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PREPARATION AND EVALUATION OF ANTIACNE HERBAL CREAM OF *TAGETES ERECTA* FLOWER PETALS

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ABSTRACT

Acne is a chronic inflammatory state. It occurs in the skin due to acne vulgaris. The number of antibiotics resistant to acne and bacterial strains is increasing daily. Herbal anti-acne cream is the best and safest method to prevent from antibiotic resistant. In the present study, we formulated and evaluated an anti-acne herbal cream using methanolic extract of *Tagetes erecta* (Marigold) flower petals. *Tagetes erecta* contains an abundant amount of flavonoids that are responsible for showing anti-microbial activity. The antimicrobial activity of methanolic extract in different concentrations (12.5%, 50%, 100% w/v) was investigated against *Streptococcus aureus* and *Escherichia coli* using the agar well diffusion method. The results of various concentration such as 12.5%, 50% and 100% w/v extracts of *Tagetes erecta* showed good antimicrobial activity. It inhibits bacterial growth which was confirmed by their zone of inhibition 22mm, 25mm and 100mm respectively. The research emphasizes flavonoids showing the anti-acne effect of extracts, significantly affecting the gram-positive and gram-negative bacteria. The present work confirmed the successful antimicrobial activity of *Tagetes erecta* flower petals. So, three batches (F1, and F3) were formulated using the o/w type emulsion method. Furthermore, the evaluation of the herbal cream was carried out based on physical appearance, pH, viscosity, homogeneity, irritancy testing, dye test, spreadability and wetness. F3 formulation showed the best results.

Keywords: *Tagetes erecta*, Antimicrobial activity, Anti-acne herbal cream, *Acne vulgaris*, Well diffusion method

1. INTRODUCTION

Antimicrobial resistance among bacterial strains poses a significant challenge, with the potential for rapid loss of effectiveness of commonly used antibiotics within just five years due to genetic mutations. According to a survey by the World Health Organization (WHO), over 80% of the global population still relies on traditional medicines for treating various illnesses **[1]**. There is a pressing need for alternative treatments with the emergence of multi drug-resistant microbes **[2][3]**. In recent years, there has been a growing demand of traditional medicines in modern societies. This interest stems from the recognition that many modern pharmaceuticals have origins in tropical flora. Traditional medicinal plants have been used for generations as it is safe, affordable and reliable alternatives to synthetic drugs **[4]**. Plant based remedies showed less adverse reactions and it is cost-effectiveness. Thus, allowed researchers to explore them as viable alternatives to conventional pharmaceuticals. The natural world offers a wealth of medicinal plants that serve as a repository of remedies for addressing various human ailments. Herbal plants represent a rich source of healthcare for both preventing and treating a range of pathological conditions. Presently, we focus on exploring the antimicrobial properties of *Tagetes erecta*, commonly known as Marigold **[5]**. Skin care products containing chemicals are becoming more concerning due to their potential negative impacts on skin and overall health. Chemical additives in these products can lead to skin irritation and allergic reactions **[6]**. There has been a growing interest in using herbal or medicinal plants as a natural alternative for skin care **[7]**. Herbal extracts are rich in antioxidants and offer benefits for maintaining youthful and healthy skin, such as compounds like omega-3, vitamins and flavonoids such as apigenin and quercetin. Plant extracts also provide essential nutrients like vitamin E, which promotes skin health **[7][8]**. Marigold, scientifically known as *Tagetes erecta* , is an aromatic herb from the Asteraceae family **[9][10]**. It contains a diverse range of chemical compounds, including saponins, triterpenes, flavonoids and essential oils. Marigold containing flavonoids which has shown significant advantages for skin health, particularly in treating acne. Moreover, *Tagetes erecta* exhibits various therapeutic effects such as antibacterial, antioxidant and anti-inflammatory properties. Also, it is used for wound healing and overall skin health **[11]**. This study aims to explore the chemical constituents and antimicrobial properties of *Tagetes erecta*, focusing on its potential in acne treatment.

2.1 MATERIALS AND METHODS

2.1 Flower Collection and Authentication: Fresh *Tagetes erecta* flowers were procured from a local market and verified by a botanist affiliated with the Department of Botany at Shardabai Pawar Mahila Mahavidyalaya (Maharashtra).

2.2 Extract Preparation: The *Tagetes erecta* flowers was dried in the shade. Then, it was finely ground and subjected to extraction using methanol in a soxhlet apparatus **[12]**. After extraction, the solvent was allowed to evaporate and the resulting extract was stored in a desiccator **[13]**. The % yield of the methanol extract was found to be 47.3%.

2.3 Preliminary Phytochemical Analysis: Analysis was conducted to detect alkaloids, flavonoids, reducing sugars, saponins, phenolic compounds, and tannins, as well as proteins and amino acids in the extract through phytochemical screening **[14]**.

2.3.1 For Carbohydrates: 1 ml of Molisch's reagent and a few drops of concentrated sulphuric acid were added to 2 ml of the plant extract. The presence of carbohydrates was confirmed by the appearance of a purple or reddish color **[15]**.

2.3.2 Test for Reducing Sugars:

i. **Fehling's test:** 1 ml of Fehling's A and 1 ml of Fehling's B solution were combined and boiled for 1 minute. The test solution was added, and the mixture was heated in a boiling water bath for 5-10 minutes, resulting in a yellow and then a brick-red precipitation **[15]**.

ii. **Benedict's test:** Equal volumes of Benedict's reagent and the test solution were combined in a test tube. It was kept for 5 minutes in a boiling water bath. The solution exhibited varying colors (green, yellow, or red) depending on the amount of reducing sugar present **[15]**.

2.3.3 Test for Terpenoids: 2 ml of chloroform and 0.5 ml of concentrated sulphuric acid were carefully added to 0.5 ml of the extract. The presence of terpenoids was confirmed by the formation of a red-brown color at the interface **[16]**.

2.3.4 Test for Saponins (Foam Test): When the extract was vigorously shaken with water, it produced a persistent and stable foam **[15]**.

2.3.5 Test for Alkaloids: Tests for alkaloids were conducted by evaporating the alcoholic extract and treating the residue with diluted HCl. Subsequently, the following tests were performed after thorough shaking and filtering **[15]**:

i. **Dragendroff's test:** When a few drops of Dragendroff's reagent were added to 2-3 ml of filtrate then the formation of orange-brown precipitation was observed.

ii. **Mayer's test:** 2-3 ml of filtrate with a few drops of Mayer's reagent yielded a precipitate.

iii. **Hager's test:** 2-3 ml of filtrate with a few drops of Hager's reagent yielded a yellow precipitate.

iv. **Wagner's test:** 2-3 ml of filtrate with a few drops of Wagner's reagent yielded a reddish-brown precipitate.

2.3.6 Tests for tannins and phenolic compounds: When a few drops of 5% $FeCl_3$ solution were added to 2-3 ml of aqueous or alcoholic extract then a deep blue-black color or discoloration with bromine water was observed **[16]**.

2.3.7 Tests for flavonoids: In the test for flavonoids **[16]**:

i. In the **Sulphuric acid test**, sulphuric acid (66%) was added, resulting in the dissolution of flavones and flavonoids and the production of a deep yellow solution. Flavones, which are responsible for the orange and red hues, dissolved accordingly.

ii. Additionally, ethanolic KOH (2ml) was added to the methanolic extract of marigold flowers, with the presence of flavonoids indicated by the yellow coloration.

2.3.8 Tests of Quinones: For the test for quinones, 1 ml of concentrated sulphuric acid was added to 1 ml of extract, and the presence of quinones was indicated by the appearance of a red color **[15]**.

2.3.9 Tests of Coumarins: In the test for coumarins, 1 ml of 10% NaOH was added to 1 ml of extract, and the presence of coumarins was signaled by the development of a yellow color **[16]**.

2.3.10 Tests of Glycosides: To test for glycosides, 2 ml of plant extract was combined with 3 ml of chloroform and a 10% ammonia solution. The presence of glycosides was revealed by the emergence of a pink color **[17]**.

2.3.11 Borntrager's test: When 1 ml of dilute H_2SO_4 was added to 2 ml of extract for the presence of anthraquinone glycosides. The mixture was then boiled and strained. Equal volume of chloroform was added to the cold filtrate. After vigorous shaking and removal of the organic solvent, ammonia was introduced. Later on, it was found to form ammoniacal layer which turns either pink or red **[12]**.

2.4 Antimicrobial Activity:

2.4.1 Preparation of Inoculum: The inoculum Gram-positive bacteria Staphylococcus aureus (SA) and Gram-negative bacteria Escherichia coli (EC) was prepared from mother culture. It was tested with the methanolic extract of *Tagetes erecta* flowers for antimicrobial properties. Both Gram-positive and Gram-negative bacteria were supplied by the Microbiology laboratory section at Pioneer Pharmacy College, Vadodara, Gujarat, India **[18]**.

2.4.2 Antimicrobial Screening: The antibacterial activity of the extract was assessed using the agar well diffusion method. Nutrient Agar was poured into sterile Petri dishes and thoroughly mixed with bacteria. A sterile cork borer having 6 mm in diameter was used to develop well in Nutrient agar medium after solidification. Then, 1 ml of each extract (12.5%, 50%, 100% w/v) was added to the corresponding wells. The concentrations of the extracts (12.5%, 50%, and 100% w/v) were determined based on preliminary experiments and previous literature. The plates were incubated at 37 °C for 24 hours. The antimicrobial activity was assessed by measuring the zone of inhibition (including the diameter of the wells) observed after the incubation period. DMSO at a concentration of 10% served as the negative control **[19]**.

2.4.3 Determination of Minimum Inhibitory Concentrations: *Tagetes erecta* flower extracts demonstrated antimicrobial activity across all concentrations, with the highest inhibitory action observed at the 100% w/v concentration. Consequently, this concentration was utilized to ascertain the minimum inhibitory concentrations (MIC) using the agar well diffusion method. The MIC was defined as the lowest concentration that inhibited the growth of the respective microorganisms. DMSO served as a control for the methanol extracts **[18]**.

2.5 Formulation of Anti-acne Herbal Cream: Stearic acid was melted in a china dish over a 70°C water bath. Potassium hydroxide and methylparaben (methyl parahydroxybenzoate) were dissolved in water in a beaker, and then glycerin was added to the mixture. This aqueous solution was heated up to 70°C on a water bath. Once both the aqueous and oily phases had reached the same temperature (70°C), the aqueous phase was added to the melted stearic acid while stirring continuously. Stirring was continued after removing the pan from the heat. When the temperature reached 40°C, perfume was added and mixed uniformly until it cooled and became a homogeneous (smooth) cream. The required percentage of extracts (12.5%, 50%, 100% w/v) was measured and added **[20]**.

Figure 1: Cream Prepared using 12.5, 50, 100%v/v *Tagetes erecta* flower extract

Table 1: Different batches of Anti-acne Herbal Cream.

Compositions	F1	F2	F3
Tagetes erecta Flower extract	2ml (12.5%)	2ml (50%)	2ml (100%)
Stearic acid	5.5g	5.5g	5.5g
Potassium hydroxide	0.176g	0.176g	0.176g
Glycerine	2.2ml	2.2ml	2.2ml
Methyl paraben	0.044g	0.044g	0.044g
Perfume	q.s	q.s	q.s
Water	14.10ml	14.10ml	14.10ml

2.6 Evaluation of Cream [20, 21, 22]:

2.6.1 pH: The pH of the prepared cream was measured using a calibrated pH meter. 0.5g of cream was weighed and dissolving in 50.0 ml of distilled water. Later on, the pH of the cream was determined.

2.6.2 Physical Appearance: The physical appearance of the cream was visually assessed against a dark background.

2.6.3 Viscosity: The viscosity of the cream was determined using a Brookfield viscometer at 20 rpm with spindle no. 64.

2.6.4 Homogeneity: Homogeneity of the formulated cream was assessed through visual inspection and touch.

2.6.5 Washability: The cream that was applied to the skin was readily removed by washing with tap water.

2.6.6 Irritation test: The irritation test involved marking a $1cm^2$ on the left dorsal surface of the skin. The cream was applied to the specified area and the time was noted. Irritation, erythema, and edema were evaluated and documented at regular intervals of up to 24 hours.

2.6.7 Dye test: Sudan red dye was mixed with the cream. A drop of the cream was placed on a microscopic slide, covered with a cover slip, and examined under a microscope. If the dispersed globules appeared red against a white background, the cream was classified as o/w type; otherwise, if the dispersed globules appeared colorless, it was categorized as w/o type.

2.6.8 Spreadability: Spreadability was assessed by applying the cream sample between two glass slides and compressing it to a uniform thickness by placing a weight of 100 gm for 5 minutes before adding weight to the weighing pan. The time taken for the upper glass slide to move over the lower glass slide was used to calculate spreadability.

2.6.9 Emollience: If the cream did not leave any residue on the skin's surface after application.

2.6.10 Wetness: Wetness was measured by applying the cream to human skin.

2.6.11 Determination of Moisture Content: Approximately 2 g of the powdered drug was weighed into a flat and thin porcelain dish. It was desiccated at 105°C in an oven. It was observed that until

two consecutive weighing it did not differ by more than 0.5 mg. After cooling in a desiccator, it was weighed again. The weight loss was recorded and moisture loss was calculated **[23]**.

2.6.12 Determination of total Ash Value: 5g of crude drug powder was heated until it forms vapors. Then the dish was lowered and heated until all carbon was burned off. After cooling in the desiccator, the ash was weighed. The percentage of total ash was calculated **[23]**.

2.6.13 Determination of Acid Insoluble Ash: The determination of acid insoluble ash value followed the procedure outlined for determining the total ash value of a crude drug. The ash from the total ash dish was washed with 25 ml of dilute hydrochloric acid. It was boiled for five minutes on Bunsen burner. The resulting mixture was filtered through 'ashless' filter paper. It was washed two times with hot water. The crucible was ignited in the flame, cooled, and weighed. The filter-paper and residue were placed in the crucible and heated gently until no vapors were produced, then more vigorously until all carbon was removed. After cooling in a desiccator, the residue was weighed. The acid-insoluble ash of the crude drug was calculated using an air-dried sample of the crude drug as a reference **[23]**.

2.6.14 Determination of water-soluble ash value: The determination of water-soluble ash value was performed in the same manner as acid insoluble ash. 25ml of water was used instead of dilute hydrochloric acid **[23]**.

2.6.15 Determination of alcohol and water-soluble extractive value: Approximately 4 g of the coarsely powdered drug was weighed and transferred to a dry 250 ml conical flask. The solvent (90% alcohol) and water were added to a 100 ml graduated flask to the delivery mark. After washing out the weighing bottle and pouring the washings and the remaining solvent into the conical flask, the flask was corked and set aside for 24 hours with frequent shaking (maceration). The mixture was filtered into a 50 ml cylinder, and once enough filtrate had collected, 25 ml of it was transferred to a thin porcelain dish used for determining ash values. It was evaporated to dryness on a water bath, then dried in an oven at 105°C for 6 hours. After cooling in a desiccator for 30 minutes, it was weighed, and the extractive percentage w/w was determined based on the air-dried drug **[23]**.

RESULTS

Table 2: Physical Characteristics of Extracts *Tagetes erecta* Flowers

Sr. No.	Solvent used	Physical Characteristics		
		Colour	Consistency	Odor
1	Methanol	Dark brown	Solid sticky	Organic

Table 3: Percentage yield of methanolic extract of *Tagetes erecta* L flowers

Sr. No.	Solvent used	Physical Characteristics	Amount(g)
1	Methanol	Weight of dry powder (g)	30
		Weight of extract (g)	14.2
		%Yield	47.3%

Table 4: Proximate analysis

Moisture content	Ash value			Extractive value	
	Total ash	Acid Insoluble Ash	Water Soluble Ash	Methanol	Water
20%	8.6%	8%	9.1%	35%	39.2%

Table 5: Phytochemical Analysis of *Tagetes erecta*

Sr. No.	Phytochemical Tests	Methanolic Extract
1	Carbohydrates	+
2	Reducing Sugar : Fehling's test	+
	Benedict's test	+
3	Terpenoids	+
4	Saponins : Foam test	+
5	Alkaloids : Dragendroff's test	+
	Mayer's test	+
	Hager's test	+
	Wagner's test	+
6	Tannins and Phenolic compounds	+
7	Flavonoids : Sulphuric acid test	+
8	Quinines	+
9	Coumarins	+
10	Glycosides	+
11	Borntrager's test	-

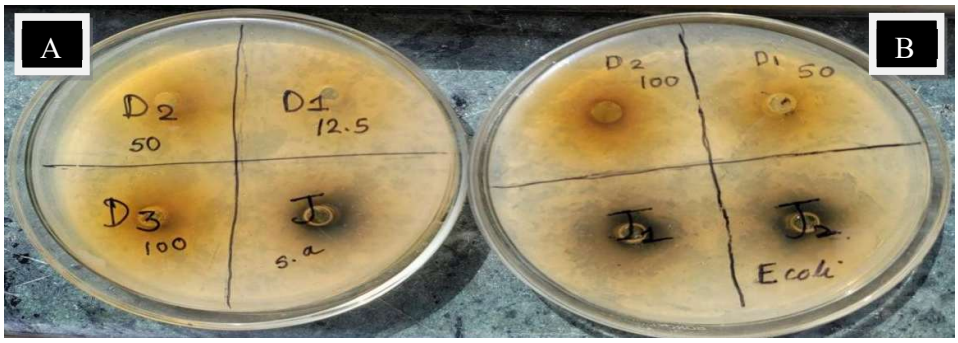

Figure 2: The Inhibition Zone (mm) of Ethanolic Extract (A, B) of *Tagetes erecta* against A- *Escherichia coli* (EC) (B) and *Staphylococcus aureus* (SA) at concentration of 12.5, 50, 100% (w/v) (D1, D2, D3) for (SA) and B- 50,100% for (EC).

Table 6: Antibacterial activity of methanolic extract of *Tagetes erecta* against different strains

Group of Bacterial Strains	The Bacterial Strain tested	Methanol Extract Concentrations	Antibacterial activity of *Tagetes erecta* in terms of inhibition zone (IZ)
Gram+	*Staphylococcus aureus*	12.5%	22mm
		50%	25mm
		100%	29mm
Gram-	*Escherichia coli*	50%	23mm
		100%	26mm

Table 7: Evaluation Parameters

Parameters	Observation
Viscosity At 20rpm, 64 no. spindle	1121 poise
pH	6.4
Colour	Yellow
Odour	Characteristics odour
Appearance	Smooth
Type of smear	Non-greasy
Dye Test	Positive; white globules appeared

CONCLUSION

The herbal anti-acne cream containing *Tagetes erecta* (Marigold) flower petal extract was formulated using the oil-in-water type emulsion method. *Tagetes erecta*, an aromatic herb rich in flavonoids, was utilized for its anti-acne properties. These flavonoids were known to reduce oil secretions from the sebaceous glands and inhibit the growth of bacteria, a key factor in acne development. The antimicrobial activity of methanolic extract at various concentrations (12.5%, 50%, 100% w/v) was investigated against Streptococcus aureus and Escherichia coli using the agar well diffusion method. The results indicated that the 12.5%, 50%, and 100% w/v extracts of *T. erecta* exhibited significant antimicrobial activity **[24]**, as evidenced by their respective zones of inhibition of 22mm, 25mm, and 100mm. The study highlighted the anti-acne effects of flavonoids present in the extracts, which affect both gram-positive and gram-negative bacteria. Overall, the research concluded the successful formulation and evaluation of an anti-acne cream using *Tagetes erecta* extracts, confirming its efficacy in addressing acne concerns.

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