

Formulation and Evaluation of Emulgel of *Calendula officinalis* and Himalayan Arnebia extract for Wound Healing

Pooja Battul^{1*}, Mithun G. Maniyar², Vrunal V. More³, Vaibhav S. Gaikwad⁴, Prajakta Khule⁵

^{1*}PG student, Department of Pharmaceutics, SVERI's College of Pharmacy, Pandharpur.
(Corresponding Author) Email - poojabattul21@gmail.com

²Principal, SVERI's College of Pharmacy, Pandharpur.

³Professor, Department of Pharmaceutics, SVERI's College of Pharmacy, Pandharpur.

⁴Assistant Professor, Department of Pharmaceutics, SVERI's College of Pharmacy, Pandharpur.

⁵Professor, Department of Pharmaceutics, SVERI's College of Pharmacy, Pandharpur

Abstract

Effective topical formulations that support healing and offer defense against microbial infections are necessary for wounds and injuries to the skin. The goal of the current study was to create and assess an emulgel for wound healing applications that contained *Calendula officinalis* flower extract and Himalayan Arnebia root extract. The prepared herbal extracts which were then integrated into an emulgel system using Design of Experiments (DoE) optimized formulation parameters. The formulation were assessed for several physicochemical parameters including total phenolic content (TPC), total flavonoid content (TFC), antimicrobial activity, drug content, in vitro release study (IVRT), and stability. The formulation showed acceptable physicochemical characteristics with strong zone of inhibition ranging from 7 to 11 and 8 to 12 mm in E.coli and S. aureus bacterial strains respectively. Because of their bioactive components linked to skin protection and regeneration, the combination of *Calendula officinalis* and Himalayan Arnebia extracts offered potential therapeutic benefits. Its suitability as a topical herbal formulation was supported by the developed emulgel's satisfactory stability and antimicrobial efficacy. According to the study's findings, the developed emulgel offers a viable method of administering herbal extracts in a practical dosage form with possible uses in wound care and wound healing.

Keywords: Wounds, *Calendula officinalis*, Himalayan Arnebia, DoE, emulgel, wound healing, antimicrobial activity, TPC, TFC, IVRT.

How to cite this article: Pooja Battul, Mithun G. Maniyar, Vrunal V. More, Vaibhav S. Gaikwad, "Formulation and Evaluation of Emulgel of *Calendula officinalis* and Himalayan Arnebia extract for Wound Healing" Int J Drug Deliv Technol. 2026;16(84s): 1538-1546; DOI: 10.25258/ijddt.16.84s.155.

INTRODUCTION

Wound healing is the natural process by which the body repairs damaged skin and tissues after an injury. Injuries such as cuts, burns, surgical incisions, pressure sores, or ulcers can damage the skin and underlying structures, triggering a series of events that help restore the affected area. The healing process begins immediately after injury with the formation of a blood clot to stop bleeding. This is followed by an inflammatory phase, during which white blood cells remove bacteria, dead cells, and other debris from the wound. As healing progresses, new blood vessels and connective tissue develop, collagen is produced, and healthy tissue gradually fills the wound. In the final stage, the repaired tissue becomes stronger as collagen fibers reorganize, although the healed area may remain as a scar.

Wounds can occur for many reasons, including accidents, falls, burns, surgical procedures, animal bites, and chronic conditions such as diabetic foot ulcers or pressure ulcers. The healing process can be affected by several factors. Infection is one of the most common causes of delayed healing, as it damages healthy tissue and prolongs inflammation. Poor blood circulation reduces the supply of oxygen and nutrients needed for tissue repair. Other local factors, such as repeated injury, excessive pressure on the wound, or the presence of dead tissue or foreign material, can also slow healing. In addition, systemic conditions such as diabetes mellitus, malnutrition, anemia, advanced age, obesity, dehydration, kidney or liver disease, a weakened immune system, smoking, and the use of

corticosteroids or chemotherapy drugs may interfere with the body's ability to repair damaged tissue.

Prolonged wound healing occurs when a wound does not heal within the expected period or shows little improvement over time. Common signs include persistent redness, swelling, pain, discharge, unpleasant odor, or failure of the wound to reduce in size. Proper wound care is essential to support healing and prevent complications. Keeping the wound clean, using appropriate dressings, maintaining a balanced diet rich in protein and essential nutrients, controlling blood glucose levels in people with diabetes, avoiding smoking, and seeking medical attention when signs of infection are present can all contribute to faster and more effective wound healing.

Current wound treatments include conventional dressings such as gauze, hydrocolloids, hydrogels, foams, alginates, and transparent films, along with debridement, topical antimicrobial agents, and antibiotics to control infection. Advanced approaches include negative pressure wound therapy (NPWT), bioengineered skin substitutes, platelet-rich plasma (PRP), growth factor therapy, stem cell therapy, and hyperbaric oxygen therapy (HBOT). Although these treatments have improved wound care, each has certain drawbacks. Conventional dressings often need frequent replacement and may not provide adequate protection against infection. Advanced therapies are costly, require specialized facilities and trained professionals, and may not be readily available. Their success also depends on factors such as infection, diabetes, blood circulation, and the patient's overall health.

Emulgels offer better spreadability, patient compliance, and effective incorporation of both hydrophilic and lipophilic constituents by combining the benefits of emulsions and gels. By offering extended skin contact, they can improve topical application and act as appropriate carriers for herbal extracts. Thus, the creation of herbal emulgel formulations is a promising strategy to aid in wound care and wound healing.

The well-known medicinal plant *Calendula officinalis*, also known as marigold, is frequently used in traditional medicine to treat skin conditions and promote wound healing. Flavonoids, triterpenoids, carotenoids, and essential oils are among the bioactive components found in its flowers that support its medicinal properties. *Calendula officinalis* has wound-healing, antioxidant, antimicrobial, and anti-inflammatory qualities. It aids in the production of collagen, encourages tissue regeneration, and lessens irritation and inflammation brought on by skin injuries. *Calendula officinalis* is regarded as a useful herbal component for topical formulations intended to enhance skin healing and repair because of these pharmacological actions.

The roots of Arnebia euchroma are used to make Himalayan Arnebia, a significant medicinal plant with healing and skin-protective qualities. Its pharmacological actions are caused by bioactive substances like naphthoquinones, phenolic components, and shikonin derivatives. Himalayan Arnebia is useful in wound healing applications due to its antimicrobial, anti-inflammatory, antioxidant, and tissue-regenerative properties. It promotes healing of injured skin, shields against microbial invasion, and aids in the repair of damaged tissues. Himalayan Arnebia is a promising herbal candidate for topical emulgel formulations for wound healing because of these qualities.

MATERIALS AND METHOD

Collection and Authentication of plant materials

The *Calendula officinalis* flower was collected from Solapur district flower market area. The Himalayan Arnebia was purchased from Ayurvedic shop at Pandharpur. The plant was authenticated by Department of Botany, K. B. P. Mahavidhyala, Pandharpur.

Soxhlet Extraction of *Calendula officinalis* flower

Using a Soxhlet apparatus, 35 g of authentic *Calendula officinalis* flowers were extracted with 350 mL of 95% ethanol after being shade-dried and coarsely powdered. A rotary evaporator was used to concentrate the extract, which was then dried to produce a semisolid mass, the percentage yield was

computed, and it was kept in an airtight, light-resistant container for additional examination.

Maceration Processes for Himalayan Arnebia roots

25 g of coarsely ground Arnebia roots were macerated with 250 mL of 95% ethanol for 15 days at room temperature while being stirred periodically. After filtering, the extract was concentrated using a rotary evaporator, dried to produce a semisolid mass, the yield percentage was computed, and it was kept in a light-resistant, airtight container.

Preliminary Phytochemical Screening

The identification of present phytochemicals was done for alkaloids, Saponins, tannins, glycosides, anthraquinone, amino acid, phenols, and flavonoids.

Total Phenolic Content

The total phenolic content of the Calendula extract was determined using the Folin–Ciocalteu method with gallic acid as the reference standard. The concentration of gallic acid was 10, 20, 40, 60, 80, 100 µg/mL. The gallic acid calibration curve exhibited excellent linearity ($R^2 = 0.9822$), confirming the accuracy and reliability of the analytical method.

Total flavonoid content

The total flavonoid content of the Calendula extract was determined using the aluminium chloride colorimetric method with quercetin as the reference standard. The concentration of gallic acid was 10, 20, 40, 60, 80, 100 µg/mL. The quercetin calibration curve exhibited excellent linearity ($R^2 = 0.9999$), indicating the reliability of the analytical method for flavonoid quantification.

Preparation of Emulgel using *Calendula officinalis* extract and Himalayan Arnebia extract using DoE software

Optimized concentrations from DoE software were used to prepare the emulgel. Span 20 was dissolved in light liquid paraffin to create the oil phase, and Tween 20 was added to purified water to create the aqueous phase. *Calendula officinalis* and Himalayan Arnebia extracts were dissolved in ethanol before being added to the aqueous phase, and methyl and propyl parabens were dissolved in propylene glycol. After heating both phases to 70°C, an emulsion was created by gradually adding the oil phase to the aqueous phase while continuously stirring. To create a homogenous emulgel, carbopol gel was made separately and combined with the emulsion (1:1).

Table no.00 Code levels of Design Expert software for Independent variables

Independent variables	Coded levels		
X1 (Liquid paraffin)	-1	0	+1
X2 (Tween 20)	-1	0	+1

X3 (Span 20)	-1	0	+1
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Experimental runs by 3³ factorial design in DoE:

Table no.03 DoE Independent variables

Formulations	X1 Liquid paraffin (ml)	X2 Tween 20 (ml)	X3 Span 20 (ml)	Prop. Glycol	Ethanol (ml)	Methyl paraben (gm)	Propyl paraben (gm)	Carbopol 934 (gm)	Dist. water (ml)
1	5	0.6	0.9	5	2.5	0.03	0.01	1	q.s.
2	7.5	0.6	0.9	5	2.5	0.03	0.01	1	q.s.
3	5	1	0.9	5	2.5	0.03	0.01	1	q.s.
4	7.5	1	0.9	5	2.5	0.03	0.01	1	q.s.
5	5	0.6	1.5	5	2.5	0.03	0.01	1	q.s.
6	7.5	0.6	1.5	5	2.5	0.03	0.01	1	q.s.
7	5	1	1.5	5	2.5	0.03	0.01	1	q.s.
8	7.5	1	1.5	5	2.5	0.03	0.01	1	q.s.
9	5	0.8	1.2	5	2.5	0.03	0.01	1	q.s.
10	7.5	0.8	1.2	5	2.5	0.03	0.01	1	q.s.
11	6.25	0.6	1.2	5	2.5	0.03	0.01	1	q.s.
12	6.25	1	1.2	5	2.5	0.03	0.01	1	q.s.
13	6.25	0.8	0.9	5	2.5	0.03	0.01	1	q.s.
14	6.25	0.8	1.5	5	2.5	0.03	0.01	1	q.s.
15	6.25	0.8	1.2	5	2.5	0.03	0.01	1	q.s.
16	6.25	0.8	1.2	5	2.5	0.03	0.01	1	q.s.
17	6.25	0.8	1.2	5	2.5	0.03	0.01	1	q.s.

Evaluation of Emulgel

Visual examination

The color, odor, homogeneity and consistency was decided to examine for the prepared emulgel.

pH

The determination of pH was done using the digital pH meter. The pH meter firstly calibrated using 4.0 and 9.2 pH capsules. The emulgel sample was made by dissolving 1 gm emulgel in 100 ml distilled water and pH was recorded. The test was carried for three times to minimize error.

Spreadability

Emulgel was placed between two glass slides under a fixed load to create a uniform film in order to measure spreadability. The upper slide was allowed to slip under a predetermined weight after the load was removed. Better spreadability was indicated by a shorter separation time, which was measured in seconds.

$$\text{Spreadability} = m \times l/t$$

Where,

m = Standard weight which is tied to or placed over the upper slide (20g)

l = length of a glass slide (5 cm)

t = time taken in seconds.

Viscosity

The formulated emulgel viscosity was measured using the Brookfield viscometer at room temperature ($37^\circ\text{C} \pm 2^\circ\text{C}$) with spindle No. 63 rotating at 2.5 RPM.

Washability

On a predetermined test area, usually the dorsal surface of the hand, apply a consistent, small amount of the emulgel (usually 1 gm). Using your fingers, gently spread the emulgel to form a thin, consistent layer which resembles a typical product use. Put the hand that has been treated under a light stream of tap water. As you rinse, gently rub the skin to see how quickly and thoroughly the emulgel separates from the skin.

Antimicrobial Activity

The agar well diffusion method was used to assess antimicrobial activity. Petri dishes were filled with sterile molten agar that had been inoculated with a standardized

microbial suspension. Following solidification, 6 mm wells were made, and each well received 100 μL of the test sample (5 mg/ml and 10 mg/mL). The positive control was a standard solution (1 mg/mL) of streptomycin, and the negative control was DMSO. The sample was allowed to diffuse through the agar by incubating the plates under the right conditions. The diameter of the clear zone of inhibition surrounding each well was measured in millimeters to determine the antimicrobial activity.

Drug content

Emulgel was precisely weighed, dissolved in methanol solvent, sonicated, and filtered using Whatmann filter paper no.1. The filtrate was diluted to the necessary concentration and subjected to spectrophotometric analysis at the 254 nm wavelength. The absorbance was used to calculate the drug content, which was then expressed as a percentage of the labeled amount.

$$\% \text{ Drug content} = \text{Absorbance} / \text{Total amount of drug} \times 100$$

In-Vitro Release Test

Franz diffusion cells were used for in vitro drug release investigations. A HIMEDIA dialysis membrane was utilized; was positioned between the glass cell top holding the sample and the lower cell reservoir to examine the diffusion. The phosphate buffer pH 6.4 solution was added to the receiving compartment, and a magnetic stirrer was used. After predetermined intervals (0, 1, 2, 4 hrs), 3ml receptor fluid was withdrawn from the receiving compartment and was replaced with the same volume of fresh solution. Samples were analyzed on UV-Visible Spectroscopy to determine the in vitro drug release from the prepared emulgel at 254 nm.

Stability Study

The formulated F2nd emulgel was subjected for the stability study for 35 days. The emulgel was kept at room temperature. The parameters like color, pH, spreadability, viscosity, phase separation and texture was evaluated.

Results

Phytochemical screening

The phytochemical screening found as per Table no.000.

Table no. 05: Phytochemical screening

Sr.no.	Test	Interference	
		<i>Calendula officinalis</i>	Himalayan Arnebia
1.	Alkaloids	-	-
2.	Flavonoid	+	+
3.	Phenol	+	+

4.	Saponins	+	+
5.	Tannins	+	+
6.	Glycoside	-	-
7.	Anthraquinone	-	+
8.	Amino acid	-	-

Visual examination

The prepared cream F2nd showed a light yellowish colour with characteristic odour. The F2nd emulgel was uniform and consistent in nature among other formulations.

Total Phenolic Content

The Calendula extract showed a concentration-dependent increase in absorbance and total phenolic content, ranging from 4.77 mg GAE/g extract at 10 µg/mL to 81.20 mg GAE/g extract at 100 µg/mL. The high phenolic content observed at higher concentrations indicates that Calendula is rich in bioactive phenolic constituents. These findings demonstrate that Calendula extract is an excellent natural source of phenolic compounds and is suitable for incorporation into herbal formulations.

Total Flavonoid Content

The total flavonoid content of Calendula extract was quantified using quercetin as the standard reference compound. The extract exhibited a progressive increase in absorbance with increasing concentration, corresponding to flavonoid contents ranging from 2.26 mg QE/g extract at 10 µg/mL to 101 mg QE/g extract at 100 µg/mL. When compared with standard quercetin, Calendula extract demonstrated a nearly parallel concentration-response profile, indicating substantial flavonoid richness.

pH

The pH was recorded using digital pH meter was found to be 6.42.

Spreadability

The F2nd formulation show less time to separate the two slides. As per the spreadability evaluation analysis less time to separate means good spreading property.

Table no. 06: Spreadability analysis of formulations

Sr.no.	Formulation	Time (sec)	Spreadability (g.cm/sec)
1.	F1	12	8.33
2.	F2	7	14.28
3.	F3	13	7.69
4.	F4	10	10
5.	F5	11	9.09
6.	F6	10	10
7.	F7	15	6.67

8.	F8	16	6.25
9.	F9	12	8.33
10.	F10	13	7.69
11.	F11	14	7.14
12.	F12	15	6.67
13.	F13	11	9.09
14.	F14	10	10
15.	F15	9	11.11
16.	F16	10	10
17.	F17	12	8.33

*n=3, all values are in average.

Viscosity

Viscosity of cream was done by using the Brookfield viscometer at room temperature using spindle No. 63 at 2.5 RPM. According to the results all the three formulations showed adequate viscosity from the range of 17850 – 25814cP.

Washability

After running under the tap water the cream removes easily. These was done for three times. To ensure easy washability of prepared cream. No any kind of film formation was observed.

Antimicrobial efficacy

The antibacterial activity of the test samples was evaluated against *Escherichia coli* using the agar well diffusion method. The standard antibiotic, **Streptomycin (1 mg/mL)**, produced the highest antibacterial activity with a **32 mm** zone of inhibition.

Table no.07: Antimicrobial activity of Emulgel in E. coli

Sr. no.	Sample	Conc.	ZOI (mm)
1.	Control		-
2.	STD. Streptomycin	1 mg/ml	32
3.	Emulgel sample	5 mg/ml	07
		10 mg/ml	11

*n=3, above values are average mean

Table no.07: Antimicrobial activity of Emulgel in Staphylococcus aureus

Sr. no.	Sample	Conc.	ZOI (mm)
1.	Control		-

2.	STD. Streptomycin	1 mg/ml	31
3.	Emulgel sample	5 mg/ml	08
		10 mg/ml	12

*n=3, above values are average mean

Emulgel exhibited minimal antibacterial activity, with inhibition zones of 7 mm and 11 mm in *E. coli* strains and 8 and 12 mm ZOI in *S. aureus* strain at the respective concentrations.

Drug content

The drug content was found to be 90.1% of the F2nd formulation.

In vitro release study

The in vitro drug release study evaluated the release behavior of *Calendula officinalis* and Himalayan Arnebia emulgel in

dissolution medium. Release data were fitted to Zero-order, First-order, Higuchi, and Korsmeyer–Peppas kinetic models to determine the release mechanism. *Calendula officinalis* emulgel exhibited excellent zero-order release ($R^2 = 0.9969$) and super case-II transport ($n = 1.0721$), indicating polymer relaxation-controlled release. Himalayan Arnebia emulgel showed strong first-order kinetics ($R^2 = 0.9968$), Higuchi diffusion ($R^2 = 0.9538$), and non-Fickian transport ($n = 0.8032$), demonstrating controlled and sustained drug release suitable for topical wound healing.

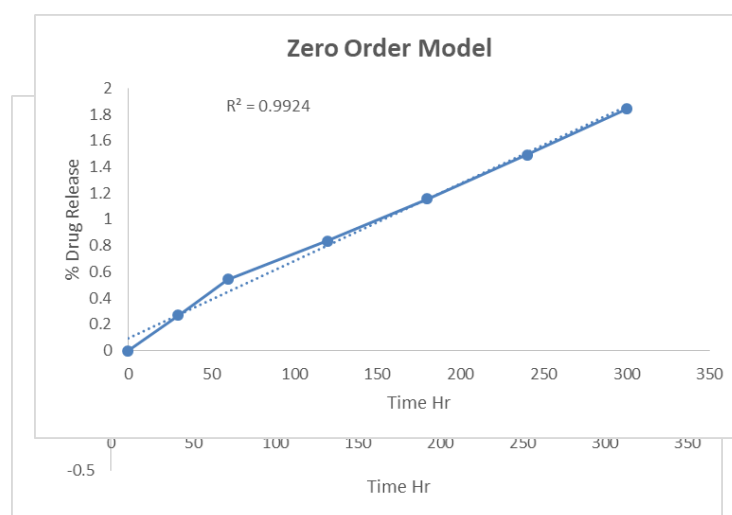


Fig. no.000 Zero order kinetics of Calendula extract

Fig. no.000 Zero order kinetics of H. Arnebia extract

Kinetic model	R2 value of <i>Calendula officinalis</i> extract	R2 value of Himalyan Arnebia extract
Zero order	0.9969	0.9924
First order	0.997	0.9968
Higuchi	0.8913	0.9538
Korsmeyer-Peppas model	0.996	0.9944

Stability Study

The optimized emulgel formulation was subjected to a stability study for 35 days under conditions ($40 \pm 2^\circ\text{C}$ and $75 \pm 5\% \text{ RH}$). Samples were evaluated periodically for appearance, pH,

viscosity, spreadability. No significant changes were observed, indicating that the formulation remained physically and chemically stable.

Table no. 08: Stability study (F2nd)

Parameters	Time (in days)
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	Initial	14	21	28	35
pH	6.42	6.43	6.47	6.49	6.50
Spreadability (Sec)	7	8	10	12	15
Viscosity	24531	24545	24562	24575	24583

Statistical Analysis

The 3D response surface plot illustrates the effect of formulation variables, liquid paraffin (Factor A) and Tween 20 (Factor B), on the drug content (%) of the emulgel. The surface indicates that drug content increased with optimized concentrations of both factors. Higher levels of liquid paraffin and Tween 20 contributed to improved drug incorporation and

uniform distribution within the formulation. The response values ranged approximately from 83–91%, showing significant variation among formulations. The curved surface suggests an interaction between the independent variables, indicating that their combined effect influences drug content. The model successfully predicts optimized formulation conditions for achieving desirable drug content.

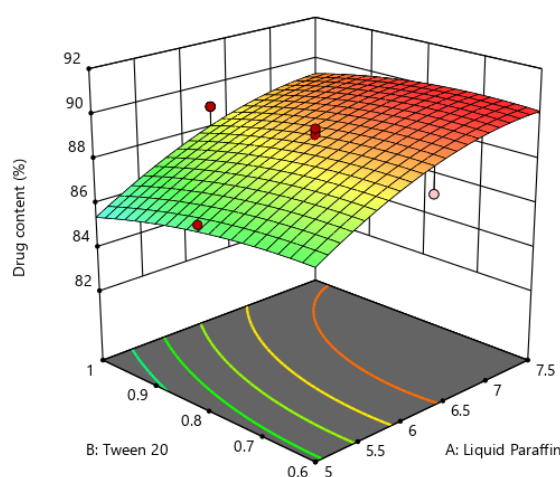


Fig.no.000 Response Surface Methodology (3D graph)

DISCUSSION:

The emulgel that was formulated using extracts from *Calendula officinalis* and Himalayan Arnebia demonstrated adequate physicochemical characteristics, such as the right pH, viscosity, spreadability, homogeneity, and appearance for topical use. The correct integration of both herbal extracts was confirmed by a uniform drug content. Additionally, the formulation showed good stability and effective antimicrobial activity with no discernible changes during storage. Overall, the optimized herbal emulgel displayed encouraging qualities.

The in vitro drug release study evaluated the release behavior of *Calendula officinalis* and Himalayan Arnebia emulgels using a suitable dissolution setup. Release data were fitted to Zero-order, First-order, Higuchi, and Korsmeyer–Peppas kinetic models to determine the release mechanism. Both formulations exhibited controlled and sustained drug release with high correlation coefficients. *Calendula officinalis* emulgel showed super case-II transport, indicating polymer relaxation and erosion-controlled release, whereas Himalayan

Arnebia emulgel exhibited non-Fickian (anomalous) transport, suggesting a combined mechanism of diffusion and polymer relaxation, supporting their suitability for prolonged topical wound healing.

CONCLUSION:

The current study effectively created and assessed an emulgel for wound healing applications that contained extracts from Himalayan Arnebia and *Calendula officinalis*. The optimized formulation's appropriate pH, viscosity, spreadability, homogeneity, and drug content were among the desirable physicochemical characteristics that demonstrated its suitability for topical application. The mixture showed promise as an antimicrobial and remained stable throughout storage. A suitable platform for delivering bioactive constituents with enhanced application properties was created by incorporating both herbal extracts into an emulgel system.

Author contribution

Pooja Battul- Data curation, original draft writing and editing.
Mithun Maniyar- Conceptualization, data validation,

reviewing and editing. Vaibhav Gaikwad- Data validation, reviewing and editing.

Collection and Authentication: The plant was authenticated by Department of Botany, K. B. P. Mahavidhyala, Pandharpur

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