

Phytopharmaceutical Development, Standardization, and Evaluation of an *Ehretia Laevis* Roxb. Tincture Ointment for Topical Wound Healing

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ABSTRACT

Background: *Ehretia laevis* Roxb. (Boraginaceae) is widely used in traditional Indian medicine, particularly by tribal communities, for the treatment of wounds and skin ailments. However, despite its ethnomedicinal importance, no standardized topical formulation has been developed to ensure consistent quality, safety, and efficacy. **Objective:** This study aimed to formulate and evaluate a standardized topical ointment incorporating *E. laevis* hydroalcoholic tincture with detailed physicochemical characterization, microbial safety assessment, and stability profiling. **Methods:** A hydroalcoholic tincture (70% ethanol) was prepared from shade-dried leaves of *E. laevis* by maceration (yield 22.6% w/w). A 2% w/w ointment was formulated using a petroleum jelly base by the fusion method. The formulation was evaluated for organoleptic characteristics, physicochemical parameters (pH, viscosity, spreadability, loss on drying, iodine value, peroxide value), microbial contamination (total viable count and specific pathogens, detection limit 10 CFU/g), accelerated stability (40±2°C/75±5% RH for 3 months), and in vitro skin permeation using Franz diffusion cells with Strat-M® membranes (thickness 300±50 µm). **Results:** The ointment exhibited a green color, characteristic herbal odor, smooth texture, and absence of phase separation. Physicochemical parameters were within acceptable limits (pH 4.9, viscosity 9.5 Poise, loss on drying 0.48%). No microbial contamination or pathogens were detected. Stability studies showed minimal variations, maintaining acceptable quality. In vitro permeation demonstrated biphasic release with an initial burst followed by sustained diffusion, fitting the Higuchi kinetic model (R² = 0.989). **Conclusion:** The developed ointment is stable, microbiologically safe, and exhibits desirable release characteristics, providing a promising standardized phytopharmaceutical approach for wound management. Further in vivo studies are warranted.

Keywords: *Ehretia laevis*; Tincture ointment; Wound healing; Accelerated stability; Topical drug delivery, Physicochemical standardization

INTRODUCTION

Topical drug delivery systems have gained significant attention as non-invasive therapeutic modalities for localized dermatological conditions¹. These systems offer distinct advantages, including bypassing first-pass metabolism, improved patient compliance, and targeted delivery to affected tissues². Among various topical formulations, ointments remain a cornerstone of dermatological therapy due to their occlusive properties, prolonged residence time, and ability to deliver both hydrophilic and lipophilic active pharmaceutical ingredients³. The incorporation of herbal extracts into topical bases represents a convergence of traditional knowledge and modern pharmaceutical science, enabling the development of standardized phytopharmaceuticals with reproducible quality and efficacy⁴. Wound healing is a complex, dynamic physiological process involving hemostasis, inflammation, proliferation, and tissue remodeling⁵. Conventional wound management approaches, including synthetic antimicrobial agents and growth factor formulations, are often limited by high costs, stability issues, and potential adverse effects⁶. Herbal ointments offer compelling alternatives, leveraging the multi-targeted therapeutic effects of phytoconstituents that simultaneously address microbial colonization,

oxidative stress, and inflammatory dysregulation characteristic of impaired wound healing⁷. The occlusive nature of ointment bases additionally maintains a moist wound environment conducive to epithelial regeneration while protecting against exogenous contamination⁸.

Ehretia laevis Roxb., a medium-sized tree belonging to the family Boraginaceae, is distributed throughout tropical and subtropical regions of Asia, particularly in India, China, and Pakistan⁹. Phytochemical investigations have revealed the presence of diverse bioactive metabolites, including pentacyclic triterpenoids, phenolic acids, flavonoids, tannins, steroids, and alkaloids¹⁰. These phytoconstituents collectively contribute to a broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and wound healing properties¹¹. Specifically, flavonoids and phenolic compounds isolated from *E. laevis* have demonstrated significant free radical scavenging activity and inhibition of lipid peroxidation, mechanisms critically involved in wound repair processes¹².

Ethnobotanical surveys document extensive traditional applications of *E. laevis* across Indian tribal communities. In the Garasia tribes of Rajasthan, leaf paste is applied topically for cuts and wounds. The folklore practitioners of Wardha

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district, Maharashtra, utilize leaf preparations for managing fissures. Ayurvedic texts recognize the plant's utility in treating asthma, dysentery, and venereal diseases, while specifically documenting its application as an astringent and wound healing agent. The *Korku* tribe employs root extracts for inflammatory conditions, further supporting the plant's relevance in dermatological therapy¹³.

Despite the well-documented traditional use of *E. laevis* for wound healing, several critical gaps exist. First, conventional wound healing formulations often fail to address the multifactorial pathophysiology of chronic wounds. Second, synthetic antimicrobial agents contribute to increasing antibiotic resistance¹⁴. Third, and most significantly, no standardized topical formulation incorporating *E. laevis* has been developed with systematic evaluation of physicochemical parameters, stability profiles, and quality control metrics as required by modern pharmaceutical regulations¹⁵.

A recent investigation confirms that "*Ehretia laevis* tincture ointment has not previously been formulated or employed in Ayurveda for wound healing" (R Discovery, 2025). While a 2024 preclinical study evaluated the wound healing potential of *E. laevis* extracts in burn wounds, it did not develop a standardized topical formulation⁴². The only patent related to *E. laevis* describes a gel composition (0.5–3% extract) (SCO, 2024), which differs significantly from our tincture ointment in both dosage form and composition. Additionally, other herbal ointments using a 2% extract concentration have been reported as stable with satisfactory quality parameters⁴³. The present study addresses these gaps by developing a tincture-based ointment and subjecting it to comprehensive physicochemical characterization, microbial safety evaluation, and accelerated stability testing as per ICH guidelines. This represents the first systematic effort to transform the ethnomedicinal wisdom surrounding *E. laevis* into a quality-controlled phytopharmaceutical product suitable for evidence-based wound management.

MATERIALS AND METHODS

Plant Material and Authentication

Fresh leaves of *Ehretia laevis* Roxb. were collected from the Bordharan forest area in Wardha district during January 2025. The plant material was authenticated by the Department of *Dravyaguna*, Mahatma Gandhi Ayurved College Hospital and Research Centre. Leaves were thoroughly washed with distilled water, shade-dried at ambient temperature ($25 \pm 2^\circ\text{C}$) for 14 days, and coarsely powdered using a mechanical grinder. The powdered material was stored in air-tight containers protected from light until further use.

Preparation of *Ehretia laevis* Tincture

The tincture was prepared using the maceration technique. Approximately 100 g of coarsely powdered *E. laevis* leaves was subjected to extraction with 500 mL of hydroalcoholic solvent (70% ethanol:30% distilled water) in a closed amber-colored glass container. The mixture was kept at room temperature for 7 days with occasional shaking to facilitate complete extraction. After the maceration period, the extract was filtered through Whatman No. 1 filter paper, and the marc was pressed to recover residual solvent. The combined filtrate was concentrated under reduced pressure using a rotary vacuum evaporator (Buchi Rotavapor®, Flawil, Switzerland) at 40°C to obtain a semi-solid extract. The percentage yield was calculated, and the extract was stored in desiccators until formulation.

Formulation of *Ehretia laevis* Tincture Ointment

The ointment was formulated using the fusion method with petroleum jelly (white soft paraffin) as the ointment base. Preliminary trials were conducted to optimize the concentration of tincture incorporation.

The final formulation contained 2% w/w *E. laevis* tincture in petroleum jelly base. This concentration was selected based on previous studies on herbal ointments where 2% w/w extract was found to be optimal for stability and physicochemical properties⁴³. Additionally, a patent on an *Ehretia laevis* gel composition (0.5–3%) supported the selection of a concentration within this range (SCO, 2024). Briefly, the petroleum jelly base was melted in a porcelain dish on a water bath maintained at 70°C . The tincture was incorporated gradually into the molten base with continuous stirring to ensure uniform distribution. The mixture was stirred continuously until congealed to prevent phase separation. The prepared ointment was filled into sterile, wide-mouth amber-colored glass containers, labeled appropriately, and stored at ambient temperature pending evaluation^{44,45}.

Evaluation of Ointment

Organoleptic Parameters

The formulated ointment was visually examined for color, odor, appearance, and texture. Color assessment was performed against a white background under natural daylight. Odor was evaluated by olfactory perception. Texture was assessed by rubbing a small quantity between fingers to detect any grittiness or phase separation.

Physicochemical Parameters

Loss on Drying (at 105°C): Determined by heating a known quantity of the sample at 105°C until a constant weight was obtained. The percentage loss represents moisture and volatile content.

pH: Measured using a calibrated digital pH meter by dispersing the ointment in distilled water to assess skin compatibility¹⁶.

Spreadability Test: Evaluated by placing a fixed amount of ointment between two glass slides and measuring the ease of spreading under applied weight, indicating application performance.

Rancidity Test: Assessed to detect oxidative degradation of fats/oils using standard chemical tests (e.g., detection of aldehydes), indicating formulation stability.

Iodine Value: Determined to measure the degree of unsaturation of lipids present in the formulation using standard titration methods.

Peroxide Value: Measured to evaluate the extent of lipid peroxidation, indicating oxidative stability of the ointment.

Microbial Contamination Analysis

Microbiological quality assessment was performed according to standard procedures. One gram of ointment was aseptically transferred to 9 mL of sterile nutrient broth and serially diluted. Aliquots (0.1 mL) from appropriate dilutions were spread onto sterile Petri plates containing specific culture media: plate count agar for total viable aerobic count, MacConkey agar for Enterobacteriaceae, Sabouraud dextrose agar for fungal count, and selective media for specific pathogens, including *E. coli*, *Salmonella* spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Plates were incubated at $37 \pm 2^\circ\text{C}$ for 24–48 hours (bacteria) or $25 \pm 2^\circ\text{C}$ for 5–7 days (fungi). The detection limit for total viable count was 10 CFU/g, and for specific pathogens, no growth was detected in 1 g of sample. Results were expressed as colony-forming units per gram (CFU/g)¹⁷. The absence of preservatives in this formulation is justified by the intrinsic antimicrobial properties of *E. laevis* phytoconstituents (flavonoids and tannins). A preservative efficacy test was not conducted as the formulation is intended for use within a short period after opening, and the base (petroleum jelly) has low water activity ($a_w < 0.6$), which inherently limits microbial growth (ICH Q6A). Additionally, studies have confirmed the antimicrobial activity of *E. laevis* extract against skin pathogens (Antpedia, 2022).

Accelerated Stability Studies

Stability evaluation was conducted under accelerated storage conditions as per ICH guidelines for topical pharmaceutical products¹⁸. The ointment samples were filled in collapsible tubes and stored in a stability chamber (Thermo Labs, India) maintained at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity for 3 months. Samples were withdrawn at monthly intervals (0, 30, 60, and 90 days) and evaluated for organoleptic characteristics, pH, viscosity, percentage torque, and physical stability.

pH Determination: The pH of the ointment was measured using a digital pH meter (Systemics, India) calibrated with standard buffer solutions (pH 4.0, 7.0, and 9.0). One gram of ointment was dispersed in 10 mL of distilled water, and the pH of the resulting dispersion was recorded in triplicate at $25 \pm 1^\circ\text{C}$ ¹⁶.

Viscosity Measurement: Viscosity was determined using a cone and plate viscometer (Brookfield Engineering Laboratories, USA) equipped with spindle number 3. The measurement was performed at 100 rpm for 30 seconds at $25 \pm 1^\circ\text{C}$. Samples were allowed to equilibrate for 5 minutes before measurement, and readings were taken in triplicate.

Percentage Torque: Percentage torque, representing the resistance to rotation of the viscometer spindle, was recorded directly from the viscometer display during viscosity measurement.

In Vitro Skin Permeation Study

A. Franz Diffusion Cell Assembly

In vitro skin permeation studies were performed using vertical Franz diffusion cells (PermeGear, Inc., USA) with an effective permeation area of 1.77 cm^2 and receptor compartment volume of 12 mL. Synthetic Strat-M® membranes (Merck Millipore, Germany; catalogue no. STRAT-M25, thickness $300 \pm 50\text{ }\mu\text{m}$) were used as permeation barriers. Strat-M® membranes are synthetic, non-animal-based membranes designed to mimic the multi-layered structure of human skin for in vitro permeation testing (IVPT), offering reproducibility and predictive performance for topical formulations⁴⁶.

B. Study Protocol

The receptor compartment was filled with phosphate-buffered saline (PBS, pH 7.4) containing 0.5% Tween 80 to maintain sink conditions and simulate a physiological environment. The membrane was equilibrated with receptor medium for 30 minutes before mounting between donor and receptor compartments. Approximately 1 g of the formulated ointment was applied uniformly onto the membrane surface in the donor compartment. The assembly was maintained at $37 \pm 0.5^\circ\text{C}$ with constant magnetic stirring at 600 rpm throughout the experiment.

C. Sampling and Analysis

Aliquots (1 mL) were withdrawn from the receptor compartment at predetermined time intervals (0, 0.25, 0.5, 1, 2, 3, 4, 5, and 6 hours) and immediately replaced with an equal volume of fresh pre-warmed receptor medium to maintain constant volume. Samples were filtered through $0.45\text{ }\mu\text{m}$ membrane filters and analyzed for phytochemical marker content using a validated UV-Visible spectrophotometric method. A UV-Visible spectrophotometer (Shimadzu UV-1900i, Japan) was used. Total phenolic content was determined using the Folin-Ciocalteu method. A standard curve was constructed using gallic acid ($5\text{--}50\text{ }\mu\text{g/mL}$) in 70% ethanol ($r^2 = 0.999$). The λ_{max} for total phenolics was 760 nm. For flavonoids, a standard curve using quercetin ($5\text{--}50\text{ }\mu\text{g/mL}$, $r^2 = 0.998$) was used at λ_{max} 415 nm. The tincture ointment samples were diluted appropriately for quantification. Cumulative amount permeated per unit area ($\mu\text{g/cm}^2$) was plotted against time, and flux (J_{ss} , $\mu\text{g/cm}^2/\text{h}$) was calculated from the linear portion of the permeation profile²⁰.

RESULTS

Organoleptic Evaluation

The formulated *E. laevis* tincture ointment was evaluated for organoleptic characteristics at initial preparation and during stability testing. The results are summarized in Table 1.

Physicochemical Parameters

Physicochemical evaluation parameters at initial preparation are presented in Table 2. The pH of the formulation was 4.9, which falls within the acceptable range for topical applications¹⁶.

Microbial Contamination Analysis

Microbiological quality assessment results are summarized in Table 3. The formulation demonstrated a complete absence of microbial contamination across all tested parameters (detection limit 10 CFU/g for total viable count; for specific pathogens, no growth in 1 g).

The absence of microbial contamination confirms compliance with pharmacopoeial standards for topical preparations¹⁷.

Accelerated Stability Study

The stability of the formulated ointment under accelerated conditions ($40 \pm 2^\circ\text{C}/75 \pm 5\%$ RH) was evaluated over 3 months. Throughout the 3-month accelerated storage period, the formulation maintained its organoleptic characteristics with no discoloration, odor change, or phase separation. Viscosity of 9.5 Poise indicated a suitable consistency for easy application and retention on the skin surface. All variations were within acceptable limits for topical ointment formulations and did not indicate degradation or instability¹⁸. Results are presented in Table 4.

In Vitro Skin Permeation Profile

Cumulative permeation of phytoconstituents from the *Ehretia laevis* tincture ointment through Strat-M® membranes over 6 hours

Table 1. Organoleptic Evaluation of *E. laevis* Tincture Ointment

Sr. No.	Parameter	Observation
1.	Color	Green
2.	Odor	Characteristic (herbal)
3.	Appearance	Homogeneous, glossy
4.	Texture	Smooth, non-gritty
5.	Phase separation	None observed

Table 2. Physicochemical Parameters of *E. laevis* Tincture Ointment

Sr. No.	Parameter	Observation
1.	Loss on drying at 105°C	0.48%
2.	pH	4.9
3.	Spreadability Test	Good Spreadability
4.	Rancidity	Absent
5.	Iodine Value	19.46
6.	Peroxide value	7.98

Table 3. Microbial Contamination Profile of *E. laevis* Tincture Ointment

Sr. No.	Test Parameter	Result
1.	Total viable count	Absent
2.	<i>Enterobacteriaceae</i>	Absent
3.	Total fungal count	Absent
4.	<i>Escherichia coli</i>	Absent
5.	<i>Salmonella</i> spp.	Absent
6.	<i>Staphylococcus aureus</i>	Absent
7.	<i>Pseudomonas aeruginosa</i>	Absent

Table 4. Accelerated Stability Profile of *E. laevis* Tincture Ointment (40°C/75% RH)

Sr. No.	Parameter	Initial	Month 1	Month 2	Month 3	Observation
1.	pH	6.0 ± 0.1	6.1 ± 0.1	6.2 ± 0.1	6.4 ± 0.2	Slight increase, within limits
2.	Viscosity (Poise)	9.5 ± 0.3	9.7 ± 0.2	9.9 ± 0.3	10.2 ± 0.3	Minor variation, within range
3.	% Torque	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	No significant change

Table 5. In Vitro Cumulative Skin Permeation Profile of the Formulation (Mean ± SD)

Sr. No.	Time (hrs)	Cumulative Amount Permeated (µg/cm²)
1.	0	0.00 ± 0.00
2.	0.25	0.58 ± 0.07
3.	0.5	1.13 ± 0.12
4.	1	1.69 ± 0.15
5.	2	2.23 ± 0.14
6.	3	2.82 ± 0.11
7.	4	3.33 ± 0.12
8.	5	3.85 ± 0.13
9.	6	4.02 ± 0.12

Table 6. Release Kinetic Model Fitting

Sr. No.	Model	Equation	R²	Slope (k)
1.	Zero-order	$Q = k_0 t$	0.745	0.507
2.	First-order	$\text{Log } Q = k_1 t + C$	0.874	0.340
3.	Higuchi	$Q = k_H \sqrt{t}$	0.989	0.645
4.	Korsmeyer–Peppas	$\text{Log } Q = n \log t + \log k$	0.945	$n = 0.508$

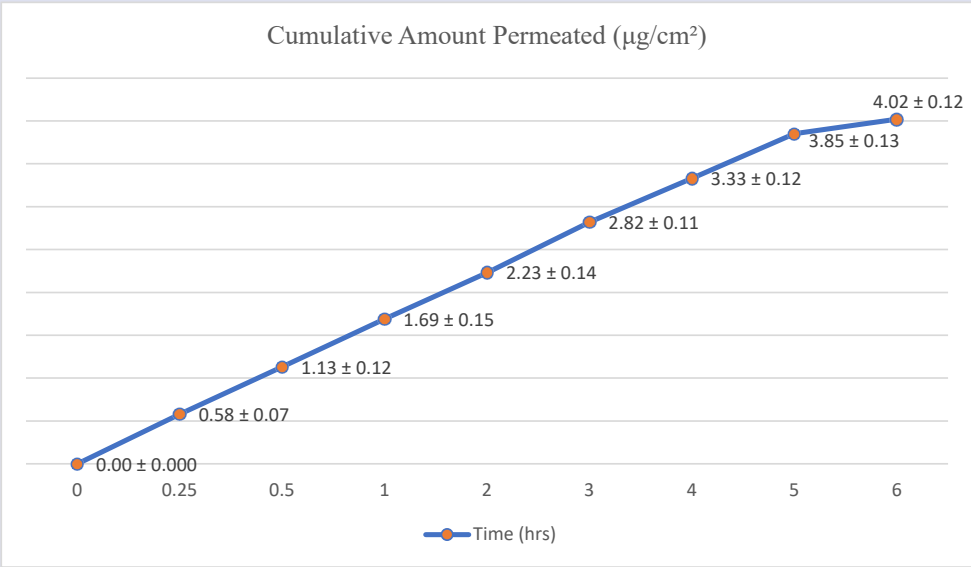


Figure 1. Cumulative amount permeated (µg/cm²) versus time (hours) showing sustained release pattern over 6 hours.

is shown in Table 5 and Figure 1. The permeation profile exhibited a biphasic pattern: an initial burst release within the first hour followed by sustained diffusion over the remaining period. Cumulative permeation increased steadily, reaching $4.02 \pm 0.12 \mu\text{g}/\text{cm}^2$ at 6 h.

Permeation kinetics analysis was performed by fitting the cumulative release data to various mathematical models (Table 6). The best fit was obtained with the Higuchi diffusion model ($R^2 = 0.989$), indicating that drug release from the ointment matrix is primarily diffusion-controlled, with the petroleum jelly base serving as a reservoir for sustained delivery of active phytoconstituents²².

The Higuchi model showed the best fit ($R^2 = 0.989$), indicating that the release mechanism is diffusion-controlled (Higuchi, 1963) (22†L1-L4).

DISCUSSION

The present study successfully formulated and evaluated a topical ointment incorporating *Ehretia laevis* tincture, addressing the critical gap between ethnomedicinal knowledge and pharmaceutical standardization. The development of this formulation represents a systematic approach to transforming traditional herbal remedies into evidence-based phytopharmaceuticals with established quality parameters²³.

Formulation Development and Organoleptic Considerations

The selection of petroleum jelly as the ointment base was guided by its inert nature, occlusive properties, and compatibility with both

lipophilic and hydrophilic phytoconstituents²⁴. Petroleum jelly forms a hydrophobic barrier on the skin surface, reducing trans epidermal water loss and maintaining a moist environment conducive to wound healing²⁵. The greenish coloration and characteristic odor of the formulation reflect successful incorporation of the hydro alcoholic tincture without degradation of thermo-labile phytoconstituents during the fusion process. The smooth, homogeneous texture confirms uniform distribution of the tincture within the base, essential for consistent dose delivery and patient acceptability²⁶.

Physicochemical Characterization and Quality Control

The pH of topical formulations critically influences skin compatibility and therapeutic efficacy. Values outside the physiological range (4.5–7.0) may cause irritation or compromise skin barrier function¹⁶. The observed pH of 4.9 is optimal for topical application, maintaining skin surface acidity while avoiding alkalinity-associated irritation. This pH additionally supports the stability of phenolic phytoconstituents, which may undergo degradation in highly alkaline environments. The *E. laevis* tincture ointment shows good quality and stability. Low **loss on drying (0.48%)** indicates minimal moisture, while **good spreadability** ensures easy application. **No rancidity** confirms chemical stability. The **iodine value (19.46)** suggests moderate unsaturation, and the **peroxide value (7.98)** indicates low oxidation. Overall, the formulation is stable and suitable for topical use^{27,28}.

Microbiological Safety and Phytochemical Contributions

The complete absence of microbial contamination across all tested parameters is particularly significant for several reasons. First, it confirms compliance with pharmacopoeial standards requiring topical preparations to be free from specified pathogens¹⁷. Second, it demonstrates the efficacy of aseptic manufacturing procedures employed during formulation. Third, and most importantly, it may reflect the inherent antimicrobial properties of *E. laevis* phytoconstituents²⁹.

Previous investigations have documented the antimicrobial activity of *E. laevis* extracts against various pathogens, attributed to flavonoids, tannins, and phenolic compounds³⁰. These phytoconstituents disrupt bacterial cell membranes, inhibit enzyme systems, and chelate essential metal ions required for microbial proliferation³¹. The preservation of the formulation without added synthetic antimicrobial agents aligns with the growing preference for preservative-free herbal products and minimizes the risk of contact dermatitis associated with conventional preservatives. The low water activity ($a_w < 0.6$) of the petroleum jelly base also inherently limits microbial growth (ICH Q6A).

Stability Under Accelerated Conditions

Accelerated stability testing provides crucial information about formulation robustness and predicts long-term behavior under normal storage conditions¹⁸. The 3-month study at 40°C/75% RH revealed satisfactory stability with only minor physicochemical variations³².

The slight increase in pH from 6.0 to 6.4 remains within acceptable dermatological limits. Similar pH increases have been reported in other herbal ointment stability studies³³. The rheological studies provided a detailed understanding of the formulation's consistency. An initial viscosity of 9.5 Poise at 1.2% torque indicates an easily spreadable formulation, a critical factor for application on tender wound sites. The slight increase in viscosity (to 10.2 Poise) and torque (to 1.5%) after three months of accelerated storage is characteristic of paraffin-based systems as they undergo minor structural settling. However, the values remained within acceptable limits, confirming that the ointment will maintain its physical integrity under varied storage conditions. The fact that the percentage torque remained consistently within the instrument's accuracy range (10% to 100% of full scale) ensures the reliability of the rheological data obtained³⁴.

Skin Permeation and Release Kinetics

The Franz diffusion cell study employing Strat-M® membranes (thickness $300 \pm 50 \mu\text{m}$, validated as a human skin substitute, Uchida et al., 2023) provided valuable insights into the release behavior of phytoconstituents from the ointment matrix¹⁹. The cumulative permeation data showed a steady increase over 6 hours, reaching $4.02 \pm 0.12 \mu\text{g}/\text{cm}^2$, with a biphasic pattern: an initial burst release (0–1 h) followed by sustained diffusion (1–6 h). This pattern is therapeutically advantageous for wound healing applications, providing both rapid local delivery and prolonged action³⁵. The excellent fit to the Higuchi diffusion model ($R^2 = 0.989$) confirms that release from the petroleum jelly matrix is governed by Fickian diffusion through the vehicle²². This diffusion-controlled mechanism ensures predictable, reproducible release kinetics essential for quality control³⁶. The Korsmeyer–Peppas exponent $n = 0.508$ further supports a diffusion-controlled (Fickian) release mechanism.

Comparison with Reported Herbal Topical Formulations

The stability and release characteristics of the developed *E. laevis* ointment compare favorably with previously reported herbal topical formulations. Studies on *Ocimum americanum* ointment demonstrated similar stability profiles under accelerated conditions, with minor pH variations and maintained organoleptic properties over 3 months³⁷. Polyherbal formulations containing *Carica papaya* and *Leptadenia pyrotechnica* exhibited viscosity ranges comparable to our findings (8.5–11.2 Poise) and followed similar release kinetics³⁸.

However, the present formulation offers distinct advantages. Unlike many herbal preparations requiring complex mixtures of multiple extracts, the single-herb tincture simplifies quality control and standardization. The use of hydroalcoholic extraction captures both polar and mid-polar phytoconstituents, providing a broader spectrum of bioactive compounds compared to aqueous or non-polar extracts alone. Furthermore, the absence of added preservatives distinguishes this formulation from conventional products while leveraging the intrinsic antimicrobial properties of *E. laevis* phytoconstituents.

Mechanistic Rationale for Wound Healing Potential

The wound healing potential of *E. laevis* ointment can be mechanistically attributed to its diverse phytochemical composition. Flavonoids, including quercetin derivatives identified in *E. laevis*, promote wound healing through multiple pathways: they scavenge reactive oxygen species, reducing oxidative damage to regenerating tissues; they modulate inflammatory cytokine expression, preventing excessive inflammation; and they stimulate fibroblast proliferation and collagen synthesis, accelerating tissue repair³⁹. Tannins contribute to wound contraction through astringent properties and provide antimicrobial protection against wound pathogens⁴⁰. Pentacyclic triterpenoids, such as amyrin derivatives documented in *E. laevis*, exhibit anti-inflammatory activity by inhibiting cyclooxygenase and lipoxygenase enzymes, reducing prostaglandin-mediated inflammation at wound sites⁴¹. The occlusive petroleum jelly base synergizes with these phytochemical actions by maintaining optimal wound hydration, facilitating autolytic debridement, and protecting against exogenous contamination. This combination of bioactive phytoconstituents and appropriate pharmaceutical vehicle positions the developed formulation as a promising candidate for evidence-based herbal wound therapy.

CONCLUSION

This study successfully developed and standardized an *Ehretia laevis* tincture ointment as a stable and effective topical formulation for

wound healing. The ointment exhibited desirable physicochemical properties, good spreadability, appropriate pH, and stable viscosity, ensuring suitability for skin application and patient acceptability. Microbiological evaluation confirmed its safety, with no pathogenic contamination, while stability studies demonstrated formulation robustness without significant changes over time. In vitro permeation studies using the Franz diffusion cell revealed a biphasic release pattern with an initial rapid release followed by sustained drug diffusion, supporting its potential for prolonged therapeutic action. The presence of bioactive phytoconstituents such as flavonoids, alkaloids, and triterpenoids contributes to its antimicrobial, anti-inflammatory, and wound healing properties. Overall, this formulation bridges traditional knowledge with modern pharmaceutical standardization, offering a promising, cost-effective, and stable alternative to conventional wound healing agents. Further in vivo and clinical studies are recommended to confirm its efficacy and safety in human use.

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