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Research Article

Development and Optimization of a *Feronia elephantum* Leaf Extract Ointment: A QbD-Based Approach for Enhanced Anti-Inflammatory Activity

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Feronia elephantum; Herbal ointment; Quality by Design (QbD); In vitro anti-inflammatory activity; HRBC membrane stabilization; Topical drug delivery.

Abstract:
Feronia elephantum possesses significant therapeutic potential owing to its rich phytochemical composition and traditional use in inflammatory disorders. The present study aimed to develop, optimize, and evaluate a topical herbal ointment containing *Feronia elephantum* leaf extract using a Quality by Design (QbD) approach. The hydroalcoholic extract was prepared by maceration and characterized using FTIR, DSC, and UV spectroscopy. FTIR analysis confirmed the presence of phenolic and flavonoid constituents, while DSC revealed a characteristic endothermic peak at 123.92°C. UV analysis showed a maximum absorption wavelength (λmax) at 275 nm. Extract–excipient compatibility studies demonstrated the absence of significant interactions between the extract and selected excipients. A 2^3 full factorial design was employed using cetostearyl alcohol, lanolin, and liquid paraffin as critical material attributes, while viscosity and percentage drug release were selected as critical quality attributes. Eight formulations were prepared and evaluated for physicochemical properties, drug content, spreadability, extrudability, homogeneity, in vitro drug release, and anti-inflammatory activity. The formulations exhibited viscosities ranging from 1450 to 2520 cP and cumulative drug release between 75.2% and 91.8%. Statistical analysis showed that cetostearyl alcohol and lanolin significantly influenced both viscosity and drug release ($p < 0.0001$). The optimized formulation (F4) demonstrated a viscosity of 2105 cP and drug release of 82.4%. In vitro anti-inflammatory evaluation using the HRBC membrane stabilization method showed that the ointment formulation produced 29.0 ± 1.0% and 46.0 ± 1.0% membrane protection at 200 and 400 mg/mL, respectively, compared with 25.0 ± 1.0% and 41.0 ± 1.0% for the crude extract. Accelerated stability studies indicated minimal changes in pH (6.45–6.61), viscosity (2105–2102 cP), and drug content (95.86–95.30%) over three months. In conclusion, the QbD-optimized *Feronia*

elephantum ointment exhibited satisfactory physicochemical characteristics, controlled drug release, enhanced anti-inflammatory activity, and good stability, indicating its potential as an effective topical herbal formulation for inflammatory conditions.

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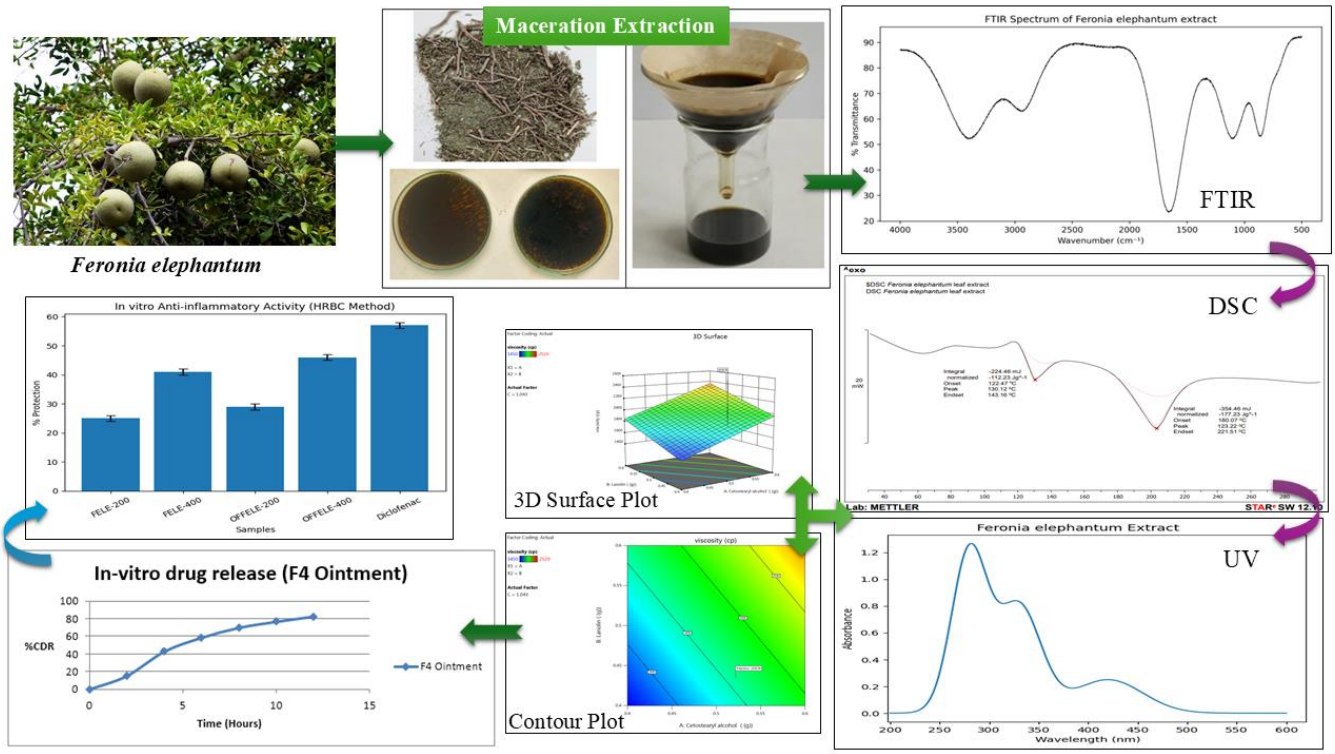
Graphical Abstract



1. Introduction

Inflammation is a complex physiological response triggered by tissue injury, infection, or exposure to harmful stimuli, serving as a protective mechanism to restore normal tissue function. Although acute inflammation is beneficial for healing and host defense, prolonged or chronic inflammation is implicated in the pathogenesis of numerous disorders, including arthritis, dermatitis, psoriasis, cardiovascular disease, and autoimmune conditions. Conventional anti-inflammatory therapies, such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, are widely used to manage inflammatory disorders; however, their long-term use is often associated with adverse effects, including gastrointestinal irritation, renal toxicity, immunosuppression, and cardiovascular complications. These limitations have encouraged the exploration of plant-derived therapeutics as safer and more sustainable alternatives [1,2].

Medicinal plants are a rich source of biologically active compounds with diverse pharmacological properties. Phytochemicals such as flavonoids, phenolics, tannins, alkaloids, and terpenoids have been extensively reported to exhibit anti-inflammatory, antioxidant, antimicrobial, and wound healing activities [3,4]. Among the medicinal plants used in traditional medicine, *Feronia elephantum* (Rutaceae), commonly

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known as wood apple, has gained considerable attention because of its therapeutic potential. Various parts of the plant have been traditionally used to treat digestive disorders, infections, diabetes, and inflammatory conditions. The leaves of *Feronia elephantum* contain several bioactive constituents that may contribute to its pharmacological activities, particularly through the modulation of inflammatory pathways and oxidative stress [5–7].

Topical drug delivery systems offer a convenient and effective approach for managing localized inflammatory conditions. By delivering therapeutic agents directly to the affected site, topical formulations minimize systemic exposure, reduce adverse effects, and improve patient compliance [1,8,9]. Among the various semisolid dosage forms, ointments are widely preferred because of their occlusive nature, prolonged residence time on the skin, ease of application, and ability to accommodate a broad range of active ingredients in their formulation. However, the therapeutic effectiveness of an ointment depends largely on its formulation characteristics, including viscosity, spreadability, homogeneity, stability, and drug release. Therefore, careful formulation design and optimization are essential for achieving consistent product performance.

The characterization of herbal extracts and pharmaceutical formulations plays a pivotal role in ensuring product quality, safety, and efficacy. Analytical techniques such as Fourier Transform Infrared (FTIR) spectroscopy, Differential Scanning Calorimetry (DSC), and UV-visible spectroscopy are widely employed to investigate the physicochemical properties of herbal materials [10–12]. FTIR spectroscopy provides information regarding characteristic functional groups and assists in confirming the presence of phytoconstituents, whereas DSC is useful for evaluating the thermal behavior, stability, and phase transitions of the extract and formulation components. UV-visible spectroscopy is an important analytical tool for the identification and quantitative estimation of phytochemical constituents. Furthermore, extract –excipient compatibility studies using FTIR and DSC helped identify potential interactions that may affect formulation stability, drug release, and overall product performance. Therefore, comprehensive characterization provides a scientific basis for developing robust and reproducible herbal formulations.

In recent years, Quality by Design (QbD) has emerged as a systematic and science-based approach for pharmaceutical product development. QbD emphasizes predefined objectives, risk assessment, identification of critical material attributes (CMAs), and understanding their influence on critical quality attributes (CQAs). Through the application of statistical experimental designs, formulation variables can be systematically optimized to achieve the desired product quality while minimizing the variability. Compared with conventional trial-and-error approaches, QbD enhances process understanding, product robustness, and regulatory compliance [13–15].

Although *Feronia elephantum* has been extensively investigated for its phytochemical composition and pharmacological activities, few studies have focused on its development as a standardized topical dosage form. Moreover, no systematic formulation strategy based on the QbD framework has been reported for optimizing a Feronia elephantum leaf extract-based ointment. In this study, a QbD-driven 2^3 full factorial design was employed to optimize the ointment formulation by evaluating the influence of critical material attributes on key quality characteristics. The developed formulation was further characterized through physicochemical evaluation, extract –excipient compatibility studies, in vitro drug release, accelerated stability testing, and HRBC membrane stabilization assay to establish a robust and reproducible herbal topical formulation with enhanced anti-inflammatory potential.

Therefore, this study aimed to formulate, optimize, and characterize a *Feronia elephantum* leaf extract-loaded ointment using a QbD approach. This study aimed to establish an optimized topical formulation through systematic evaluation of formulation variables and comprehensive physicochemical characterization to ensure desirable quality attributes and suitability for topical application.

2. Materials and Methods

2.1 Collection and Authentication of Plant Material

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Leaves of *Feronia elephantum Correa* (Family: *Rutaceae*) were collected from Buldhana, Maharashtra, India, in December 2025. The plant material was authenticated by the Department of Botany, Government Degree College, Kukatpally, Telangana, India, and a voucher specimen (No. 0409) was deposited for future reference at the same institution. Following authentication, the collected leaves were shade-dried, coarsely powdered, and processed for hydroalcoholic extraction. Representative images of authenticated plants are presented in Figure 1.

Figure 1. The *Feronia elephantum* plant

2.2 Maceration Extraction

The dried and coarsely powdered plant material was subjected to cold maceration using a hydroalcoholic solvent (ethanol 70:30 v/v). The powder was immersed in the solvent in a closed container and maintained at room temperature for 72 h with occasional shaking. The mixture was then filtered through a muslin cloth, followed by a Whatman No. 1 filter paper. The filtrate was then concentrated under reduced pressure and dried to obtain a semisolid extract. The resulting extract was stored in an airtight container at 4°C until further analysis [16–18].

Figure 2. Maceration extraction process of *Feronia elephantum* showing the dried powdered plant material, filtration of the hydroalcoholic extract through filter paper, and the obtained concentrated extract after filtration.

2.3 Preformulation Study

2.3.1 Physical Characterization and Identification of Extract

The *Feronia elephantum* extract was subjected to physicochemical characterization using Fourier-transform infrared (FTIR) spectroscopy and Differential Scanning Calorimetry (DSC) to determine its functional

groups and thermal properties. FTIR measurements were carried out using a Shimadzu FTIR spectrometer (version 7.03) over a spectral range of 4000–400 cm^{-1}. For analysis, approximately 10 mg of the sample was finely blended with potassium bromide (KBr), compressed into a pellet, and exposed to an infrared beam to obtain the corresponding absorption spectra. The thermal behavior of the extract was further evaluated using a Mettler DSC 1 Star System. The sample was enclosed in a perforated aluminum pan and subjected to controlled heating at a rate of 10°C/min within a temperature range of 30–350°C under a nitrogen atmosphere maintained at a flow rate of 20 mL/min. The resulting thermogram provides insights into the thermal stability and phase transitions of the extract [19,20].

2.4 Analytical methods

2.4.1 Standard Calibration Curve in Methanol

A stock solution of *Feronia elephantum* extract was prepared by dissolving 100 mg of the extract in 5 mL of methanol and diluting the solution to 100 mL with the same solvent. From this stock, a series of working solutions were obtained by appropriate dilution to achieve concentrations ranging from 10–80 µg/mL. The solutions were analyzed using a UV spectrophotometer to identify the wavelength of maximum absorbance (λmax). The absorbance values of each dilution were recorded at the selected λmax values. A calibration curve was generated by plotting the concentration (µg/mL) on the X-axis against the corresponding absorbance on the Y-axis. The linear relationship confirmed adherence to Beer–Lambert's law within the selected concentration range. This calibration plot was used to quantify the amount of drug released during in vitro dissolution studies [21,22].

2.5 Extract–Excipients Compatibility Study

The compatibility between *Feronia elephantum* extract and the selected excipients was investigated to identify any potential physicochemical interactions using FTIR spectroscopy and DSC. FTIR spectra were recorded using a Shimadzu FTIR spectrometer (version 7.03) over a wavenumber range of 4000–400 cm^{-1}. For analysis, approximately 10 mg of the pure extract and its physical mixtures with excipients were blended with potassium bromide (KBr), compressed into pellets, and scanned to compare the characteristic absorption peaks. Thermal compatibility was further assessed using a Mettler DSC 1 Star System. The extracts and their mixtures were placed in perforated aluminum pans and subjected to a controlled heating program at a rate of 10°C/min within the temperature range of 30–350°C under a nitrogen atmosphere maintained at 20 mL/min. The resulting thermograms were analyzed to detect any changes in thermal behavior, indicating possible interactions between the extract and excipients [23,24].

2.6 Preparation of Herbal Ointment Containing *Feronia Elephantum* Leaf Extract

The herbal ointment was formulated using the fusion method within a Quality by Design (QbD) framework to ensure reproducibility and controlled product quality. The formulation variables were selected based on a prior risk assessment, identifying excipient composition as a critical determinant of product performance. The ointment base was prepared by accurately weighing lanolin (0.52 g), cetostearyl alcohol (0.52 g), hard paraffin (0.52 g), and white soft paraffin (8.44 g), which were melted together with continuous stirring to obtain a uniform molten phase. The processing temperature and mixing were controlled as critical process parameters (CPPs) to maintain the consistency of the semisolid matrix. The concentrated *Feronia elephantum* leaf extract (0.12 g), considered the active pharmaceutical ingredient (API), was levigated with a small portion of the base to form a smooth paste, ensuring uniform dispersion and minimizing aggregation. This step was identified as a critical step influencing the content uniformity and drug release. The paste was gradually incorporated into the remaining molten base under continuous stirring to achieve a homogeneous mixture. The formulation was then allowed to cool under controlled conditions to obtain a uniform semisolid ointment (10 g). The final product was transferred to suitable containers for further evaluation. This systematic approach ensured that critical quality attributes (CQAs), such as viscosity, spreadability, and drug release, were consistently achieved [5,25].

2.7 Factorial Design

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A Quality by Design (QbD)-based Design of Experiments (DoE) approach was employed using a 2^3 full factorial design to systematically evaluate the influence of formulation variables on critical quality attributes. Based on a preliminary risk assessment, three independent variables were identified as critical material attributes (CMAs): cetostearyl alcohol (X_1: 0.40–0.60 g), lanolin (X_2: 0.40–0.60 g), and liquid paraffin (X_3: 1.0–2.0 g), as these excipients significantly affect the rheological properties and drug diffusion behavior of the ointment.

The dependent variables selected as critical quality attributes (CQAs) were viscosity (Y_1) and percentage drug release (Y_2), which directly influence the product's performance and therapeutic efficacy. Eight experimental runs (F1–F8) were generated to represent all possible combinations of the factor levels. Each formulation was prepared using a standardized fusion method and evaluated for the selected responses [26,27].

The experimental data were subjected to statistical analysis using ANOVA and regression modeling to determine the significance of the individual factors and their combined effects. The results demonstrated that cetostearyl alcohol and lanolin exerted a significant positive effect on viscosity, whereas all three variables showed an inverse relationship with drug release. The developed models exhibited high predictability, confirming their robustness. Response surface methodology was employed to establish a design space, defining the optimal range of formulation variables that ensure the desired product quality. The optimized formulation was selected based on the desirability criteria, achieving a balance between adequate viscosity and maximum drug release. This QbD-driven approach enabled a deeper understanding of the formulation behavior, minimized variability, and ensured consistent product performance. The detailed levels of the variables and factorial design batches are presented in Tables 1 and 2, respectively.

Table 1. Levels of variables for optimization

Sr. No.	Factor	Low level (%)	High Level (%)
1	Cetostearyl alcohol (g) (X_1)	0.40	0.60
2	Lanolin (g) (X_2)	0.40	0.60
3	Liquid paraffin (g) (X_3)	1	2

Table 2. Factor level for 2^3 Full Factorial Designs

Batch Code	X_1	X_2	X_3
F1	0.4	0.4	1
F2	0.6	0.6	1
F3	0.4	0.4	2
F4	0.6	0.4	2
F5	0.6	0.6	2
F6	0.4	0.6	2
F7	0.4	0.6	1
F8	0.6	0.4	1

2.8 Characterization of Ointment

2.8.1 FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy was performed using a Shimadzu FTIR spectrometer (version 7.03) to identify the characteristic functional groups of the sample. The spectrum was recorded over a wavenumber range of 4000–400 cm^{-1}. For analysis, approximately 10 mg of the sample was finely blended with potassium bromide (KBr) and compressed into a pellet using a suitable holder. The prepared pellet was then exposed to an infrared beam, and the resulting spectrum was recorded and analyzed using the instrument software [23,24].

2.8.2 Differential Scanning Calorimetry

Thermal analysis of the optimized formulation was performed using a Mettler DSC 1 Star System. The sample was placed in a perforated aluminum pan and subjected to controlled heating at a rate of 10°C/min over a temperature range of 30–350°C. The analysis was conducted under a nitrogen atmosphere maintained at a

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flow rate of 20 mL/min, and the resulting thermogram was recorded to evaluate the thermal behavior of the formulations.

2.8.3 Measurement of pH

The pH of the ointment formulations was determined at 25°C using a calibrated digital pH meter. Prior to measurement, the instrument was standardized using buffer solutions of pH 4.0 and 7.0. A 10% w/w dispersion was prepared by dispersing 1 g of ointment in 10 mL of distilled water, followed by thorough mixing to obtain a uniform solution. The pH of each formulation was recorded using a calibrated instrument.

2.8.4 Measurement of Viscosity

The viscosity of the ointment formulations was evaluated at 25°C using a Brookfield DV-E Viscometer. The measurements were performed using spindle number 6 at varying rotational speeds of 10, 20, 30, and 60 rpm to assess the rheological behavior of the formulations [28].

2.8.5 Drug Content Determination

The amount of drug present in the ointment formulation was quantified using UV spectrophotometry. Approximately 1.0 g of the formulation was accurately weighed and dissolved in 100 mL of a methanol: phosphate buffer mixture (2:8). The resulting solution was filtered to remove any undissolved particles and diluted further when necessary. The absorbance of the prepared sample was measured at 255 and 275 nm using a UV spectrophotometer, and the drug content was calculated accordingly [29].

2.8.6 Spreadability

The spreadability of the ointment formulation was evaluated using a slip-and-drag technique. A measured quantity of ointment (0.5 g) was placed between two glass slides, and a weight of 500 g was applied for 5 min to form a uniform film and eliminate entrapped air. The excess material was carefully removed from the edges. Subsequently, an additional weight of 20 g was attached to the upper slide to allow it to move over the lower slide. The time required for the upper slide to travel a fixed distance (5 cm) was recorded.

Spreadability (S) was calculated using the formula:

$S = (M \times l)/t$, ------ (1)

Where M is the applied weight (g), l is the distance moved (cm), and t is the time (s). Higher S values indicate better spreadability.

2.8.7 Extrudability

The extrudability of the ointment formulations was assessed using the collapsible aluminum tube method. Approximately 20 g of the prepared ointment was placed in a lacquered aluminum tube and sealed. The tube was then placed between two glass slides, and a constant load of 500 g was applied to it. The quantity of ointment extruded from the tube within 10 s was measured. Extrudability was expressed in terms of the weight of the extruded ointment or qualitatively categorized as excellent, good, or poor, depending on the ease with which the formulation was expelled from the tube.

2.8.8 Homogeneity

The homogeneity of the ointment formulation was assessed through visual examination. A small portion of the prepared ointment was gently pressed between the thumb and index fingers to evaluate its uniformity, smooth texture, and consistency. The formulation was also observed to be free of lumps, aggregates, and any signs of phase separation.

2.9 *In-vitro* Release Study of Ointment Loaded Ointment Formulations

In *in-vitro* drug release from the ointment formulation was evaluated using a Franz diffusion cell assembly. A pre-hydrated dialysis (cellophane) membrane with a molecular weight cut-off of approximately 12,000 Da was employed to permit the diffusion of the released drug while restricting larger components. The receptor compartment was filled with 25 mL of phosphate buffer (pH 7.4) and maintained at 37 ± 0.5°C with continuous stirring at 100 rpm. An accurately weighed quantity of the ointment (approximately 20 mg) was

placed in the donor compartment. To maintain sink conditions, the concentration of the drug in the receptor medium was kept significantly below its saturation solubility, and each withdrawn sample was replaced with an equal volume of fresh buffer. This ensured a constant concentration gradient and enabled a reliable assessment of the drug release behavior over time [30].

2.10 Kinetics of drug release

2.10.1 Zero order

The zero-order kinetic model is commonly applied to describe drug release from modified dosage forms, such as transdermal systems, coated formulations, and matrix systems containing poorly soluble drugs. In this model, the drug is released at a constant rate over time, independent of its concentration, resulting in a uniform amount of drug delivery per unit time.

The relationship is represented by the following equation:

$$Q_t = Q_0 + K_0 t \text{.....(2)}$$

where $Q_t$denotes the amount of drug released at time t, Q_0represents the initial amount of drug present in the solution, and $K_0$is the zero-order release rate constant. This model indicated a steady and controlled drug release profile throughout the study period [31].

2.10.2 First order

The first-order kinetic model describes drug release from dosage forms, where the rate of release depends on the amount of drug remaining in the formulation. In these systems, the drug is released at a rate proportional to its residual concentration, resulting in a gradual decrease in the amount released per unit time.

This relationship is expressed by the following equation:

$$\log Q_t = \log Q_0 + \frac{K_t \cdot t}{2.303} \text{..... (3)}$$

where $Q_t$represents the amount of drug released at time t, $Q_0$is the initial drug concentration, and $K_t$denotes the first-order release rate constant. This model typically indicates a concentration-dependent release.

2.10.3 Higuchi Model

This model helps study the release mechanism of water-soluble and low water-soluble drugs incorporated in semi-solid and solid matrices. The mathematical expression for drug release is as follows:

Q = [D (2C-CS) CS.t] ½ --------- (4)

where $Q$is the cumulative percentage of drug released per unit area at time t, Cdenotes the initial drug concentration, $C_s$is the solubility of the drug in the matrix, and Drepresents the diffusion coefficient.

Assuming that the diffusion coefficient and other parameters remain constant during release, the above equation reduces to Q = k.t ½ Thus, for a diffusion-controlled release mechanism, a plot of cumulative % of drug released vs. the square root of time should be linear. The linearity of the plots was checked by performing a linear regression analysis and determining the regression coefficient of the plot.

2.10.4 Korsmeyer Peppas

Peppas used this n value to characterize the different release mechanisms. The drug release data were plotted according to the Peppas equation, in which log % cumulative release is plotted against log time. According to the Peppas equation, the rate of drug release can be expressed as Q = Ktn. Taking the log on both sides of the equation:

Log Q = log K + n log t --------- (5)

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where Q represents the cumulative percentage of drug released at time t, K is the release rate constant, and n is the release exponent corresponding to the slope of the linear plot of $\log Q$ versus $\log t$. The value of n provides insight into the mechanism governing drug release from the formulation [31].

2.11 Accelerated Stress Stability Study

The stability of the optimized ointment formulation was evaluated following the International Council for Harmonization guidelines (ICH Q1A(R2)). The formulation was filled in amber-colored glass containers and stored under accelerated conditions at 40 ± 2°C and 75 ± 5% relative humidity for three months. At specified time intervals, samples were collected and analyzed for key parameters such as pH, viscosity, and drug content to monitor any changes and determine the overall stability of the formulation under stress conditions [32].

2.12 *In- vitro* anti-inflammatory activity

The in vitro anti-inflammatory activity of *Feronia elephantum* leaves extract (FELE) and its ointment formulation (OFFELE) was evaluated using the Human Red Blood Cell (HRBC) membrane stabilization method, following the protocol described in a previous study. Fresh human blood was collected from healthy volunteers and mixed with an equal volume of Alsever solution. The mixture was centrifuged at 3000 rpm, and the packed cells were washed with an isotonic saline solution. A 10% v/v HRBC suspension was prepared and used in the assay [33].

Test samples (FELE and OFFELE) were evaluated at concentrations of 200 and 400 mg/mL, and diclofenac sodium (5 mg/mL) served as the standard drug. Each sample was mixed with phosphate buffer, hyposaline solution, and HRBC suspension and incubated at 37°C for 30 min. After incubation, the mixtures were centrifuged, and the absorbance of the supernatant was measured at 560 nm wavelength. The experiment was performed in triplicate (n = 3), and the percentage protection was calculated using the standard formula (Figure 3).

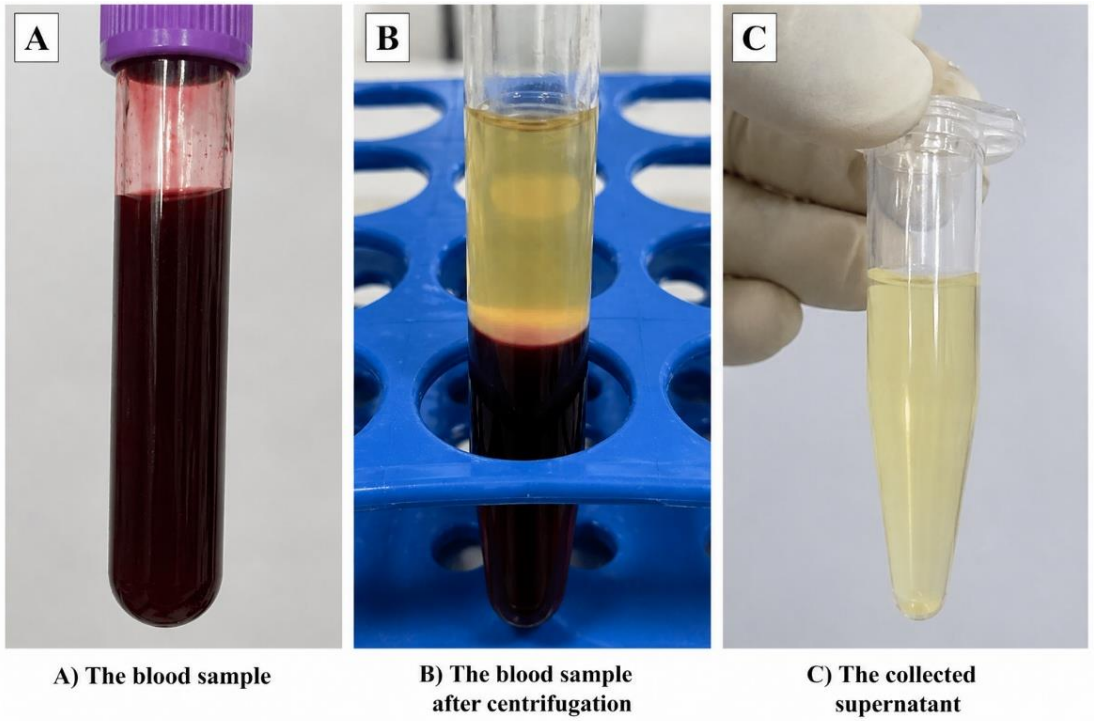

Figure 3. The working images of *in vitro* anti-inflammatory activity, A) The blood sample; B) The blood sample after centrifugation; C) The collected supernatant

3. Results and Discussion

3.1 Preformulation Study

3.1.1 Identification and Confirmation of Drug

The drug was identified and confirmed by melting point determination using the capillary method and DSC, FTIR, and UV spectroscopy.

3.1.2 FTIR Spectroscopy

The FTIR spectrum of *Feronia elephantum* leaf extract shows characteristic absorption bands, indicating the presence of various functional groups (Figure 4). A broad peak observed at approximately 3400 cm^{-1} corresponds to O–H stretching vibrations, suggesting the presence of phenols and alcohols. Peaks near 2920 cm^{-1} indicate C–H stretching of aliphatic compounds. A strong absorption band around 1650–1700 cm^{-1} is attributed to C=O stretching, indicating the presence of carbonyl groups (such as flavonoids or ketones). The band near 1400–1450 cm^{-1} represents the C–C stretching or bending vibrations of the aromatic rings. Additionally, peaks around 1050–1150 cm^{-1} correspond to C–O stretching, confirming the presence of alcohols, ethers and/or esters. The fingerprint region below 1000 cm^{-1} further supports the presence of complex phytoconstituents. Overall, the spectrum confirmed the presence of phenolic and flavonoid compounds in the extract.

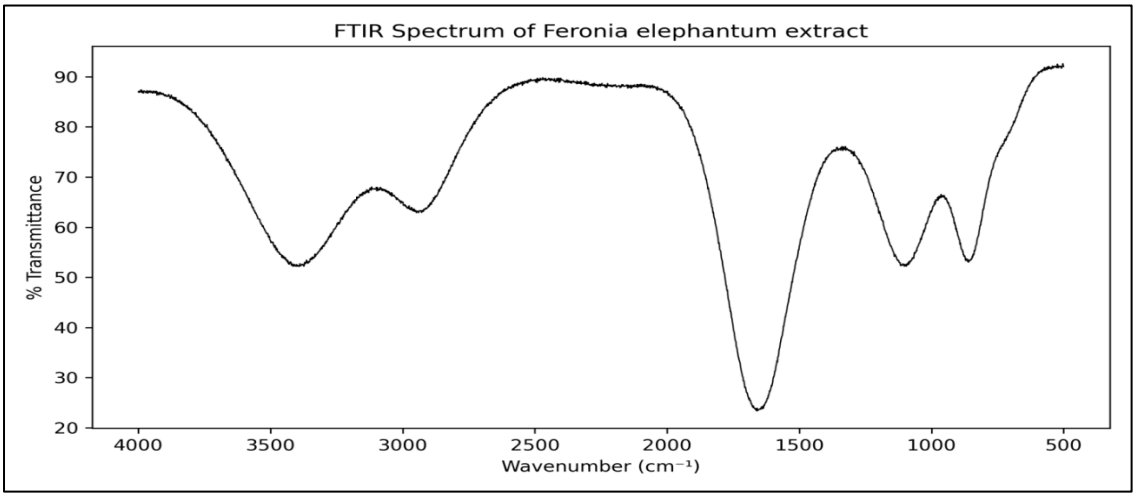

Figure 4. FTIR of Leaf Extract

3.1.3 Differential Scanning Calorimetry

DSC analysis of leaf extract performed at a 10ºC/min of scanning rate with continuous nitrogen splurging revealed a sharp endothermic peak at 123.922 °C, confirming the specified melting point range of 180.07-221.51ºC (Figure 5).

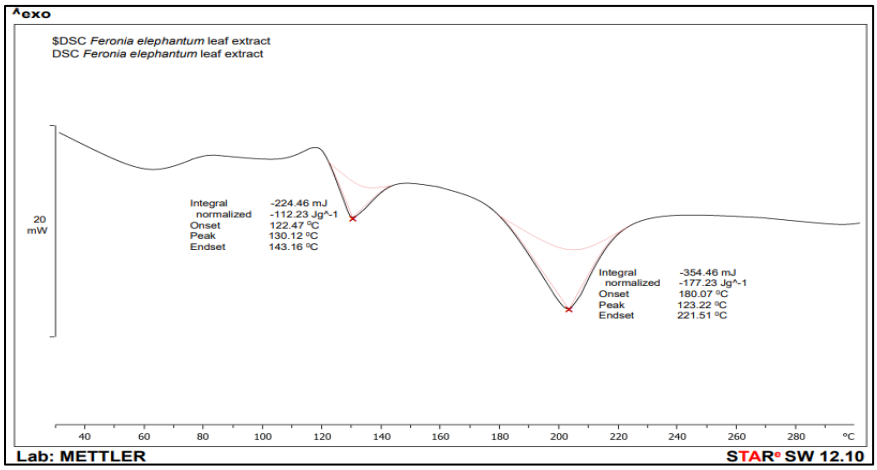

Figure 5. DSC of Leaf Extract

3.1.4 UV-Visible spectrophotometer

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The UV spectrum of the leaf extract exhibited absorption maxima at 275 nm, corresponding to the π→π* transitions of the aromatic system (Figure 6). This observation was in agreement with the previously documented and published UV-Visible spectrum of leaf extract, which also indicated a maximum absorption at 275 nm (λ max).

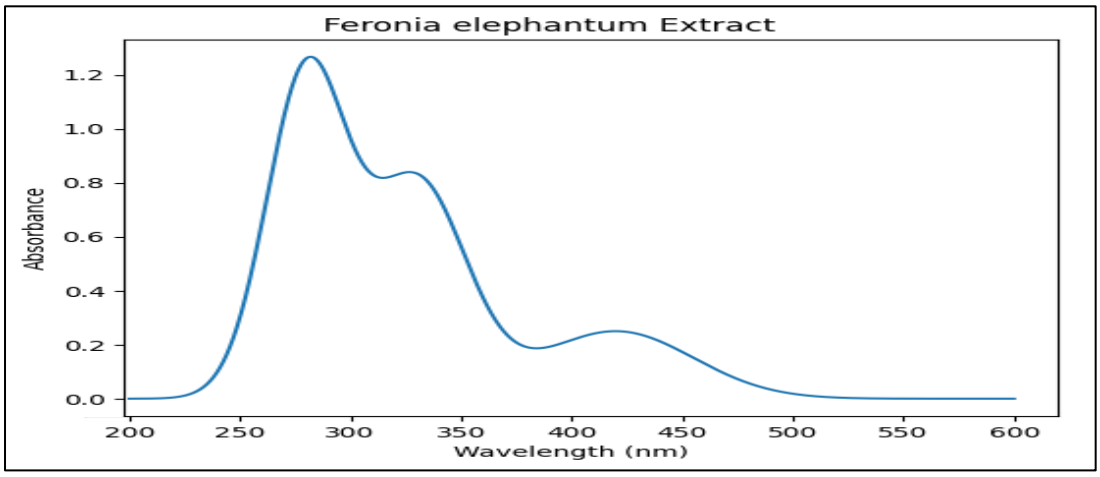

Figure 6. UV spectrum of Leaf Extracts

3.2 Determination of extract-polymer compatibility

3.2.1 FTIR Spectroscopy

The extract–excipient compatibility study is crucial for confirming the identity of the product and ensuring its reproducibility with guaranteed therapeutic efficacy. This method was used to identify any interactions between the leaf extract, excipients, and their physical mixtures. The spectra of the leaf extract, excipients, and physical mixtures are shown in Figures 4 and 7-11. A broad peak observed at approximately 3400 cm^{-1} corresponds to O–H stretching vibrations, suggesting the presence of phenols and alcohols. The peaks near 2920 cm^{-1} indicate C–H stretching of aliphatic compounds. A strong absorption band around 1650–1700 cm^{-1} is attributed to C=O stretching, indicating the presence of carbonyl groups (such as flavonoids or ketones). The band near 1400–1450 cm^{-1} represents the C–C stretching or bending vibrations of the aromatic rings. Additionally, peaks around 1050–1150 cm^{-1} correspond to C–O stretching, confirming the presence of alcohols, ethers and/or esters. The fingerprint region below 1000 cm^{-1} further supports the presence of complex phytoconstituents. A similar result for the pure extract was observed in the thermogram of the physical mixture. It showed no interaction and was compatible.

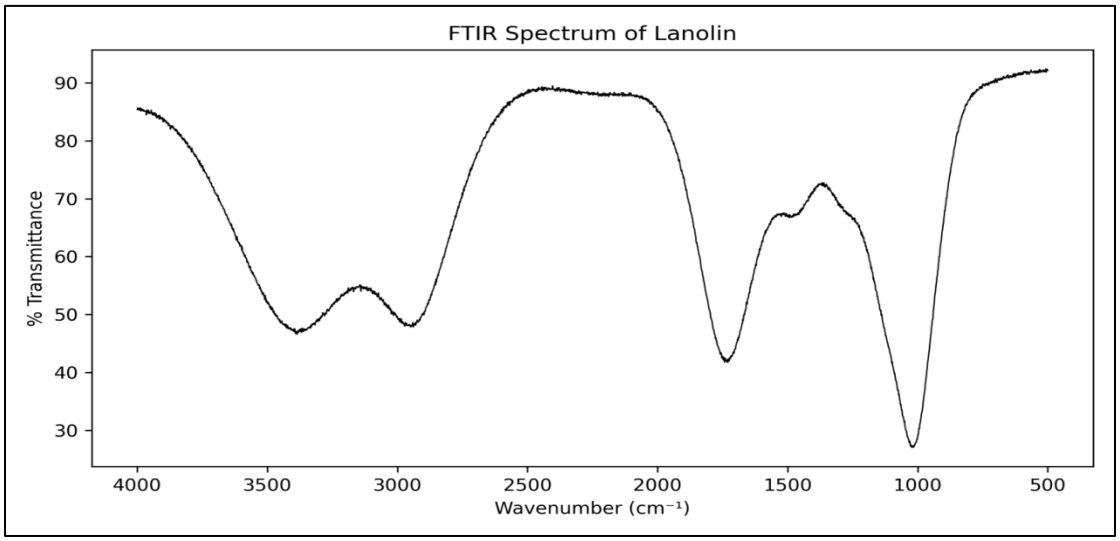

Figure 7. FTIR graph of Lanolin

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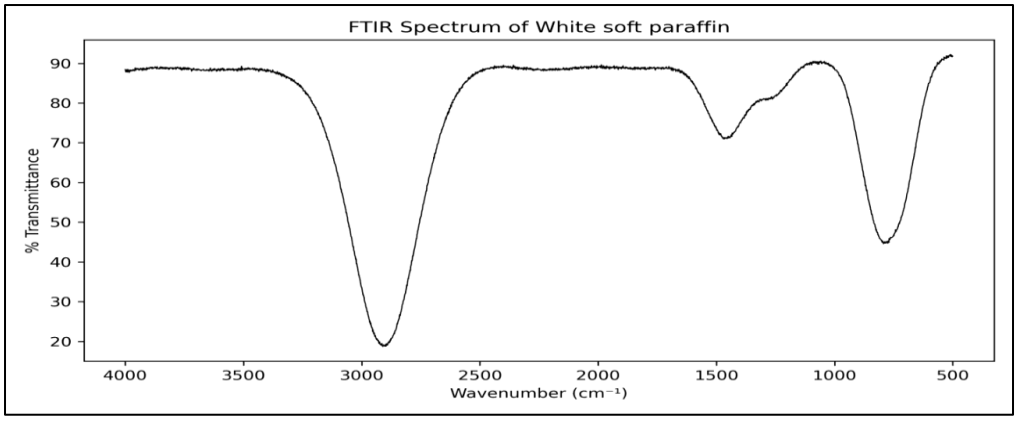

Figure 8. FTIR graph of White soft paraffin

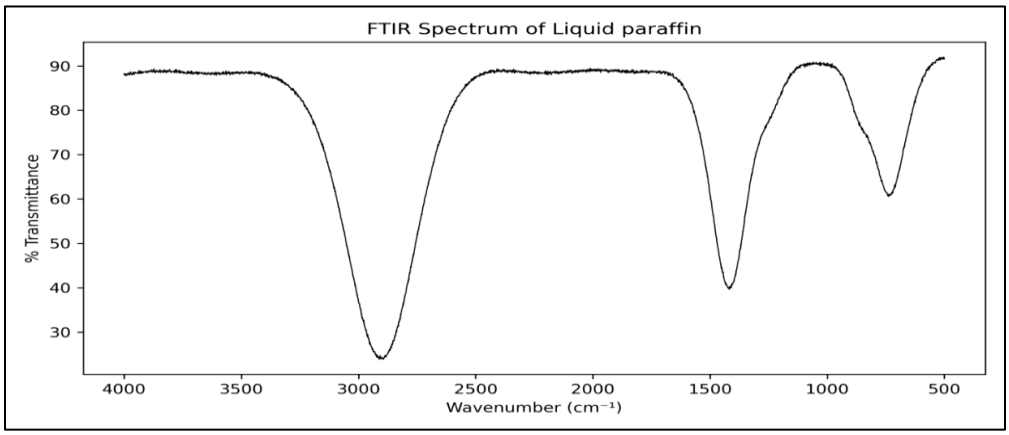

Figure 9. FTIR graph of Liquid paraffin

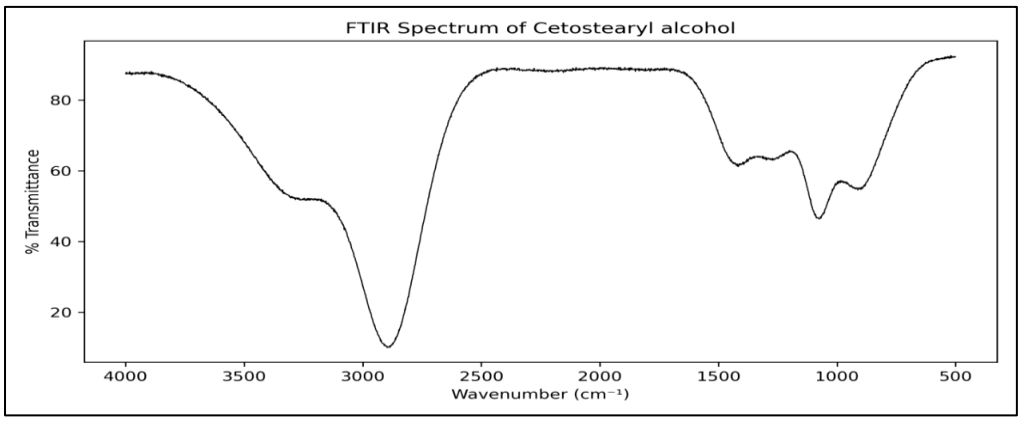

Figure 10. FTIR graph of Cetostearyl alcohol

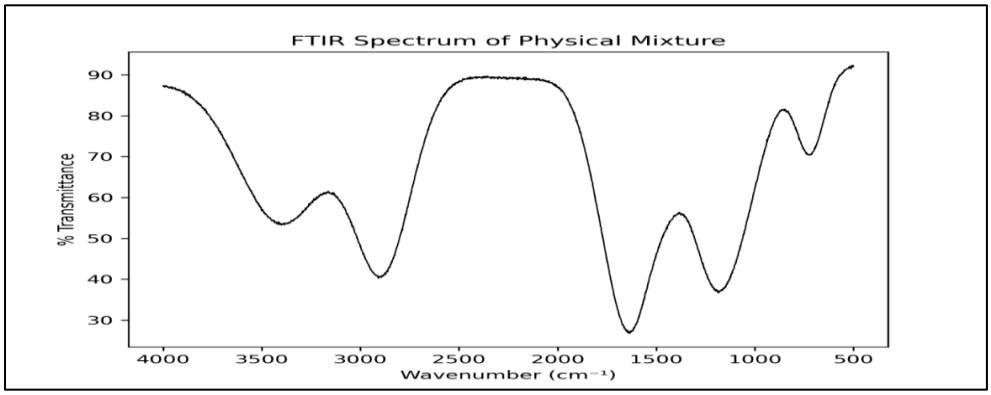

Figure 11. FTIR graph of Physical Mixture

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3.2.2 DSC

The melting point of the extract was determined using DSC. Thermograms for leaf extracts with other polymers and physical mixtures were obtained using a Mettler DSC 1-star system (Mettler-Toledo, Switzerland). The extract, enclosed in a perforated aluminum pan, was heated at a constant rate of 10°C/min in the 30-300°C temperature range, as shown in Figure 12-6. DSC analysis of the leaf extract performed at a 10ºC/min of scanning rate with continuous nitrogen splurging revealed a sharp endothermic peak at 123.922 °C (Figure 5), confirming the specified melting point range of 180.07-221.51ºC. A similar result for the pure extract was displayed in the thermogram of the physical mixture. No interaction was observed, and the results were compatible.

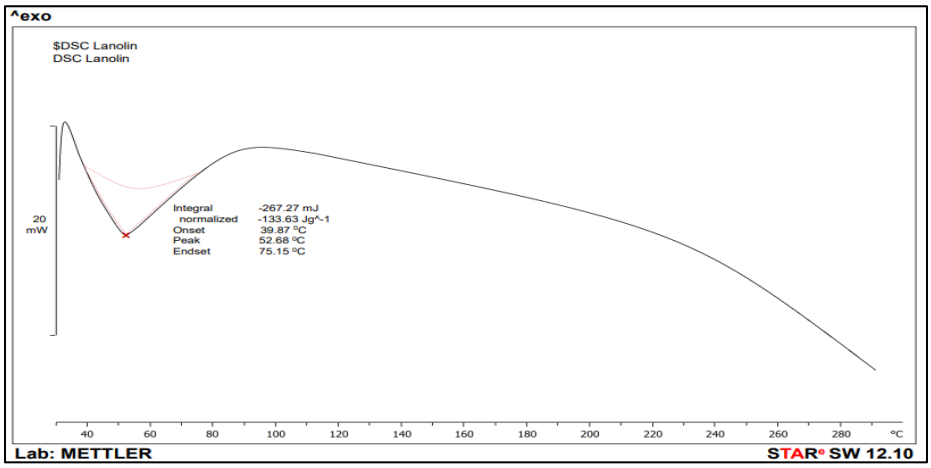

Figure 12. DSC graph of Lanolin

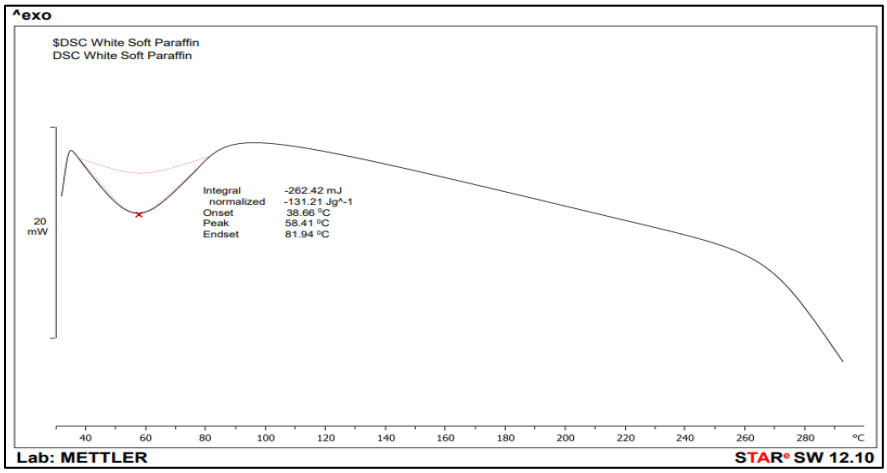

Figure 13. DSC graph of White soft paraffin

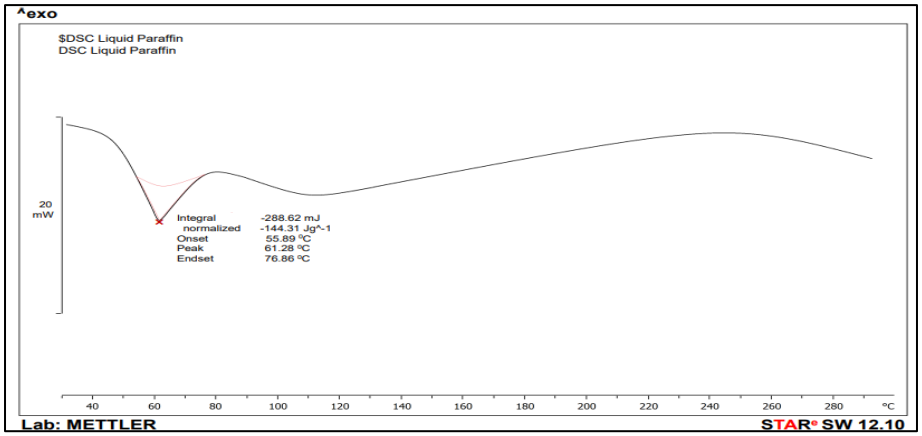

Figure 14. DSC graph of Liquid paraffin

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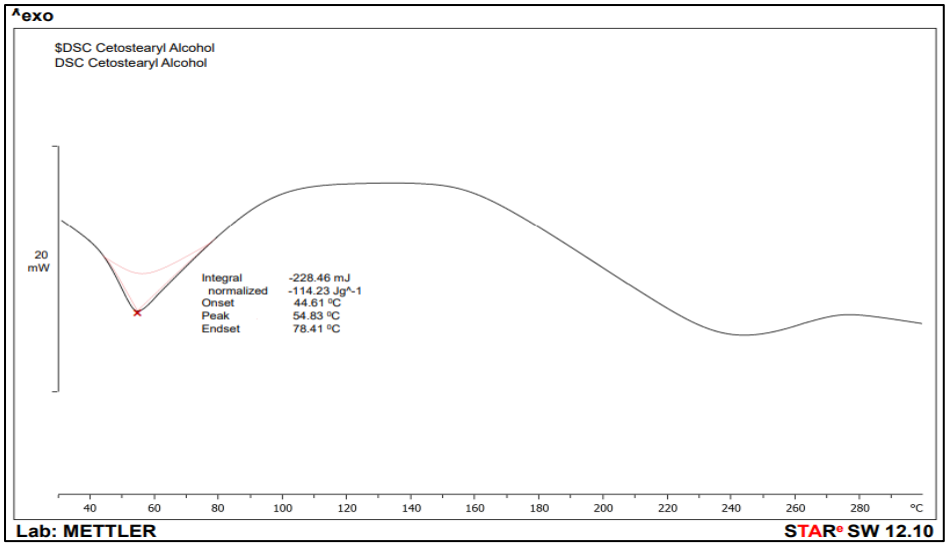

Figure 15. DSC graph of Cetostearyl alcohol

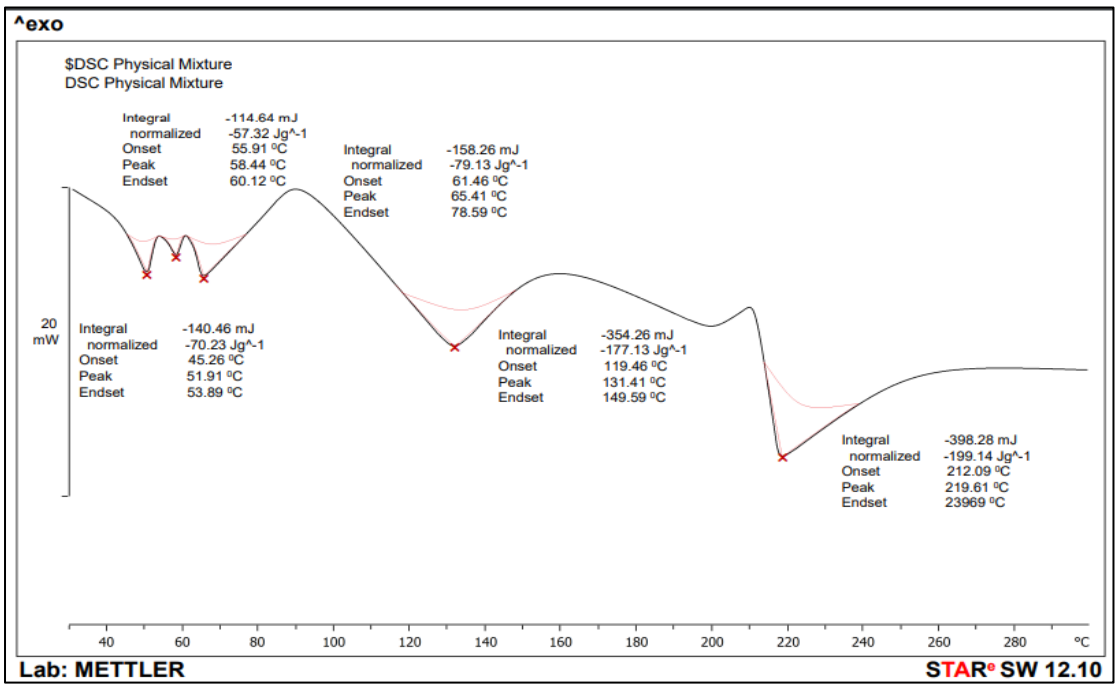

Figure 16. DSC graph of Physical Mixture

3.3 Formulation Design

The formulation of *Feronia elephantum* leaf extract ointment was developed using a QbD approach, ensuring a systematic and science-based understanding of the formulation variables and their impact on product quality. In this study, formulation development focused on identifying CMAs and evaluating their influence on CQAs.

A 2^3 full factorial design was employed using Design-Expert® software to optimize the ointment formulation. Three independent variables—cetostearyl alcohol concentration (X_1 = 0.40–0.60 g), lanolin concentration (X_2 = 0.40–0.60 g), and liquid paraffin concentration (X_3 = 1.0–2.0 g)—were selected as CMAs based on their significant influence on the structural and release characteristics of the formulations. The dependent variables considered as CQAs were viscosity (Y_1) and percentage drug release (Y_2), representing the formulation performance and therapeutic efficiency.

Eight experimental runs were generated, covering all possible combinations of the factor levels. Each formulation was prepared and evaluated based on the selected CQAs. The experimental design and observed

responses are listed in Table 3. The results indicated that variations in excipient levels significantly affected both viscosity and drug release, confirming their critical role in formulation optimization.

Table 3. Design Batches

Batch Code	X_1	X_2	X_3	Y1 (CP)	Y2 (%)
F1	0.4	0.4	1	1450	91.8
F2	0.6	0.6	1	2380	78.6
F3	0.4	0.4	2	1625	88.9
F4	**0.6**	**0.4**	**2**	**2105**	**82.4**
F5	0.6	0.6	2	2520	75.2
F6	0.4	0.6	2	1980	80.7
F7	0.4	0.6	1	1820	84.5
F8	0.6	0.4	1	1950	85.9

3.3.1 Optimization data analysis and model-validation

The experimental data obtained from the factorial design batches (Table 3) were analyzed using the Design-Expert® software to establish a mathematical relationship between the CMAs and CQAs. Different statistical models, including the mean, two-factor interaction (2FI), and main effect models, were evaluated for the best fit. Based on statistical significance and model adequacy, the main effect model was selected for both the responses.

The summary of the regression analysis is presented in Table 4, which shows high correlation coefficients ($R^2 > 0.99$), indicating an excellent agreement between the predicted and experimental values. The low standard deviation and percentage coefficient of variation (%CV) confirm the model's precision and reliability.

Table 4. Summary of results of regression analysis for responses Y_1 and Y_2

Models	R^2	Adjusted R^2	Predicted R^2	SD	%CV
Response (Y_1) Main Effect	0.9977	0.9959	0.9906	22.98	1.16
Response (Y_2) Main Effect	0.9984	0.9972	0.9935	0.2894	0.3466

Regression Equations

$$Y1=1978.75+260.00A+196.25B+78.75C \text{---------- (6)}$$

$$Y2=83.50-2.98A-3.75B-1.70C \text{---------- (7)}$$

3.3.2 Model Assessment for Dependent Variables

Model validation was performed using Analysis of Variance (ANOVA) to determine the statistical significance of the developed models. The ANOVA results for viscosity (Y_1) and drug release (Y_2) are presented in Tables 5 and 6, respectively. The results showed that both models were statistically significant ($p < 0.0001$), confirming the adequacy of the model. For viscosity (Y_1), cetostearyl alcohol (A) had the greatest influence, followed by lanolin (B) and liquid paraffin (C). Similarly, for drug release (Y_2), lanolin (B) had the most significant effect, followed by cetostearyl alcohol (A) and liquid paraffin (C). The low residual error values indicate minimal experimental deviation, confirming the robustness and predictive capability of this model. The regression coefficients further suggest that the effects of the formulation variables are not strictly linear and may vary depending on their combined levels, supporting the importance of factorial design in QbD-based optimization.

Table 5. Results of Analysis of Variance for Measured Response (Y1)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	8.985E+05	3	2.995E+05	567.12	< 0.0001	significant

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A-Cetostearyl alcohol	5.408E+05	1	5.408E+05	1024.00	< 0.0001	
B-Lanolin	3.081E+05	1	3.081E+05	583.41	< 0.0001	
C-Liquid paraffin	49612.50	1	49612.50	93.94	0.0006	
Residual	2112.50	4	528.13			
Cor Total	9.006E+05	7				

Table 6. Results of Analysis of Variance for Measured Response (Y2)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	206.43	3	68.81	821.59	< 0.0001	significant
A-Cetostearyl alcohol	70.80	1	70.80	845.43	< 0.0001	
B-Lanolin	112.50	1	112.50	1343.28	< 0.0001	
C-Liquid paraffin	23.12	1	23.12	276.06	< 0.0001	
Residual	0.3350	4	0.0837			
Cor Total	206.76	7				

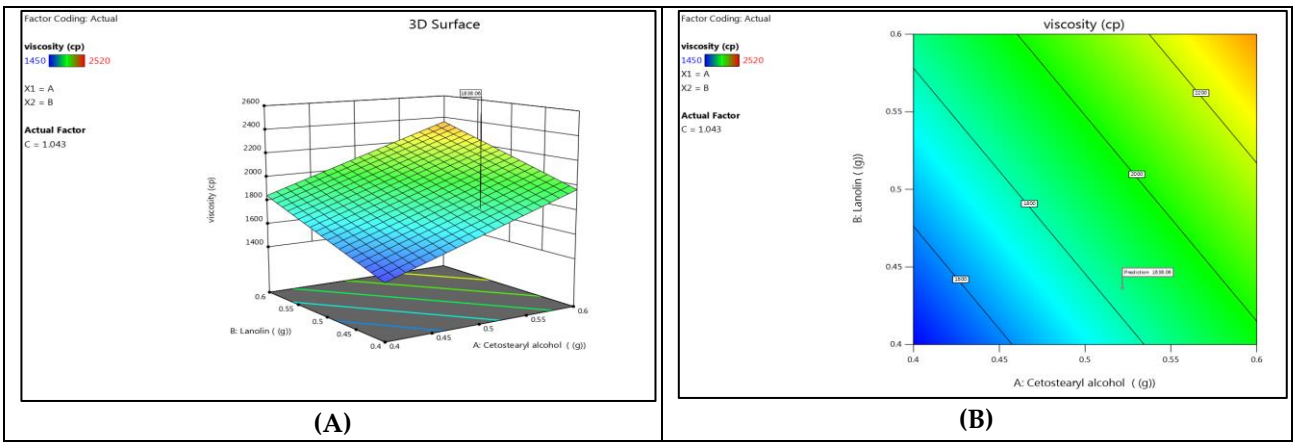
3.3.3 Response Surface Plot Analysis

Three-dimensional response surface plots and contour plots generated using the Design-Expert® software (Figure 17 and 18) provide a graphical representation of the relationship between the CMAs and CQAs. These plots are essential for defining the design space in the QbD.

The response surface plots demonstrated that viscosity (Y_1) increased with increasing concentrations of cetostearyl alcohol (A) and lanolin (B), indicating a synergistic thickening effect. This can be attributed to the enhanced structural network formation within the ointment base. In contrast, the percentage drug release (Y_2) decreased with increasing concentrations of these components, likely due to the increased matrix density, which restricts drug diffusion.

Thus, a clear trade-off between viscosity and drug release was observed in this study. Higher viscosity improves formulation stability and retention but reduces drug diffusion, whereas lower viscosity enhances drug release but may compromise the consistency.

Among all formulations, batch F4 was identified as the optimized formulation based on desirability criteria, showing a balanced combination of viscosity (2105 cP) and drug release (82.4). This formulation demonstrated optimal rheological properties and satisfactory drug release behavior, making it suitable for further development. The statistical significance of the model ($p < 0.05$) confirmed that the selected CMAs had a direct and predictable influence on the CQAs, validating the applicability of the QbD approach in formulation optimization.



(A) **(B)**

Figure 17. 3D surface plot (A) and Contour Plot (B) for Viscosity

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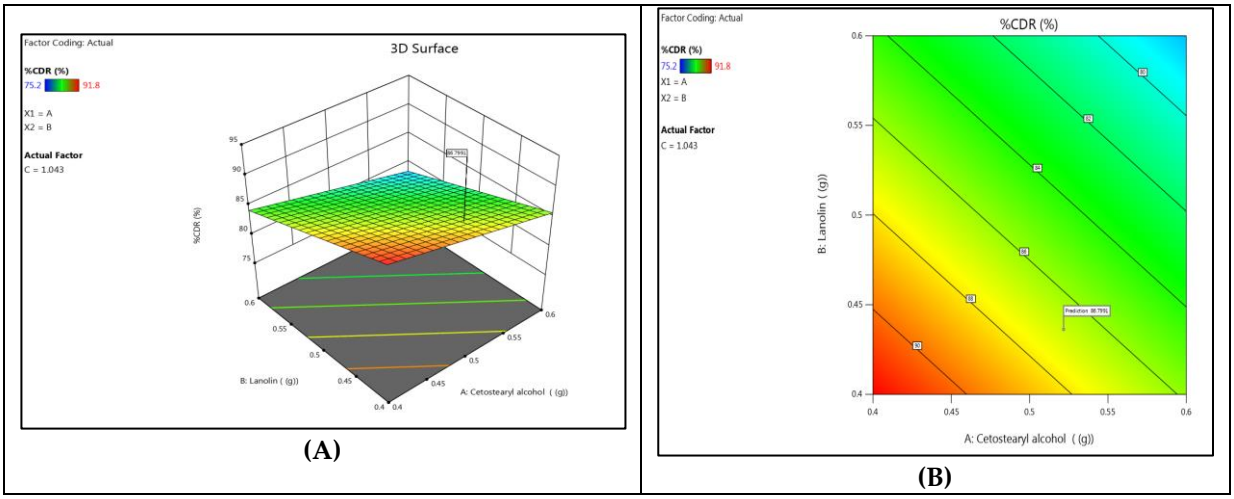

Figure 18. 3D surface plot (A) and Contour Plot (B) for %CDR

The Main effect model demonstrated significant results with a p-value less than 0.05. ANOVA was conducted, and the results were significant.

3.4 Preparation of Ointment

The ointment formulation (F4 batch) of *Feronia elephantum* leaf extract was successfully prepared using a hydrocarbon base system. The composition is presented in Table 7. The formulation strategy was designed to ensure adequate consistency, spreadability, stability, and uniform distribution of the active extract within the base of the gel.

Table 7. Formulation of Ointment

Sr. No.	Ingredients (F4 Batch)	Quantity (g)
1	Cetostearyl alcohol	0.6
2	Lanolin	0.4
3	Liquid paraffin	2
4	White soft paraffin	6.88
5	*Feronia Elephantum* leaf extract	0.12
	Total	10

3.5 Characterization of Ointment

3.5.1 Measurement of pH

The pH value of the selected ointment (F4) formulation was 6.45 ± 0.04, as shown in Table 8. The pH of the ointment was found to be within the range of pH of skin (5-7) and would not cause any irritation to the skin. Thus, prepared Ointment formulations are suitable for skin application and the formulated Ointment is also suitable for skin application.

3.5.2 Measurement of Viscosity

A Brookfield viscometer was used to measure the viscosity of the ointment at different spindle speeds. Viscosity reveals the rheological properties of all ointment formulations. All formulations showed a shear-thinning effect, wherein the viscosity decreased as the shear stress increased. Formulation F4 was found to be more viscous than the other formulations.

3.5.3 Drug Content of Ointment

The drug content in the ointment is supposed to be decreased to some extent because the agent occupies some volume as it swells in the formulations; therefore, it was determined using a UV spectrometer at 275 nm. The percentage drug content of the ointment was 95.86 ± 0.52%, as shown in Table 8. The percentage drug content of the formulations was within the permissible range.

3.5.4 Spreadability

Spreadability, determined as the percentage increase in the area of the ointment upon pressing with a certain weight, was found to be 93.42 ± 0.10%.

3.5.5 Extrudability

Formulation (F4) showed good extrudability (18.95 ± 0.5g/10 s), indicating ease of extrusion from the collapsible tube.

3.5.6 Homogeneity

Formulation (F4) was homogeneous with no lumps, phase separation, or grittiness, indicating a uniform distribution of components.

Table 8. Characterization of Ointment

Formulation code	Drug Content (%)	Spreadability (%)	Extrudability	Homogeneity	pH value	Viscosity (cps)
F4	95.86 ± 0.52%	93.42 ± 0.10%	18.95 ± 0.5g/10 sec	Homogenous	6.45 ± 0.04	2105 ± 1.15

3.6 In-vitro Release Study

The *in-vitro* drug release from the ointment of all batches was investigated using a Franz diffusion cell. For batch F4, the maximum drug release was approximately 82.49 ± 0.01%, as indicated in Table 9. The *in-vitro* release of the formulated ointment in phosphate-buffered saline (PBS) at pH 7.4 and 37 °C was examined, with the ointment undergoing a 60-minute dialysis. The quantity of the released drug was assessed using a UV-visible spectrophotometer to measure the absorbance. An initial burst drug release was observed, likely attributed to the smaller particle size, leading to a larger surface area of the ointment. Additionally, the diffusion of the drug from the outer shell of the ointment may have contributed to the observed initial burst release (Figure 19).

Table 9. *In-vitro* release profile of Ointment

Sr. No.	Time (Hours)	Ointment (F4)
1	0	0
2	2	15.17 ± 0.05
3	4	42.75 ± 0.09
4	6	58.46 ± 0.03
5	8	69.76 ± 0.04
6	10	76.61 ± 0.06
7	12	82.49 ± 0.01

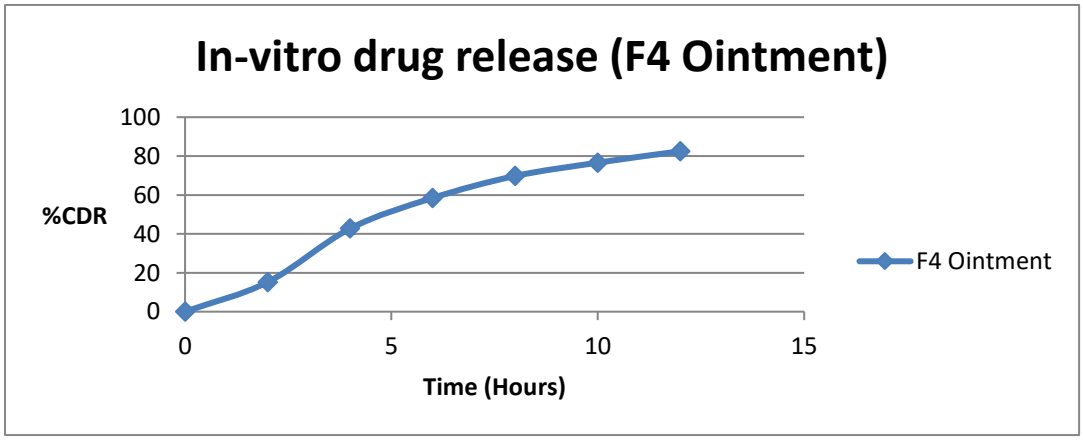

Figure 19. *In-vitro* drug release study of F4 formulation

3.7 Kinetics of drug release

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The release kinetics of the optimized F4 ointment were evaluated using various mathematical models to understand the drug release mechanism (Table 10 and Figure 20). The regression (R^2) values indicated that the formulation showed the best fit with the first-order model (0.9951), followed by the Higuchi (0.9529) and zero-order models (0.9417), while the Korsmeyer–Peppas model showed the lowest fit (0.8634). These results suggest that drug release from the F4 ointment predominantly follows first-order kinetics, with a significant contribution from diffusion-controlled release.

Table 10. R^2 values of various Kinetic Models

Kinetic Model	R^2 Value (F4 Ointment)
Zero Order	**0.9417**
First Order	**0.9951**
Higuchi Order	**0.9529**
Korsmeyer-Peppas Equation	**0.8634**

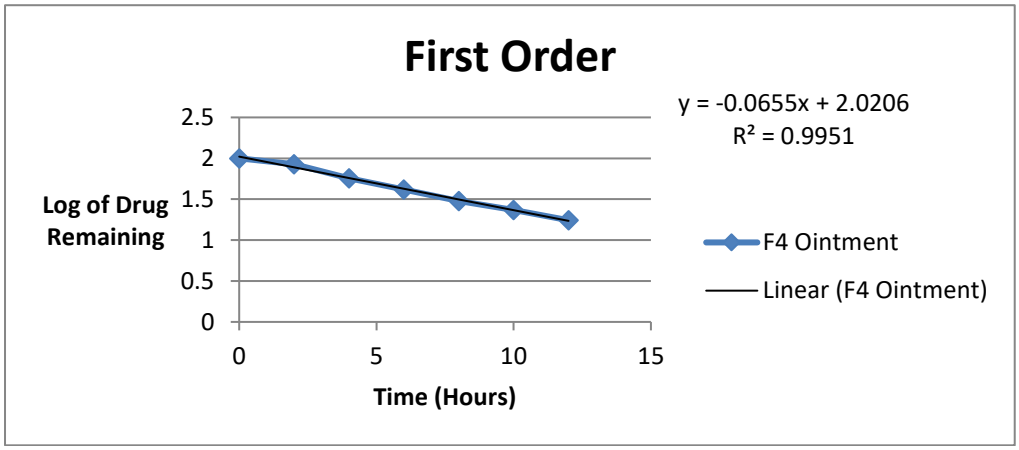

Figure 20. Kinetics of F4 Ointment

3.7 Accelerated Stability Study

The optimized ointments were subjected to stability studies, and the results are presented in Table 11. The optimized F4 ointment was subjected to stability studies over three months, and the results indicated good formulation stability with minimal variations in the physicochemical parameters. The pH showed a slight increase from 6.45 ± 0.04 to 6.61 ± 0.03, remaining within the acceptable range for topical applications. The viscosity remained nearly constant, with only minor changes from 2105 ± 1.15 to 2102 ± 2.14 cps, suggesting no significant alteration in the ointment matrix. Similarly, the drug content showed a negligible decrease from 95.86 ± 0.52% to 95.30 ± 0.6%. Overall, these findings confirm that the F4 formulation remained stable under the conditions studied.

Table 11. Stability study of parameters of the optimized formulation (F4)

Parameters	Initial Month	1st Month	2nd Month	3rd Month
pH	6.45 ± 0.04	6.61 ± 0.04	6.64 ± 0.02	6.61 ± 0.03
Viscosity (cps)	2105 ± 1.15	2110 ± 2.20	2103 ± 2.39	2102 ± 2.14
Drug content (%)	95.86 ± 0.52%	95.25 ± 0.5	95.19 ± 0.3	95.30 ± 0.6

3.9 *In-vitro* anti-inflammatory activity

The in vitro anti-inflammatory activity, evaluated using the HRBC membrane stabilization method, demonstrated a clear concentration-dependent increase in membrane protection for both *Feronia elephantum* leaf extract (FELE) and its ointment formulation (OFFELE), with the formulation consistently showing superior efficacy. FELE exhibited 25.0 ± 1.0% protection at 200 mg/mL, which increased to 41.0 ± 1.0% at 400 mg/mL, indicating a marked enhancement in activity with increasing concentrations. In comparison, OFFELE produced 29.0 ± 1.0% and 46.0 ± 1.0% protection at 200 and 400 mg/mL, respectively, reflecting a consistent improvement

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of approximately 4–5% over the crude extract at the corresponding concentrations. This enhancement suggests that incorporation into an ointment base improves the functional availability of bioactive constituents, likely through improved dispersion, increased interaction with the membrane surface, and stabilization of the phytochemicals.

The HRBC model is considered analogous to lysosomal membranes; thus, membrane stabilization is indicative of the prevention of lysosomal enzyme release, which is a critical event in inflammatory processes. The observed protective effect may be attributed to phytoconstituents such as flavonoids, phenolics, and triterpenoids, which interact with membrane proteins and phospholipids via hydrogen bonding and hydrophobic interactions, preventing protein denaturation and osmotic lysis. The ointment formulation likely enhances these interactions by promoting closer and more uniform contact between the active constituents and the erythrocyte membrane.

The standard drug diclofenac sodium exhibited the highest membrane stabilization effect, ranging from 53.0 ± 1.0% to 61.0 ± 1.0%, which was significantly higher than that of both FELE and OFFELE under comparable conditions. This superior activity is consistent with its established mechanism involving the inhibition of cyclooxygenase and subsequent suppression of prostaglandin synthesis. Nevertheless, the ointment formulation demonstrated appreciable activity (46.0% at 400 mg/mL), highlighting its potential as a natural anti-inflammatory alternative with fewer adverse effects. The consistently low standard deviation (±1.0) across all samples indicates the high precision and reproducibility of the experimental results.

Overall, these findings confirm that formulating the extract into an ointment significantly enhances its anti-inflammatory efficacy of *Feronia elephantum* extract and supports its potential application as a topical therapeutic agent, as evidenced by the comparative data presented in Table 12. and Figure 21.

Table 12. *In vitro* Anti-inflammatory Activity (Mean ± SD, n = 3)

Sample	Concentration (mg/mL)	% Protection
FELE	200	25.0 ± 1.0
FELE	400	41.0 ± 1.0
OFFELE	200	29.0 ± 1.0
OFFELE	400	46.0 ± 1.0
Diclofenac	05	53.0 ± 1.0 – 61.0 ± 1.0

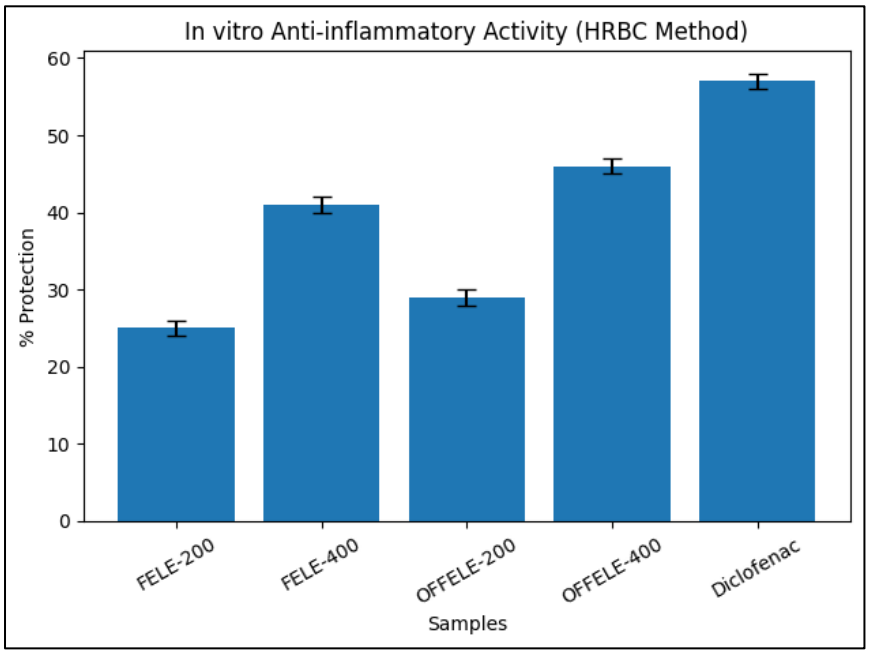

Figure 21. Anti-inflammatory activity of FELE and OFFELE by HRBC membrane stabilization method (mean ± SD, n = 3); OFFELE > FELE, while Diclofenac sodium showed highest activity.

4. Conclusion

In conclusion, an herbal ointment containing *Feronia elephantum* leaf extract was successfully formulated and optimized using a QbD-based 2^3 full factorial design. Preformulation studies, including FTIR, DSC, and UV analyses, confirmed the identity of the extract and its compatibility with the selected excipients. The developed formulations exhibited acceptable physicochemical characteristics, including suitable pH, viscosity, spreadability, extrudability, homogeneity, and drug content values. Optimization studies revealed that cetostearyl alcohol, lanolin, and liquid paraffin significantly influenced the critical quality attributes of the formulation. Among the prepared batches, formulation F4 was identified as the optimized formulation, providing an appropriate balance between viscosity and drug release rate. The optimized ointment exhibited satisfactory in vitro drug release behavior and demonstrated notable anti-inflammatory activity in the HRBC membrane stabilization assay. Furthermore, the formulation remained stable under accelerated storage conditions, without significant changes in its quality attributes. These findings suggest that the developed *Feronia elephantum* ointment is a promising topical herbal formulation for anti-inflammatory applications and warrants further in vivo and clinical investigation.

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None

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

Conceptualization; methodology; software; validation; formal analysis; investigation; resources; data curation; writing—original draft preparation; writing—review and editing; visualization; supervision; project administration by I. A. Shaikh, S. D. Tayade, A. S. Tamboli, K. K. Ganjare, V. S. Borkar, and J. B. Khedekar. All authors have read and agreed to the published version of the manuscript.

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