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Research Article

Development of a Calendula Officinalis-Based Emulgel as a Topical Herbal Therapy for Leg Ulcer Management: Formulation and In Vivo Assessment

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ABSTRACT

Objectives: The study aimed to develop and evaluate a Calendula officinalis-based emulgel as a topical herbal therapy for chronic leg ulcer management, emphasizing anti-inflammatory and antimicrobial activity, stability, and patient acceptability. Methods: A 3^2 full factorial design was employed to optimize the emulgel formulations using varying Smix ratios (Span 20:Tween 20), oil, and water phase compositions. The optimized batch was selected based on drug release, antimicrobial, and anti-inflammatory activity. Formulations were assessed for organoleptic properties, pH, viscosity, spreadability, drug content, In-vitro drug release, skin irritancy, washability, and accelerated stability. Antimicrobial activity was evaluated against Staphylococcus aureus, and anti-inflammatory activity was assessed using the protein denaturation assay. Results: The optimized formulation, F5 (Smix 3:1, 1 mL oil, 1 mL water), demonstrated superior performance with the highest drug release (90.5 ± 3.4%), zone of inhibition (22.4 ± 0.5 mm), and protein denaturation inhibition (75.6 ± 1.1%). All batches exhibited pH within 5.76–5.78, viscosity between 15,800–16,500 cP, and spreadability around 5.5 cm. No irritation was observed during skin tests, and all batches were easily washable. The formulations remained stable over 3 months under accelerated conditions. Conclusion:The optimized Calendula officinalis emulgel exhibited excellent physicochemical stability and potent bioactivity, highlighting its potential as a costeffective and natural alternative for chronic leg ulcer treatment. These findings support future clinical translation of the formulation in wound care therapeutics.

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INTRODUCTION

Leg ulcers recur chronically impacting 1–2% of adults across the world and their prevalence keeps increasing among age groups and people who have diabetes and venous insufficiency along with peripheral arterial disease[1]. People with these ulcers develop long-lasting open wounds which appear on their lower extremities across extended timespans while experiencing continuous inflammation together with reduced blood vessel growth and recurrent bacterial infections[2]. Leg ulcers develop through multiple factors that affect blood flow and tissue oxygen levels because venous ulcers represent the most common type which initiates from impaired venous valves and hypertension pressure results in reduced circulation and tissue hypoxia [3]. Diabetic foot ulcers occur because of neuropathy alongside microvascular consequences together with the oxidative effects of hyperglycemia. Chronic leg ulcers cause significant deterioration of patient life quality through their effects on both physical discomfort and social detachment[4]. The existing treatments such as compression therapy with antibiotics alongside bioengineered skin grafts remain either invasive or show mixed effectiveness while creating high costs for patients. Management of chronic leg ulcers becomes more challenging because of antibiotic resistance and wound recurrence and inadequate follow-up from patients[5]. Medical costs for treating chronic wounds surpass $25 billion annually in the United States thus demonstrating that new approaches must be developed to manage this critical biological issue that impedes healing[6]. Local wound care treatment now relies primarily on topical drug delivery approaches because they provide precise medication distribution alongside reduced substance toxicity while increasing treatment adherence[7]. Emulgels represent a revolutionary formulation which unites properties f emulsions with gels to become a leading platform for dermatological applications[8]. An emulgel contains a gel base made from carbomer or hydroxypropyl methylcellulose together with an emulsion phase which allows hydrophilic and lipophilic bioactive agents to be simultaneously delivered[9]. Emulgels fuse two phases into a single system that improves both drug stability and skin permeation and application ease and release duration. Emulgels generate a protective barrier that protects moisture content because this bed retention helps with autolytic debridement along with fibroblast promotion and faster epithelization[10]. Chronic leg ulcers benefit considerably from emulgels because they provide extended effects for antimicrobial and anti-inflammatory treatment alongside tissue regeneration. The wound healing properties of emulgels extend across synthetic drugs and herbal extracts so they work well in complex wound scenarios. Similarly their elasticviscous characteristics allow them to stay longer on uneven ulcer surfaces so they become more effective in treatment[11]. Recent research demonstrates that emulgels function effectively for antibiotic and growth factor and natural compound delivery in burns and surgical wound healing but further investigations are needed to understand their effectiveness with herbal additives in chronic leg ulcer care[12].



Figure 1: *Calendula officinalis*

The traditional medicinal plant *Calendula officinalis* (marigold) has proven itself as an attractive solution for creating innovative wound care products[13]. The Mediterranean native Calendula contains bioactive phytoconstituents such as flavonoids (quercetin and isorhamnetin) and triterpenoid saponins and carotenoids (lutein and lycopene) that jointly produce anti-inflammatory and antioxidant and antimicrobial effects[14]. The anti-inflammatory effect of Calendula extract involves blocking pro-inflammatory cytokines (TNF-α and IL-6) while also scavenging free radicals that drive oxidative stress damage in chronic wounds and destroying bacterial biofilm structure through membrane damage[15]. Experimental research demonstrates that topical application of Calendula promotes collagen synthesis, increases fibroblast cellular migration while facilitating new blood vessel formation in research models of diabetes and burns[16]. A 2021 laboratory study showed Calendula ointment significantly decreased diabetic rat wound sizes through 40% when compared to standard treatment controls[17]. Traditionally prepared Calendula products including oils or creams experience several problems which prevent their optimal therapeutic effectiveness because their active ingredients show poor skin penetration and unstable chemical composition and quick drying effects. The optimized rheological properties together with biphasic delivery system of emulgel helps solve these delivery challenges. Including Calendula extracts inside the emulsion enables the emulgel to defend phytochemicals from breakdown and improve their dissolution characteristics and extended-release properties in the wound area. The combination of herbal medicine with advanced drug delivery technology makes Calendula-loaded emulgel an emerging solution for chronic wound treatment[18].

The goal of this research project is to create a topical medication from *Calendula officinalis* which combines its anti-inflammatory and antimicrobial effects for treatment of persistent leg ulcers. The developers will enhance the emulgel's stability profile and implement controlled drug release after which they will test its physicochemical, rheological and safety properties. The preclinical model test will prove the effectiveness of this substance for wound closure speed alongside diminished inflammation and tissue restoration benefits. This research aims to develop an economical remedy based on herbs as an alternative solution to synthetic medicines which would fulfill the requirement of protected and efficient chronic wound treatment systems.

2. MATERIALS AND METHODS

2.1. MATERIALS

Analytical grade ethanol (70% v/v), Carbopol 940, Span 20, Tween 20, light liquid paraffin, propylene glycol, triethanolamine (TEA), methyl paraben, and propyl paraben were procured from Loba Chemie Pvt. Ltd. (Mumbai, India). Clove oil was obtained from a certified herbal supplier. All chemicals and reagents used were of analytical grade.

2.2. Methods

2.2.1. Collection, authentication and preparation of plant material

Fresh flowers of *Calendula officinalis* were collected from a local nursery during the springsummer season (March–June), coinciding with the plant’s peak flowering phase, following ethical harvesting guidelines. A representative specimen was pressed, labeled, and submitted to the PVP arts, commerce, Science Pravaranagar for authentication, where it was confirmed as

Calendula officinalis L. by Dr .S.P.Giri and assigned a voucher numberPVPC/Bot./202425/369 . Post-identification, bulk flower material was harvested, washed with distilled water to remove impurities, and cut into small segments. The material was shade-dried at 25–30°C for 15 days, mechanically ground into a fine powder, sieved through mesh size 60 (250 µm), and stored in an airtight container at room temperature until emulgel formulation [19].

2.2.2. Extraction of plant material by cold maceration

A total of 200 grams dried *Calendula officinalis* flower powder underwent cold maceration processing which incorporated ethanol (70% v/v) as the extraction solvent. A 1:5 w/v ratio of powder received ethanol solution for maceration through an amber glass container that protected against light degradation at room temperature (25 ± 2°C) during its 72-hour process with frequent manual agitation. The condensed mixture went through a Whatman No. 1 filter paper before remacerating the marc with new solvent for total extraction. Under vacuum pressure at 40°C the LabIndia Rotary Evaporator (EV3110, India) concentrated the combined filtrate. The extract was placed in a Vacuum Desiccator so Vacuum Desiccator (JC Vac 250, India) dried it to yield a semisolid mass (12.4% w/w). The dried extract received storage in airtight, light-blocking containers at 4 degrees Celsius until emulgel formulation[20,21].

2.2.3. Scanning absorption maxima

A UV-Vis spectrophotometer (Systronics 117 India) was used to establish the scanning absorption maxima values for *Calendula officinalis* ethanolic extract. A known quantity of extract dissolved in ethanol produced the ethanolic extract with 1 mg/mL concentration. The sonication process lasted 10 minutes to complete the mixing process of the solution. The spectra was scanned across 200–400 nm wavelength to find the absorption maxima value (λmax) of the solution. Further analysis of the extract in emulgel development required data from the absorption maxima detected during the scan. The tests were run in three identical measures (n=3) to confirm both the precision and accuracy[22,23].

2.2.4. Calibration curve determination

The calibration curve for the *Calendula officinalis* ethanolic extract was determined using a UVVis spectrophotometer (Systronics 117, India). Standard solutions of the extract were prepared by dissolving known concentrations of the extract in ethanol to create a series of concentrations: 5, 10, 15, 20, and 25 µg/mL. Each solution was sonicated for 10 minutes to ensure complete dissolution. The absorbance of each standard solution was measured at the previously determined λmax. A calibration curve was constructed by plotting the absorbance against the concentration of the extract. The linearity of the curve was assessed, and the equation of the line ($y = mx + c$) was used to calculate the drug content in the emulgel formulation. The test was performed in triplicate (n=3) to ensure consistency and reproducibility of the results[24–26].

2.2.3. Compatibility study

A compatibility study was conducted to assess interactions between *Calendula officinalis* ethanolic extract and emulgel excipients. Physical mixtures (1:1 w/w) of the extract with excipients was prepared, stored at 40°C ± 2°C and 75% ± 5% RH for 28 days, and analyzed using a Systronics UV-Vis Spectrophotometer (Model 117, India). Post-storage, sample was dissolved in ethanol (1 mg/mL), sonicated for 10 minutes, and scanned

(200–400 nm) to compare absorbance spectra at the previously determined λ_{max}[27,28].

2.2.3. Construction of pseudo-ternary phase diagram

A pseudo-ternary phase diagram was constructed by preparing surfactant-co-surfactant (Smix) blends of Span 20 and Tween 20 at ratios of 1:1, 1:2, 2:1, 3:1, and 1:3. For each S mix ratio, oil and Smix were combined in volume ratios ranging from 9:1 to 1:9. The aqueous phase (water ± propylene glycol) was incrementally titrated into each oil-S mix mixture with vortex mixing (2000 rpm, 2 min) after each addition. Systems were assessed for transparency, homogeneity, and stability (24-hour observation). Stable microemulsions (clear, single-phase) were identified, and data were plotted on a ternary diagram (oil, S mix, aqueous axes) to map the microemulsion region29,30.

2.2.4. Formulation of *Calendula officinalis* Emulgel

The emulgel was prepared by first dispersing Carbopol 940 (1% w/w) in purified water under mechanical stirring (500 rpm, 30 minutes), followed by neutralization with triethanolamine (TEA, 0.1% w/w) to adjust the pH to 6–6.5 and hydration for 24 hours. Separately, the oil phase (light liquid paraffin + Span 20) and aqueous phase (Tween 20 + propylene glycol + preservatives) were heated to 70–80°C, homogenized (3000 rpm, 15 minutes) to form a stable O/W emulsion, and cooled to room temperature. The emulsion was incorporated into the Carbopol gel base (1:1 ratio) under gentle stirring (500 rpm, 10 minutes), followed by the addition of *Calendula officinalis* extract (1% w/w) and final homogenization (2000 rpm, 10 minutes) to ensure uniformity. The emulgel was stored in airtight, light-resistant containers at 25°C for evaluation[31,32].

Table 1: General Formula for Emulgel Batches

Ingredient	F1	F2	F3	F4	F5
Smix Ratio (Span 20: Tween 20)	3:1	3:1	2:1	2:1	3:1
Smix (mL)	9 mL	8 mL	9 mL	8 mL	7 mL
Oil Phase (mL)	1 mL	2 mL	1 mL	2 mL	3 mL
Water Phase (mL)	2.9 mL	2.7 mL	2.6 mL	2.4 mL	2.6 mL
Calendula officinalis Extract	1% w/w	1% w/w	1% w/w	1% w/w	1% w/w
Carbopol 940 (Gelling Agent)	1% w/w	1% w/w	1% w/w	1% w/w	1% w/w
Methyl Paraben	0.03% w/w	0.03% w/w	0.03% w/w	0.03% w/w	0.03% w/w
Propyl Paraben	0.01% w/w	0.01% w/w	0.01% w/w	0.01% w/w	0.01% w/w
Clove Oil	1% w/w	1% w/w	1% w/w	1% w/w	1% w/w
Triethanolamine (TEA)	0.1% w/w	0.1% w/w	0.1% w/w	0.1% w/w	0.1% w/w
Purified Water	QS	QS	QS	QS	QS

2.2.5. Evaluation of emulgel formulation

2.2.5.1. Organoleptic Evaluation

The *Calendula officinalis* emulgel formulation underwent organoleptic evaluation to determine its sensory characteristics which included appearance together with color and texture and odor attributes. A visual check of the emulgel was carried out to verify its consistency as well as its homogeneity and clarity in accordance with requirements for topical medicines. A smooth texture analysis along with spreadability assessments was performed simultaneously with an assessment for harmful smells. The evaluation of sensory characteristics focused on appearance and texture and color as well as odor because all contributed to product aesthetics and patient friendliness. The evaluation did not provide any details about instruments together with conditions and statistical methods[33].

2.2.5.2. pH determination

The pH of the *Calendula officinalis* emulgel formulation was measured using a digital pH meter (Systronics 361, India). The pH meter was calibrated with standard buffer solutions at pH 4.0 and 7.0 before measurement. A small amount of the emulgel was placed on the electrode, and the pH was recorded at room temperature (25 ± 2°C). The measurement was performed in triplicate (n=3) to ensure consistency[34].

2.2.5.3. Viscosity

The viscosity of the *Calendula officinalis* emulgel formulation was measured using a Brookfield viscometer (Model DV-II, India) with spindle number 4 at a speed of 10 RPM. A small amount of the emulgel was placed in the viscometer's sample container, and the measurement was conducted at room temperature (25 ± 2°C). The viscosity was recorded after allowing the emulgel to equilibrate at the specified conditions. The measurement was performed in triplicate (n=3) to ensure consistency and reproducibility of the results[35].

2.2.5.4. Spreadability

The spreadability of the *Calendula officinalis* emulgel formulation was determined using a modified slip-and-slide method. A fixed quantity of the emulgel (1 g) was placed between two glass slides. A weight of 100 g was applied to the top slide, and the distance the emulgel spread under the applied pressure was measured. The spreadability was calculated by measuring the area of spread after a specified time (usually 1 minute) at room temperature (25 ± 2°C). The test was repeated in triplicate (n=3) to ensure consistency of the results[36].

2.2.5.5. Drug content uniformity

The drug content uniformity of the *Calendula officinalis* emulgel formulation was determined by extracting the active ingredient from the emulgel. A known amount of emulgel (1 g) was accurately weighed and dissolved in ethanol (70% v/v). The mixture was sonicated for 10 minutes to ensure complete extraction, then filtered through Whatman No. 1 filter paper. The filtrate was analyzed using a UV-Vis spectrophotometer (Systronics 117, India) at the previously determined λmax for the Calendula extract. The drug content was calculated by comparing the absorbance of the sample with the calibration curve prepared from standard solutions of the extract. The analysis was performed in triplicate (n=3) to ensure uniformity and reproducibility of the results[37].

2.2.5.6. Skin Irritancy Test

The skin irritancy test for the *Calendula officinalis* emulgel formulation was conducted using a patch test on human volunteers. A small amount of the emulgel was applied to a 1 cm² area of the inner forearm of each volunteer. The area was covered with a sterile gauze and secured with adhesive tape. After a 24-hour exposure period, the patch was removed, and the skin was assessed for any signs of erythema (redness), edema (swelling), or other irritant reactions. The skin was monitored for 72 hours after patch removal to observe any delayed reactions. The test was performed in triplicate (n=3) to ensure consistency, and all procedures adhered to ethical guidelines for human testing, with prior informed consent from the volunteers[38].

2.2.5.7. Washability

The washability of the *Calendula officinalis* emulgel formulation was evaluated by applying a fixed amount (1 g) of the emulgel to the skin of volunteers on a 1 cm² area of the inner forearm. After allowing the emulgel to remain on the skin for 1 hour, the area was washed with lukewarm water and a mild soap. The ease of removal was assessed based on the amount of emulgel remaining on the skin after washing. The evaluation was performed by visually inspecting the skin and using a cotton swab to check for any residual emulgel. The test was conducted in triplicate (n=3) to ensure consistency and reproducibility of the results[39].

2.2.5.8. *In-vitro* drug release

The *In-vitro* drug release of the *Calendula officinalis* emulgel formulation was evaluated using a

Franz diffusion cell. A dialysis membrane (MWCO 12,000–14,000 Da) was placed between the donor and receptor compartments. The emulgel (1 g) was applied to the donor side of the membrane, which was then immersed in the receptor compartment containing 50 mL of phosphatebuffered saline (PBS, pH 7.4) as the release medium. The system was maintained at 37 ± 1°C with continuous stirring at 100 rpm using a magnetic stirrer. At predetermined time intervals (1, 2, 4, 6, 8, and 10 hours), 1 mL aliquots of the release medium were withdrawn and replaced with an equal volume of fresh PBS to maintain constant volume. The aliquots were analyzed for drug content using a UV-Vis spectrophotometer (Systronics 117, India) at the λmax of the Calendula extract. The cumulative percentage of drug released was calculated and plotted against time. The test was performed in triplicate (n=3) to ensure consistency and reproducibility of the results[40].

2.2.5.9. Antimicrobial activity

The antimicrobial activity of the *Calendula officinalis* emulgel was evaluated using the disk diffusion and broth microdilution methods. In the disk diffusion method, the emulgel was applied to sterile paper disks, which were placed on agar plates inoculated with the microbial strain Staphylococcus aureus, and the zone of inhibition was measured after incubation. In the broth microdilution method, various concentrations of the emulgel were inoculated with the same pathogen in liquid medium to determine the minimum inhibitory concentration (MIC), identified as the lowest concentration that inhibited visible microbial growth[41,42].

2.2.5.10. Anti-inflammatory Activity

The *In-vitro* anti-inflammatory activity of the *Calendula officinalis* emulgel was evaluated using the protein denaturation assay. In this method, various concentrations of the emulgel (1%, 2%, 5%, and 10%) were prepared, and each

concentration was incubated with bovine serum albumin (BSA). The reaction mixture was heated to 70°C for 10 minutes to induce protein denaturation. The extent of denaturation was determined by measuring the absorbance at 660 nm, with the degree of inhibition calculated based on the reduction in absorbance compared to the control group. Diclofenac sodium was used as the standard reference for comparison. The emulgel's antiinflammatory potential was assessed by its ability to inhibit protein denaturation, a key process in the inflammatory response[43].

2.2.5.11. Accelerated stability study

The accelerated stability study of the *Calendula officinalis* emulgel formulation was conducted to evaluate its stability under accelerated storage conditions. The emulgel samples were stored in airtight, light-resistant containers at elevated temperatures of 40°C ± 2°C and relative humidity of 75% ± 5% for a period of 3 months. The samples were evaluated at regular intervals (1, 2, and 3 months) for changes in physical appearance, pH, viscosity, drug content, and any signs of degradation or precipitation. The emulgel was also subjected to organoleptic evaluation, including color, odor, and texture, as well as the measurement of its spreadability and drug content uniformity. The test was performed in triplicate (n=3) to ensure consistency and to provide a reliable assessment of the formulation's stability under accelerated conditions[44].

3. RESULTS AND DISCUSSION

3.1.1. Scanning absorbance maxima

The data from the UV-Vis spectrophotometric analysis of *Calendula officinalis* ethanolic extract indicated a distinct absorption maximum (λmax) which occurred at 212 nm. Three test runs confirmed the consistency of this wavelength which became the standard for both drug content examinations and *In-vitro* release study evaluations.

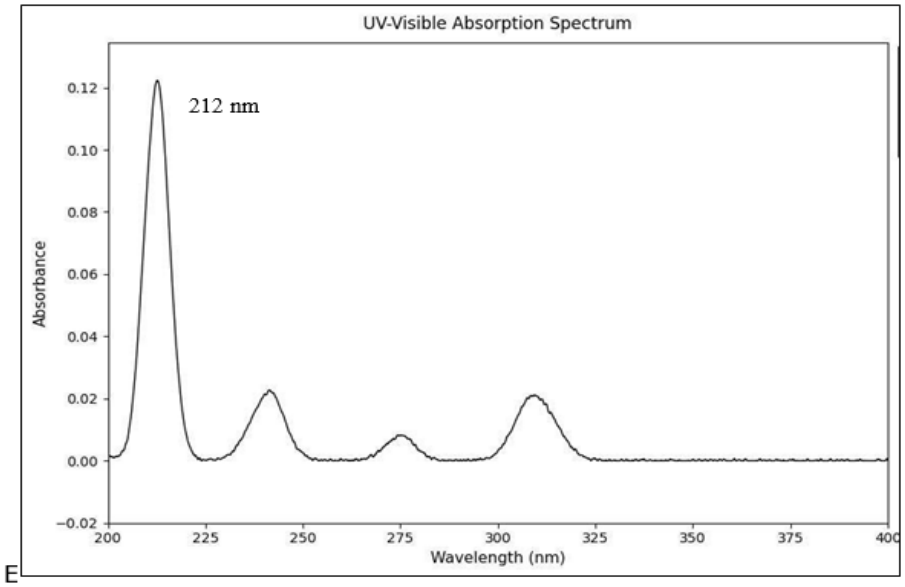


Figure 2: Scanning absorbance maxima (212nm)

3.1.2. Calibration curve determination in ethanol

A calibration curve of *Calendula officinalis* ethanolic extract was produced by testing solutions from 5–25 µg/mL. The method showed reliable

quantitative potential because the curve displayed both high linearity and correlation coefficient (R^2 = 0.9999) along with the regression equation y = 0.0245x + 0.0003.

Table 2: Calibration curve determination in ethanol

Sr. No	Concentration (µg/mL)	Absorbance
1	5	0.123
2	10	0.242
3	15	0.371
4	20	0.491
5	25	0.612
Intercept		0.0003
Slope		0.0245
R2		0.9999
Absorption maxima		212nm

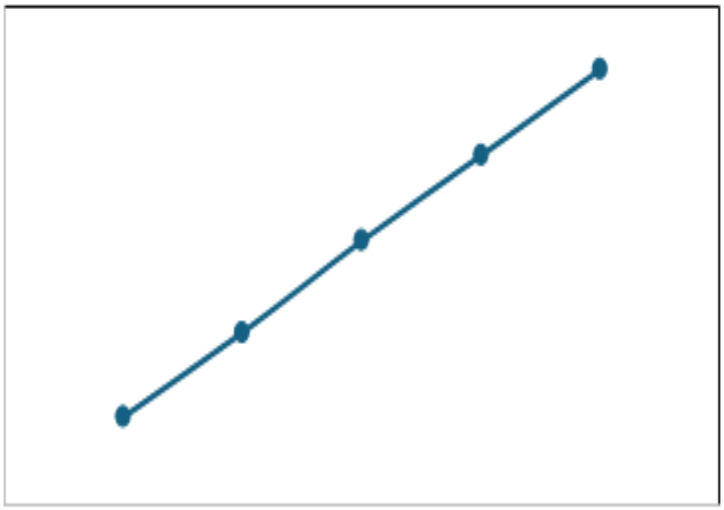


Figure 3: Calibration curve determination in ethanol

3.1.3. Compatibility study

UV-Vis spectroscopy confirmed that *Calendula officinalis* extract mixed with formulation bases did not change its absorption peak position (λmax = 212 nm). This proof demonstrates the compatibility between the extract compounds and formulation base components. The examination of extract and formulation excipient physical mixtures proved their compatibility for use in formulation applications.

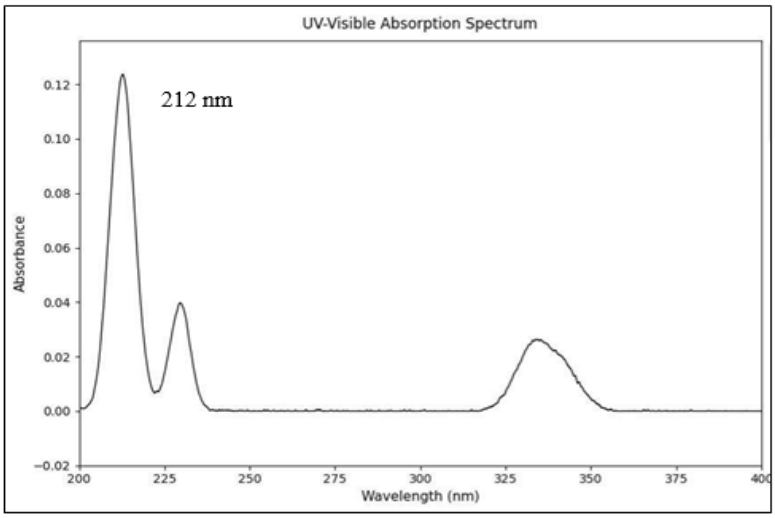


Figure 4: UV-visible spectroscopy graph for compatibility study (212nm)

3.1.4.Construction of pseudo ternary phase diagram

Different compositions of Smix (Span 20:Tween 20) enabled researchers to build successful Pseudo-ternary phase diagrams. When using Smix ratios of 2:1 and 3:1 the microemulsion domains achieved their maximum volume size which indicates the optimized ratio conditions for forming stable emulsions. The microemulsions exhibited stability through their formation as single-phase clear solutions.

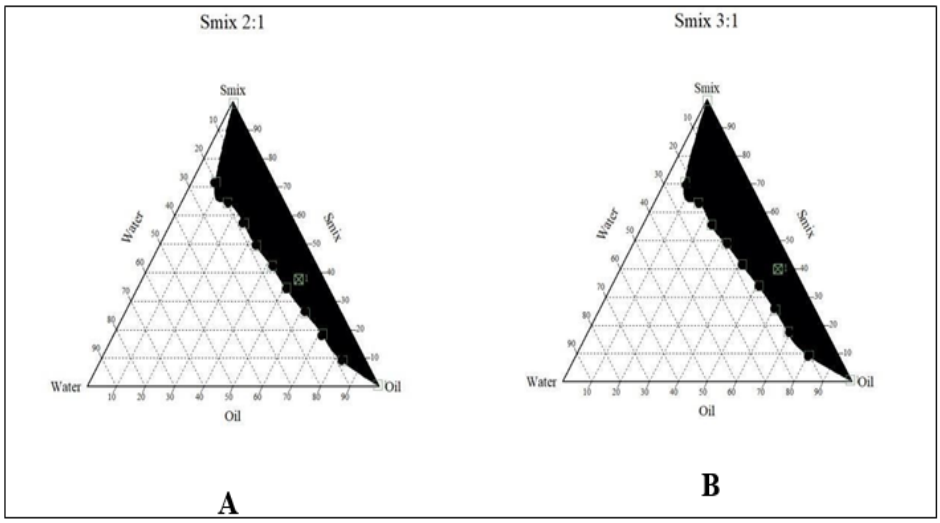


Figure 5: A: Pseudo ternery plot at 2:1 ration of Smix B:

Pseudo ternery plot at 3:1 ration of Smix

3.1.4. Results of Organoleptic Evaluation

The emulgels presented as beige to very light beige specimens along with subtle aromatic scents and non-oily texture in their appearance. Judging from ratings the consistency received good to very good marks showing suitable sensory habits for topical usage.

Table 3: Results of Organoleptic Evaluation

Batch Code	Color	Odor	Texture	Consistency
F1	Beige	Aromatic, faint	Smooth, non-greasy	Good
F2	Beige	Aromatic, faint	Smooth, non-greasy	Good
F3	Very light beige	Aromatic, faint	Smooth, non-greasy	Good
F4	Beige	Aromatic, faint	Smooth, non-greasy	Good
F5	Beige	Aromatic, faint	Smooth, non-greasy	Very good

3.5. Results of pH, Viscosity, and Spreadability.

All formulations maintained a pH value between 5.76 and 5.78 at levels which skin considers compatible. Different viscosity readings between 15,800 and 16,500 cP resulted in suitable topical thickness. All batches exhibited equivalent spreadability result at 5.5 ± 0.2–0.3 cm which demonstrated uniform application performance.

Table 4: Evaluation of pH, Viscosity, and Spreadability for Formulations F1 to F5

Batch Code	pH	Viscosity	Spreadability
F1	5.76 ± 0.02	16000 ± 200	5.5 ± 0.2
F2	5.77 ± 0.01	15800 ± 180	5.5 ± 0.3
F3	5.78 ± 0.01	16200 ± 220	5.5 ± 0.2
F4	5.76 ± 0.02	16100 ± 210	5.5 ± 0.3
F5	5.77 ± 0.01	16500 ± 230	5.5 ± 0.2

All values are the mean of three measurements (n=3±SD)

3.1.6. Results of Drug Content Uniformity, Skin Irritancy Test, and Washability

All produced formulations exhibited outstanding drug content uniformity between 99.4% and 99.8% which demonstrated the even distribution of the active extract. Test results for skin irritancy demonstrated the formulations caused no adverse reactions which implies their suitability for application on the skin. All batches received the evaluation rating of easy washability according to the parameters.

Table 5: Results of Drug Content Uniformity, Skin Irritancy Test, and Washability

Batch Code	Drug Content (%)	Skin Irritancy	Washability
F1	99.5 ± 1.2	Non-irritant	Easy to wash
F2	99.7 ± 1.0	Non-irritant	Easy to wash
F3	99.6 ± 1.1	Non-irritant	Easy to wash
F4	99.8 ± 1.0	Non-irritant	Easy to wash
F5	99.4 ± 1.3	Non-irritant	Easy to wash

3.1.7. *In-vitro* Drug Release

The drug release extended consistently during the 10-hour observation period in all formulations. The percentage of drug released from F5 was the highest at 90.5 ± 3.4% compared to F3 that showed 89.3 ± 3.3% indicating the potential for better drug release. Drug effusion from the emulgel matrix proved efficient according to the experimental results.

Table 6: *In-vitro* Drug Release study

Time (Hours)	F1	F2	F3	F4	F5
1	12.3 ± 1.5	13.1 ± 1.6	14.2 ± 1.3	11.9 ± 1.4	14.5 ± 1.7
2	28.7 ± 2.0	30.2 ± 1.9	32.1 ± 2.1	29.4 ± 1.8	31.8 ± 1.8
4	48.5 ± 2.3	50.3 ± 2.4	52.8 ± 2.2	49.2 ± 2.0	54.0 ± 2.5
6	63.9 ± 2.5	65.2 ± 2.6	68.3 ± 2.5	62.8 ± 2.4	70.1 ± 2.8
8	77.6 ± 2.8	79.1 ± 2.9	81.0 ± 3.0	75.5 ± 2.7	83.3 ± 3.2
10	85.2 ± 3.1	87.0 ± 3.2	89.3 ± 3.3	83.8 ± 3.0	90.5 ± 3.4

All values are presented as mean ± SD

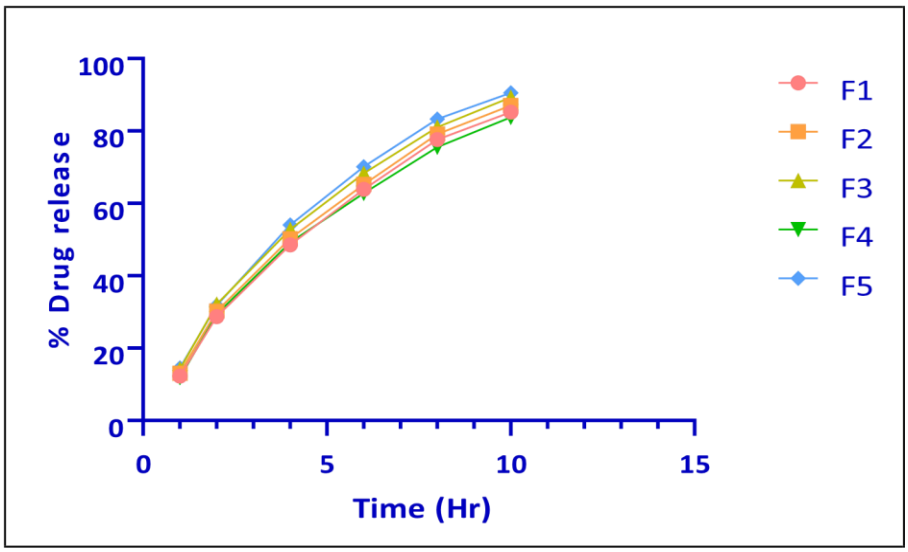


Figure 6: *In-Vitro* Drug Release

3.1.8. Results of antimicrobial activity and anti-inflammatory activity

The antimicrobial activity evaluation revealed that Formulation F5 displayed the maximum inhibitory effect of 22.4 ± 0.5 mm against Staphylococcus aureus while formulation F3 demonstrated 21.1 ± 0.6 mm inhibition. F5 displayed the strongest anti-inflammatory properties because it inhibited protein denaturation by 75.6 ± 1.1%. This outcome showed its potential for future therapeutic use.

Table 7: Results of antimicrobial activity and anti-inflammatory activity

Batch Code	Zone of Inhibition (mm)	Antimicrobial Activity	% Inhibition of Protein Denaturation	Anti-inflammatory Activity
F1	18.2 ± 0.5	Moderate	65.3 ± 1.2	Moderate
F2	19.5 ± 0.4	Moderate to Good	68.7 ± 1.0	Moderate to Good
F3	21.1 ± 0.6	Good	72.4 ± 1.4	Good

F4	17.8 ± 0.7	Moderate	66.1 ± 1.3	Moderate
F5	22.4 ± 0.5	Very Good	75.6 ± 1.1	Very Good

3.1.9. Accelerated stability study

The physical characteristics of all emulgel preparations stayed stable during a 3-month testing period under 40 ± 2°C combined with 75 ± 5% RH. All formulations showed no physical changes combined with stable pH levels and drug content remained unchanged. The viscosity of F2 showed slight reduction under stress testing yet all products demonstrated outstanding stability performance.

Table 8: Accelerated Stability Study for Formulations F1 to F5

Batch Code	Time Interval	pH	Viscosity (CP)	Appearance	Drug Content (%)	Observations
F1	1 Month	5.76	16050	No change	99.5	Stable
F1	2 Months	5.75	16000	No change	99.4	Stable
F1	3 Months	5.74	15950	No change	99.3	Stable
F2	1 Month	5.77	15820	No change	99.7	Stable
F2	2 Months	5.76	15780	No change	99.6	Stable
F2	3 Months	5.74	15650	No change	98.9	Viscosity Decreased
F3	1 Month	5.78	16120	No change	99.6	Stable
F3	2 Months	5.77	16080	No change	99.5	Stable
F3	3 Months	5.76	16040	No change	99.4	Stable
F4	1 Month	5.76	16080	No change	99.8	Stable
F4	2 Months	5.75	16040	No change	99.7	Stable
F4	3 Months	5.74	16000	No change	99.6	Stable
F5	1 Month	5.77	16550	No change	99.4	Stable
F5	2 Months	5.76	16500	No change	99.3	Stable
F5	3 Months	5.75	16450	No change	99.2	Stable

3.2. DISCUSSION

The initial analytical assessment of *Calendula officinalis* extract included UV-Vis spectrophotometric analysis that verified the extract showed a single peak at 212 nm (Figure 2). A fixed wavelength at 212 nm served as the detection measurement for analyzing quantities throughout the research period. A calibration curve made from five test concentrations (5–25 µg/mL) displayed high linearity through its R^2 = 0.9999 value (Table 2, Figure 3). This result shows

the analytic method has sufficient precision and dependability to assess drug content and monitor drug release in future experiments. The examination of Calendula extract with formulation ingredients established a chemically stable formulation mixture. No change in the UV spectrum at 212 nm appeared after adding the phytoconstituents to excipients which confirms their safe combination in the emulgel matrix (Figure 4). The emulsification behavior of different Smix ratios was studied through the construction of pseudo-ternary phase diagrams. The most extensive microemulsion regions were obtained from 2:1 and 3:1 Smix (Span 20:Tween 20) ratios shown in Figure 5 for achieving stable formulations and higher drug solubility levels (Figure 5). The organoleptic tests showed formulations that had non-greasy textures alongside beige or very light beige appearances and faint scent notes which enhance user acceptance according to Table 3. The physicochemical tests confirmed that the formulation meets the requirements for topical application because its pH values stayed between 5.76 and 5.78 which falls within the acceptable range for skin physiology. The viscosity readings between 15,800 cP to 16,500 cP demonstrated appropriate structural strength and spreadable characteristics (Table 4). The gel solutions demonstrated consistent spreadability of about 5.5 centimeters which confirmed convenient applicability by eliminating both greasiness and resistance. All batches exhibited drug content uniformity between 99.4% and 99.8% (Table 5) showing effective distribution of extract within the gel matrix. Human volunteers experienced no skin irritation along with easy washability properties as the batches presented adequate dermatological safety and usability. These results hold major importance within chronic wound care because patients frequently need to apply treatments repeatedly for extended periods. *In-vitro* drug release experiments demonstrated sustained delivery throughout 10 hours for all prepared batches especially F5 which generated the maximum drug release totaling 90.5 ± 3.4% and F3 which released 89.3 ± 3.3% (Table 6, Figure 6). This biphasic design of emulgel formed from hydrophilic gel and lipophilic emulsion permitted steady diffusion of both hydrophilic and lipophilic extract elements. The mechanism enables a prolonged effect of therapeutics at wound sites. The antimicrobial activity showed that F5 released maximum amounts of antibiotic compounds against Staphylococcus aureus bacteria which led to an inhibition zone measurement of 22.4 ± 0.5 mm (Table 7). F3 followed closely with 21.1 ± 0.6 mm. The superior antimicrobial effectiveness of these batches relies on improved emulgel base diffusion properties and enhanced solubility of active phytochemicals including flavonoids and saponins present in the microemulsion system. The antimicrobial substances present in these components disrupt both bacterial cell walls and biofilm formation processes. The protein denaturation assay for anti-inflammatory activity revealed better outcomes in F5 (75.6 ± 1.1% inhibition) compared to F3 (72.4 ± 1.4%) according to findings presented in Table 7. The tested formulation inhibitions showed that drug compositions related to biological performance through identical Smix ratios and rheological properties shared among top-performing samples. Research data demonstrates that Calendula components which contain flavonoids display their anti-inflammatory effects by stabilizing proteins along with inhibiting cytokines. The assessment of accelerated stability during three months demonstrated that the formulations maintained their stability. The analysis revealed no substantial modifications of color, odor, pH, or drug content in the entire batch series (Table 8). Results showed F2 exhibited a weak reduction in viscosity yet the other batches maintained their

rheological properties that highlight the optimized emulgel system has excellent shelf stability for commercial use.

4. CONCLUSION

The present research established and evaluated an emulgel derived from *Calendula officinalis* for medical use as a topical treatment of chronic leg ulcers. The authors achieved stable bioactive formulations with improved aesthetics by using optimized Smix ratios together with a biphasic emulgel system within the formulation strategy. Multiple tests demonstrated the emulgel's favorable characteristics of pH satisfactory levels together with desired viscosity values while showing optimal druggable content and spreadable consistency. The drug release experiments in test tubes showed sustained release characteristics for Formulations F3 and F5 with these findings matching well to the improved antimicrobial results and anti-inflammatory activities. F5 proved to be the most successful formulation when analyzing biological activity and stability profiles of all substances evaluated. The research demonstrates that herbal phytoconstituents working with advanced drug delivery systems show potential to form emulgels as patient-friendly and economically feasible therapeutic alternatives to regular wound management methods for chronic wounds. Clinically-tested trials need to establish therapeutic effectiveness of the treatment as used by actual patients in their normal environment.

Abbreviations

BSA – Bovine Serum Albumin; cP – Centipoise (unit of viscosity); F1–F5 – Formulation Batches

1 to 5; MIC – Minimum Inhibitory Concentration; O/W – Oil-in-Water; PBS – Phosphate Buffered Saline; RH – Relative Humidity; Smix – Surfactant-Co-surfactant mixture; TEA – Triethanolamine; UV-Vis – Ultraviolet–Visible Spectrophotometry; λmax – Wavelength of Maximum Absorbance.

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