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FORMULATION AND IN VITRO EVALUATION OF *TAGETES ERECTA* ANTIAGING GEL CREAM

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ABSTRACT

Aging of the skin is a complicated biological process that is impacted by both external and internal factors. Fine lines and wrinkles, changes in skin pigmentation, and photoaging—the generation of free radicals that disrupt DNA production—are all indicators of aging caused by UV-A and UV-B radiation. The flowers of Tagetes have flavonoids which has properties such as photoprotective, depigmentation, and anti-aging, all of which are promising in the treatment of a variety of skin problems. In the current research work the ethanolic extract of Tagetes erecta was prepared and subjected to phytochemical screening. Results confirmed presence of flavonoids. Further this extract along with lemon oil was incorporated in gel cream base. Optimized formulation was evaluated for parameters like homogeneity, water washability, spreadability, pH, Viscosity, irritancy and in vitro drug release. Results conclusively demonstrated that developed herbal based formulation would have great market potential and would exhibit superior antiaging properties.

Keywords – *Antiaging, Tagetes erecta, Lemon oil, Flavonoids, Gel cream.*

1. INTRODUCTION

Free radicals have the potential to prematurely age the skin. Skin aging can cause melanin damage and a decrease in skin flexibility. Aging is a process that is inevitable. Both intrinsic and extrinsic elements cause cutaneous aging. Medicinal and aromatic plants (MAP), as well as other natural products, contain hundreds of antioxidant compounds. Plants have a number of chemicals that can trap free radicals, including phenolic compounds, nitrogen compounds, vitamins, and others. These natural antioxidants have drawn a lot of attention lately since they are an efficient way to reduce and eliminate the activity of free radicals that cause oxidative stress. [1-4]

1.1 Gel vs Cream

Gels and creams work in slightly different ways, so many people use them in combination. Gels are often water-based, and creams are often oil-based. An oil-based cream is rich and slightly heavier than gel. It often provides greater moisturizing benefits and is better for mature or dry skin. Gel is lighter than cream, which makes it easy for the skin to absorb. Gels are particularly useful for targeting specific areas, such as the sensitive eye area, where heavier creams might build up. [5]

1.2 What are Gel-Creams?

Gel-creams are water-based formulations. They deliver hydration and moisture quickly to skin unlike oil-based moisturizers, which can take time to be absorbed by your skin. With their light formulations gel-creams are non-comedogenic and can make your skin look fresh and dewy without it being oily or shiny. Gel-creams are compatible with all skin types, but especially effective with skin types that are on the oilier side as they would not add any additional oil to skin.[6]

Table-1: Gel versus Cream

Gel	Cream
Water-based	Oil-based
Light and watery	Thicker
Gel evaporates easily Suitable for oily skin	Cream do not evaporate easily Suitable for dry skin
Rapidly absorbed	Slowly absorbed
Easier to prepare than cream	Complex to prepare than gel

1.3 *Tagetes erecta*

Table-2: Details of *Tagetes erecta*

Biological source	Family	Chemical constituents	Pharmacological activities	Photo
Tagetes erecta (Petals)	Asteraceae	Flavonoids Thiophenes Carotenoids Triterpenoids	Anti-bacterial Anti-inflammatory Antioxidant Hepatoprotective Wound healing Anticancer Antidiabetic	

1.4 Lemon oil (limonene)

Lemon essential oil can be diluted and applied directly on the skin. Lemon oil is used as an anti-exhaustion, anti-depression, skin-clearing, anti-viral, anti-bacterial, and anti-inflammatory component. It contains terpenes, sesquiterpene, aldehyde (Citral and citronellal). Antioxidant activity of oil extracted from lemon peel was because of limonene, alpha pinene. [7-10]

2. MATERIALS AND METHODS

2.1 Materials

Tagetes erecta was collected from our college's botanical garden, and the same was authenticated lemon oil was obtained from Alpha Pharmaceuticals. Beeswax, stearic acid, glycerol, carbopol 974P, triethanolamine, and liquid paraffin was purchased from Molychem, Mumbai.

2.2 Methodology

Extraction of Flavonoids: Dried flowers (5g) of *Tagetes erecta* were boiled in 150 mL of ethanol for 1 hour. The ethanolic extract was then evaporated on an electronic water bath and the residue obtained was collected.

Fig. 1: Extract of Tagetes erecta

2.3 Tests on the residue obtained [11-14]

Table -3: Chemical test

Sr. no.	Test	Observation	Inference
1	Alkaline reagent test Test solution + Few drops of NaOH solution	Intense yellow colour	Flavonoids present
2	Sulphuric acid test Test solution + Sulphuric acid	Orange to red colour	Flavonoids present
3	Zinc Hydrochloride test Test solution + Mixture of Zinc dust and conc. HCl	Deep red colour after few minutes	Flavonoids present
4	Shinoda's Test Test solution _ Few Magnesium turnings + conc. HCl dropwise	Crimson red after few minutes	Flavonoids confirmed

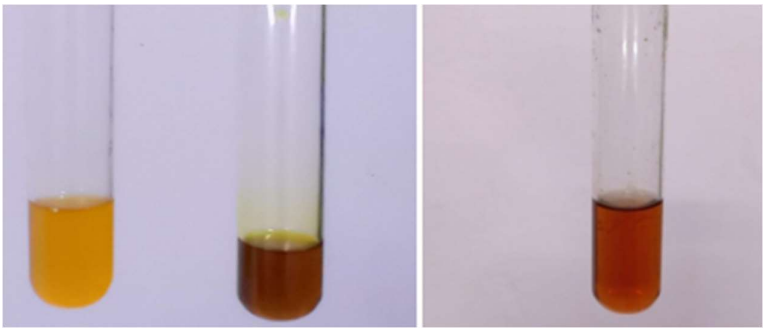

Fig. 2: Test for Alkaline reagent, Sulphuric acid test, & Zinc HCl test

Fig. 3: Shinoda test

2.4 Total Phenolic Content

The total phenolic content was determined quantitatively using the Folin Ciocalteu reagent (FCC), with Gallic acid as the standard. The total phenolic content of the alcoholic extracts was calculated in mg/g of the Gallic acid equivalent (GAE). The wavelength of the maximum absorption in the UV spectrum was 630nm.

Preparation of Standard Gallic Acid:

Stock solution: Folin Ciocalteu assay method was used for the determination of the total phenolic content. 100mg of gallic acid is dissolved in 100ml of methanol to prepare a 1000 ppm solution which was further diluted to prepare a 100 ppm solution. A set of standard solutions of gallic acid (2, 4, 6, 8 and 10 ppm) were prepared and the absorbance was taken at 630 nm with an UV/visible spectrophotometer.

Working std: 100 ppm

Table - 4: Standard Solutions

Concentration (ppm)	Volume(ml)	Volume of FCC (ml)	Volume of 7% Na2CO3 (ml)	Volume makeup with DW upto (ml)	Absorbance
2	0.5	1	10	25	0.098
4	1	1	10	25	0.2817
6	1.5	1	10	25	0.4061
8	2	1	10	25	0.5727
10	2.5	1	10	25	0.6854

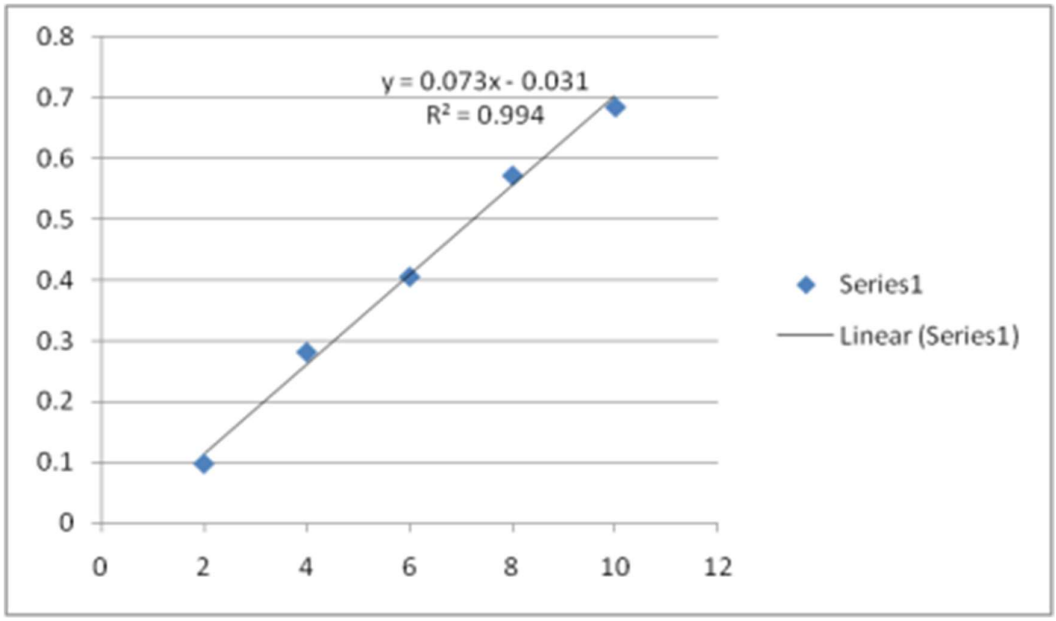

Fig. 4: Calibration curve

Linear equation obtained by standard Gallic acid: y= 0.073x - 0.031

Sample: Extract

Stock solution- 10 mg of extract is dissolved in 10 ml of methanol to make 1000 ppm solution and from this further concentration was prepared.

Table-5: Sample solutions

Concentration (ppm)	Volume(ml)	Volume of FCC (ml)	Volume of 7%Na_2CO_3 (ml)	Volume makeup with DW up to (ml)	Absorbance
1000	1	1	10	25	0.761
2000	2	1	10	25	0.782
3000	3	1	10	25	0.8108

From the linear equation obtained by standard Gallic acid y= 0.073x - 0.031, we calculated the total phenolic content present in our formulation which is found to be 57.64 %w/w [15].

2.3 Formulation Development

Oil phase:

Table-6

Ingredients	Quantity	Activity
Beeswax	2%	Humectant
Stearic acid	3%	Emulsifier, emollient
Cetyl alcohol	5%	Emulsifier
Liquid paraffin	10%	Emollient

Aqueous phase:[16]

Table-7

Ingredients	Quantity	Activity
Carbopol 974P	1%	Gelling agent
Triethanolamine	0.50%	Stabilizer
Sorbitol	5%	Humectant/moisturizer
Methyl paraben	0.20%	Preservative
Propyl paraben	0.25%	Preservative
Propylene Glycol	5%	Moisturizer
Water	q.s to 100 ml	Vehicle

Formulation of gel cream [11]

Table-8

Ingredients	Quantity	Activity
Marigold extract (Flavonoid) extract	3%	Antioxidant
Lemon oil	3%	Boost collagen production
Gel-cream base	qs	Gel-cream base

Procedure

Step 1-Preparation of oil phase

The oil phase ingredients were weighed and blended with continuous stirring using a mechanical stirrer at 1000 rpm at 80°C.

Step 2-Preparation of water phase

The Aqueous phase constituents were weighed and blended with continuous stirring using a mechanical stirrer at 1000 rpm at 80°C.

Step 3-Preparation of gel-cream base

The oil phase was incorporated in the water phase at 80°C with continuous stirring using a mechanical stirrer at 1000 rpm for 30 mins.

Step 4-Preparation of gel-cream formulation

The extracted flavonoid was incorporated to gel-cream base with continuous stirring using a mechanical stirrer at 1000 rpm until the components were uniformly dispersed. At room temperature, it was allowed to equilibrate for 24 hours. A wide-mouth container was used to fill and store the created gel cream. Further the formulation was evaluated.

Fig. 5: Gel cream Formulation

2.4 Evaluation

2.4.1 Physical Parameters

The prepared gel-cream was evaluated for the following parameters:

a) Colour

The colour of the formulation was checked by visual inspection. [16]

b) Odor

Odor was checked by mixing gel-cream with water.[16]

c) Homogeneity

Homogeneity was confirmed by appearance and by touch. [16]

Fig 6. Test for Homogeneity

d) Appearance

When formulation was kept for a long time, it was found that there was no change in the color of cream.

e) Water washability

The ease and extent of washing with water were personally examined after the formulations were applied to the skin.

2.5 Pharmaceutical Parameters

a) Spreadability

The spreadability of the gel-cream was determined by spreading 0.25 g of the gel on a 1 cm diameter circle premarked on a glass plate,

followed by a second glass plate. For 5 minutes, a half kg of weight was allowed to lie on the upper glass plate. The circle's diameter was measured after the gel had been spread out. [19]

b) pH determination

pH of the gel- cream formulation was determined using a pH meter. The glass electrode was calibrated using standard buffer solutions. A 1% solution of gel-cream sample was prepared using distilled water and the readings were done in triplicate and average was taken.

c) Viscosity

The viscosity of gel-cream formulation was determined using Brookfield viscometer. A 50 gm of formulation was weighed and transferred to beaker and viscosity of formulation was determined with the help of Brookfield Viscometer using spindle number **6** was rotated at three different rpm - **2.5, 1 and 0.5 rpm** and result was obtained in centipoise (cps).

2.6 In vivo Irritancy test

An area (1sq.cm) was marked on the left-hand dorsal surface. The gel-cream was applied to the specified area and time was noted. Presence of irritancy, erythema and edema were checked at regular intervals up to 24 hrs. [18]

Numerous cytokines are released when a chemical penetrates the multiple layers of the skin, especially the dermis and the epidermis .and chemokines produced by various cell types that correspond to functions in the inflammatory process for Primary irritation tests were performed. The skin irritation was measured using a numerical scale to determine how much the irritation of the skin (visual score). The reactions were evaluated according to the following arbitrary scale. No erythema: 0, Light erythema (hardly visible): 1, Clearly visible erythema: 2, Moderate erythema: 3, Serious erythema (dark red with possible formation of light eschars): 4, No edema: 0, Very light edema (hardly visible): 1, Light edema: 2, Moderate edema (about 1 mm raised skin): 3, Strong edema (extended swelling even beyond the application area): 4

Table-9

Irritancy	Erythema	edema
5min	0	0
30 min	0	0
1hr	0	0
2hr	0	0
4hr	0	0
6hr	0	0
12 hr	0	0
18hr	0	0
24hr	0	0

2.7 In Vitro Drug Diffusion Studies

In vitro skin permeation was carried out using the Franz diffusion cell. The receptor compartment consisted of a 10 ml phosphate buffer solution (PBS) with pH 6.5, which was prepared by referring to the Indian Pharmacopoeia 2007, Volume 1, page no. 480 and was constantly stirred by a magnetic stirrer at 100 R.P.M. The hairless goat skin from the abdominal areas was obtained from a local shop. The skin was mounted on the receptor compartment with a stratum corneum layer facing upwards. The gel-cream formulation was applied onto the skin in the donor compartment (250 µL). Samples were withdrawn at specific time intervals over a period of 2hrs which were immediately replaced with an equal volume of the fresh buffer. The samples were then analyzed for UV/Vis spectrophotometry at 254 nm.[20-22]

3. RESULTS AND DISCUSSION

3.1 Results

Physical Parameters

The formulation was found to be homogeneous and smooth in nature. There was no change in the yellow colour of the gel-cream, when kept for longer time periods. The formulated cream was o/w type emulsion, hence can easily washed with water and gives better consumer compliance.

Pharmaceutical parameters

Spreadability- The diameter of the circle after spreading was 3.8 cm. Thus, the formulation exhibited good spreadability.

Fig 7: Test for Spreadability

pH - The pH of gel-cream was found to be 4.55 which is considered good as the skin pH is below.

Table-10

pH	Average
4.48	4.55
4.67	

Viscosity

Table-11

R.P.M	Dial Reading	Factor	Viscosity (cp) = Dial reading*Factor
2.5	45	4000	180000
1	39.5	10000	395000
0.5	33	20000	660000

Irritancy test - The formulation showed no irritation, edema or redness during the study. Hence it is safe to use for skin

Table-12

Volunteer	1	2	3	4	5 6	7 8	9	10
Irritancy	-	-	-	-	- -	- -	-	-
Edema	-	-	-	-	- -	- -	-	-
Redness	-	-	-	-	- -	- -	-	-

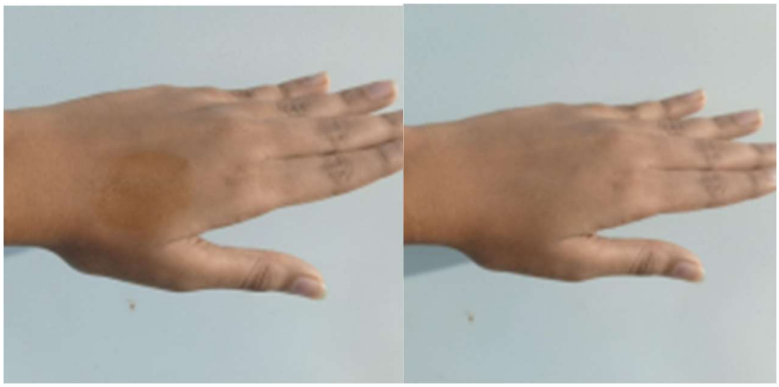

Fig. 8 Test for Irritancy

3.2 Discussion

Herbal medicines and nutraceuticals are becoming increasingly popular around the world, with many people turning to these products for treatment of a variety of health problems. Natural remedies have clearly seen a huge increase in public acceptability and interest in both developing and developed countries over the last decade. Apart from it, herbal cosmetics are relatively safe, easy availability and economical.

The herbal anti-aging gel-cream was formulated with the aim to have good spreadability which contain flavonoids that possess anti aging properties. The presence of flavonoids was confirmed by Shinoda's test, zinc hydrochloride test etc. The total phenolic content was confirmed by the Folin Ciocalteu test. Various evaluation tests were being performed such as color, odor, homogeneity etc. Pharmaceutical parameters like spreadability, viscosity, irritancy test, pH was considered. With the aim to study permeability, Franz diffusion was performed.

In future, there is a scope to evaluate the safety and efficacy of this gel-cream formulation by conducting stability studies, in vitro, in vivo studies, antioxidant potential by bioassay and phytoconstituent evaluation. If this formulation proves to be beneficial, a variety of phytoconstituents can be incorporated to further enhance the effectiveness.

4. CONCLUSION

In the present research work, an attempt has been made to formulate a novel herbal anti-aging gel-cream which overcomes the lacunae of existing anti-aging formulations. The prepared formulation displayed good texture and homogeneity, spreadability, no irritation, ease of water washability and acceptable pH. This combination of gel and cream forms a light weight product which is easily absorbed from the skin and delivers hydration.

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