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Photoprotective and anti-inflammatory potential of *Tagetes erecta* L. from southern coastal region of Pakistan: A study on psoriasis-prone skin

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Abstract: **Background**: Psoriasis represents a chronic autoimmune dermatosis that necessitates the implementation of both anti-inflammatory measures and UVA-UVB photoprotection. **Objectives**: This study aimed to evaluate the phytochemical profile, antioxidant capacity , photoprotective efficacy, and anti-inflammatory potential of *Tagetes erecta* flower extracts (TEFE) prepared in different solvents. **Methods**: In this study phytochemical analysis and antioxidant properties were performed in various solvent extracts of *Tagetes erecta* flowers. Photoprotective efficacy was assessed using critical wavelength (λ_c) and SPF measurements under different exposure conditions. Anti-inflammatory activity was evaluated via heat-induced protein denaturation, heat-induced hemolysis, and hypotonicity- induced hemolysis assays. **Results**: The result indicated that TEFE contain all the secondary metabolites known for their skin-protective properties. The highest TPC was observed in the methanolic and ethyl acetate extracts, measuring (0.7733 ± 0.0024 and 0.7729 ± 0.0007 mg GAE/mg), respectively. The acetone and ethanolic extracts exhibited the highest flavonoid contents (17.553 ± 0.1064 and 14.904 ± 0.506 mg Rutin/mg) and flavanol contents (4.279 ± 0.268 and 3.829 ± 0.1002 mg Rutin/mg), respectively. At all concentrations, TEFE exhibited λ_c greater than 370 nm, indicating broad-spectrum photoprotective properties. At 800 ppm, TEFE demonstrated SPF values of 29 without exposure, while SPF of 40.2 under sunlight and 32.80 under UV exposure were measured. The Statistical analysis revealed significant differences in photoprotective activity across all tested concentrations ($P<0.05$).TEFE showed considerable inhibition of protein denaturation, with IC_{50} = 527.845 µg/mL and exhibited concentration-dependent membrane stabilization, with a maximum inhibition of 69% in heat-induced RBC lysis and 22% in hypotonicity-induced RBC lysis. **Conclusion**: These results advocate for a novel phototherapeutic strategy for addressing photoprotection in psoriatic skin and require further clinical research in dermatological preparations.

Keywords: Anti-inflammatory; Photo protection; Psoriasis, SPF; *Tagetes erecta* flowers

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INTRODUCTION

Psoriasis is a persistent inflammatory autoimmune skin disorder characterized by excessive growth of keratinocytes and immune system imbalance (Zhou *et al*., 2022; Sieminska *et al*., 2024), often driven by a strong genetic tendency (Sieminska *et al*., 2024). According to the WHO, it impacts about 0.09-11.43% of the world's population, with variations based on geographic, genetic and methodological factors (Wang *et al*., 2024). Clinically, it manifests as red, scaly plaques on body surfaces (elbows, knees and scalp) (Gaurav *et al*., 2024). Besides genetic susceptibility, various exogenous factors including psychological stress, pathogenic diseases, injuries and certain drugs can worsen the condition (Zhou *et al*., 2022; Yang *et al*., 2025).

Although UV exposure, especially narrowband UVB, plays a therapeutic role in treating psoriasis, excessive and uncontrolled sun exposure can exacerbate symptoms in some people. This may be attributed to factors such as the Koebner phenomenon triggered by sunburn, the presence of photosensitive disorders, or severe photosensitive psoriasis, a rare form that worsens with sunlight. Additionally, extended UV exposure heightens the risk of photoaging, actinic damage and non-melanoma skin cancers (Roszkiewicz *et al*., 2020; Adler and DeLeo, 2020). Research indicates that even an hour of UV radiation can cause genomic damage, highlighting the need for photoprotection in patients with psoriasis (Lee *et al*., 2020). Although phototherapy is a common psoriasis treatment, paradoxically, approximately 5.5% of patients experience symptom aggravation due to sun exposure, which is known as photosensitive psoriasis (Ros and Eklund, 1987). This subtype is more common among those with fair skin, older age, or a family history of photosensitivity (Nicholas *et al*., 2020;Serpone, 2021). Data from the NHANES survey (2009–2014) showed a complex relationship between sun-protective behaviors and psoriasis prevalence, suggesting that moderate photoprotection measures, such as wearing long sleeves, might offer protection by reducing UV-induced skin inflammation (Xuan *et al*., 2025). Traditional psoriasis

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treatments, including topical corticosteroids, vitamin D analogs (Papp *et al*., 2021), calcineurin inhibitors and systemic therapies, provide temporary symptom relief but often result in side effects, tachyphylaxis, or high costs (Papp *et al*., 2021; Lo *et al*., 2024).

Topical sunscreens act as primary defenses against skin damage caused by UV rays, but traditional formulations often depend on synthetic chemical filters, such as titanium dioxide, zinc oxide (Sharma *et al*., 2021), oxybenzone, octinoxate and avobenzone (Opriş *et al*., 2022). These substances have been linked to negative dermatological effects such as allergic reactions, endocrine disruption and environmental harm (Serpone, 2021). Synthetic sunscreens seldom provide additional therapeutic benefits for skin conditions such as psoriasis and Vitamin D deficiency along with immune imbalance may increase the risk of sunburn in patients with psoriasis (Osborne *et al*., 2025;Yang *et al*., 2025). Recently, natural products have gained significant attention in sunscreen development owing to their dual function in photoprotection and antioxidant activity, which not only aids in preventing skin cancer but also supports inflammatory skin conditions. Historically, plant-based ingredients like curcumin and liquorice have been utilized for their skin-protective qualities and modern *in vivo* and *in-vitro* research continues to validate their therapeutic advantages in dermatologic applications (Sharma *et al*., 2021;Batista *et al*., 2022). As noted by (Ratajczak *et al*., 2023), more than 60% of global skincare consumers now favor products labeled as "natural" or "plant- based," especially those with antioxidant properties, indicating a clear shift in consumer preference towards safer and more bioactive formulations.

Plants containing phytochemicals, particularly flavonoids, alkaloids, polyphenols and carotenoids, have demonstrated significant capacity in neutralizing free radicals induced by ultraviolet radiation and in modulating the inflammatory pathways pertinent to psoriatic dermatological conditions (Ghazi, 2022;Gupta *et al*., 2024). Among the noteworthy botanical candidates, *Tagetes erecta,* or African marigold, has attracted considerable scholarly attention due to its substantial concentration of flavonoids, carotenoids and essential oils (Peralta-Sánchez *et al*., 2023). Historically, this species has been employed in ethnomedicine for its wound-healing and anti-inflammatory properties (Bohatu *et al*., 2024). Taxonomically, *T. erecta* is an angiospermic plant belonging to the family Asteraceae, genus *Tagetes* and species *T. erecta* L., which comprises over 50 cultivated and wild species. Native to Mexico, the plant was introduced to Europe in the 16th century. It is now cultivated worldwide because of its ease of growth and low ecological demands. In Pakistan, the species is widely cultivated across all provinces, with particularly vigorous growth in the southern coastal belt, where warm and humid climate favors lush flowering and higher phytochemical yields, making it an ideal region for cultivation. The flowers exhibit considerable antioxidant activity and UV-absorbing capabilities, indicating its prospective application in topical photoprotective formulations. Limited research has investigated the *in-vitro* SPF effectiveness of such botanical formulations alongside their antioxidant properties.

While the conventional applications of TEFE (*Tagetes erecta* flower extract) have demonstrated therapeutic efficacy, scientific inquiries into standardized *T. erecta*-based topical formulations, particularly in relation to photoprotection for psoriasis, remain insufficiently examined. The antioxidant-rich botanical extracts with skin-soothing components may present a safer and more biocompatible alternative for individuals exhibiting sensitive skin barriers, including those with psoriasis.

This research endeavor aimed to evaluate *T. erecta* antioxidant properties and UVB-UVA values through spectrophotometric methodologies, the study aspires to ascertain its potential as a natural therapeutic agent for skincare, particularly in the context of managing photo damage and psoriatic inflammation.

MATERIALS AND METHODS

Chemicals and reagents

For the current research, all the chemicals of analytical-grade were purchased from reputable international chemicals distributors. Ethanol, Methanol, Ethyl acetate, Hexane, Acetone, Gallic Acid (extra pure), were purchased from Duksan Pure Chemicals. NaCl, Na_2CO_3, $AlCl_3$, L-ascorbic acid was bought from BDH Laboratory Chemicals. Folin-Ciocalteu phenol reagent, $NaH_2PO_4.2H_2O$, Na_2HPO_4 was procured from Sisco Research Laboratories Pvt Ltd. (+)-Rutin Trihydrate was obtained from Alfa Aesar suppliers. Fehling Solution A and B were bought from General Purpose Reagent while HCl was purchased from Merck Chemicals.

Collection of flowers

The *T. erecta* flowers were sourced from a flower market in Karachi in December 2024. A voucher specimen (No. TEF-11-24) has been archived at the Department of Pharmacognosy, Jinnah University for Women. The flowers chosen for the study were thoroughly washed under running tap water and then rinsed with distilled water. They were left to air dry at room temperature for a short period before being shade-dried for approximately 3 to 4 weeks. Once dried, the plant material was ground using an electric grinder.

Preparation of extract in different solvents

A total of 200 g of *Tagetes erecta* flower powder was subjected to extraction using various solvents: hexane, ethyl acetate, ethanol, methanol and acetone. For each solvent, 40g of powder was extracted using a sample

to solvent ratio of 1:20 (w/v). The mixtures were shaken frequently for the first 6 hours at 40°C ±2 and then left to stand undisturbed for an additional 18 hours at room temperature. After maceration, the mixtures were first filtered through muslin cloth and subsequently through Whatman No. 41 filter paper with a pore size of 20 to 25µm. A 25 mL aliquot of each filtrate was taken and evaporated to dryness in an oven at 105°C to determine the extractive yield, expressed in milligrams of dried extract per gram of plant material (mg/g). The remaining filtrate was concentrated under reduced pressure using a rotary evaporator (IKA RV05) and stored in airtight containers at ± 4°C for further use (Khantwal *et al*., 2025;Tourabi *et al*., 2025).

Phytochemical investigation

The qualitative screening was performed for the identification of various primary and secondary metabolites such as carbohydrate, fat/oil, alkaloids, flavonoids, phytosterols, terpenoids, glycosides, tannins and polyphenols, by following the standard methods in all extracts.

Test for alkaloids

Hager's test

For the identification of an alkaloid few ml of the extract, was taken in a test tube and 1-2 mL Hager's reagents were added a creamy white precipitate appeared indicating the presence of alkaloid (Shaikh and Patil, 2020).

Test for flavonoid

Ferric chloride test 10%

For the test of flavonoid, 1 to 2ml of extract (aqueous solution) was added to a few ml of 10% $FeCl_3$ solution. A green precipitate formed, indicating the presence of flavonoid (Bhandary *et al*., 2012).

Lead acetate solution

1mL of plant extract was treated with few drops of 10% lead acetate solution that gave yellow precipitate, indicated the presence of flavonoid (Bhandary *et al*., 2012).

H_2SO_4

By adding 1 to 2 ml of conc. sulphuric acid to the extract a yellowish orange colour was appeared, indicating the presence of flavonoid (Shaikh and Patil, 2020).

Test for phenols and tannins

Ferric chloride test

Few ml of extract was added with 1 mL of water in a test tube followed by addition of 1 to 2 drops of 5 % Iron III chloride ($FeCl_3$). A dark green or bluish black colour appeared, indicating the presence of phenols/tannins (Shaikh and Patil, 2020).

Iodine test

A Few drops of diluted iodine solution was added with 1 ml of extract a bluish black color was formed, indicating the presence of phenols/ tannins (Shaikh and Patil, 2020).

Test for glycoside

Concentrated H_2SO_4 test

Few ml plant extract was mixed with 2mL glacial acetic acid by addition of a drop of 5% $FeCl_3$ and conc. H_2SO_4. A brown ring appeared, indicated presence of glycoside (Shaikh and Patil, 2020).

Test for cardiac glycoside

Keller-kilani test

TEFE was added with 1-2ml of glacial acetic acid, contained 1 to 2 drops of 2% iron chloride solution. The mixture was subsequently transferred to a different test tube that held 2ml of concentrated sulphuric acid. The appearance of brown ring at the interphase indicated the presence of cardiac glycosides (Yadav and Agarwala, 2011).

Baljet test

2mL extract was added with few drops of Baljet's reagent. A yellowish orange colour was formed, indicated the presence of glycoside (Shaikh and Patil, 2020).

Test for phytosterol

Hesse's response

5ml extract was added with 2ml chloroform and 2ml sulphuric acid. A red colour was obtained in chloroform layer, indicating the presence of phytosterols (Shaikh and Patil, 2020).

Test for quinone

Conc. HCl test

1 to 2 ml of Extract was added with 1 ml conc. HCl. A green colour was appeared, indicated the presence of Quinone (Shaikh and Patil, 2020).

Test for carbohydrate

Molisch test

Few ml of extract was added with Molisch reagent, followed by few drops of conc. H_2SO_4 along the sidewall of the test tube. A violet ring was formed, indicating the presence of carbohydrate (Shaikh and Patil, 2020).

Test for fixed oil and fat

spot test

A spot of herbal extract is pressed between the folds of filter paper, the presence of oil stain on the filter paper indicating, the presence of fixed oils in the plant extract (Shaikh and Patil, 2020).

Test for terpenoids

Few ml of flower extract was first dissolved in 2ml of CCl4 and allowed to evaporate. To this solution, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour was indicating the presence of terpenoids (Shaikh and Patil, 2020).

Total phenolic content

Total phenolic content was assessed using a modified version of the method described by (Rizvi *et al*., 2023). 1

ml sample extract (0.1g /2.5 ml) was combined with 2.5 ml of 10% Folin reagent and incubated for 5 minutes. 2.5 ml of 7.5% Na_2CO_3 was added with continuous shaking. The solution was kept in the dark for 2 hours and absorbance was measured at 765 nm. Gallic acid (10µg/ml-100µg/ml) was used as standard and results were expressed as Gallic acid equivalent (GAE) per 100 grams of dry extract.

Total flavonoid content

The total flavonoid content was assessed using the method outlined by (Farooqi *et al*., 2024), with some modifications. 1 ml sample extract (0.1g / 2.5ml) 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 absorbance was measured at 415 nm using a UV spectrophotometer, with methanol and distilled water serving as the blanks. Rutin (Rt) 10 µg/ml -100 µg/ml was utilized as the standard.

Total flavonol

Total flavonol in the samples was determined by using the Aluminum chloride colorimetric technique, as described previously. After the incubation of 150 min, the absorbance was measured at 440 nm (Zhang and Elomaa, 2024).

Estimation of the broad spectrum coverage (UVA-UVB) of Extract at different concentration

UV spectrophotometric analysis for broad-spectrum assessment

Three different concentrations of *T. erecta* flower extract (200 ppm, 400 ppm and 800 ppm) were prepared using analytical grade ethanol. The UV absorption of these test samples were recorded in the wavelength range of 290–400 nm using UV-Visible spectrophotometry in order to determine the critical wavelength (λc). The λc is defined as the wavelength below which 90% of the total UV absorbance of a substance occurs. According to regulatory guidelines, a formulation must exhibit a critical wavelength of ≥370 nm to be labeled as providing "broad-spectrum" protection, which ensures significant protection against both UVA and UVB radiation. To determine λc, area under the absorbance curve was calculated and the wavelength corresponding to 90% of the cumulative area was identified—following the method recommended by the U.S. FDA and COLIPA guidelines. The Sun Protecting Factor calculation was carried out according to the Mansur equation, with EE x I representing a constant factor (Kant *et al*., 2016).

$$SPF = CF \times \Sigma\, EE(\lambda) \times I(\lambda) \times ABS(\lambda)$$

Where, CF = Correction Factor; EE = Erythema Effect; I = Sunlight Intensity; ABS = sample absorbance

Photostability test

The photosensitivity test of the extract was done by exposing the sample in direct sunlight and under UV Lamp (290-400 nm) at 1 m W/cm² intensity for more than 1 hour, adapted from the approach described by (Monsalve-Bustamante *et al*., 2023) with slight modifications. After exposure, the sample were measure for absorbance at the same wave length using spectrophotometer for the assestment of of the stability of the extract under irradiation.

In-vitro anti-inflammatory assay

Heat-induced protein denaturation method

Protein denaturation is a key factor in inflammation and NSAIDs reduce this by inhibiting COX enzymes. Natural compounds can similarly be tested for anti-inflammatory activity using *in-vitro* egg albumin denaturation assays by measuring % inhibition through absorbance.

The anti-inflammatory activity of TEFE can be determined *in-vitro* for inhibition of the denaturation of egg albumin (protein) followed by (Chandra *et al*., 2012) with some minor modification, also adapted in recent studies (HDT, 2023). 0.2 mL of fresh egg albumin, 2.0 mL of test sample extract at varying concentration (100, 200, 400, 800 µg/mL and 2.8 mL of phosphate buffered saline (pH 7.4) were mixed to form a reaction mixture of a total volume of 5.0 mL. The test samples were then incubated at 37°C for 15 min and then heated in a water bath at 70°C ± 2 for 5 minutes. After the sample became cooled, the absorbance was measured at 660 nm by using UV spectrophotometer (SHIMADZU, UV 1800). Control sample consisted of albumin and buffer without extract or standard and distilled water was used as the blank to zero the spectrophotometer . For the standard sample, diclofenac sodium, at varying concentrations was treated in the same manner for the determination of absorbance. The IC50 of the extract was calculated by plotting 50% inhibition with respect to control against corresponding treatment concentration (HDT, 2023;Chandra *et al*., 2012).

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

Where, TE1= Control sample; TE2 = Test sample

Membrane stabilization assay of human RBC (In-vitro)

Anti-inflammatory action of the extract was evaluated using the membrane stabilization method based on human red blood corpuscles, following the protocol by (Yesmin *et al*., 2020b). Acetyl salicylic acid used as standard drug and Anti-inflammatory activity was expressed as the percentage of lysis of human red blood cell. As the membrane of the red blood cells resembles the lysosomal membrane, its stabilization by the extract suggests the stabilization of the lysosomal membrane as well. Absorbance was taken by Spectrophotometer at 560 nm range for estimation of hemoglobin content in the test sample suspension. Blood was collected in anticoagulant test tube (EDTA tube) from healthy volunteer that didn't use any anti-inflammatory drug for two weeks. The blood sample was stored at ± 2 to 4 °C for 24 h before use. The blood sample was centrifuged using (Centrifuge DUAB

DM 0412S) for 10 mins at 3000 rpm, the plasma was separated and skimmed off to obtain red blood cells pack. The RBC pack was then washed 3 times by normal saline solution (0.9% w/v) by removing the supernatant in centrifugation machine at 3000 rpm for 5 minutes. 40% suspension of RBC pack was prepared by adding 10mM phosphate-buffered saline (pH 7.4), (260 mg of $NaH_2PO_4.2H_2O$; 1150 mg of Na_2HPO_4 and 9 x 10^3 mg of NaCl).

RBC lysis assay by heat induce method

Extracts at varying concentrations (100 µg/mL, 200 µg/mL, 400 µg/mL and 800 µg/mL) were mixed with 5 mL of isotonic buffer (pH 7.4) in centrifuge tubes, followed by the addition of 50 µL of RBC suspension to each tube. The tubes were gently mixed by inversion. The same procedure was followed for the control (without extract). First set of tubes was incubated at 54 °C for 20 minutes in a water bath, while the other set was kept at 0–4 °C in an ice bath. After incubation, the mixtures were centrifuged at 4500 rpm for 5 minutes. The absorbance of the supernatant was measured at 540 nm using a spectrophotometer. Acetylsalicylic acid (ASA) at different concentrations (µg/mL) was used as the reference standard (Yesmin *et al*., 2020a).

The percent inhibition of hemolysis was calculated according to the equation:

$$\text{Hemolysis inhibition rate (\%)} = \frac{\text{TE3} + \text{TE1} - 2(\text{TE2})}{\text{TE3 - TE2}} \times 100$$

Where, TE1 = Test Sample at 0-4℃ ; TE2 = Test Sample at 54ºC and TE3 = Control Sample at 54ºC.

RBC lysis assay by hypotonic solution method

The isotonic buffer solution of pH 7.4 was made by adding 0.154M (NaCl) in 0.01M sodium phosphste solution. 50 µl from the stock RBC suspension (40% v/v) was mixed with 5.0 ml of the hypotonic solution containing the TEFE at concentrations (100, 200, 400 and 800) µg/ml. The control sample was mixed by adding the hypotonic buffer and RBC suspension without extract. Incubating all the samples for 10 min at ambient temperature (25±2℃) and the whole mixture was centrifuged 4500 rpm for 5 min and the absorbance of the supernatant was measured at 540 nm, UV-spectrophotometer. Acetyl salicylic acid of different concentration was used as standard drug and followed the same procedure (Yesmin *et al*., 2020b). The hemolysis inhibition rate (%) was calculated using the following equation:

$$\text{Hemolysis inhibition rate (\%)} = \frac{\text{TE3} + \text{TE1-2(TE2)}}{\text{TE3 - TE2}} \times 100$$

Where, TE1 = Test sample in isotonic solution; TE2 = Test sample hypotonic solution and TE3 = Control sample in hypotonic solution.

Statistical analysis

All statistical tests were performed three times for accuracy and the data was statistically reported as average ± standard deviation (SD) using the SPSS 20.0 software. Kruskal-Wallis test and post hoc Mann-Whitney test was applied to compare absorbance values across different concentrations (200, 400, 800 ppm) under each exposure type (No exposure, sunlight, UV), due to non-normal distribution of data. The test was also used to compare exposure types within the same concentration. A significance level of $P < 0.05$ was considered.

RESULTS

Extractive values of T. erecta in different solvents

The extractive values (mean ± standard deviation) and standard error means (SEM) for various solvents are presented in table 1. Among the different solvents, methanol had the highest extraction value (53.333 ± 5.508 mg/g, SEM = 3.180), followed by ethanol (40 ± 8.000 mg/g, SEM = 4.619). In contrast, hexane had the lowest extraction value (5.333 ± 2.309 mg/g, SEM = 1.333). These findings highlight a significant difference in extraction potential across solvents, with polar solvents (methanol and ethanol) generally showing greater extraction efficiency than non-polar solvents, such as hexane.

Phytochemical screening

The phytochemical analysis of TEFE in different solvents indicates the presence of both primary and secondary metabolites, with varying intensity across solvents. Alkaloids were moderately present in ethyl acetate, ethanol and acetone, but absent in hexane, indicating that the polar solvents are more effective in extracting alkaloids. Flavonoids are present in high amounts among all solvents except hexane, confirming polarity-dependent solubility. Phenols and tannin showed strong positivity in all solvents except hexane, which showed no response to $FeCl_3$ but for iodine test, it showed positive , which indicates limited presence of phenol in hexane. Glycosides and cardiac glycosides were strongly present across all solvents, quinones were absent in all solvents. Phytosterols are highly present in all solvents except hexane, showing preference for mid-to high- polarity solvents. Fixed oils and fats were present in all solvents except acetone which showed slightly lower response. Terpenoids were present across all extracts with moderate to high presence, showing strong extractability in hexane, ethanol and acetone. Carbohydrates were strongly detected in hexane, ethanol and acetone, whereas moderately present in ethyl acetate and methanol as shown in table 2.

Total phenolic content of different solvent extract

Methanol and Ethyl acetate solvent has high phenolic content (0.7733 ± 0.0024 mg GAE/mg and 0.7729 ± 0.0007 mg GAE/mg) respectively. While Hexane contains the lowest (0.0488 mg GAE/mg). Polar solvents were better in extracting the phenolic compounds as expected. Ethanol (0.7615 ± 0.0017 mg GAE/mg) showed moderately high values, followed by Acetone (0.7355 ± 0.0024 mg GAE/mg) shown in table 3.

The solvent efficiency order for TPC is M>EA>E>A>H. Calibration curve for gallic acid ($r^2 = 0.9978$) displaying a linear relationship, (fig. 1) to determine the total phenolic content expressed as Gallic acid equivalent (GAE) in miligram per gram of dry extract.

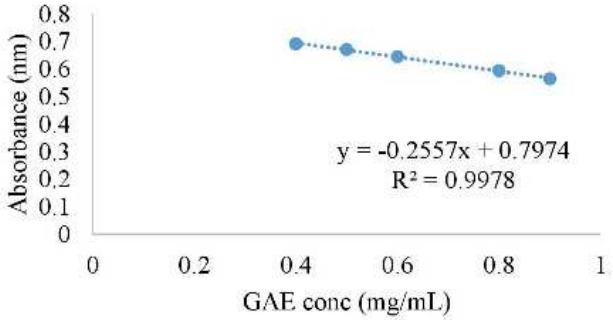

Fig. 1: Standard calibration curve of Gallic acid expressed as GAE (TPC)

Total flavonoid content of different solvent extracts

Acetone extract showed the highest Total Flavonoid Content (17.553 ± 0.106 mg Rt/mg), followed by ethanol (14.904 ± 0.050 mg Rt/mg) and methanol (10.614 ± 0.015 mg Rt/mg), reflecting its significance towards the flavonoid due to polarity. Hexane extract exhibited the lower TFC (6.724 ± 0.020 mg Rt/mg) and ethyl acetate extract showed the lowest (1.2042 mg Rt/mg), indicating low affinity towards flavonoids. The solvent efficiency order for TFC is A>E>M>H>EA. Calibration curve for Rutin ($r^2 = 0.9797$) displayed a linear relationship, (fig. 2), to determine the total flavonoid content in miligram per gram of dry extract as shown in table 4.

Total flavonol content

Acetone extract showed the highest flavonol Content (4.279 ± 0.268 mg Rt/mg), followed by ethanol (3.829 ± 0.100 mg Rt/mg) and hexane (2.939 ± 0.023 mg Rt/mg). Methanol had a lower flavonol content (1.978 ± 0.025 mg Rt/mg) while ethyl acetate (0.774 ± 0.022 mg Rt/mg) showed the least extraction efficiency. These results indicate that acetone and ethanol are the most effective solvents for flavanol extraction represented in table 5. The solvent efficiency order for flavonol content is A > E > H > M > EA. A calibration curve for Rutin ($r^2 = 0.9797$) was constructed to determine flavonol content in miligram per gram of dry extract as shown in fig. 2.

UV spectrophotometric analysis for broad-spectrum assessment (In-vitro)

The UV spectrophotometric analysis represented in table 6 revealed that increasing concentrations of TEFE (200-800 ppm) shifted the critical wavelength (λc) slightly from 386.84 nm to 388.04 nm, maintaining values above 386 nm, indicative of broad-spectrum coverage. The SPF values increased with concentration from 16.5 at 200 ppm to 29 at 800 ppm, while UVB absorbance increased significantly from 1.648 ± 0.035 to 2.882 ± 0.033. UVA absorbance remained minimal across concentrations (0.010–0.021) as shown in fig. 3.

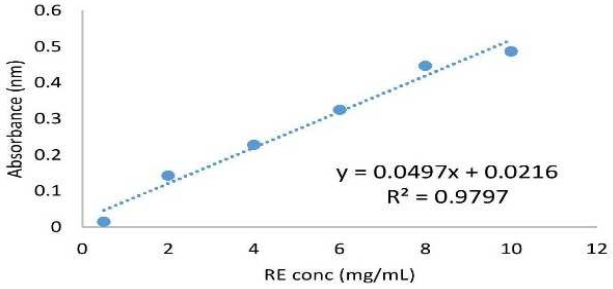

Fig. 2: Standard calibration curve of rutin equivalent (TFC)

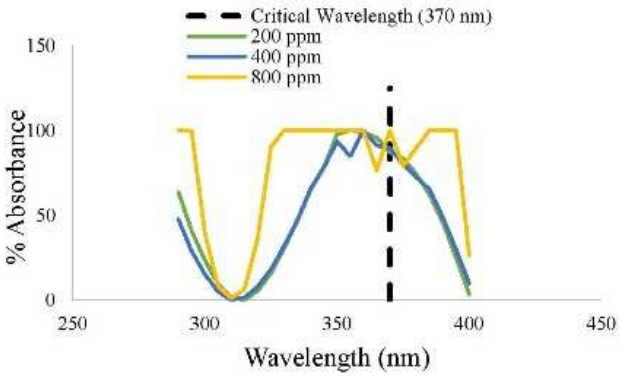

Fig. 3: UV absorbance spectra of *T. erecta* extract at different concentrations (200-800) across 290–400 nm. The vertical line indicates the critical wavelength (370 nm), which marks the threshold for broad-spectrum UV protection.

Photosensitivity test of T. erecta at different concentrations

The photosensitivity test represented in table 7 further demonstrated concentration-dependent variations under direct sunlight and UV exposure. Under direct sunlight, the extract showed critical wavelengths around 385–390 nm, UVB absorbance ranging 3.905-4.310 and SPF values between ≥ 39 to ≥ 43. Under UV chamber testing, absorbance values were lower for both UVB (1.419–3.39) and UVA (1.136-3.91), with SPF increasing from 14.2 to 32.8 as concentration increased.

Absorbance vs. wavelength graphs

The absorbance spectra plot of *T. erecta* extract at 200 ppm, 400 ppm and 800 ppm concentrations across 290-400 nm. It clearly shows: All three concentrations have high absorbance in the UVB (290-320 nm) range. 800 ppm shows consistently high absorbance in the UVA range (320-400 nm) as well. The vertical line at 370 nm marks the broad-spectrum threshold. Absorbance maintained beyond this wavelength qualifies the formulation as broad-spectrum according to ISO/FDA standard. The extract shows good UVB-UVA absorbance at 800 ppm concentration while at low dose (200 ppm) the UVA protection becomes significantly low. The photo stability

test revealed that the extract has better stability absorbance in direct sunlight as compared to UV exposure. The Sun protection factor showed moderate to high SPF with UVA-UVB protection at high dose (800 ppm). The result shown in fig. 4 (a, b, c).

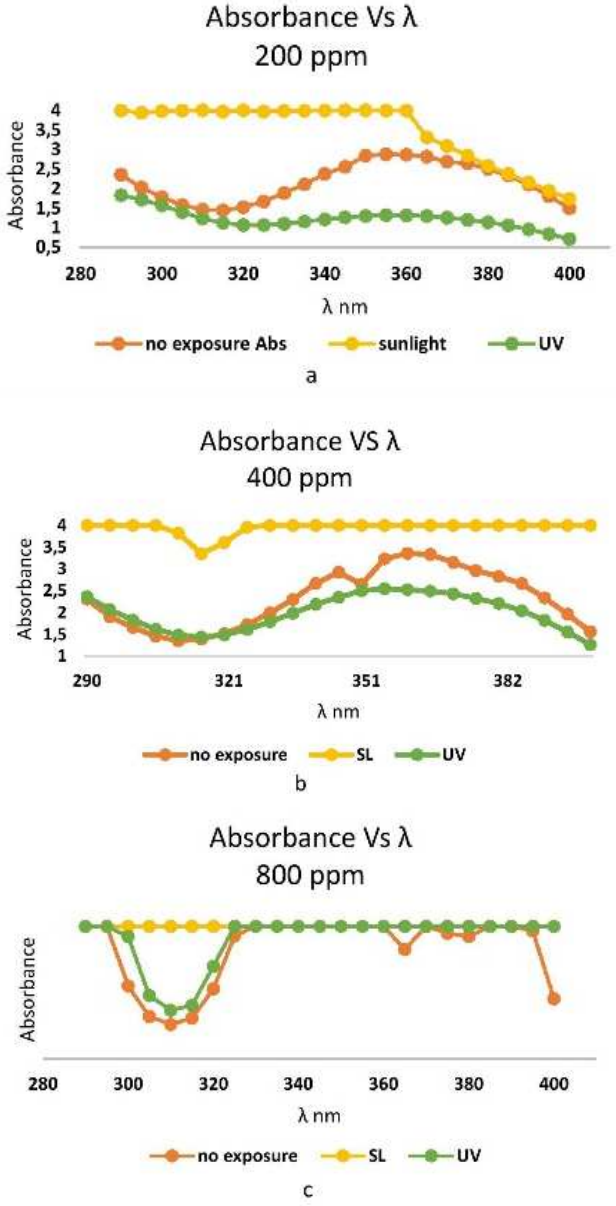

Fig. 4(a-c): Absorbance spectra of *Tagetes erecta* extract (200-800 ppm) from 290-400 nm

Kruskal-Wallis test

The Kruskal-Wallis test revealed significant differences in photoprotective activity across all tested concentrations (200, 400 and 800 µg/mL) under no exposure, direct sunlight exposure and UV exposure ($P < 0.05$). Mean rank values consistently increased with concentration, indicating a dose-dependent enhancement of photoprotection as represented in table 8.

Mann-whitney test

Pairwise Mann-Whitney U-tests with Bonferroni correction (adjusted significance = 0.0167) confirmed significant differences between the groups, particularly between 200 vs. 800 µg/mL and 400 vs. 800 µg/mL in all exposure conditions shown in table 9.

In-vitro anti-inflammatory effect (Heat-induced protein denaturation method)

The heat-induced protein denaturation assay is a widely accepted method for evaluating the anti-inflammatory potential of plant extracts and compounds. The test extract was evaluated at concentrations ranging from 100 to 800 µg/mL and its effect was compared with a standard drug Diclofenac sodium. At 100 µg/mL, the extract showed only 10.198 % inhibition, which gradually increased to 43.30 % at 800 µg/mL indicating enhanced inhibition of protein denaturation. The IC50 for the extract was 940.921 µg/mL while the standard showed significantly lower IC50 of 527.845 µg/mL as represented in table 10. These results suggest that the extract possesses notable anti-inflammatory properties through the stabilization of proteins under heat stress.

Human RBC membrane stabilization assay

The membrane-stabilizing potential of the extract was assessed using both heat-induced and hypotonic solution-induced hemolysis methods. In the heat-induced RBC lysis assay, the extract exhibited concentration-dependent membrane stabilization with a maximum inhibition of 69% at 800 µg/mL, while the standard drug showed 92% inhibition at the same concentration. At the lowest tested concentration (100 µg/mL), the extract demonstrated 40% inhibition compared to 47% inhibition by the standard represented in table 11.

In the hypotonic solution-induced hemolysis assay, the extract showed comparatively lower activity. The inhibition ranged from 11.6% to 22% across the tested concentrations, while the standard drug exhibited inhibition from 10% to 65%, with the highest activity observed at 800 µg/mL presented in table 12.

DISCUSSION

Tagetes erecta flowers are medicinal herbs known for therapeutic effects on a number of diseases and are particularly in dermatological preparations. (Burlec *et al*., 2021). The extractive values in different solvents reflect the differential solubility of phytoconstituents in *T. erecta*. The polar solvents methanol and ethanol extracted a greater portion of compounds, indicating that the flower contains mostly the polar constituents. In contrast, lower extractive value observed with hexane suggests a smaller presence of non polar compounds. This trend aligns with previous studies on how solvents polarity affects extraction efficiency during the isolation of specific phytochemicals (Ghaffar and Perveen, 2025).

Table 1: The reported values are Mean ± SD (n=3) and SEM of extractive values of the *T.erecta* flower in different solvents

S. no	Solvent extracts	Extractive values (Mean ± SD)	SEM
1.	Hexane	5.333 ± 2.309	1.333
2.	Ethyl acetate	21.333 ± 4.619	2.667
3.	Ethanol	40 ± 8.000	4.619
4.	Methanol	53.333 ± 5.508	3.180
5.	Acetone	21.333 ± 4.619	2.667

Table 2: Phytochemical investigation of different solvent extracts; +: present, ++: moderately present, +++: highly present, -: not present

	Tests	Hexane	Ethyl acetate	Ethanol	Methanol	Acetone
1.	Alkaloid					
	Hager's test	-	+	+	++	+
	Picric acid	-	-	+	-	+
2.	Flavonoid					
	$FeCl_3$10% test	-	+++	+++	+++	+++
	Lead acetate 10%	+++	+++	+++	+++	++
	H_2SO_4	+++	+++	+++	+++	+++
3.	Phenol and tannin					
	$FeCl_3$ 5% test	-	+++	+++	+++	+++
	Iodine test	+++	+++	+++	+++	+++
4.	Glycoside					
	Conc. H_2SO_4 test	+++	+++	+++	+++	+++
5.	Cardiac glycosides					
	Baljet test	+++	+++	+++	+++	+++
	Keller kiliani	+++	+++	+++	+++	+++
6.	Quinone					
	Conc. HCl	-	-	-	-	-
7.	Phytosterol					
	Hesse's response	-	+++	+++	+++	+++
8.	Fixed oil and Fat					
	Using filter paper	+++	+++	+++	+++	++
9.	Terpenoid					
	Chloroform test	+++	++	+++	+	+++
10.	Carbohydrate					
	Molisch's test	+++	++	+++	+	+++

Table 3: Total phenolic content of *T.erecta* flower extracts

S.no	Extract	GAE Conc (µg/mL)	GAE Conc (mg/ml)	TPC ± SD (mg GAE/mg)
1.	Hexane	64	0.064	0.0488 ± 0.0033
2.	Ethyl acetate	3095	3.095	0.7729 ± 0.0007
3.	Ethanol	3053	3.053	0.7615 ± 0.0017
4.	Methanol	3082	3.082	0.7733 ± 0.0024
5.	Acetone	2946	2.946	0.7355 ± 0.0024

Table 4: Total Flavanoid content of *T.erecta flower* extracts

S.no	Extract	Rutin Conc (µg/mL)	Rutin Conc (mg/ml)	TFC ± SD (mg Rutin /mg)
1.	Hexane	13448	13.448	6.724 ± 0.020
2.	Ethyl acetate	4817	4.817	1.204 ± 0.005
3.	Ethanol	14897	14.897	14.904 ± 0.050
4.	Methanol	14696	14.696	10.614 ± 0.015
5.	Acetone	17553	17.553	17.553 ± 0.106

Table 5: Total flavanol content of *T.erecta flower* extracts

S.no	Extract	Rutin Conc (µg/mL)	Rutin Conc (mg/ml)	Total Flavanol Content Mean ± SD (mg Rutin /mg)
1.	Hexane	1175	11.75	2.939 ± 0.0230
2.	Ethyl acetate	3099	3.099	0.774 ± 0.0226
3.	Ethanol	1531	15.31	3.829 ± 0.1002
4.	Methanol	7915	7.915	1.978 ± 0.0251
5.	Acetone	1711	17.11	4.279 ± 0.2680

Table 6: UV spectrophotometric snalysis for broad-spectrum assessment (*In-vitro*)

T.erecta Extract conc.(ppm)	Critical Wavelength (λc nm)	UVB	UVA	SPF
200	386.84	1.648 ± 0.035	0.010 ± 0.067	16.5
400	386.13	1.538 ± 0.017	0.010 ± 0.041	15.49
800	388.04	2.882 ± 0.033	0.021 ± 0.074	29

Table 7: Photosensitivity test of *T.erecta* at different concentrations

Photosensitivity Test	*T.erecta Extract* conc(ppm)	Critical Wavelength (λc nm)	UVB	UVA	SPF
Direct sunlight	200	385	4.310 ± 0.037	0.0218 ± 0.007	43.3
	400	390	3.905 ± 0.057	0.0218 ± 0.019	39.3
	800	390	4.001 ± 0.00	0.022 ± 0.00	40.2
UV	200	385	1.419 ± 0.297	1.136 ± 0.178	14.2
	400	390	1.754 ± 0.35	2.1 ± 0.397	17.0
	800	390	3.39 ± 0.54	3.91 ± 0.32	32.8

Table 8: Kruskal-Wallis test showing the mean rank values of photo protective activity at different concentrations under no exposure, sunlight exposure, and UV exposure.

No exposure	WL(nm)	Concentration	Mean Rank	Chi-square	df	P-value
	290-400	200	23.04	35.711	2	<0.001
		400	26.70			
		800	55.26			
Sunlight	290-400	200	14.57	47.802	2	<0.05
		400	41.93			
		800	48.50			
UV	290-400	200	13.22	58.461	2	<0.001
		400	33.78			
		800	58.00			

Table 9: Post hoc mann-whitney test

Non exposure				
200 vs 400	235.000	-0.648	0.517	Not significant
200Vs 800	19.000	-5.430	<0.001	significant
400 Vs 800	44.000	-4.877	<0.001	significant
Sunlight				
200 vs 400	59.000	-4.682	<0.001	Significant
200Vs 800	0.000	-6.212	<0.001	significant
400 Vs 800	218.500	-2.068	>0.005	significant
UV				
200 vs 400	28.000	-5.196	<0.001	Significant
200Vs 800	0.000	-5.993	<0.001	Significant
400 Vs 800	0.000	-5.993	<0.001	Significant

Table 10: Heat-induced protein denaturation method

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	0.211	1.656	10.198		30.34879	
200	0.200	1.525	14.872	527.845	36.43657	940.921
400	0.174	1.330	25.920		48.19689	
800	0.121	1.111	43.300		59.72013	

Table 11: RBC lysis assay by heat induce method

Conc (µg/mL)	Absorbance of Extract (mean ± SD)	Absorbance of standard (mean ± SD)	% inhibition of Extract	% inhibition of standard
100	0.881 ± 0.002	0.233 ± 0.001	40	47
200	0.756 ± 0.022	0.271 ± 0.001	42	72
400	0.533 ± 0.198	0.280 ± 0.006	62	75
800	0.455 ± 0.002	0.296 ± 0.012	69	92

Table 12: RBC lysis assay by hypotonic solution method

Conc (µg/mL)	Absorbance of Extract (mean ± SD)	Absorbance of standard (mean ± SD)	% inhibition of Extract	% inhibition of standard
100	0.209 ± 0.003	0.722 ± 0.062	11.6	10
200	0.205 ± 0.001	0.636 ± 0.006	12	19
400	0.188 ± 0.001	0.579 ± 0.017	17	27
800	0.177 ± 0.001	0.290 ± 0.014	22	65

The phytochemical screening of *Tagetes erecta* flower extract identified a wide range of primary and secondary metabolites, indicating its potential therapeutics in various ailments (Bocso and Butnariu, 2022). The phytoconstituents such as flavonoids, tannins, phenols, glycosides, cardiac glycosides, phytosterols and terpenoids are found mainly in polar solvents and are widely known for their antioxidant, anti-inflammatory and UV-absorbing properties. Flavonoids are responsible for the neutralization of reactive oxygen species that are generally produced by UV exposure, reduce inflammation and produce anti-aging effects, supporting the flower's therapeutic relevance in psoriasis. The abundance of fixed oils and carbohydrate support their potential role in topical formulations. (Anbualakan *et al*., 2022).

Methanol and ethyl acetate extracts represented the highest TPC (0.7733 ± 0.00242 and 0.7729 ± 0.00074 mg GAE/mg), indicating the greatest capacity to extract polar phenolic compounds. Ethanol and acetone flower extracts indicated comparatively high total phenolic content values as compare to hexane (0.0488 mg GAE/mg). Acetone and ethanol demonstrated the highest TFC and total flavonol content (17.553 ± 0.1064 and 14.904 ± 0.0506 mg Rutin/mg). These findings indicate that the extract has the capability to absorb UVA and UVB rays, thus providing both antioxidant and photoprotective benefits and is widely incorporated into sunscreens and anti-aging products as harmless, more viable substitutes to conventional photoprotective agents. The study reveals that the flavonoids, with other polyphenols and carotenoids, support the avoidance of sunburn, photoaging and UV exposure skin malignancies by obstructing oxidative stress and promoting cellular repair mechanisms (Budzianowska *et al*., 2025; Anbualakan *et al*., 2022).

The spectrophotometric analysis revealed that in all tested concentrations of TEFE, the critical wavelength measurements exceed 386 nm. This result indicated that *T. erecta* extract provided broad-spectrum UV filter. According to the FDA and COLIPA guidelines, the critical wavelength above 370 nm threshold demonstrated for broad-spectrum classification.(Al-Sadek and Yusuf, 2024) Additionally, the extract exhibited enhanced UVA protection at higher concentrations, although UVB absorption remained dominant. As UVA exposure plays a crucial role in modulating inflammation and abnormal keratinocyte proliferation in psoriasis, the UVA coverage of *T. erecta* extract is particularly relevant for therapeutic use. (Zhou *et al*., 2022;Zhang *et al*., 2024b) Importantly, the extract was tested for photosensitivity and did not exhibit phototoxic effects, demonstrating both stability and safety under UV and sunlight exposure. Notably, the higher SPF values observed under sunlight exposure (SPF = 20) showed the broad-spectrum protection and higher intensity for solar radiation compared to artificial UV filters. Pairwise comparisons using the Mann-Whitney U test further confirmed a statistically significant dose-dependent enhancement of photoprotection.

In addition to photoprotection, the anti-inflammatory potential of the extract was demonstrated through the heat-induced protein denaturation assay. The phytoconstituents in TEFE are known to inhibit inflammatory enzymes and melanogenesis; these bioactives help in supporting wound healing and skin protection, including UV-induced damage. The extract showed concentration-dependent inhibition, comparable to the standard drug diclofenac sodium at higher concentrations (800 µg/mL). Protein denaturation is a critical event in the inflammatory cascade and compounds that stabilize proteins under heat stress are considered effective anti-inflammatory agents. Similarly, the extract displayed significant inhibition of heat-induced hemolysis in the RBC membrane stabilization assay, with up to 69% protection at the highest tested concentration (Singh *et al*., 2024). This effect is likely due to the interaction of flavonoids and polyphenols with membrane phospholipids, enhancing erythrocyte resistance to thermal stress. These observations are in agreement with existing literature suggesting that plant-based polyphenols provide lysosomal membrane stabilization, an important mechanism in anti-inflammatory therapy (Zhang *et al*., 2024a). Conversely, moderate activity was observed against hypotonicity induced RBC lysis (22% inhibition) which suggests that the extract exert its primary anti-inflammatory effects via protein and membrane stabilization pathways rather than osmoregulatory mechanisms. The result indicated that extract showed efficacy against oxidative and thermal stress, which are commonly associated with psoriatic skin inflammation (Bilski *et al*., 2024; Bakić *et al*., 2024).

The current findings strongly support the photoprotective and anti-inflammatory potential of *T. erecta* and translation of these results requires in vivo studies and human trials. However, the dual efficiency of *T. erecta* as a UV-protective and anti-inflammatory agent establish it a viable botanical for developing cosmeceutical creams intended in managing psoriasis and photo-induced skin damage.

CONCLUSION

From the result it has been revealed that *T. erecta* from flora of Pakistan is considered a promising plant source for use as a sunscreen as the extract showed the high content of flavonoid. Moreover, it is the first report that the extract is safely used as a sun protection for the psoriatic patient, a finding not previously reported. The findings also suggest that *T. erecta* possesses bioactive compounds with promising anti-inflammatory activity along with Sun Protecting Factor, could be further explored for topical use in conditions involving irritated or damaged skin barriers in psoriasis.

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Authors' contributions
[Sanober Kamal]: Designed and executed the study, conducted experiments, and analyzed the results.
[Fatima Qamar]: Supervision, Conceptualization.
[Aymen Owais]: Critically reviewed and refined the manuscript.
[Faiza Akhtar]: Provided substantial intellectual input during the drafting and revision of the manuscript.
[Maria Rahat]: Participated in manuscript review, revision, and editing.

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Conflict of interest
This is an original research article. All authors have reviewed and approved the final manuscript. The authors declare that there are no conflicts of interest related to this work.

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