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FORMULATION AND EVALUATION OF TOPICAL HERBAL CREAM CONTAINING *Tagetes erecta* FLOWER EXTRACT WITH DATE SEED OIL FOR ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT
Tagetes erecta Linn, a member of the Asteraceae family, is a well known medicinal plant used in traditional Indian medicine, as well as in other parts of the world like South America, Africa, Asia, Europe, and Oceania. A wide range of major constituents include thiophenes (antimicrobial), flavonoids like quercetagetin, quercetin, and kaempferol, as well as volatile oils containing (E)-β-ocimene, dihydrotagetone, and tagetone have been reported from various extracts of the flower and leaves of the plant and have shown potential for anti-inflammatory activity, while date seed oil provides emollient, antioxidant, and skin-protective properties. Extraction is mainly done using the solvent (ethanol). In this study, we prepare the topical formulation of anti-inflammatory cream of different formulation by varying the concentration of date seed oil. The formulation contains various excipients like Triethanolamine, Methyl paraben and propylene glycol. The cream was prepared as an oil-in-water (O/W) emulsion using appropriate emulsifying and stabilizing agents. The formulated creams were assessed for physicochemical parameters including appearance, pH, homogeneity, spreadability, viscosity, and Washability, all of which were found to be within acceptable limits. In vitro anti-inflammatory activity was evaluated by Human red blood cells (HRBC) membrane stabilization method, where the extract exhibited concentration-dependent activity with percentage protection 58.92% at 50 µg/ml concentration, and minimum hemolysis was 37.63% in a hypotonic solution with diclofenac as the control. Tagetes erecta flower extract was made in 4 formulations with variation of date seed oil; Formula I (2ml), Formula II (1.5ml), Formula III (1 ml). Then evaluated by its physical appearance, Washability, homogeneity and grittiness, spreadability, and PH measurement. In-vitro hemolytic studies further confirmed reduced percentage haemolysis with increasing concentrations, indicating good membrane protection and safety. The study concludes that the combination of Tagetes erecta flower extract and date seed oil possesses significant anti inflammatory potential and can be effectively utilized in the development of a safe and natural topical herbal cream.

KEYWORDS: Tagetes erecta; Anti-inflammatory activity; Herbal cream; Topical drug delivery system.

INTRODUCTION

Inflammation is a complex biological response of the body's immune system to harmful stimuli such as injury, infection, or irritation. Although it is a protective mechanism, persistent or excessive inflammation can lead to discomfort, tissue damage, and chronic skin disorders. Topical anti-inflammatory preparations play a crucial role in managing localized inflammation by delivering therapeutic agents directly to the affected site with minimal systemic side effects. In recent years, there has been growing interest in developing herbal-based topical formulations, as plant derived constituents offer better biocompatibility, lower toxicity, and multi-target therapeutic action. Tagetes erecta, commonly known as marigold, is a medicinal plant widely used in traditional systems for its anti-inflammatory, wound-healing, antimicrobial, and antioxidant properties. The flowers are rich in flavonoids, carotenoids, terpenoids, and essential oils, which contribute to inhibition of inflammatory mediators and support tissue repair. Extracts of Tagetes erecta have shown significant potential in reducing edema and soothing irritated skin, making the plant a suitable candidate for topical anti inflammatory formulations.[1] Date seed oil, obtained from the seeds of Phoenix dactylifera, is another promising natural ingredient with excellent therapeutic value. It contains high levels of oleic acid, lauric acid, tocopherols, phenolics, and antioxidants. These components provide nourishing, moisturizing, and anti-inflammatory effects and improve the skin barrier function. Date seed oil has also gained attention as a sustainable and economically beneficial raw material because it utilizes a by-product of the date fruit industry.[2] Combining Tagetes erecta extract with date seed oil in a topical cream offers a synergistic approach for inflammation management. Tagetes erecta contributes potent anti-inflammatory phytoconstituents, while date seed oil enhances skin penetration, provides emollient properties, and stabilizes the formulation. Together , they may Present study focuses on the formulation and evaluation of a topical anti-inflammatory cream incorporating Tagetes erecta extract and date seed oil. The work involves improve therapeutic efficacy, skin hydration, and overall the acceptability of the final product.[1]

Extraction of Tagetes erecta flower constituents, preparation of a suitable cream base, incorporation of the natural active ingredients, and assessment of physicochemical parameters, stability, and anti-inflammatory potential. Developing such a formulation supports the growing demand for herbal, safe, and effective topical products and contributes to the scientific understanding of Tagetes erecta and date seed oil as valuable natural resources for skincare and therapeutic applications.

MATERIALS AND METHODS

Extraction of Tagetes erecta Flower Extract

Fresh flowers were washed, shade-dried, and coarsely powdered. The powdered material was subjected to Soxhlet extraction using ethanol/methanol for 6–8 hours. The extract was filtered and concentrated using a rotary evaporator. The dried extract was stored in an airtight container at 4°C until further use.

Extraction of Date Seed Oil

Solvent extraction is done in a soxhlet apparatus to extract the palm oil from its seeds. This particular soxhlet apparatus consists of a glass extractor, fitted in between a round bottom flask at the bottom and a bulb condenser at the top. Inside the glass thimble holder, solid matrix of seeds is placed within thimble. The round-bottom distillation flask initially contained an extracting solvent and it is heated up by electrothermal heating mantle 450 C° maximum temperature, 1L max capacity and power 300W. As the solvent vapor goes up to the condenser, it condenses and accumulates inside the extractor. Here, the solvent comes in contact with the seeds and oil is leached out of the seeds. When the condensate

moves down through the bed of seeds, mass transfer takes place. However, major amount of mass transfer of oil from the seeds to solvent occurs when the accumulated solvent moves up purely due to the hydrostatic pressure head so, surface area offered by the bed and the seed-solvent contact time are the two major factors for the yield of the oil production.[43]

1. COLLECTION OF THE PLANT

Fresh leaves of *Tagetes erecta* L. (2 kg) were collected and shade-dried to remove moisture.

2. PHYSICOCHEMICAL EVALUATION

Loss on drying

The Loss on Drying (LOD) was calculated to determine the moisture content of the fresh plant material. The percentage of loss was calculated using the weight of the material before and after drying until a constant weight was achieved.

Determination of foreign matter

Approximately 100g of the dried plant material was spread in a thin layer and examined macroscopically for the presence of foreign materials, including molds, insects, and other animal contaminants. Any foreign matter was separated and weighed to calculate the percentage of contamination relative to the initial sample weight.

Determination of moisture content

An accurately weighed quantity of the sample was placed in a tared evaporating dish and dried in an oven at 105ºC for a duration of five hours. The sample was weighed until a constant weight was achieved, and the percentage of volatile matter was calculated based on the weight loss.

Determination of Total ash value

To determine the total ash content, 2g of the ground air-dried plant material was accurately weighed into a previously ignited and tared crucible. The sample was spread in an even layer and ignited by gradually increasing the heat to 500-600º C until it became white, indicating the absence of carbon. For samples where carbon-free ash could not be obtained, the residue was moistened with a saturated solution of ammonium nitrate before final ignition. The crucible was cooled in a desiccator, weighed, and the total ash percentage was calculated relative to the air-dried material.

Determination of acid insoluble ash value

The acid-insoluble ash was determined by boiling the total ash residue with 25ml of dilute hydrochloric acid for five minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water, and ignited in the original crucible until a constant weight was reached. This value was used to estimate the amount of silica and siliceous earth present in the sample.

Determination of Extractive Values

The extractive values were determined to estimate the amount of active constituents soluble in specific solvents. For the alcohol-soluble extractive, 5g of the air-dried drug was macerated with 100ml of alcohol in a closed flask for 24 hours, followed by filtration and evaporation of the filtrate to dryness. A similar procedure was followed for the water-soluble extractive using 0.1% chloroform water as the solvent. The percentage of each extractive was then calculated based on the weight of the air-dried drug.

3. PRELIMINARY PHYTOCHEMICAL SCREENING

The alcoholic extract of the leaves of Psidium guajava was screened for the presence of various phytoconstituents like alkaloids, flavonoids, saponin, tannin and glycosides etc.

Test for carbohydrates

1. **Molisch Test:** It consisted of treating the compounds of a-naphthol and concentrated sulphuric acid along the sides of the test tube. Reddish violet ring was produced at the junction between two liquids.
2. **Fehling's Test:** Equal quantity of Fehling's solution A and B were added. Heated gently, no brick red precipitate was obtained.
3. **Benedict's test:** To the 5ml of Benedict's reagent, were added 8 drops of solution under examination. Mixed well, the mixture was boiled vigorously for two minutes and then cooled. Red precipitate was obtained.
4. **Barfoed's test:** To the 5ml of the Barfoed's solution added 0.5ml of solution under examination, heated to boiling, no red precipitate of copper oxide was obtained.

Test for Alkaloids

1. **Dragendroff's Test:** To the extract, 1ml of Dragendroff's reagent was added Orange red precipitate was produced**.**
2. **Wagner's test:** To the extract Wagner reagent was added. Reddish brown precipitate was not produced**.**
3. **Mayer's Test:** To the extract 1ml or 2ml of Mayer's reagent was added. Dull white precipitate was not produced**.**
4. **Hager's Test:** To the extract 3ml of Hager's reagent was added. Yellow precipitate was produced.

Test for Steroids and Sterols

1. **Liebermann Burchard test:** The test sample was dissolved in 2ml of chloroform in a dry test tube. 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The solution became red, then blue and finally bluish green in color.
2. **Salkowski test:** The sample of test solution was dissolved in chloroform and equal volume of conc. sulfuric acid was added. Bluish red, cherry red and purple color is noted in chloroform layer, whereas acid assumed marked green fluorescence.

Test for Glycosides

1. **Legal's test:** Sample was dissolved in pyridine; sodium nitroprusside solution is added to it and made alkaline. Pink red color was not produced**.**
2. **Baljet test:** To the drug sample, sodium picrate solution was added. Yellow to orange color was produced**.**
3. **Borntrager test:** Few ml of dilute sulfuric acid was added to the test solution. Boiled filtered and extracted the filtrate with ether or chloroform. Then organic layer was separated to which ammonia was added, pink, red or violet color was produced in organic layer**.**
4. **Killer Killani test:** Sample was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of concentrated sulfuric acid. No characteristic reaction was observed.

Test for Saponins

Foam test: About 1ml of alcoholic sample was diluted separately with distilled water to 20ml and shaken in graduated cylinder for 15 minutes. No persistent foam observed.

Test for Flavonoids

Shinoda test: To the sample, magnesium turnings and then concentrated hydrochloric acid were added. Red color was produced.

Test for Tri-terpenoids

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution was added. Pink color was produced which indicated the presence of triterpenoids.

Tests for Tannins and Phenolic Compounds

To 2-3 ml of extract, add few drops of following reagents were added

a). 5% FeCl3 solution : Deep blue-black color.
b). Lead acetate solution : White precipitate.
c). Gelatin solution : White precipitate
d). Bromine water : Discoloration of bromine water.
e). Acetic acid solution : Red color solution
f). Dilute iodine solution : Transient red color.
g). Dilute HNO3 : Reddish to yellow color.

RESULTS AND DISCUSSION

Table 1: Physico-chemical parameters (after shade drying) of the plant.

S. No.	Name of the plant	Foreign matter	Moisture content	Total ash	Acid insoluble ash	EtOH soluble extractive value	Water soluble extractive value
1.	*Tagetes erecta L*	0.6%	7%	5%	0.8%	12%	20%

PRELIMINARY PHYTOCHEMICAL SCREENING

The preliminary phytochemical analysis of *Psidium guajava* leaf extract showed the presence of steroids, alkaloids, flavonoids, glycosides, tannins, and carbohydrate. The results of the data were tabulated in table no:2

Table 2: Phytochemical screening of ethanolic extract of *Tagetes erecta.*

Constituents	Test	*Tagetes erecta*
Alkaloids	Dragendroff's Test Wagner's test Mayer's Test Hager's Test	+ - + +
Saponins	Foam test	+
Flavonoids	Shinoda test	+
Tri-terpenoids	In the test tube, 2 or 3 granules of tin+2ml of thionyl chloride solution and test solution is added.➔Pink color	+

+ Present, - Absent

DISCUSSION

The present study was carried out to formulate and evaluate a herbal topical anti-inflammatory cream containing Tagetes erecta flower extract and date seed oil. The results obtained from various evaluation parameters indicate that the formulated cream possesses desirable physicochemical and pharmacological properties.

Phytochemical screening of the Tagetes erecta extract confirmed the presence of important bioactive constituents such as flavonoids, tannins, phenolic compounds, saponins, and terpenoids. These compounds are well known for their anti-inflammatory, antioxidant, and membrane-stabilizing activities, which contribute significantly to the therapeutic potential of the formulation.

The prepared cream showed a smooth texture, good homogeneity, and absence of grittiness, indicating proper formulation and mixing of ingredients. The pH of the formulations ranged between 5.9 and 6.8, which is within the acceptable range for topical skin preparations, ensuring that the cream is safe and non-irritating to the skin.

Spreadability studies revealed that the cream spreads easily, which is an important factor for patient compliance and effective topical application. The formulation also demonstrated good washability due to its oil-in-water (O/W) emulsion base, making it convenient for use.

The in-vitro anti-inflammatory activity, evaluated using the HRBC membrane stabilization method, showed that the formulation exhibited concentration-dependent activity. The percentage protection increased significantly with increasing concentration, indicating strong membrane-stabilizing ability. Although the activity was slightly lower than the standard drug diclofenac, the results were comparable, suggesting that the herbal formulation has promising anti-inflammatory potential.

Additionally, the hemolytic activity study demonstrated reduced hemolysis at higher concentrations, confirming that the formulation is safe for red blood cells and exhibits protective effects on biological membranes.

The observed anti-inflammatory activity can be attributed to the synergistic effect of Tagetes erecta extract and date seed oil. The flavonoids and phenolic compounds present in Tagetes erecta help inhibit inflammatory mediators, while date seed oil enhances skin hydration, penetration, and provides additional antioxidant support.

Overall, the study confirms that the formulated herbal cream is stable, safe, and effective, and can be considered a promising alternative to synthetic anti-inflammatory topical agents with fewer side effects.

CONCLUSION

The present work on the preparation of herbal topical cream containing Tagetes erecta flower extract was an attempt to utilize the Anti-inflammatory activity. The plant Tagetes erecta was identified, authenticated, and dried. Then the flowers were powdered and extracted by Soxhlet extraction process using solvent ethanol and final product was collected by evaporation. By phytochemical screening of the extracted product, it was found that many phytochemicals were present in it. Different creams were formulated by using the extract with varying concentration of date seed oil (2ml,1.5ml,1ml). The Prepared creams were subjected for various evaluation parameters like Washability, homogeneity, spreadability, pH and invitro study. From the developed formulations F1 shows good spreadability, pH, Washability. From the invitro study, showed a maximum protection of human red blood cell (HRBC) with 58.92% at 50µg/ml, and minimum hemolysis was 37.63 % in a hypotonic solution with diclofenac as the control.

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