointment base was incorporated.

The resulting formulation was mixed continuously for approximately 15–20 min to achieve complete homogeneity and eliminate the possibility of aggregation or localized concentration gradients. The prepared ointment was allowed to cool to room temperature and was then transferred into sterile, wide-mouth amber glass containers for further evaluation.

Preparation of Optimized Formulation

Based on the experimental design matrix, multiple formulation batches were prepared and evaluated for critical quality attributes including spreadability, viscosity, extrudability, homogeneity, pH, and in vitro drug release. The formulation variables selected for optimization included extract concentration, cetostearyl alcohol concentration, and hard paraffin concentration.

Each experimental batch was prepared using the same fusion method under identical manufacturing conditions to minimize process variability. The batch size for each experimental run was maintained at 100 g.

Following statistical analysis and numerical optimization, the software-generated optimized formulation was prepared independently for validation purposes. The optimized batch was manufactured according to the predicted formulation composition and subjected to comprehensive physicochemical evaluation.

To ensure reproducibility, the optimized formulation was prepared in triplicate under identical experimental conditions. The prepared batches were stored in well-closed containers at room temperature ($25 \pm 2^\circ\text{C}$) and subsequently utilized for physicochemical evaluation, stability studies, and in vitro drug release investigations.

6. Evaluation of Ointment Formulation

The prepared ointment formulations were evaluated for various physicochemical parameters to assess their suitability for topical administration.

Organoleptic Evaluation

The prepared ointment formulations were subjected to organoleptic evaluation immediately after preparation and during storage studies.

Approximately 2 g of ointment was withdrawn from each formulation batch and examined visually under normal daylight conditions. The formulations were evaluated for color, odor, texture, smoothness, grittiness, and overall appearance.

The texture of the formulation was assessed by gently rubbing a small quantity of ointment between

the thumb and index finger. The presence of coarse particles, phase separation, lump formation, or any undesirable characteristics was carefully observed.

Each formulation was evaluated independently by three observers to minimize subjective bias. The observations were recorded and compared among the different formulation batches.

Color and Appearance

Color and appearance of the formulations were evaluated visually under standardized lighting conditions.

Approximately 1 g of ointment was spread uniformly on a clean glass slide to form a thin film and examined against both white and black backgrounds. The formulations were assessed for:

- Uniformity of color
- Surface smoothness
- Glossiness
- Presence of particulate matter
- Evidence of phase separation
- Surface cracking
- Syneresis

The observations were recorded immediately after preparation and periodically during stability studies to monitor any changes in appearance.

Homogeneity

Homogeneity of the ointment formulations was evaluated by visual inspection and tactile examination.

Approximately 0.5 g of formulation was spread uniformly on a clean glass slide and observed against a light source. The presence of lumps, aggregates, coarse particles, and non-uniform distribution of extract was examined.

The formulations were further rubbed gently between the thumb and index finger to assess smoothness and detect any grittiness. The evaluation was performed in triplicate to confirm consistency of observations.

Consistency

Consistency of the ointment formulations was evaluated manually by assessing firmness, spreadability behavior, and resistance to deformation.

Approximately 2 g of ointment was placed on a glass plate and subjected to gentle pressure using a stainless-steel spatula. The resistance offered by the formulation during spreading was observed.

The formulations were evaluated for:

- Softness
- Firmness
- Ease of spreading
- Structural integrity

Consistency was further assessed at room temperature ($25 \pm 2^\circ\text{C}$) to determine suitability for topical application and storage stability.

Each batch was evaluated in triplicate, and observations were recorded comparatively.

pH Determination

The pH of the ointment formulations was determined using a calibrated digital pH meter. One gram of ointment was accurately weighed and dispersed in 10 mL of distilled water. The dispersion was allowed to stand for 2 h at room temperature with intermittent stirring to facilitate equilibration. The pH meter was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.2 before analysis. The electrode was immersed into the prepared dispersion, and the pH was recorded after stabilization of the reading. Three independent measurements were performed for each formulation, and the mean value was calculated.

Spreadability

Spreadability of the ointment formulations was evaluated using the parallel-slide method.

Approximately 1 g of ointment was placed between two glass slides of equal dimensions (20 cm × 20 cm). A weight of 500 g was placed on the upper slide for 5 min to produce a uniform film of the formulation between the slides and remove entrapped air.

After removal of excess ointment, a weight of 50 g was attached to the upper slide through a pulley arrangement.

The time required for the upper slide to move a fixed distance of 7.5 cm was recorded.

Spreadability was calculated using the equation:

$$S = M \times L / T$$

Where:

S = Spreadability (g·cm/sec)

M = Weight tied to the upper slide (g)

L = Length moved by the glass slide (cm)

T = Time taken to separate the slides (sec)

Each measurement was performed in triplicate.

Extrudability

Extrudability was determined by measuring the ease with which the ointment could be extruded from collapsible aluminum tubes.

Approximately 15 g of ointment was filled into lacquered aluminum collapsible tubes and sealed properly.

The tubes were placed between two glass slides and subjected to a constant force of 1 kg/cm².

The quantity of ointment extruded from the nozzle within 10 s was collected and weighed. Extrudability was calculated as the percentage of ointment extruded relative to the total content present in the tube.

Three independent determinations were performed for each formulation batch.

Washability

Washability studies were performed to assess the ease of removal of the formulation from the skin surface.

Approximately 0.5 g of ointment was applied uniformly over a marked area (4 cm²) on the dorsal surface of the hand. The formulation was allowed to remain in contact with the skin for 5 min.

The treated area was subsequently washed with running tap water without the use of soap or detergents.

The ease of removal of the formulation and the presence of residual greasiness were evaluated visually and recorded qualitatively.

The procedure was repeated three times for each formulation.

Viscosity Determination

Viscosity measurements were performed using a Brookfield rotational viscometer.

Approximately 50 g of ointment formulation was transferred into the sample container and allowed to equilibrate at 25 ± 1°C for 30 min before analysis.

The viscosity was measured using spindle No. 64 at a rotational speed of 10 rpm. Measurements were recorded after stabilization of the instrument reading.

To evaluate rheological behavior, viscosity was also measured at different rotational speeds ranging from 5–50 rpm.

All measurements were performed in triplicate, and the average value was calculated.

The obtained viscosity data were used for comparative evaluation of formulation batches and assessment of semisolid flow behavior.

7. Stability Studies

Stability studies of the optimized *Parmotrema perlatum* ointment formulation were performed to evaluate the effect of storage conditions on the physical integrity, pharmaceutical quality, and overall stability of the formulation over time.

The studies were conducted in accordance with the International Council for Harmonisation (ICH) guidelines for stability testing of pharmaceutical products. The optimized ointment formulation was packed in lacquered aluminum collapsible tubes and amber-colored glass containers to minimize exposure to environmental factors such as light, moisture, and air.

Three independent batches of the optimized formulation were prepared and subjected to stability evaluation under accelerated storage conditions.

Accelerated Stability Studies

Accelerated stability studies were performed according to ICH Q1A(R2) guidelines.

Approximately 100 g of the optimized ointment formulation was filled into collapsible aluminum tubes and properly sealed. The packaged formulations were placed in a stability chamber maintained at:

Accelerated Storage Condition

- Temperature: 40 ± 2°C
- Relative Humidity: 75 ± 5%

The formulations were stored under these conditions for a period of 3 months.

Samples were withdrawn at predetermined intervals:

- Initial (0 month)
- 1 month
- 2 months

- 3 months

At each sampling interval, the formulations were evaluated for changes in physicochemical properties and overall product stability.

In addition to accelerated stability testing, samples were also maintained under room-temperature conditions:

Long-Term Storage Condition

- Temperature: $25 \pm 2^\circ\text{C}$
- Relative Humidity: $60 \pm 5\%$

for comparative observation throughout the study period.

All evaluations were performed in triplicate to ensure reproducibility and reliability of observations.

Evaluation Parameters During Storage

At each storage interval, the withdrawn samples were examined for any changes in physical appearance, consistency, and performance characteristics.

The following parameters were evaluated:

Organoleptic Characteristics

The formulations were visually inspected for:

- Color changes
- Odor changes
- Surface appearance
- Phase separation
- Liquefaction
- Syneresis
- Cracking

Homogeneity

The formulations were examined for:

- Uniformity
- Presence of lumps
- Aggregation
- Particle formation
- Grittiness

pH Determination

The pH of the stored formulations was determined using a calibrated digital pH meter following the procedure described previously.

Measurements were performed in triplicate and the mean value was recorded.

Spreadability

Spreadability measurements were carried out at each storage interval using the parallel-slide method to determine whether storage affected the ease of application of the formulation.

Extrudability

Extrudability testing was performed to evaluate the ability of the formulation to be dispensed from the collapsible tube after prolonged storage.

The quantity of ointment extruded under standardized pressure conditions was determined and compared throughout the study period.

Viscosity Determination

The viscosity of the stored formulations was measured using a Brookfield rotational viscometer.

Measurements were performed under identical experimental conditions used during initial evaluation to assess any rheological changes occurring during storage.

Drug Release Study

The in vitro drug release behavior of the stored formulation was evaluated at the beginning and completion of the stability study using the Franz diffusion cell method.

The release profile was compared to determine whether storage conditions influenced the release characteristics of the formulation.

Physical Stability Assessment

The formulations were also observed for:

- Oil separation
- Crystallization
- Hardening
- Softening
- Drying of formulation
- Container compatibility issues

The absence of significant changes in these parameters was considered indicative of satisfactory formulation stability.

All observations and analytical measurements obtained during stability testing were recorded systematically and subsequently subjected to statistical evaluation.

8. In Vitro Drug Release Studies

In vitro drug release studies were performed to evaluate the release behavior of phytoconstituents from the optimized *Parmotrema perlatum* ointment formulation. The study was conducted using a Franz diffusion cell apparatus under controlled laboratory conditions. The release profile was investigated to assess the ability of the formulation to provide sustained delivery of bioactive constituents across a semipermeable membrane.

Franz Diffusion Cell Method

The in vitro release study was carried out using a vertical Franz diffusion cell apparatus having an effective diffusion area of 3.14 cm^2 and a receptor compartment capacity of 20 mL.

A dialysis membrane (molecular weight cut-off 12,000–14,000 Da) was used as the diffusion membrane. Prior to use, the membrane was soaked overnight in phosphate buffer solution (pH 6.8) to ensure complete hydration and removal of preservatives.

The receptor compartment was filled with freshly prepared phosphate buffer (pH 6.8) and maintained at $37 \pm 0.5^\circ\text{C}$ using a thermostatically controlled water bath to simulate physiological skin temperature. Continuous stirring was maintained at 100 rpm using a magnetic stirrer throughout the experiment.

Approximately 1 g of the optimized ointment formulation was accurately weighed and uniformly spread onto the surface of the hydrated membrane mounted between the donor and receptor compartments.

At predetermined time intervals of:

- 0.5 h
- 1 h
- 2 h
- 4 h
- 6 h
- 8 h
- 10 h
- 12 h

1 mL aliquots were withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh phosphate buffer maintained at the same temperature to maintain sink conditions. The collected samples were filtered through a 0.45 μ m membrane filter and analyzed using a UV–Visible spectrophotometer at the previously determined analytical wavelength.

The cumulative percentage drug release was calculated using the measured absorbance values and corresponding calibration curve data.

The release study was continued for 12 h to evaluate the sustained-release characteristics of the formulation.

Release Kinetics Analysis

The in vitro release data obtained from the Franz diffusion cell study were subjected to kinetic modeling to determine the mechanism of drug release from the ointment matrix.

The cumulative drug release data were fitted into various mathematical models commonly used for semisolid formulations, including:

Zero-Order Model

This model was used to evaluate whether the drug release occurred at a constant rate independent of concentration.

The cumulative percentage drug released was plotted against time.

First-Order Model

The first-order model was used to determine whether drug release was dependent upon the concentration of active constituents remaining within the formulation.

The logarithm of the percentage drug remaining was plotted against time.

Higuchi Model

The Higuchi diffusion model was applied to evaluate drug release through diffusion-controlled mechanisms.

The cumulative percentage drug released was plotted against the square root of time.

Korsmeyer–Peppas Model

The Korsmeyer–Peppas equation was employed to investigate the release mechanism of phytoconstituents from the semisolid matrix.

The logarithm of cumulative percentage drug release was plotted against the logarithm of time.

The release exponent (n) was determined from the slope of the regression line to characterize the mechanism of release.

Hixson–Crowell Model

The Hixson–Crowell cube root model was also evaluated to assess the influence of changes in surface area and particle dimensions on the release process.

Appropriate plots were generated using the experimental release data.

For each kinetic model, the coefficient of determination (R^2) was calculated using linear regression analysis. The model exhibiting the highest R^2 value was considered the best-fit model describing the release kinetics of the optimized formulation.

All calculations and kinetic analyses were performed using GraphPad Prism® software and Microsoft Excel®. The generated release profiles and kinetic plots were subsequently utilized for comparative evaluation of the formulation performance.

9. Statistical Analysis

Statistical analysis was performed to evaluate the significance, reliability, and reproducibility of the experimental data obtained during extract characterization, formulation development, physicochemical evaluation, stability studies, and in vitro drug release investigations.

All experiments were carried out in triplicate ($n = 3$), and the results were expressed as mean $\pm$ standard deviation (SD). The mean values and standard deviations were calculated using standard statistical procedures.

The data generated during formulation optimization studies were analyzed using Design-Expert® software (Version 13, Stat-Ease Inc., Minneapolis, USA). Experimental runs generated through the Design of Experiments (DoE) approach were evaluated using multiple regression analysis and Analysis of Variance (ANOVA).

For the Quality by Design (QbD)-based optimization studies, the influence of independent variables, including extract concentration, cetostearyl alcohol concentration, and hard paraffin concentration, on the selected response variables was assessed using a second-order polynomial model. The adequacy of the developed mathematical models was evaluated based on:

- Regression coefficient (R^2)
- Adjusted R^2
- Predicted R^2
- Adequate precision
- Lack-of-fit test

Analysis of variance (ANOVA) was employed to determine the statistical significance of the model terms and interaction effects. A p -value of less than 0.05 was considered statistically significant.

Response surface plots and contour plots were generated using Design-Expert® software to visualize the effect of formulation variables on critical quality attributes and to identify the optimum formulation region.

For physicochemical evaluation studies, including pH, spreadability, viscosity, extrudability, and stability parameters, the obtained values were analyzed descriptively and compared among formulation batches.

The in vitro drug release data were analyzed using GraphPad Prism® (Version 9.0, GraphPad Software Inc., San Diego, USA) and Microsoft Excel®. The cumulative drug release profiles were subjected to kinetic modeling using Zero-order, First-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell equations.

Linear regression analysis was performed for each kinetic model, and the coefficient of determination (R^2) was calculated. The model exhibiting the highest R^2 value was considered the best-fit model for describing the release kinetics of the optimized formulation.

For comparative evaluation of formulation batches, one-way Analysis of Variance (One-Way ANOVA) was applied wherever appropriate. When significant differences were observed, post hoc multiple comparison analysis was performed using Tukey's test.

The confidence level for all statistical analyses was maintained at 95%, and differences were considered statistically significant when $p < 0.05$.

All statistical analyses, graphical representations, response surface plots, regression models, and kinetic plots were generated using validated software packages to ensure accuracy and reproducibility of the experimental findings.

2. RESULTS AND DISCUSSION

Solubility Study Results

The solubility behavior of *Parmotrema perlatum* powder was evaluated in various solvents of different polarities to identify a suitable solvent for extraction. The extract exhibited maximum solubility in ethanol, followed by methanol, while lower solubility was observed in acetone, chloroform, and distilled water.

Table 2. Solubility Profile of *Parmotrema perlatum*

Solvent	Solubility Observation
Distilled Water	Slightly Soluble (+)
Chloroform	Moderately Soluble (++)
Acetone	Moderately Soluble (++)
Methanol	Highly Soluble (+++)
Ethanol	Very Highly Soluble (++++)

The superior solubility observed in ethanol indicated its suitability for efficient extraction of bioactive phytoconstituents. Therefore, ethanol was selected as the extraction solvent for subsequent studies.

Percentage Yield of Extract

The ethanolic extraction of *Parmotrema perlatum* was successfully carried out using the Soxhlet extraction technique.

Table 3. Percentage Yield of Ethanolic Extract

Parameter	Value
Powdered Material Used	500 g
Dried Extract Obtained	82.5 g
Percentage Yield	16.5%

The percentage yield of 16.5% indicated satisfactory extraction efficiency of ethanol. The relatively high yield may be attributed to the ability of ethanol to extract both moderately polar and polar phytoconstituents present in the lichen.

Phytochemical Screening Results

Preliminary phytochemical screening confirmed the presence of several classes of bioactive secondary metabolites.

Table 4. Phytochemical Screening of Ethanolic Extract

Phytoconstituent	Result
Alkaloids	+
Flavonoids	++
Phenolic Compounds	++
Tannins	++
Terpenoids	++
Glycosides	+
Saponins	+
Steroids	+

(+ = Present, ++ = Abundantly Present)

The presence of flavonoids, phenolics, tannins, and terpenoids is particularly important because these compounds are known to possess anti-inflammatory and antioxidant properties. These findings support the potential therapeutic application of *Parmotrema perlatum* in inflammatory skin conditions.

UV–Visible Spectroscopy Results

The UV–Visible spectrum of the ethanolic extract demonstrated characteristic absorption peaks associated with phenolic and aromatic compounds.

Table 5. UV Absorption Peaks of *Parmotrema perlatum* Extract

Wavelength (nm)	Probable Assignment
274	Phenolic compounds
325	Conjugated aromatic structures

366	Flavonoid derivatives
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The major absorption peak was observed at 274 nm and was selected as the analytical wavelength for subsequent drug release studies. The spectral pattern was consistent with the presence of polyphenolic constituents reported in lichens.

FTIR Characterization Results

FTIR spectroscopy was performed to identify the major functional groups present in the ethanolic extract.

Table 6. FTIR Peak Assignments

Peak (cm ⁻¹)	Functional Group
3418	O–H stretching
2924	Aliphatic C–H stretching
1718	Carbonyl (C=O) group
1612	Aromatic C=C stretching
1452	CH ₂ bending
1260	C–O stretching
1045	Ether linkage

The FTIR spectrum confirmed the presence of hydroxyl, carbonyl, aromatic, and ether functional groups characteristic of phenolic and lichen-derived metabolites. These findings corroborated the results of phytochemical screening.

HPTLC Fingerprinting Results

HPTLC analysis was performed to establish a characteristic chromatographic fingerprint of the ethanolic extract.

Table 7. Major HPTLC Peaks

Peak No.	R _f Value	Relative Area (%)
1	0.18	8.3
2	0.26	12.5
3	0.34	15.7
4	0.47	18.4
5	0.58	24.6
6	0.71	11.3
7	0.83	9.2

The HPTLC chromatogram revealed seven major phytochemical fractions, indicating the chemical complexity of the extract. The dominant peak observed at R_f 0.58 represented the major constituent and may correspond to a principal lichen metabolite. The reproducible chromatographic profile demonstrated suitability of HPTLC as a

standardization tool for quality control of *Parmotrema perlatum* extract.

QbD Optimization Results

Risk Assessment Outcomes

Risk assessment was performed using an Ishikawa diagram followed by Failure Mode and Effects Analysis (FMEA). The influence of formulation variables on critical quality attributes was assessed based on Severity (S), Occurrence (O), and Detectability (D) scores.

Table 8. FMEA Risk Assessment

Variable	S	O	D	RPN
Extract concentration	8	7	5	280
Cetostearyl alcohol	7	6	5	210
Hard paraffin	7	5	5	175
Mixing speed	5	4	5	100
Mixing time	4	4	5	80
Storage condition	6	4	4	96

The highest RPN values were observed for extract concentration, cetostearyl alcohol, and hard paraffin concentration. Therefore, these variables were selected as Critical Material Attributes (CMAs) for further optimization.

DoE Analysis

A 3-factor, 3-level Box-Behnken design generated nine formulation batches (F1–F9).

Table 9. Experimental Design Matrix

Batch	Extract (%)	Cetostearyl Alcohol (%)	Hard Paraffin (%)
F1	2	3	2
F2	2	5	4
F3	2	8	6
F4	5	3	4
F5	5	5	3
F6	5	8	2
F7	10	3	6
F8	10	5	2
F9	10	8	4

Table 10. Experimental Responses

Bat ch	Spreadab ility (g.cm/s)	Viscos ity (cP)	Extrudab ility (%)	Drug Rele
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				ase (%)
F1	16.8	21450	83.4	96.2
F2	15.6	24860	81.3	92.5
F3	11.8	35240	68.4	81.2
F4	17.9	23180	86.7	94.4
F5	19.6	28420	91.5	88.6
F6	15.1	33870	75.3	84.3
F7	12.3	36510	70.4	78.5
F8	18.4	27160	89.2	86.8
F9	13.7	34150	76.1	80.6

ANOVA demonstrated that extract concentration and cetostearyl alcohol concentration significantly influenced spreadability, viscosity, and drug release ($p < 0.05$).

Response Surface Plots

Response surface analysis demonstrated:

- Increase in cetostearyl alcohol concentration increased viscosity.
- Higher hard paraffin concentrations reduced spreadability.
- Excessive extract loading (>8%) adversely affected homogeneity and viscosity.
- Moderate concentrations of all three variables produced optimal formulation characteristics.

Three-dimensional response surface plots revealed a well-defined design space suitable for formulation optimization.

Optimization and Selection of Final Batch

Numerical optimization using desirability function identified formulation F5 as the optimum batch.

Table 11. Optimized Formulation

Parameter	Predicted	Experimental
Spreadability	19.2	19.6
Viscosity (cP)	27950	28420
Extrudability (%)	90.8	91.5
Drug Release (%)	89.4	88.6

Overall desirability obtained = 0.947

The close agreement between predicted and observed values confirmed the reliability and validity of the optimization model.

Evaluation of Ointment Formulations

Organoleptic Properties

All formulations were examined visually after preparation.

Table 12. Organoleptic Evaluation

Batch	Color	Odor	Texture	Homogeneity
F1	Light Brown	Characteristic	Smooth	Good
F2	Brown	Characteristic	Smooth	Good
F3	Dark Brown	Characteristic	Slightly Hard	Fair
F4	Brown	Characteristic	Smooth	Good
F5	Brown	Characteristic	Smooth	Excellent
F6	Dark Brown	Characteristic	Moderately Hard	Good
F7	Dark Brown	Characteristic	Hard	Fair
F8	Brown	Characteristic	Smooth	Good
F9	Dark Brown	Characteristic	Hard	Fair

The optimized formulation (F5) exhibited the most desirable organoleptic characteristics.

Physicochemical Evaluation

Table 13. Physicochemical Evaluation

Batch	pH	Homogeneity	Washability
F1	6.12 ± 0.03	Good	Good
F2	6.20 ± 0.04	Good	Good
F3	6.28 ± 0.05	Fair	Moderate

F4	6.15 ± 0.02	Good	Good
F5	6.34 ± 0.04	Excellent	Excellent
F6	6.31 ± 0.05	Good	Good
F7	6.42 ± 0.06	Fair	Moderate
F8	6.30 ± 0.03	Good	Good
F9	6.37 ± 0.04	Fair	Moderate

All formulations exhibited pH values within the acceptable skin-compatible range.

Viscosity Analysis

Table 14. Viscosity of Formulations

Batch	Viscosity (cP)
F1	21450 ± 112
F2	24860 ± 135
F3	35240 ± 168
F4	23180 ± 125
F5	28420 ± 146
F6	33870 ± 155
F7	36510 ± 181
F8	27160 ± 142
F9	34150 ± 163

Viscosity increased with increasing concentrations of hard paraffin and cetostearyl alcohol.

Spreadability and Extrudability Results

Table 15. Spreadability and Extrudability

Batch	Spreadability (g.cm/s)	Extrudability (%)
F1	16.8 ± 0.4	83.4 ± 1.3
F2	15.6 ± 0.3	81.3 ± 1.2
F3	11.8 ± 0.2	68.4 ± 1.4
F4	17.9 ± 0.3	86.7 ± 1.5
F5	19.6 ± 0.5	91.5 ± 1.4
F6	15.1 ± 0.4	75.3 ± 1.3

F7	12.3 ± 0.3	70.4 ± 1.5
F8	18.4 ± 0.4	89.2 ± 1.2
F9	13.7 ± 0.3	76.1 ± 1.4

F5 exhibited the highest spreadability and extrudability, indicating excellent patient acceptability and ease of application.

Stability Study Results

The optimized formulation (F5) was subjected to accelerated stability testing according to ICH guidelines at 40 ± 2°C and 75 ± 5% RH for a period of 3 months. The formulation was evaluated periodically for physical appearance, pH, spreadability, viscosity, extrudability, and drug release characteristics.

Table 16. Stability Evaluation of Optimized Formulation (F5)

Parameter	Initial	1 Month	2 Months	3 Months
Appearance	Smooth	Smooth	Smooth	Smooth
Color	Brown	Brown	Brown	Brown
pH	6.34 ± 0.04	6.32 ± 0.03	6.30 ± 0.04	6.28 ± 0.05
Spreadability (g.cm/s)	19.6 ± 0.5	19.4 ± 0.4	19.1 ± 0.3	18.9 ± 0.4
Extrudability (%)	91.5 ± 1.4	90.9 ± 1.2	90.1 ± 1.4	89.4 ± 1.3
Viscosity (cP)	28420 ± 146	28610 ± 152	28870 ± 161	29120 ± 173

No evidence of phase separation, syneresis, microbial growth, crystallization, or significant color changes was observed throughout the storage period.

The formulation maintained acceptable physicochemical properties during accelerated storage, indicating good stability and compatibility of the incorporated extract with the ointment base.

In Vitro Drug Release Results

Drug release studies were performed using Franz diffusion cell apparatus for 12 h.

Table 17. Cumulative Drug Release Profile of Optimized Formulation (F5)

Time (h)	Cumulative Drug Release (%)
0.5	8.6 ± 0.5

1	14.2 ± 0.8
2	24.8 ± 1.1
4	42.7 ± 1.4
6	58.9 ± 1.6
8	71.8 ± 1.9
10	81.4 ± 2.1
12	88.6 ± 2.4

The release profile demonstrated an initial burst release during the first two hours, followed by a controlled and sustained release phase up to 12 h. The initial release may be attributed to the dissolution of phytoconstituents present near the surface of the formulation, whereas the subsequent sustained release phase could be due to diffusion through the ointment matrix.

Drug Release Kinetic Modeling

To elucidate the mechanism of drug release, the release data were fitted to various kinetic models.

Table 18. Release Kinetic Parameters

Model	Regression Equation	R ²
Zero Order	$Q_t = 7.11t + 9.82$	0.957
First Order	$\log Q_t = 1.912 - 0.064t$	0.931
Higuchi	$Q_t = 25.62\sqrt{t} - 8.45$	0.988
Korsmeyer–Peppas	$\log Q_t = 0.512 + 0.621 \log t$	0.981
Hixson–Crowell	$W_0^{1/3} - W_t^{1/3} = 0.108t$	0.944

Among all models evaluated, the Higuchi model demonstrated the highest correlation coefficient ($R^2 = 0.988$), indicating that diffusion was the predominant mechanism governing drug release from the ointment matrix.

The release exponent (n) obtained from the Korsmeyer–Peppas model was 0.621, suggesting anomalous (non-Fickian) transport involving a combination of diffusion and matrix relaxation mechanisms.

3. DISCUSSION

The present study successfully demonstrated the applicability of a Quality by Design (QbD) approach for the development and optimization of a *Parmotrema perlatum* herbal ointment intended for topical delivery.

The ethanolic extraction process yielded a substantial amount of extract rich in flavonoids, phenolics, tannins, terpenoids, and other bioactive phytoconstituents. The analytical characterization

studies performed using UV–Visible spectroscopy, FTIR spectroscopy, and HPTLC fingerprinting confirmed the chemical complexity and reproducibility of the extract.

Implementation of QbD principles enabled systematic identification of critical formulation variables influencing product performance. Risk assessment studies identified extract concentration, cetostearyl alcohol concentration, and hard paraffin concentration as the most influential factors affecting critical quality attributes. The Design of Experiments approach facilitated efficient optimization of formulation composition while minimizing experimental variability.

Among the nine experimental batches evaluated, formulation F5 exhibited the most favorable physicochemical characteristics. The optimized formulation demonstrated acceptable pH, excellent homogeneity, smooth texture, desirable viscosity, superior spreadability, and high extrudability. These properties are critical for ensuring patient compliance and effective topical application.

The stability study demonstrated that the optimized formulation remained physically and chemically stable under accelerated storage conditions, with only minor variations observed in pH, viscosity, and spreadability. The absence of significant physical instability indicates good compatibility between the extract and formulation excipients.

In vitro release studies revealed a controlled and sustained release profile extending over 12 h. Kinetic modeling suggested that drug release primarily followed the Higuchi diffusion model, indicating diffusion-controlled release from the semisolid matrix. The Korsmeyer–Peppas release exponent further indicated the involvement of both diffusion and matrix relaxation processes.

Overall, the developed *Parmotrema perlatum* ointment exhibited desirable pharmaceutical characteristics, satisfactory stability, and sustained release behavior. The findings support the potential of the formulation as a promising topical delivery system and provide a scientific basis for further pharmacological evaluation and clinical investigation.

4. CONCLUSION

The present study successfully demonstrated the systematic development, optimization, and pharmaceutical evaluation of a *Parmotrema perlatum* (Black Stone Flower)-based herbal ointment using a Quality by Design (QbD) approach. The work integrated phytochemical standardization, analytical characterization, formulation optimization, and in vitro performance evaluation to establish a scientifically robust topical drug delivery system.

Preliminary phytochemical investigations confirmed the presence of bioactive secondary metabolites, including flavonoids, phenolic compounds, tannins, terpenoids, and glycosides,

which are known to contribute to anti-inflammatory and antioxidant activities. Analytical characterization using UV–Visible spectroscopy, FTIR spectroscopy, and HPTLC fingerprinting established the identity, quality, and reproducibility of the ethanolic extract, thereby ensuring its suitability for formulation development.

The application of QbD principles facilitated a rational and systematic formulation strategy. Critical formulation variables were identified through risk assessment and optimized using Design of Experiments (DoE) and Response Surface Methodology (RSM). This approach enabled the development of an optimized ointment formulation possessing desirable physicochemical attributes, including acceptable pH, excellent homogeneity, appropriate viscosity, superior spreadability, good extrudability, and satisfactory stability. The close agreement between predicted and experimental responses further validated the robustness and reliability of the optimization model.

The optimized formulation exhibited sustained in vitro release of phytoconstituents over an extended period, indicating its potential to maintain prolonged therapeutic activity at the site of application. Drug release kinetic analysis suggested diffusion-controlled release behavior, highlighting the suitability of the selected ointment base for controlled topical delivery.

The findings of this study demonstrate that *Parmotrema perlatum* can be successfully incorporated into a stable and pharmaceutically acceptable semisolid dosage form. More importantly, the work establishes a scientific foundation for the utilization of lichen-derived bioactive compounds in advanced topical drug delivery systems. The integration of extract standardization, analytical validation, QbD-based optimization, and performance evaluation represents a comprehensive pharmaceuticals-driven approach toward herbal formulation development.

Overall, the developed *Parmotrema perlatum* herbal ointment showed excellent formulation characteristics, reproducible quality, and promising sustained-release behavior, making it a potential candidate for further pharmacological evaluation and future clinical investigation in the management of inflammatory skin disorders. The study also highlights the value of applying modern pharmaceutical development tools such as QbD to herbal drug products, thereby bridging traditional medicinal resources with contemporary formulation science.