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Original Article

Evaluation of a *Tagetes erecta* Flower Extract Cream for Psoriasis-Relevant Anti-Inflammatory and Antioxidant Effects

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ABSTRACT

Background: Psoriasis is a chronic inflammatory skin disease in which the use of conventional therapies is often associated with adverse effects; therefore, medicinal plants with antioxidant and anti-inflammatory properties represent a promising alternative for the development of safer topical treatments. **Objectives:** To develop and characterize a topical anti-psoriatic cream formulated with *Tagetes erecta* (TE) flower extract, due to its anti-inflammatory property. **Materials and Methods:** The study consisted of a qualitative and a quantitative assessment of TE flower, antioxidant assessment. Analytical quantitation and validation carried out via HPLC. Development of the topical anti-psoriatic cream followed the determination of the *in vitro* anti-inflammatory activity of the cream. **Results:** The ethanolic extract exhibited higher flavonoid content (17.80 mg RE/g) than phenolic content (0.389 mg GAE/g). An anti-psoriatic cream was formulated with varying concentrations of TE and subjected to physicochemical characterization. In the DPPH assay, TE showed an IC_{50} of 11.88 mg/mL and an AAE of 46.3 mM/100 g, compared to the cream's IC_{50} of 30.83 mg/mL and AAE of 17.83 mM/100 g. HPLC analysis confirmed the highest quercetin concentration in formulation F3 (1.625 mg/g), with a validated linearity range (R^2=0.9949) and %RSD between 0.49-5.69%. The RBC membrane stabilization assay showed significant inhibition of hemolysis 74.78% at 1000 µg/mL (p<0.05) as compared to standard. TE showed considerable inhibition of protein denaturation, showing highly significant effect of concentration (p<0.01), and a significant difference between Standard and Extract treatments (p<0.05). **Conclusion:** The extract-enriched formulation showed better anti-inflammatory activity, which shows that it has potential as a new herbal treatment for psoriasis.

Keywords: Anti-inflammatory, Antioxidant, DPPH, Psoriasis, *Tagetes erecta*.

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INTRODUCTION

Skin is the largest organ of our body which serves as a protective barrier against environmental hazards and various pathogens. It also saves us from oxidative stressor and toxic substances. The barrier function of the skin is essential for maintaining homeostasis and protecting internal organs from microbial invasion and mechanical injury (Jiao *et al*., 2024). But in various autoimmune and chronic inflammatory skin disorders such as Psoriasis, this barrier becomes weakened and it results in transepidermal water loss, immune imbalance, and persistent inflammation (Dong *et al*., 2024). In this disease, the patient not only suffers from the trauma but also it rises a financial burden globally. The recent investigation indicates that the number of individuals living with psoriasis has nearly doubled over the past three decades. A study conducted by (Li *et al*., 2025) reported number of individuals living with psoriasis reached approximately 43 million cases worldwide in 2021, with more than 5 million new diagnosed that year. This increase is reflected in growing disabilities and comorbidities such as psoriasis arthritis (Damiani *et al*., 2021). The Global economic costs for curing and managing the disease were estimated at nearly USD 149 billion in 2021(Wei *et al*., 2025). Asia accounts for more than half of global incident and prevalent cases and a substantial proportion of psoriasis-related

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DALYs, highlighting the need for research focused on Asian populations where disease burden continues to rise (Xiong *et al.*, 2025). As Pakistan lies within Asia, where the Southern coastal region, covering the coastal belt of Sindh and Balochistan. This represents a distinct phytogeographical zone characterized by arid to semi-arid climate, saline soils, high temperatures, and low rainfall (Haider *et al.*, 2025). Plants growing under these region frequently accumulate higher levels of secondary metabolites, including flavonoids, terpenoids, and phenolic acids, as compensatory biochemical responses (Zahedi *et al.*, 2021). According to Ethnobotanical surveys, the coastal region reports several local plants that are being used for various skin diseases as traditional or complementary medicine. This reflects both the cultural reliance on natural remedies as well as phytochemical richness of the regional flora (Zainab *et al.*, 2025). Despite this potential, rigorous studies on the chemical composition and dermatological applications of coastal medicinal plants remain limited. We selected *Tagetes erecta* flower for our research due to its local availability, traditional usage, environmental conditions and rich phytoconstituents that support to reduce inflammation and oxidative stress.

There are various medicinal plants such as *Aloe barbadensis* (Jales *et al.*, 2022), *Mahonia aquifolium* (Anheyer *et al.*, 2025), *Salvia officinalis* (Hamzic *et al.*, 2022), *Glycyrrhiza glabra* (Khorshidian *et al.*, 2024), *Centella asiatica* (Lin *et al.*, 2023) that have been explored for the treatment and management of psoriasis. Many herbs have been reported for their anti-inflammatory and anti-proliferative potential in psoriasis-related models but clinical findings remain uneven and still limited (Ojha *et al.*, 2024). According to previous studies it has been noted that that only a few extracts, including *Mahonia aquifolium, Indigo naturalis*, and Aloe vera, which have progressed to evaluation in randomized clinical trials but there are limited evidence for routine clinical use and formulation challenges (Anheyer *et al.*, 2025). Despite encouraging preclinical results, translation into meaningful clinical benefit has been slow, underscoring the ongoing need for well-designed studies on promising plant-derived agents that can target the key pathological pathways of psoriasis.

Tagetes erecta L. (*T. erecta*), a member of the Asteraceae family, is native to Mexico and Central America but is now cultivated worldwide. Among the 56 species of the Tagetes genus, *T. erecta* has gained prominence because of its diverse applications in the food industry as a natural colorant and condiment. It is also used for medicinal ornamental purpose (Estrada *et al.*, 2025; Bohatu *et al.*, 2024). Various phytoconstituents including flavonoids, terpenoids, phenols, tannins, alkaloids, quinones, coumarins, and carbohydrates have been reported as antibacterial, antifungal, anti-inflammatory, insecticidal, and antioxidant properties (Tomar *et al.*, 2025; Javed *et al.*, 2025).

Traditionally in many Asian countries, the flowers of *T. erecta* have been used to treat wounds, menstrual irregularities, and various skin conditions (Chaudhari *et al.*, 2024). The highest carotenoid such as lutein reported in managing visual impairments, age-related macular degeneration, and cancer prevention (Estrada *et al.*, 2025). The highest phenolic content emphasize its strong free radical scavenging activity (Rivas-García *et al.*, 2023) that exhibit the suppression of LPS-induced inflammatory responses through regulation of the TLR4/MD2 signaling pathway (Sanjaya *et al.*, 2024). This potential makes it a strong candidate for Psoriasis management.

According to (Vaz *et al.*, 2024), the flowers of *T. erecta* extracts shows regulation of cytokine release, migration of neutrophil, and inflammatory markers in *in vivo* studies. However, no prior study has investigated on *T. erecta* formulation effect on psoriasis such as keratinocyte hyper proliferation.

Psoriasis is driven by persistent autoimmune activation, characterized by cytokines such as TNF-α, IL-17, and IL-23, along with downstream signaling molecules including NF-κB and STAT3, which together stimulate keratinocyte proliferation and recruit inflammatory cells (Chhabra *et al.*, 2022). Flavonoids such as Quercetin and kaemferol in *T. erecta* possess inhibitory effects on pro-inflammatory mediators, it can interfere with inhibition of NF-κB and TLR4-associated pathways that mitigate the oxidative stress and inflammatory signaling in psoriatic tissue (El-Gazzar *et al.*, 2024).

Given the absence of previous research examining *T. erecta* within psoriasis-relevant biological systems there is need for novel plant therapies with validated mechanisms. This study aims to formulate and evaluate a *T. erecta* flower extract-based topical cream for the management of psoriasis.

Methods for standardization and quality parameter determination

Collection of flowers

The flowers of *Tagetes erecta* L. were collected from the botanical garden of Ziauddin University located at the southern coastal region of Pakistan. The plant was identified and authenticated by Dr. Muhammad Mohtasheem Ul Hasan. A voucher specimen (No. TEF-11-24) was deposited at the Department of Botany, University of Karachi. The flowers selected for the investigation were thoroughly washed under running tap water, followed by rinsing with distilled water. They were then allowed to air dry for a period at room temperature before being shade dried for approximately 3-4 weeks. The dried plant material was ground via an electric grinder (Anex AG-6042).

Physicochemical evaluation

Determination of extractive value

The dried powder of *T. erecta* (500 g) was measured and macerated with analytical grade ethanol at a sample/ solvent ratio of 1:20 (w/v) for seven days. The flask was shaken at regular

interval during maceration. The macerate was then filter by muslin cloth and then filter by means of Whatman No. 41 filter paper with a pore size of 20 to 25 µm. The solvent was removed from filtrate by means of rotary evaporator (IKA RV05) and the extractive value was determined. The semi solid extract was stored in an air tight container at 4°C (Mohammedi *et al.*, 2019).

Determination of loss on drying

The degree of loss during drying was determined by heating the sample to a temperature below its melting point, which included water and volatile substances such as alcohol. 2 g of powdered drug was weighed and heated for 3 hr at 105°C. From the difference in weight, the percentage loss of drying with respect to the air-dried substance was calculated via the following formula (Sagar *et al.*, 2022).

$$\%loss\ on\ drying = \frac{Initial\ weight\ - Final\ weight}{Initial\ weight} \times 100$$

Total ash

2 g of powder drug was taken from preweighed crucibles and placed in a muffle furnace (SX-2.5-10) for 5 hr at 600°C until all the carbon from the powder drug was completely removed. The crucible and the amount of ash were weighed. The procedure was repeated for three samples to determine the mean reading (Mandal *et al.*, 2017).

pH

For the determination of pH, an aqueous solution of the powder drug was prepared by weighing 1 g of the powder drug with 10 mL of water (10% w/v), filtering the solution and determining the pH via a digital pH meter (Mushtaq *et al.*, 2014).

Phytochemical investigation

Qualitative screening was performed for the identification of various primary and secondary metabolites, such as carbohydrates, fat/oil, alkaloids, flavonoids, phytosterols, terpenoids, glycosides, tannins and polyphenols, following standard methods.

Test for alkaloids

Hager test

When alkaloid react with Hager reagent it forms yellow or yellowish orange ppt. For the identification of alkaloid 1-2 mL of the extract was taken in a test tube, and few mL of Hager's reagent was added, which yielded yellow precipitate indicating the presence of alkaloids (Maheshwaran *et al.*, 2024).

Test for flavonoids

Shinoda's Test

When magnesium reacts with flavonoids and concentrated hydrochloric acid it undergoes a reduction reaction that converts the flavonoids into the aglycone forms resulting in the formation of colored complex. For the identification of flavonoid, a small piece of magnesium ribbon is added to the sample in the presence of concentrated HCl, resulting in appearance of red color (Maheshwaran *et al.*, 2024).

Test for Phenols and Tannin

Ferric chloride test

The phenolic OH group react when react with $FeCl_3$, a colored complex is formed between the ferric ions and OH^- group. For the flavonoid test, 1-2 mL of extract was added to a few millilitres of $FeCl_3$, resulting in the formation of green precipitate, which indicates the presence of flavonoids (Maheshwaran *et al.*, 2024).

Test for Cardiac Glycoside

Keller-Kilani test

In this test, the Ferric Ions (Fe^{+3}) in the reagent react with the deoxy sugars (cardiac glycosides) in the plant extract to form colored complex, while glacial acetic acid stabilizes the glycoside. The addition of sulfuric acid produces distinct color changes at the interface in form of ring.

T. erecta flower extract (2 mL) was added to 1 mL of glacial acetic acid, followed by 1-2 drops of iron chloride solution and 2 mL of concentrated sulfuric acid. A brown ring was appeared at interphase indicated the presence of cardiac glycosides (Maheshwaran *et al.*, 2024).

Test for Phytosterols

Hesse response

Add 5 mL of extract to 2 mL of chloroform and 1 mL of sulphuric acid. The red color was appeared in the chloroform layer which indicates the presence of phytosterols (Shaikh and Patil, 2020).

Test for Quinone

Conc. Acid test

In this test, the acid undergoes deprotonation when react with the quinones, resulting in a color change. 1-2 mL of extract was added to 1 mL of concentrated HCl. A green-brown color appeared, indicating the presence of Quinone (Maheshwaran *et al.*, 2024).

Test for Carbohydrates

Molish test

In this test when concentrated acid react with the carbohydrates present in plant, it undergoes a dehydration reaction followed by a condensation reaction with phenol molecules, resulting in the formation of violet colored ring. 2 mL of plant extract was added to 1 mL of Molish reagent, followed by few drops of conc. H_2SO_4 at the inner surface of the test tube. A violet ring formed at the interface, indicating the presence of carbohydrates (Maheshwaran *et al.*, 2024).

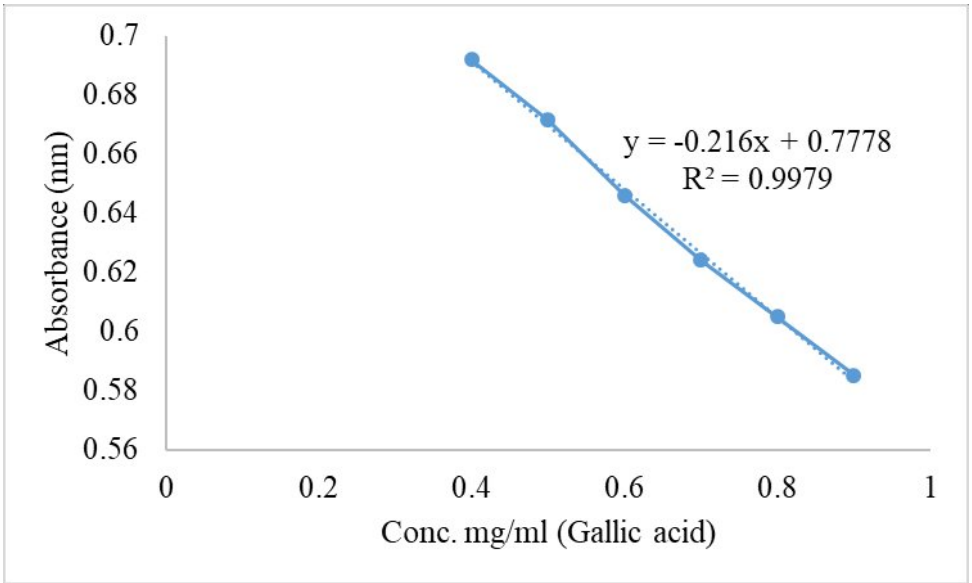

Figure 1: Total Phenolic Content.

Test for Terpenoids

A few millilitres of extract was first dissolved in 2 mL of CCl4 and allowed to evaporate. To this mixture, 2 mL of concentrated H_2SO_4 was added, and the mixture was heated for approximately 2 min. A grayish color indicates the presence of terpenoids (Shaikh and Patil, 2020).

Total phenolic content

The total phenolic content was assessed via a modified version of the method described by (Rizvi *et al*., 2023). 1 mL sample extract (0.10 g/2.5 mL) was combined with 2.5 mL of 10% Folin reagent and incubated for 5 min. Next, 2.5 mL of 7.5% Na_2CO_3 was added with continuous shaking. The solution was kept in the dark for 2 hr, and the absorbance was measured at 765 nm via a V-730 spectrophotometer. Gallic acid (10-100 µg/mL) was used as a standard, and the results are expressed as Gallic Acid Equivalent (GAE) per 100 g of dry extract.

Total flavonoid content

The total flavonoid content was assessed via the method outlined by Farooqi *et al*., (2024) with some modifications (Farooqi *et al*., 2024). 1 mL of sample extract (0.10 g/2.5 mL) was combined with 0.5 mL of 10% Aluminum Chloride ($AlCl_3$), 0.5 mL of 1 M potassium acetate ($C_2H_3O_2K$), and 4.15 mL of distilled water, and the absorbance was measured at 415 nm via a UV spectrophotometer, with methanol and distilled water serving as blanks. Rutin (10-100 µg/mL) was utilized as the standard.

Determination of antioxidant activity

DPPH radical scavenger for the extract

The antioxidant activities of the TE extract were assessed by measuring the DPPH radical scavenging activity, following the methods of Meda *et al*., and Liu *et al*., (2007), with slight modifications. In this method, 0.5 mL of flower extract was mixed with 3.5 mL of DPPH solution prepared in methanol. The concentration of the sample extracts ranged from 60 to 100 mg/mL. Each sample was transferred to a separate test tube, wrapped in aluminum foil to prevent light exposure, and placed in the dark for 30 min. The absorbance of each sample was measured at 517 nm in triplicate via UV-visible spectrophotometer. Methanol was used as the blank, and the control consisted of DPPH solution and methanol without extract. A calibration curve was constructed using ascorbic acid solutions ranging from 1 mg/mL to 10 mg/mL. The antioxidant activity of the extracts was calculated in terms of Ascorbic Acid Equivalents (AAE), expressed as g/100 g of dry extract. The free radical scavenging activity of each extract was expressed as the percentage inhibition and IC_{50} values (Siddiqa *et al*., 2025).

$$\%\text{inhibition} = \frac{[Tc - Ts]}{Tc} \times 100$$

where Tc is the absorbance of the control sample and Ts is the absorbance of the sample.

Formulation of anti-psoriatic cream

The flowers of *T. erecta* were selected for the formulation of a cream intended for the treatment of psoriasis. The cream was formulated via the Oil-in-Water (O/W) emulsion method and consisted of two phases: an oil phase and an aqueous phase. The water-soluble ingredients, including glycerol, propylene glycol, triethanolamine, and methyl paraben, were added to one beaker. Similarly, the oil-phase ingredients stearic acid, beeswax, cetyl alcohol, and the preservative propyl paraben were placed in separate beakers. Both phases were heated in a water bath at 72±2°C. The aqueous phase was then gradually added to the oil phase with continuous stirring until a smooth emulsion formed (Dhamale *et al*., 2023).

Experimental design and optimization of anti-Psoriatic cream

A 3^2 factorial design was employed to investigate the combined effect of *Tagetes erecta* extract (Factor A) and stearic acid (Factor B) on the physicochemical properties of a topical cream intended for psoriasis treatment. A total of 4 experimental formulations were prepared. The formulations were coded F1 to F4 accordingly. Each factor was studied at three levels-low, medium, and high coded as −1, 0, and +1, respectively. Finally, TE was incorporated into the cream base (Okafo *et al.*, 2023).

Characterization of the formulated cream

Organoleptic properties

The organoleptic test of a cream preparation was performed visually, including color, texture, odor, appearance and consistency.

Stability of a formulation

The stability of each formulation was evaluated by placing the formulation at different storage conditions ranging from 25°C to 40°C. The parameters used were pH, homogeneity, viscosity and spreadability at different time intervals (Singh *et al.*, 2024).

pH of the cream

To determine the pH of the cream, 1 g of the formulation was dispersed in 10 mL of distilled water, and the pH was measured via a calibrated digital pH meter (Sirsat *et al.*, 2022).

Homogeneity

The prepared cream formulation was evaluated for its visual appearance because of its homogeneity and because its texture was compressed between fingers (Ijaz *et al.*, 2022).

Viscosity

The viscosity of the creams was evaluated with a Rotary viscometer (NDJ-8S) at a temperature of 25°C±2. Spindle 3 was inserted into the creams and rotated at 30 rpm. The displayed values were measured (Okafo *et al.*, 2023).

Spreadability

The method was used with slight modifications. A quantity of 0.1 g of cream was placed between two glass slides. A weight of 100 g was placed on top of the upper plate for 5 min. Spreadibility was assessed by measuring the diameter of the cream in both lengthwise and widthwise directions using a digital caliper, and the average of these measurements was taken for each reading (SE *et al.*, 2022).

HPLC quantification of querecitin in anti-psoriatic cream

The quantification of quercetin in the formulated cream of *Tagetes erecta* was performed via a validated High-Performance Liquid Chromatography (HPLC) method adapted from the standard method of Deshmukh *et al.*, 2024. HPLC analysis was carried out via a Shimadzu SPD-20A Prominence UV/vis detector with an LC-20 AT pump, a manual injector with a loop volume of 20 µL (Rheodyne), and a Welchorm C18 column (4.6 × 250 mm, 5 µm). GC-10 software (Shimadzu Japan) was used to acquire and process the data. The mobile phase consisted of HPLC-grade acetonitrile and 0.1% (v/v) orthophosphoric acid in deionized water at a 1:1 ratio and was delivered at a flow rate of 0.8 mL/min, and the column was maintained at room temperature (25°C). The detection wavelength was set to 370 nm.

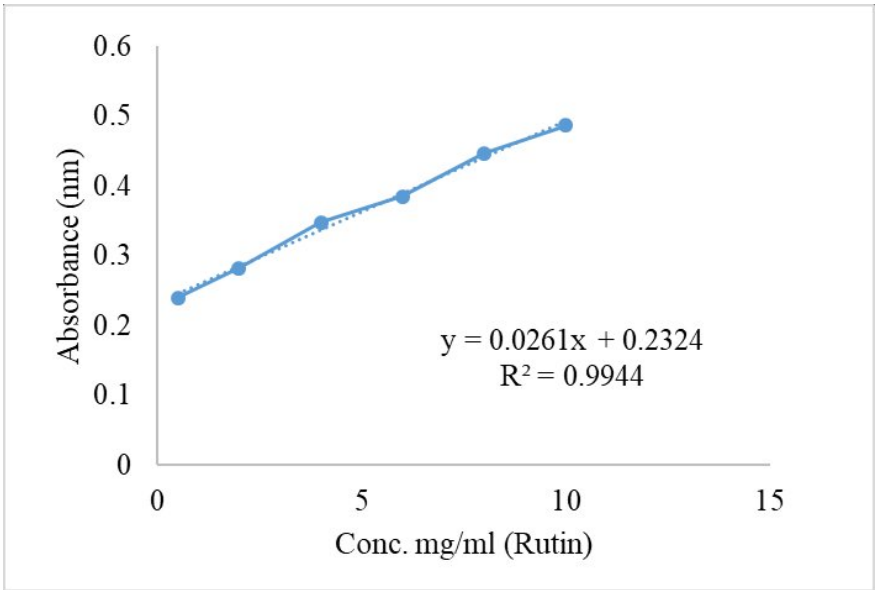

Figure 2: Total Flavonoid Content.

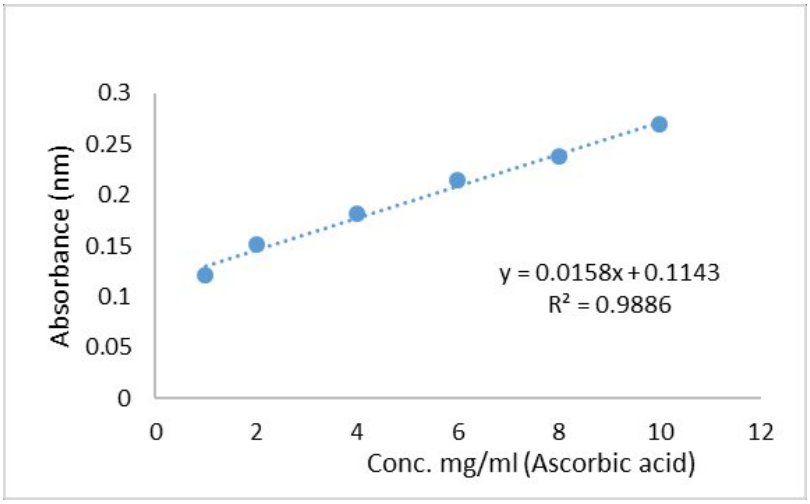

Figure 3: DPPH activity (AAE conc. mg/mL).

Table 1: Physicochemical evaluation of the crude drug.

Sl. No,	Parameters Value (w/w)	Mean±SD
1	Extractive value	47.66±0.57
2	Loss on drying	0.133±0.03
3	pH of 1% aqueous solution	4.61±0.04
4	pH of 10% aqueous solution	4.23±0.03

The reported values are expressed as Mean±(SD), where *n*=3 independent values

Sample preparation for quantification

For sample preparation, 1 g of cream was mixed with 10 mL of HPLC-grade methanol and subjected to sonication (Elmasonic E-60 H) for 30 min to ensure complete solubilization of the bioactive compound. The sample was then centrifuged at 4,500 rpm for 10 min (DUAB DM 0412S). The resulting supernatant was carefully separated and filtered through a 0.45 µm syringe filter. The filtered extract was transferred into HPLC vials for analysis. Identification of the bioactive compound was achieved by comparing the Retention Time (RT) and UV absorption spectra of the sample peak with those of the standard quercetin (Deshmukh and Patil, 2024).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The Detection Limit and Quantification Limit was determined using the standard deviation of the regression line (σ) from the calibration curve and the slope, based on ICH Q2 (R1) guidelines (Guideline, 2022).

In vitro anti-inflammatory test

Membrane stabilization assay

The anti-inflammatory effect of the extract on membrane stabilization (human red blood corpuscles) was evaluated following the methods of Sakkaa *et al.*, (2022) (Sakkaa *et al.*, 2022) with slight modifications. Diclofenac sodium was used as a standard drug, and the anti-inflammatory activity was expressed as the percentage of human red blood cell lysis. As the membrane of red blood cells resembles the lysosomal membrane, its stabilization by the extract suggests the stabilization of the lysosomal membrane as well. The absorbance was measured with a spectrophotometer in the 560 nm range to estimate the RBC content in the test sample suspension. Blood was collected in anticoagulant test tubes (EDTA tubes) from healthy volunteer (the author) who had not used any anti-inflammatory drugs for two weeks under the ethical approval no 2023-07/SK/FoP. The blood sample was centrifuged (centrifuge DUAB DM 0412S) for 10 min at 3000 rpm, and the plasma was separated and skimped to obtain packed red blood cells. The RBC mixture was then washed 3 times with normal saline solution (0.9% w/v) by removing the supernatant via centrifugation at 4500 rpm for 5 min. A 10% (v/v) suspension of the RBC pack was prepared by adding 1 mL of washed packed RBCs to 9 mL of Phosphate Buffered Saline (PBS).

Acid Hemolysis-Induced RBC Membrane Stabilization Assay

Extracts at various concentrations (200 µg/mL, 400 µg/mL, 600 µg/mL, 800 µg/mL and 1000 µg/mL) were mixed with 5 mL of PBS (pH 7.4) in centrifuge tubes. A total of 0.5 mL of the stock RBC suspension (10% v/v) was mixed with 2 mL of the hyposaline solution (0.36% NaCl, 4.5 pH), 1.0 mL of PBS (0.15 M) and 1 mL of test sample. The control sample was mixed by adding hyposaline solution (pH 5.0) and an RBC suspension without extract. All the samples were incubated for 30 min at 37°C. The whole mixture was centrifuged at 4500 rpm for 5 min, and the absorbance of the supernatant liquid was measured at 560 nm with a UV spectrophotometer. Different concentrations of diclofenic sodium were used as standard drugs following the same procedure (Sakkaa *et al.*, 2022). The hemolysis inhibition rate (%) was calculated via the following equation:

$$\text{Inhibition rate \% (RBC lysis)} \frac{\text{TE3} + \text{TE1} - 2\text{TE2}}{\text{TE3} - \text{TE2}} \times 100$$

ROS Generation Inhibition Assay (Egg Albumin-H_2O_2 Model)

Reduction of Reactive Oxygen Species (ROS) by a test sample indicates antioxidant and anti-inflammatory activities, which suggests the potential anti-psoriatic effect of the sample. The anti-inflammatory potential of *Tagetes erecta* (TE) extract was evaluated using an *in vitro* egg albumin-Hydrogen Peroxide (H_2O_2) model, which mimics oxidative protein damage (antioxidant activity). The assay was adapted from (Osman *et al.*, 2016) with some minor modification, as described in recent studies (Madhuranga and Samarakoon, 2023). Hydrogen Peroxide (H_2O_2) was used as an oxidizing agent to generate ROS and induce protein denaturation (Hambly and Gross, 2009). We prepared 0.5% (w/v) H_2O_2 stock solution by diluting 0.5 mL of H_2O_2 in 29.5 mL phosphate buffer. TE extract was prepared at varying concentrations of 100, 200, 400, and 800 µg/mL. For the assay, 0.2 mL of fresh egg albumin was mixed with 2.8 mL phosphate buffer in each test tube. H_2O_2 (0.5 mL, 0.5%) was added to all tubes except the normal control, and test samples (1.5 mL) were added according to the experimental group. Each test tube contains final volume of 5 mL of the reaction mixture. Ascorbic acid served as a positive standard, while albumin along with H_2O_2 served as the negative control. Tubes were gently mixed by inversion and incubated at 37°C for 20 min. After cooling to room temperature, absorbance was measured at 660 nm using a UV-vis spectrophotometer, with buffer used as the blank.

The percentage inhibition of ROS-induced protein denaturation was calculated as:

$$\text{\% ROS Inhibition} = \frac{\text{TE1} - \text{TE2}}{\text{TE1}} \times 100$$

Where, TE1= Control sample; TE2 = Test sample.

RESULTS

Physicochemical evaluation

The extractive values, effects of loss on the drying and pH values of the flower extracts are shown in Table 1.

Phytochemical investigation of different solvent extracts

The ethanolic extract of *Tagetes erecta* showed the strong presence of the various phytoconstituents including flavonoid, phenolic compounds and tannins, phytosterol, Quoinone and Terpenoid, while showed a weak presence of alkaloid. It also demonstrates a significant presence of carbohydrate as shown in Table 2.

Table 2: Phytochemical screening of the crude extract.

Sl. No.	Phytoconstituents	Result
1	Alkaloid	
2	Hager test	+
3	flavonoids	
4	Shinoda's Test	+++
5	Phenols and Tannin	
6	Ferric chloride test	+++
7	Cardiac Glycoside	
8	Keller-Kilani test	+++
9	Phytosterol	
10	Hesse response	+++
11	Quinone	
12	Conc. Acid test	+++
13	Test for Carbohydrates	
14	Molish test	+++
15	Test for Terpenoids	+++

Where, '+++' indicates strong presence of the corresponding phytoconstituent.

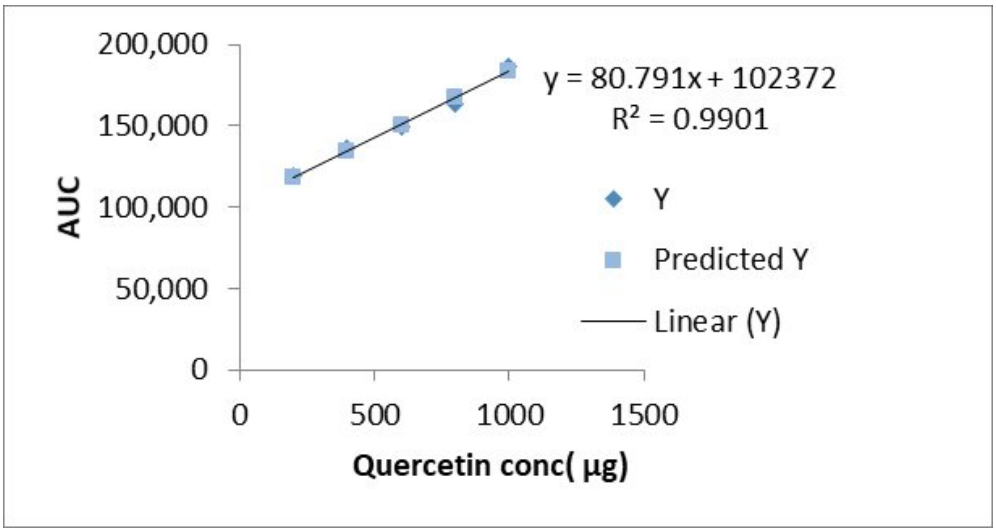

Figure 4: Standard calibration curve for Quercetin used for quantifying flavonoid content.

Total phenolic content and Total flavonoid content

The data presented in the table indicate that the Total Phenolic Content (TPC) of the extract was 0.389 mg GAE/g, indicating a moderate presence of phenolic compounds, which are known for their antioxidant properties (Figure 1). These findings may contribute to the therapeutic potential of the extract. The TFC value was significantly greater, at 17.80 mg RE/g, suggesting that the extract is rich in flavonoids (Figure 2). Flavonoids such as quercetin and rutin derivatives are key bioactive compounds with potent antioxidant, photoprotective, and skin-protecting effects (see Table 3).

Formulation of anti-psoriatic cream (*Tagetes erecta*)

The flower extract at different concentrations are used in the formulations in the range of 1-5% as the active ingredient. The quantity of all the ingredients are mentioned in Table 4. The concentrations of *T. erecta* extract and stearic acid were optimized based on a factorial design.

Factorial design matrix

A total of four cream formulations (F1 to F4) were developed via a factorial design approach, where the concentrations of TE and stearic acid were systematically varied, while the rest of the formulation components were held constant shown in Table 5. The goal was to evaluate the effects of these two variables on the physicochemical properties, viscosity, and spreadability of the herbal cream

Organoleptic and physicochemical properties of the anti-psoriatic cream

Organoleptic characteristics provide crucial preliminary insights into the aesthetic and sensory qualities of topical formulations, which influence patient acceptability and compliance. The evaluated parameters included color, texture, odor, appearance, and consistency, as summarized in the table below. All formulations presented shades of yellow consistent with the natural pigmentation of *T. erecta* extracts, indicating stable incorporation of plant constituents. F2 appeared slightly brighter, possibly due to differences in the extracts. F1 to F3 presented a uniform texture, suggesting effective emulsification and blending of the cream base with the active ingredients. However, F4 exhibited a nonuniform texture**,** indicating inadequate homogenization during the formulation process. All formulations had a characteristic floral aroma, likely attributable to the volatile aromatic compounds inherent in *T. erecta*. All formulations were described as nondripping, which is optimal for topical creams. This indicates a suitable viscosity that ensures ease of application. In terms of thermal stability, all formulations remained stable at 25°C, indicating good shelf stability under accelerated conditions. The pH values ranged from 6.29 to 6.79, all within the acceptable range for skin application, ensuring compatibility with the natural pH of the skin. Viscosity measurements at 30 rpm revealed that F4 had the highest viscosity and poor spreadability followed by all remaining formulations, F3 is best overall it shows optimal balance of extract, stearic acid which make the formulation stable, uniform and spreadable (Table 6).

DPPH activity

The antioxidant potential of both the TE extract and the anti-psoriatic cream formulation was evaluated via the DPPH assay, and their IC_{50} values and Ascorbic Acid Equivalent (AAE) capacities were determined (Figure 3). The TE extract exhibited an IC_{50} of 11.88 mg/mL, with a corresponding AAE of 46.3 mM/100 g, indicating that the extract retained a significant level of free radical scavenging potential, comparable to that of standard ascorbic acid. In contrast, the anti-psoriatic cream formulation had an IC_{50} value of 30.83 mg/mL**,** and the calculated AAE for the cream was 17.83 mM/100 g, indicating a lower antioxidant capacity than that of the raw extract (Table 7).

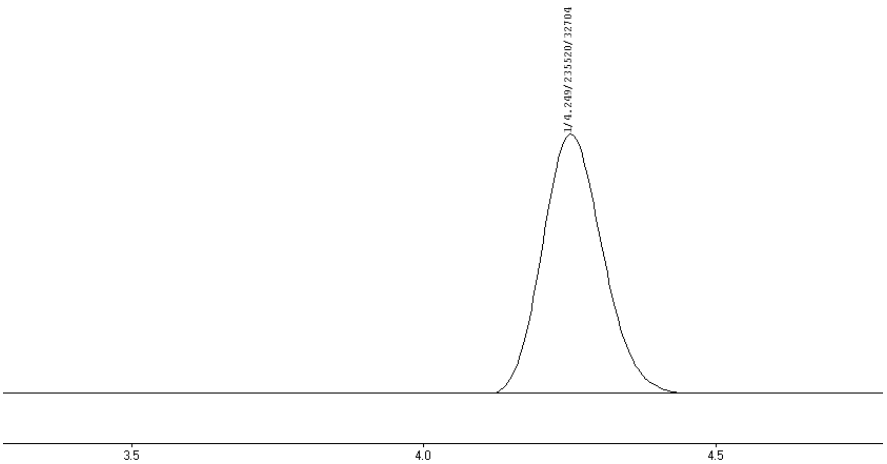

Figure 5: HPLC chromatogram showing quercetin peak in formulation F3 at 370 nm.

Table 3: TPC and TFC of *Tagetes erecta* flowers extract.

Assay	Sample	Absorbance	Dilution factor	C (mg/mL)	V (mL)	M (mg)	Result (mg/g)
TPC	TE	0.620	2	0.778	10	40	0.389 GAE/g
TFC	TE	0.769	2	35.601	10	40	17.80 RE/g

Where, C=Concentration of sample solution (mg/mL), V=Volume of solution used (mL), M=Mass of sample (mg).

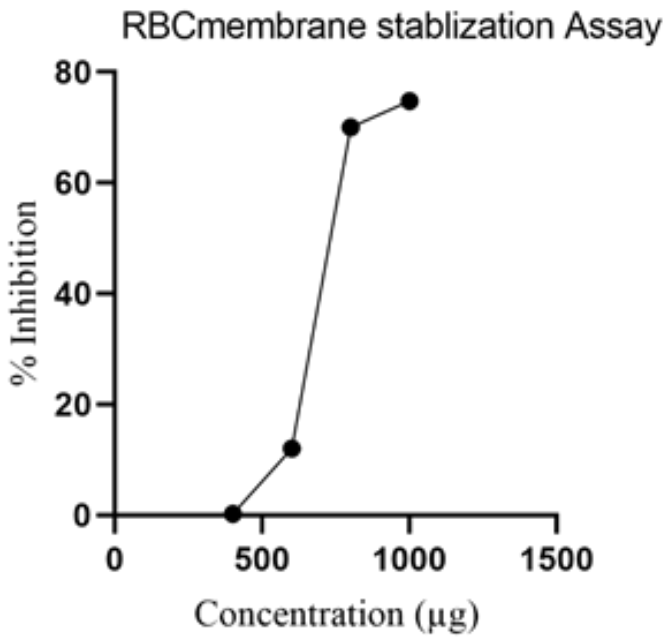

Figure 6: % inhibition of Acid hemolysis of T. erecta at different concentration.

HPLC method for Quercetin determination in *T. erecta* anti-psoriatic cream

The method development for Quercetin as marker was based on HPLC. To get the better separation and resolved peak, different types of columns and mobile phase were employed. The column was selected on the basis of good resolutions of peaks. Absorbance was selected using UV spectra of standard and extract samples overlaid. The best response was found at 370 nm.

The aim of this study is to establish an accurate, precise and reproducible method with sensitivity and cost effectiveness to quantify Quercetin in *Tagetes erecta* cream.

Method Validation

The developed method was validated following ICH guidelines.

Linearity

A standard calibration curve for quercetin was established using five concentrations ranging from 200 µg/mL to 1000 µg/mL. The relationship between the concentration and peak area was linear, following the equation:

$$y = 80.791x+102372$$

With a correlation coefficient of R^2=0.9901, indicating excellent linearity (Figure 4).

Precision

In this study the intraday variations were evaluated at different concentration. The % RSD values for the standard concentrations ranged between 0.17% and 1.32%, demonstrating the acceptable repeatability of the method (Table 8). The percentage recovery of quercetin ranged between 97.74% and 101.63%, which falls within the acceptable range (98-102%) as per ICH Q2 (R1) guidelines, confirming the accuracy of the developed method.

Accuracy

The accuracy of the developed HPLC method was determined through the analysis of quercetin at three concentration levels corresponding to 100±20% of the target concentration (200 µg/mL). The percentage recovery was calculated by comparing the measured concentration with the nominal concentration, while the precision was expressed as % RSD.

The obtained results, as mentioned in Table 9, reveal that the %RSD values ranged from 0.18-0.25%, which indicate excellent repeatability, and the %Recovery values were between 99.58%

Table 4: Formulation and function of ingredients of anti-psoriatic cream.

Ingredients	Quantity (%)	Function
Phase A (oil phase)		
T. erecta extract	1-5%	Active ingredient; provides therapeutic and antioxidant effects for psoriasis treatment.
Beeswax	1.5%	Emollient and contributes to cream thickness and stability.
Stearic Acid	2-6%	Emulsifying agent and thickener.
Cetyl Alcohol	5%	Emollient and improves texture and smoothness.
Propyl Paraben	0.15%	Lipophilic preservative.
Phase B (Aqueous phase)		
Glycerol	5%	Humectant and moisturizer.
Propylene Glycol	5%	Humectant.
Methyl Paraben	0.25%	Hydrophilic preservative.
Triethanolamine (TEA)	1%	Emulsifying and pH-adjusting agent.
Distilled Water	q.s	Vehicle and dispersion medium.

*Concentrations of *T. erecta* extract and stearic acid were varied according to the factorial design (see Table 5).

and 100.60%, confirming the accuracy and reliability of the developed analytical method for the quantification of quercetin in the cream formulation.

Quercetin content in the F1 and F3 formulations

The F1 and F3 *T. erecta* cream formulations were analyzed under identical HPLC conditions. The F1 cream yielded a mean AUC of 161576.7with % RSD of 0.98%, and the F3 cream showed a mean AUC of 234717.3 with % RSD of 0.55%. On the basis of the calibration equation represented in Figure 5, the quercetin content was calculated to be 0.732 mg/g in the F1 cream and 1.638 mg/g in the F3 cream (Table 10). Both formulations were above the LOQ and therefore, considered reliably quantifiable represented in Table 11.

Acid Hemolysis-Induced RBC Membrane Stabilization Assay

Comparative analysis of the standard and treated samples at various concentrations (200-1000 µg/mL) revealed a concentration-dependent trend toward inhibition. Compared with the standard group, the 1000 µg/mL and 800 µg/mL groups presented highly significant reductions in absorbance values, with % inhibition values of 74.78% ($p<0.0001$) and 70.0% ($p=0.0000223$), respectively. A statistically significant inhibition of 12.05% was also observed at 600 µg/mL ($p=0.00303$). In contrast, at 400 µg/mL and 200 µg/mL, no significant difference was noted between the standard and treated samples ($p>0.05$), and no inhibition was recorded. These results are summarized in Table 12 and Figure 6.

ROS Generation Inhibition Assay (Egg Albumin-H_2O_2 Model)

The ROS generation inhibition assay demonstrated that *Tagetes erecta* extract exhibits a concentration-dependent inhibition of H_2O_2-induced albumin denaturation. At 800 µg/mL, TE extract achieved 81.445% inhibition, indicating significant antioxidant and anti-inflammatory potential, approaching the activity of the standard antioxidant (ascorbic acid) mention in Table 13. These findings suggest dose-dependent increase in % inhibition for both the Standard and Extract. Two-way ANOVA revealed that concentration had a highly significant effect ($p<0.01$), while treatment type also differed significantly ($p<0.05$). IC_{50} analysis further confirmed the superior potency of the Standard (IC_{50}=391.38 µg/mL) relative to the Extract (IC_{50}=561.20 µg/mL) as shown in Figure 7.

DISCUSSION

Tagetes erecta flowers are medicinal herbs that have therapeutic effects on a number of diseases because of their high flavonoid and phenolic contents (Burlec *et al.*, 2021). The solvent Ethanol, extract polar compounds such as flavonoids, phenolics, tannins and glycosides (Ng *et al.*, 2020, Bartnik and Facey, 2024). This explains why the antioxidant and antimicrobial potential of medicinal plants varies according to the extraction solvent used (Agidew, 2022). The total phenolic content measured for TE was 0.389 mg GAE/g, suggesting the traditional use of *T. erecta* in the treatment of various skin diseases, such as wound healing (Vaz *et al.*, 2024), allergies and eczema, as phenolic compounds are reported to be involved in fibroblast-mediated responses, collagen production, and tissue regeneration, help in wound healing and inhibit scar formation (Fernandes *et al.*, 2023). These phenolic compounds protect against UV-induced skin damage, reduce signs of aging, and reduce the risk of skin carcinogenesis (Baloghová *et al.*, 2023, Carrara *et al.*, 2021). Notably, the Total Flavonoid Content (TFC) in the extract was significantly greater, i.e., 17.80 mg RE/g, which represents a substantial presence of flavonoid compounds,

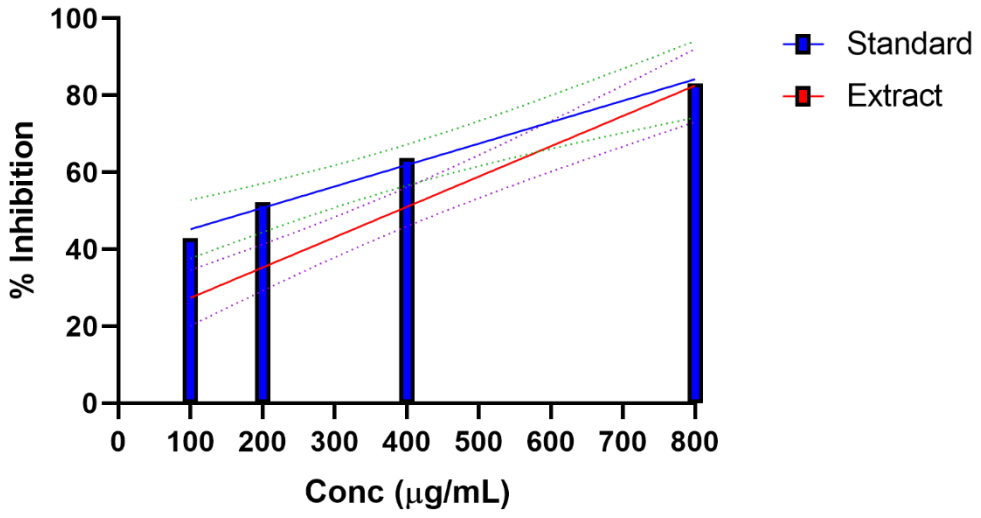

Figure 7: Dose-dependent % inhibition of protein denaturation by extract and standard.

Table 5: Factorial design matrix showing composition (% w/w) of ingredients in anti-psoriatic cream formulation (F1-F4).

Ingredient	F1	F2	F3	F4
Extract	1	3	5	5
Stearic acid	2	4	6	2
Bees wax	1.5	1.5	1.5	1.5
Cetyl alcohol	2.5	2.5	2.5	2.5
Triethanolamine	1	1	1	1
M.paraben	0.15	0.15	0.15	0.15
p. paraben	0.25	0.25	0.25	0.25
Glycerol	5	5	5	5
Propylene glycol	5	5	5	5
Water	q.s	q.s	q.s	q.s

All values are expressed as percentage by weight (% w/w).

Table 6: Organoleptic evaluation of the cream formulations.

Organoleptic analysis	F1	F2	F3	F4
Color	Light golden yellow	Slighter brighter yellow	Golden yellow	Golden yellow
Texture	Uniform	Uniform	Uniform	Non uniform
Odor	Characterstic floral aroma	Characterstic floral aroma	Characterstic floral aroma	Characterstic floral aroma
Appearance	Glossy	Glossy	Glossy	Glossy
Consistency	Non dripping	Non dripping	Non dripping	Non dripping
Thermal Stability at room temperature	Stable	Stable	Stable	Stable
pH	6.35±0.01	6.76±0.05	6.29±0.04	6.79±0.1
Viscosity (30 rpm)	1937.78±563.37	994.41±207.94	1108.62±166.17	2179.14±57.04
Homogeneity	Uniform	Uniform	Uniform	Non uniform
Spreadibility	2.56±0.10	2.5±0.08	2.26±0.14	2.25±0.05

Table 7: DPPH assay of the *Tagetes erecta* extract and F3 formulation.

Extract	Conc (mg/mL)	Percent inhibition %	IC_{50} (mg/mL)	AAE (mM/100 g)
TE	8	31.5	11.88	46.3
	10	42		
	12	50.5		
	14	52		
	16	54.5		
	18	53.25		
Anti-psoriasis cream F3	20	43.06	30.83	17.83
	40	55.88		
	60	61.17		
	80	65.76		
	100	77.39		

Table 8: Precision (Intraday) for Standard Quercetin Calibration Curve.

Quercetin conc. (µg/mL)	Mean (AUC)	%RSD	% Recovery
200	118,949	0.17	100.35
400	136047	0.49	101.01
600	149854	0.56	99.34
800	163236	1.32	97.74
1000	186145	0.48	101.63

Values represent mean of replicate injections. % RSD values less than 2% indicate excellent precision, and %Recovery between 97.74-101.63% confirms the accuracy of the analytical method.

predominantly quercetin derivatives (Kusumiyati K, 2025), which are known for their broad-spectrum pharmacological activities. A high total flavonoid content in plant extracts is closely associated with amended management of skin disorders and psoriasis, mainly via antioxidant, anti-inflammatory, and immunotherapeutic actions (Rivera-Yanez CR, 2021). Flavonoids reduce the activation of the proinflammatory cytokines IL-6, IL-17A, and TNF-α and downregulate signaling pathways that help reduce the symptoms of psoriatic lesions (Baloghová *et al.*, 2023). In a previous study, the extract of *Scrophularia desrti* 16.85 mg QE was shown to help reduce inflammatory mediator levels and enhance skin repair (Patidar *et al.*, 2024). Phytotherapeutics high in flavonoids are promising for both prophylaxis and the management of such dermatological disorders. The antioxidant activity of TE and the formulated anti-psoriatic cream was further proven through a DPPH free radical scavenging assay. TE presented a relatively low IC_{50} value of 11.88 mg/mL, accompanied by an Ascorbic Acid Equivalent (AAE) concentration of 46.3 mM/100 g, revealing efficient suppression of oxidative stress. However, the anti-psoriatic cream formulation demonstrated a higher IC_{50} of 30.83 mg/mL and an AAE value of 17.83 mM/100 g. These results support the antioxidant-rich nature of *T. erecta*, with the extract offering stronger free radical scavenging activity than the final formulation does (Okafor *et al.*, 2023), although both have substantial antioxidant potential that is helpful for managing psoriasis (Zhu, 2024). A factorial design-based approach was adopted to optimize the anti-psoriatic cream formulation by varying the concentrations of the TE extract and stearic acid in the four formulations (F1-F4). The assessment of physicochemical parameters revealed significant differences in the ratios of these components. All formulations demonstrated appropriate organoleptic properties, including a uniform and stable color with the natural pigmentation of *T. erecta*, homogenous texture (except F4), floral essence, and glossy appearance. These parameters are critical for patient adherence to and acceptance of topical dermatological preparations (Imaz *et al.*, 2020). Viscosity measurements indicated formulation-dependent variation, with F4 exhibiting the highest viscosity (40.5%), likely due to the stearic acid content and potential interaction with the extract concentration. In contrast, F3 exhibited relatively lower viscosity and better handling properties, possibly making it a more favorable formulation from a consumer perspective (Imaz *et al.*, 2020; Shaikh *et al.*, 2020). The pH values of all formulations are within the range of the skin's natural range (6.36-6.9), which falls within the dermatologically acceptable range for topical products and the matrix (Burlec *et al.*, 2021). For the analysis of the delivery and stability of the principle active constituents in post formulation, the quercetin content was quantified in selected formulated creams (F1 and F3) via a validated HPLC method (U, 2024; S, 2024). The calibration curve displayed excellent linearity (R^2=0.9949). Quercetin levels were found to be 0.746 mg/g and 1.625 mg/g in F1 and F3, respectively, with both values exceeding

Table 9: Accuracy of Quercetin (% Recovery).

Concentration (%)	Mean conc. (µg/mL)	% RSD	% Recovery
80	160	0.25	99.75
100	200	0.18	100.55
120	240	0.22	99.67

Table 10: Analysis of Quercetin in F1 and F3 creams formulation.

Cream formulation	AUC	%RSD	Quercetin Content (µg/g)	Quercetin Content (mg/g)
F1	161576.7	0.98	732.822	0.732
F3	234717.3	0.55	1638.131	1.638

Table 11: LOD and LOQ Calculated from regression data.

	SD	LOD (µg)	LOQ (µg)
Calculated from Residual standard deviation (from regression)	2949.724	120.486	365.108

Table 12: Acid Hemolysis-Induced RBC membrane stabilization assay.

Concentration (µg)	Standard Mean ± SD	Treated Mean Mean ± SD	% inhibition
1000	0.2023	0.0510*	74.78
800	0.2228	0.0671*	70.0
600	0.2307	0.2029	12.05
400	0.2317	0.2311	0.25
200	0.2352	0.2471	-

Acid Hemolysis-Induced RBC membrane stabilization assay, n=3, p value (two-tailed)<0.05, * highly significant.

Table 13: ROS Generation Inhibition Assay.

Conc (µg/mL)	Absorbance of Extract (mean ±SD)	Absorbance of standard (mean ±SD)	% inhibition of Extract	IC_{50} Standard	% inhibition of Standard	IC_{50} Extract
100	0.175 ±0.05	1.330±0.04	25.354	391.38	42.855	561.20
200	0.150±0.06	1.111±0.20	36.119		52.257	
400	0.110±0.00	0.843±0.47	53.258		63.754	
800	0.150±0.05	0.391±0.06	81.445		83.163	

All experiments were performed in triplicate (n=3). IC_{50} values were determined from dose-response curves from a nonlinear regression of % inhibition versus concentration.

the method's LOQ (489.41 µg), confirming reliable quantification. The higher concentration in F3 is consistent with its increased extract percentage, verifying that quercetin is retained effectively in the final formulation (Chaudhari *et al.*, 2024). These results highlight that formulation parameters directly influence the physicochemical characteristics and phytoconstituent retention of the final product. Compared with all formulations, F3 demonstrated an optimal balance of quercetin content, viscosity, texture, and pH, suggesting its superiority for further pharmacological validation and clinical consideration (Lin *et al.*, 2023; Anheyer *et al.*, 2025). The anti-inflammatory potential of the *Tagetes erecta*-based formulation was further evaluated via an acid-induced hemolysis assay, which serves as a model for evaluating the membrane-stabilizing properties of therapeutic agents (Ibrahim *et al.*, 2023). The extract clearly inhibited red blood cell destruction in a concentration-dependent manner, with significant therapeutic effects at relatively high concentrations. At 1000 µg/mL and 800 µg/mL, the treated samples presented highly significant reductions in hemoglobin release, with % inhibition values of 74.78% and 70.0% (p<0.0001), respectively. These findings revealed strong membrane-stabilizing and anti-inflammatory properties, potentially linked to the presence of flavonoids and phenolic compounds such as quercetin, which are known to inhibit lysosomal enzyme release and prevent red blood cell lysis under acidic conditions (Ajazuddin, 2024). These results support the role of the extract in membrane stabilization

and justify its inclusion in natural topical anti-psoriatic formulations.

In *in vitro* study Egg Albumin-H_2O_2 Model, Hydrogen peroxide was used to generate oxidative stress, as H_2O_2 is an established oxidant for inducing protein oxidation and structural modification. Previous studies have shown that millimolar concentrations of H_2O_2 (e.g., ~75-100 mM) cause significant protein oxidation in model proteins. This supporting the use of a 0.5% H_2O_2 working solution in our assay to induce albumin denaturation (Davies, 2016).

CONCLUSION

The results of the present study indicate that *Tagetes erecta* is a rich source of valuable phytochemicals, namely, flavonoids and phenolics that have antioxidant and anti-inflammatory effects. The extract demonstrated significant DPPH scavenging activity and membrane stabilization activity and thus warrants application in the treatment of oxidative stress and inflammation-associated cutaneous disorders. Experiments with formulations confirmed that the concentration of the extract influences the texture, pH, and quercetin recovery of the final formulation. HPLC analyses further ensured the precise quantification of the quercetin content in the finished formulation. These findings support the use of *T. erecta* as a promising candidate herbal topical cream formulation for the management of psoriasis.

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ABBREVIATIONS

AAE: Ascorbic Acid Equivalent; **AUC:** Area Under the Curve; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **GAE:** Gallic Acid Equivalent; **HPLC:** High-Performance Liquid Chromatography; **IC_{50}:** 50% Inhibitory Concentration; **ICH:** International Council for Harmonisation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **O/W:** Oil-in-Water; **PBS:** Phosphate Buffered Saline; **q.s:** Quantum Satis; **RBC:** Red Blood Cell; **RE:** Rutin Equivalent; **ROS:** Reactive Oxygen Species; **RSD:** Relative Standard Deviation; **RT:** Retention Time; **TE:** *Tagetes erecta* Extract; **TEA:** Triethanolamine; **TFC:** Total Flavonoid Content; **TPC:** Total Phenolic Content; **UV:** Ultraviolet.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHOR CONTRIBUTIONS

Sanober Kamal: Conceptualized and designed the study, carried out the experimental work and data collection; **Fatima Qamar:** Performed data analysis and statistical interpretation; **Aymen Owais:** Assisted in literature review and methodology optimization; **Hoor Nasir:** Contributed to formatting, proofreading, and manuscript submission; **Asma Wazir:** Assisted in critical review and proofreading; **Syed Shafqat Ali Shah:** Provided critical review and final approval of the manuscript; **Maria Rahat:** Supported in final review and proofreading; **Iqra Kanwal:** Assisted in formatting and proofreading.

SUMMARY

This study highlights the anti-inflammatory and antioxidant potential of *Tagetes erecta* flower extract cream in psoriasis-relevant models. The findings support its possible role as a complementary topical therapeutic option. Further clinical investigations are recommended to validate its efficacy and safety.

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