RESEARCH PAPER

Formulation Development, Phytochemical Characterization and In Vitro Pharmacological Assessment of Calendula officinalis Extract for Dermatological Applications

Ms. Neha Tamta[1*], Ms. Rachana Belwal[2], Dr. Rita Mourya[3], Ms. Tripti Padalia[4], Dr. Sachin Tyagi[5], Dr. Amit Singh[6], Ms. Neha Gupta[7], Ms. Ritika Arora[8], Mr. Kapil Dev Tiwari[9]

[1*] Assistant Professor, Pharmacy Academy, Faculty of Pharmacy, IFTM University, Moradabad, Uttar Pradesh, India - 244102 (Corresponding Author). Phone: 9634696336. Email: neha.pharmacy18@gmail.com

[2] Assistant Professor, Department of Pharmaceutical Chemistry, School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India - 248007

[3] Professor and Principal, School of Pharmacy, SAM Global University, Raisen, Bhopal, Madhya Pradesh, India - 464551

[4] Assistant Professor, Faculty of Pharmaceutical Sciences, Amrapali University, Haldwani, Nainital, Uttarakhand, India - 263139

[5] Professor and Director, Bharat Institute of Technology, School of Pharmacy, Meerut, Uttar Pradesh, India - 250103

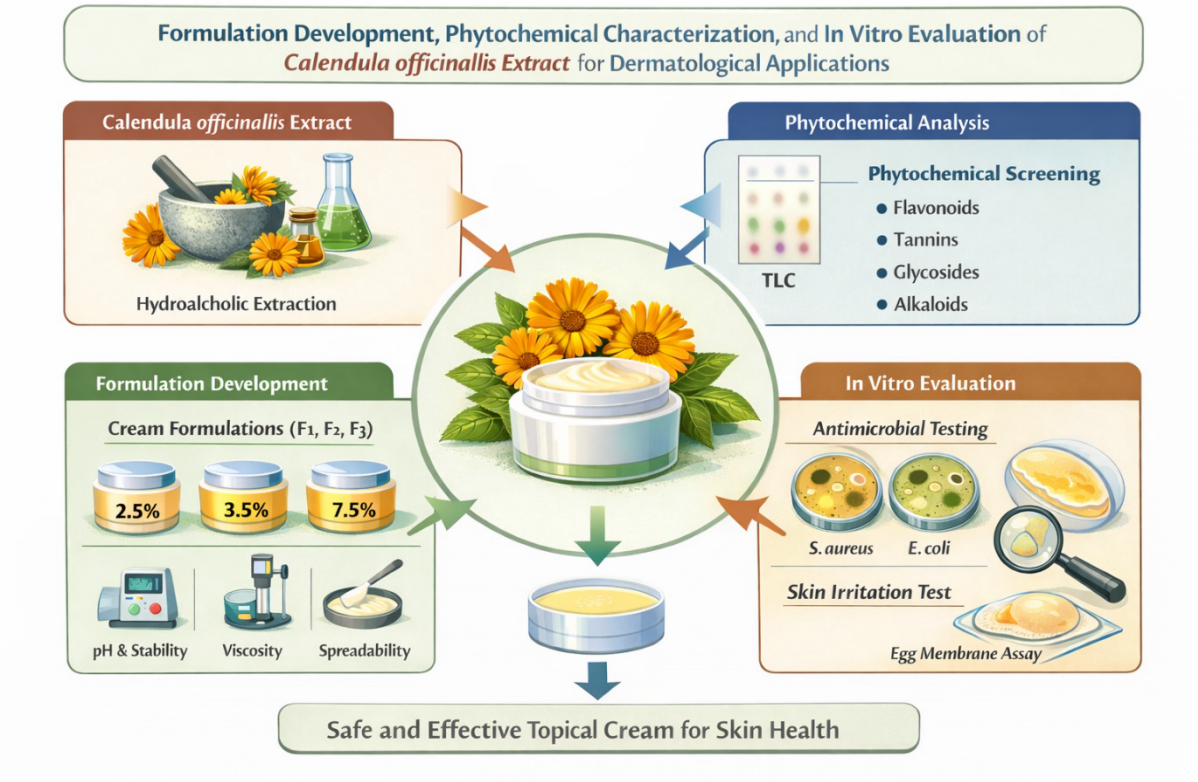
[6] Principal, Panchwati College of Pharmacy, Roorkee-Haidwar Bypass, Ghat Road, Near Subharti University, Meerut, Uttar Pradesh, India - 250004

[7] Assistant Professor, College of Pharmacy, COER University, Roorkee, Uttarakhand, India - 247667

[8] Research Scholar, Department of Pharmaceutical Sciences, Kumaun University, Bhimtal Campus, Bhimtal, Nainital, Uttarakhand, India - 263136

[9] Assistant Professor, Kamla Nehru Institute of Management & Technology, Sultanpur, Ayodhya Prayagraj Highway, Faridipur, Sultanpur, Uttar Pradesh, India - 228119

Abstract



Formulation Development, Phytochemical Characterization and In Vitro Pharmacological Assessment of Calendula officinalis Extract for Dermatological Applications

Calendula officinalis is a medicinal plant traditionally used for treating skin disorders due to its anti-inflammatory, antimicrobial, and wound-healing properties. This study focused on the formulation of a topical cream containing *Calendula officinalis* extract, its phytochemical characterization, and in vitro evaluation. The developed formulations showed good stability, antimicrobial activity, and skin compatibility, supporting the potential of *Calendula officinalis* as a natural dermatological agent.

Keywords- *Calendula officinalis*, Wound healing, Skin disorders, Dermatological agent and In vitro evaluation.

How to cite this article: Tamta N, Belwal R, Mourya R, Padalia T, Tyagi S, Singh A, Gupta N, Arora R, Tiwari KD. Formulation Development, Phytochemical Characterization and In Vitro Pharmacological Assessment of Calendula officinalis Extract for Dermatological Applications. Int J Drug Deliv Technol. 2026;16(23s): 347-353. DOI: 10.25258/ijddt.16.23s.35

Introduction

Skin disorders remain one of the most common health concerns worldwide, affecting individuals of all ages and significantly impacting quality of life. Conditions such as inflammation, microbial infections, delayed wound healing, and oxidative skin damage necessitate the development of safe and effective dermatological therapies. Although synthetic drugs are widely used, their prolonged application is often associated with adverse effects, drug resistance, and high cost. Consequently, there has been a growing interest in plant-based formulations as alternative or complementary therapeutic options due to their better safety profile, biocompatibility, and multi-targeted pharmacological actions [1].

Calendula officinalis, a well-known medicinal plant, has been used for a long time and belongs to the plant kingdom and the Asteraceae family. It's also called English marigold, pot marigold, Bride of the Sun, bull flower, and butterwort [2]. This plant grows well in sunny places and can adapt to different soil types.*Calendula officinalis* is a tall plant with stems that grow upright and have few branches. Its leaves are long and narrow, and it has tubular disc florets. The plant's seeds are curved and have thorns, and they are typically yellow or orange. The plant's leaves and flowers contain carotenoids, flavonoids, saponins, sterols, phenolic acids, lipids, and other biologically active compounds, which are used in both laboratory and living organism studies. The plant is thought to have healing properties and is often used for its anti-inflammatory, diaphoretic, analgesic, and antiseptic effects. It's used to treat digestive problems, women's health issues, oral health problems, eye diseases, skin injuries, and certain types of burns, among other things [3]. Researchers found fifteen amino acids in the leaves, stems, and flowers. The flowers were made into extracts, tinctures, and balms for external use, and were used to treat skin inflammation, open wounds, and bleeding wounds. *Calendula officinalis* possesses medicinal properties recognized in both the Ayurvedic and Unani medical traditions. Two recent treatments derived from *Calendula officinalis* are Carophyllenic ointment (which includes carotenoids extracted from the flowers) and pot marigold tincture. It is a component of a homeopathic remedy used to alleviate pain and swelling associated with acute musculoskeletal injuries. Herbal ear drop formulations that contain *Calendula* flowers have shown effectiveness in treating ear pain in children suffering from acute otitis media. Extracts from *Calendula* flowers of varying polarity have demonstrated antioxidative effects on liposomal lipid peroxidation caused by ferrous ions and ascorbic acid. Isorhamnetin 3-glycosides derived from *Calendula* flowers have been found to inhibit lipoxygenase. *Calendula officinalis* is rich in medicinally active constituents and is known to enhance blood and lymphatic circulation, thereby facilitating the removal of toxins from the body [4]. No contraindications or drug interactions have been reported; however, individuals with known sensitivity to plants of the Compositae (Asteraceae) family may be more prone to allergic reactions. *Calendula*-based mouthwashes exhibit notable anti-inflammatory activity, helping to reduce swollen and irritated gums, along with antibacterial properties effective against periodontopathic microorganisms. Bioactive phytochemical compounds such as isorhamnetin, rutin, and quercetin glucosides have been isolated using advanced analytical techniques, and these compounds are known to possess diverse biological activities [5].This review paper highlights the traditional uses and clinical significance of *Calendula* species and aims to draw the attention of natural product researchers worldwide to their vast therapeutic potential and diverse biological activities. Furthermore, the authors emphasize the important role of *Calendula* in both general healthcare and oral therapeutic applications.Recent advances in phytopharmaceutical research emphasize the importance of formulation development to enhance the stability, bioavailability, and therapeutic efficacy of

herbal extracts. Incorporating plant extracts into suitable topical formulations can improve skin penetration, prolong drug release, and ensure patient compliance. However, the pharmacological potential of *C. officinalis* depends not only on formulation design but also on thorough phytochemical characterization to ensure quality, consistency, and reproducibility of the extract. Advanced analytical techniques play a crucial role in identifying and quantifying biologically active constituents responsible for therapeutic outcomes.In vitro pharmacological evaluation serves as a fundamental step in validating the biological efficacy and safety of herbal formulations prior to in vivo and clinical studies. Assessments such as antioxidant, anti-inflammatory, antimicrobial, and cytoprotective assays provide scientific evidence supporting traditional claims and help establish the therapeutic relevance of plant-based dermatological products.Therefore, the present study focuses on the formulation development, phytochemical characterization, and in vitro pharmacological assessment of *Calendula officinalis* extract for dermatological applications. This research aims to scientifically substantiate the traditional use of *C. officinalis*, explore its bioactive potential, and contribute to the development of effective, safe, and natural topical formulations for skin health management.

Fig.1 ***Calendula officinalis***

Materials and Methods

Plant Material and Authentication

Fresh flowers of *Calendula officinalis* L. were collected during the flowering season from a local area nursery. The plant material was authenticated by a qualified botanist, and a voucher specimen was preserved for future reference. The flowers were washed thoroughly with distilled water to remove extraneous matter and dried under shade at room temperature to prevent degradation of thermolabile constituents. The dried material was pulverised into coarse powder and stored in airtight containers protected from light and moisture until further use.

Preparation of Plant Extract

The powdered flower material was subjected to solvent extraction using an appropriate organic solvent (ethanol/methanol/hydroalcoholic solvent). The extraction was carried out by Soxhlet apparatus for 48 hours.The extract was filtered and concentrated under reduced pressure using a rotary evaporator at controlled temperature. The concentrated extract was dried to obtain a semi-solid mass and stored at 4°C until formulation and analysis.

Preliminary Phytochemical Screening

Preliminary phytochemical analysis of the *Calendula officinalis* extract was carried out to identify the presence of major secondary metabolites using standard qualitative chemical tests. These tests help in establishing the phytochemical profile of the extract and provide insight into compounds that may contribute to its pharmacological activity.

Alkaloids

The presence of alkaloids in the extract was evaluated using Dragendorff's test. A few milliliters of the filtrate were treated with 1–2 mL of Dragendorff's reagent. The formation of a reddish-brown precipitate indicated a positive result, confirming the presence of alkaloids in the extract.

Flavonoids

Flavonoids were detected by the lead acetate test. 1mL of the plant extract was treated with a few drops of 10% lead acetate solution. The appearance of a yellow precipitate confirmed the presence of flavonoid compounds, which are known for their antioxidant and anti-inflammatory properties.

Tannins

Tannins were identified using Braymer's test. 1mL of the filtrate was diluted with 3 mL of distilled water, followed by the addition of three drops of 10% ferric chloride solution. The development of a blue-green coloration indicated a positive test for tannins.

Glycosides

The presence of glycosides was confirmed by the Keller–Killiani test. 1mL of the filtrate was mixed with 1.5 mL of glacial acetic acid containing one drop of 5% ferric chloride solution. Concentrated sulfuric acid was then carefully added along the side of the test tube. The appearance of a blue-colored solution indicated the presence of glycosides in the extract.

Carbohydrates:

Carbohydrates were detected using Molisch's test. 2 mL of the filtrate were treated with two drops of alcoholic α-naphthol solution, followed by the careful addition of 1 mL of concentrated sulfuric acid along the sides of the test tube. The formation of a violet ring at the junction of the two liquids confirmed the presence of carbohydrates.

Thin Layer Chromatography of *Calendula officinalis*

Thin layer chromatography of the hydroalcoholic extract of *Calendula officinalis* was carried out using toluene : ethyl acetate : formic acid (5 : 4 : 1) as the mobile phase. This solvent system is considered suitable for the effective separation of flavonoids, phenolic compounds, triterpenoids, and glycosides, which are reported as major phytochemical constituents of *Calendula officinalis*. The TLC analysis revealed the presence of multiple well-resolved spots on the chromatogram, indicating the occurrence of diverse phytoconstituents in the extract. The calculated Rf values were approximately 0.12, 0.26, 0.32, and 0.48, reflecting compounds with varying degrees of polarity. The appearance of several distinct spots confirms the complex phytochemical composition of the hydroalcoholic extract, with a predominance of flavonoids and phenolic constituents that are likely responsible for the observed pharmacological activities.

Fig.2 TLC of hydroalcoholic extract of *calendula officinalis*

Formulation Development

A topical formulation was developed using the *Calendula officinalis*hydroalcoholic extract as the active ingredient. Suitable excipients such as emulsifying agents, emollients, humectants, preservatives, and purified water were selected based on dermatological compatibility. The oil phase and aqueous phase were prepared separately and heated to the same temperature. The aqueous phase was slowly incorporated into the oil phase with continuous stirring until a uniform formulation was obtained. The formulation was allowed to cool to room temperature and stored in suitable containers for further evaluation.

Formulation of Emollient Cream

Three different formulations (F1, F2, and F3) were prepared with varying concentrations of marigold extract—2.5%, 3.5%,and 7.5% respectively. The cream base was composed of standard cosmetic ingredients including emulsifying wax, stearic acid, acetyl alcohol, liquid paraffin, glycerin, and distilled water. The oil phase (waxes, paraffin, and extract)was heated to 70°C while the aqueous phase (glycerin and water) was heated separately to the same temperature. The aqueous phase was gradually added to the oil phase with constant stirring using a mechanical stirrer until a homogenous cream was formed. The final cream was allowed to cool at room temperature before being transferred into sterile glass containers.

Fig 3. Formulation F1, F2 and F3

Physicochemical Evaluation of Formulated Creams

All three formulations were subjected to a range of physicochemical tests to assess quality, stability, and performance.

- **pH Measurement:** The pH of the creams was measured using a digital pH meter at room temperature. A 1g sample of cream was dispersed in 10 mL of distilled water for accurate reading.
- **Viscosity:** A Brookfield viscometer was used to determine the viscosity of each formulation. Measurements were taken at a constant spindle speed and recorded in centipoise.
- **Spread ability:** Spread ability was evaluated by placing 1g of cream between two glass slides and applying a known weight. The diameter of the spread cream was measured after a fixed time.

▪ **Stability** Testing: Accelerated stability studies were conducted by storing the cream at three different temperature conditions (4°C, room temperature, and 40°C) for 30 days. Observations for colour change, phase separation, and odour were recorded weekly.

▪ **Homogeneity and Texture:** Each cream was visually and tactilely evaluated for uniformity, smoothness, and ease of application.

In Vitro Antimicrobial Testing

To assess the antimicrobial activity of the formulated creams, the agar well diffusion method was used. Test organisms included Staphylococcus aureus and Escherichia coli. Sterile nutrient agar plates were inoculated with bacterial suspensions, and wells were filled with the cream formulations. After 24 hours of incubation at 37°C, the zone of inhibition around each well was measured in millimetres.

In Vitro Skin Irritation Test

To ensure the formulations were safe for human use, a preliminary irritation test was conducted using egg membrane as a surrogate for human skin. Cream samples were applied to isolated egg membranes and observed over 24 hours for signs of irritation such as discolouration or structural degradation.

.

Physicochemical Properties

1. pH Evaluation

The pH values of the three cream formulations remained within the acceptable range for topical application (pH 4.5–7.0). F1 recorded a mean pH of 6.45, F2 had a pH of 6.38,and F3 showed a slightly lower pH of 6.30. These values indicate that all formulations are suitable suggesting a denser formulation at higher extract loads.Despite these variations, all formulations maintained consistency suitable for smooth topical application.

2. Spreadability

Spreadability was inversely related to viscosity, with F1 demonstrating the highest spreadability (6.2 cm), followed by F2 (5.8 cm), and F3 (5.5 cm) under the same applied force. The results indicate that while the creams were all easily spreadable, higher extract concentrations slightly reduced this property, possibly due to increased thickness.

3. Homogeneity and Texture

All formulations were visually homogenous with no signs of phase separation, clumping, or granulation. The creams appeared smooth and uniform in colour, with F1 and F2 being light yellow, while F3 appeared more intensely yellow due to the higher concentration of extract. Textural analysis by hand confirmed that all samples were smooth, non-gritty, and non-sticky, making them cosmetically acceptable.

4. Stability Testing

Over a 30-day period, creams were stored at three different conditions (4°C, room temperature, and 40°C). There was no noticeable change in colour, odour, or consistency across all storage conditions. No phase separation or microbial growth was observed. These findings confirm that the formulations were chemically and physically stable undervarying environmental conditions.

Antimicrobial Activity

Antimicrobial testing was performed against Staphylococcus aureus and Escherichia coli using the agar well diffusion method. The zones of inhibition (in mm) are presented below:

Against Staphylococcus aureus and Against Escherichia coli

Antimicrobial activity of the formulated creams was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the agar well diffusion method. For *S. aureus*, the zones of inhibition were 10.5 ± 0.2 mm for F1, 13.8 ± 0.3 mm for F2, and 15.6 ± 0.4 mm for F3. Against *E. coli*, the inhibition zones measured 9.2 ± 0.2 mm for F1, 11.5 ± 0.3 mm for F2, and 13.3 ± 0.3 mm for F3. A clear positive correlation was observed between extract concentration and antimicrobial efficacy. F3, containing the highest concentration of marigold extract, demonstrated the largest inhibition zones for both organisms, while F1, with the lowest extract concentration, exhibited the smallest but still significant antimicrobial effect compared to the control base cream, which showed no activity.

A direct correlation was observed between extract concentration and antimicrobial activity. F3, which contained the highest amount of marigold hydroalcoholic extract, demonstrated the largest zones of inhibition against both organisms. F1 exhibited the lowest antimicrobial effect, although still significant compared to the control (base cream with no extract), which showed no antimicrobial activity.

In Vitro Skin Irritation Test

The egg membrane irritation test showed no signs of membrane damage, discolouration, or reaction after 24 hours of exposure to any of the formulations. The results confirm that all three formulations were non-irritant and safe for skin application. The base cream (without extract) also showed no irritation, indicating that both the extract and base ingredients were non-

reactive.All formulations exhibited acceptable pH, good homogeneity, and stability under different storage conditions. Antimicrobial studies demonstrated a concentration-dependent inhibitory effect, with the 7.5% formulation showing the highest zones of inhibition against both test organisms. None of the formulations irritated the in vitro skin irritation test.

Results

The present study was designed to formulate and evaluate emollient creams containing varying concentrations of Calendula officinalis (marigold) Hydroalcoholic extract. Three formulations—F1 (2.5%), F2 (3.5%), and F3 (7.5%)—were prepared and tested to assess their physicochemical characteristics, antimicrobial activity, skin compatibility, The findings are presented below in a structured format, covering each set of evaluations

Conclusion

The present research successfully formulated and evaluated topical emollient creams containing hydroalcoholic extract of *Calendula officinalis*. Phytochemical characterization confirmed the presence of key bioactive constituents responsible for dermatological benefits. The developed formulations exhibited desirable physicochemical properties, good stability, and excellent skin compatibility. In vitro antimicrobial evaluation revealed a concentration-dependent activity against *Staphylococcus aureus* and *Escherichia coli*, with higher extract concentrations demonstrating enhanced efficacy. The absence of skin irritation further supports the safety of the formulations for topical use.Overall, the findings validate the traditional use of *Calendula officinalis* in skin care and highlight its potential as a natural, effective, and safe alternative for the management of skin infections and related dermatological conditions.

References

1. Kasolang, S., Adlina, W. A., Rahman, N. A., & Nik, N. R. (2020). Common skin disorders: a review. JurnalTribologi, 25(April), 59-82.
2. Patil, K., Sanjay, C. J., DoggALLI, N., Devi, K. R., & Harshitha, N. (2022). A Review of Calendula Officinalis-Magic in Science. Journal of Clinical & Diagnostic Research, 16(2).
3. Arora, D., Rani, A., & Sharma, A. (2013). A review on phytochemistry and ethnopharmacological aspects of genus Calendula. Pharmacognosy reviews, 7(14), 179.
4. Shahane, K., Kshirsagar, M., Tambe, S., Jain, D., Rout, S., Ferreira, M. K. M., ... & Lima, R. R. (2023). An updated review on the multifaceted therapeutic potential of Calendula officinalis L. Pharmaceuticals, 16(4), 611.
5. AshwlayanVD, K. A., & Verma, M. (2018). Therapeutic potential of Calendula officinalis. Pharm Pharmacol Int J, 6(2), 149-155.
6. Preethi, K. C., Kuttan, G., &Kuttan, R. (2008). Antioxidant potential of an extract of Calendula officinalis flowers in vitro and in vivo. Pharmaceutical Biology, 44(9), 691–697. PMC
7. Niclaus, C., Junghanns, S., Hartmann, A., Murillo, R., Ganzera, M., &Merfort, I. (2017). In vitro studies to evaluate the wound healing properties of Calendula officinalis extracts. Journal of Ethnopharmacology, 196, 94–103. PMC
8. Oliva-Rodríguez, C., Nomizo, A., & Gerlach, R. F. (2010). Protective effect of Calendula officinalis extract against UVB-induced oxidative stress in skin: Evaluation of reduced glutathione levels and matrix metalloproteinase secretion. Journal of Ethnopharmacology, 127(2), 596–601. PubMed
9. Akhtar, N., Uz Zaman, S., Khan, B. A., Amir, M. N., & Ebrahimzadeh, M. A. (2011). Effects of a newly formulated topical cream of Calendula officinalis extract on mechanical skin parameters in healthy volunteers. PubMed PMID: 21928714. PubMed
10. Fatima, S. S., Govekar, S. U., Satardekar, K. V., Barve, S. S., & Dhawal, P. P. (2018). In vitro analysis of ethanolic extract of flowers of Calendula officinalis for antioxidant, antimicrobial and UV-H_2O_2 induced DNA damage protection activity. Journal of Pharmacognosy and Phytochemistry, 7(5), 2378–2383. Phyto Journal
11. Characterization of biologically active substances from Calendula officinalis. (2018). Journal of Medicinal Chemistry & Phytochemistry. PubMed
12. Kumar, R., Zilate, S. R., & Varma, S. (2019). A pharmacological study on the effect of Calendula officinalis extract on wound healing in animals. International Journal of Basic & Clinical Pharmacology. IJBCP

13. Givol, O., et al. (2019). Calendula officinalis and wound healing: A systematic review of clinical and animal studies. Wound Repair and Regeneration. PubMed
14. Calendula extract in topical formulations for UVB irradiation-induced skin damage: formulation stability and skin penetration. PubMed PMID: 21491442. PubMed
15. Dhingra, G., Dhakad, P., & Tanwar, S. (2022). Review on phytochemical constituents and pharmacological activities of plant Calendula officinalis Linn. Biological Sciences, xx, 1-9. IR Research Publication
16. Arora, D., Rani, A., & Sharma, A. (2013). A review on phytochemistry and ethnopharmacological aspects of genus Calendula. Pharmacognosy Reviews, 7(14), 179–187. PMC
17. Formulation and evaluation of hydrophilic cream with antioxidant activity using Calendula officinalis extract. ResearchGate. ResearchGate
18. Comparison of different concentrations of Calendula officinalis gel on wound healing. Biomedical & Pharmacology Journal. Biomed Pharma Journal
19. Calendula in modern medicine: Advancements in wound healing and topical applications. ScienceDirect Review.