

A RESEARCH PEPAR ON FORMULATION AND EVALUTION OF A POLYHERBAL WOUND HEALING CREAM CONTAINING TRIDAX PROCUMBENS, TULSI AND TITHONIA DIVERSIFOLIA

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ABSTRACT

In India, there is a common weed called Tridax procumbens (also known as coat buttons). People have been using its leaves for centuries to treat wounds. The leaves are crushed into a paste and applied to the injured area. It is believed to help with cuts, wounds, and skin infections because it contains helpful chemicals like alkaloids, tannins, flavonoids and saponins. These chemicals have antioxidant, antibacterial and anti-inflammatory properties, which help wounds heal. However, some studies have shown that it might not always help with healing in some cases. Tulsi (also called Holy Basil) is another plant widely used in India for thousands of years. It has many health benefits because it contains various chemicals like flavonoids, saponins, and phenols. These chemicals are known to be antiseptic, pain-relieving, anti-inflammatory, and antioxidant. It's often used in

traditional medicine for treating infections and improving overall health. Tithonia diversifolia (a shrub or weed), also has medicinal properties. It has been found to have anti-bacterial, anti-inflammatory, and anti-fungal effects. This plant is used by some ethnic communities to treat wounds and reduce inflammation. The study is focused on creating a cream that combines the extracts of Tridax procumbens, Tulsi and Tithonia diversifolia to help wounds heal faster. The plant extracts were mixed into a cream base, making sure the cream has the right texture, stability, and pH for safe use on the skin. Early results show that the cream is effective in killing bacteria and healing wounds. This cream could be a good, natural alternative to synthetic creams for wound care. However, more clinical tests are needed to confirm if it is completely safe and effective for widespread use.

KEYWORDS: Polyherbal cream, *Tridax procumbens*, Tulsi, *Tithonia Diversifolia*, Wound Healing Properties.

INTRODUCTION

A wound is defined as an injury to the skin's epithelial layer, which compromises its integrity. According to the World Health Organization (WHO), wounds can lead to the breakdown or opening of the skin, disrupting its structure, function, and physiological processes. The wound-healing process involves the regeneration of tissue to restore the skin's normal structure and function. Proper healing is crucial for restoring the skin's anatomical continuity and functional state. One of the common and difficult challenges in wound care is impaired wound healing. The most effective wound-healing methods minimize tissue damage, optimize blood flow to the area, remove nonviable tissue, and maintain a moist healing environment. Topical treatments that promote blood vessel growth, collagen production, and skin regeneration are particularly beneficial.

In this context, ancient herbs with known medicinal properties, such as *Tridax procumbens*, Tulsi and *Tithonia diversifolia*, have gained attention for their potential in wound healing. *Tridax procumbens*, also known as "coat buttons" in English and "Ghamra" in Hindi, is commonly found in tropical and subtropical regions. It is widely used in Ayurvedic medicine for various conditions, including wound healing, due to its anti-inflammatory, antimicrobial, and wound-healing properties. Tulsi, a fragrant perennial herb from the *Ocimum* species, has been recognized since ancient Vedic times for its therapeutic benefits, including its use in wound healing, as well as for respiratory and digestive issues. Lastly, *Tithonia diversifolia*, also known as the Mexican sunflower, is a subtropical plant native to Central America but widely found in other regions as well. Traditionally, its extracts have been used for a range of ailments, including wound healing, diabetes, and malaria.

This study focuses on the formulation of a polyherbal cream combining *Tridax procumbens*, Tulsi and *Tithonia diversifolia*, with the aim of creating a natural and effective treatment for common skin conditions. The research includes an in-depth analysis of the active compounds in these herbs that contribute to the cream's medicinal properties, followed by the formulation process using validated techniques. The final cream undergoes rigorous testing to assess its stability, efficacy, and other essential parameters, ensuring its therapeutic potential. The study seeks to contribute to the growing body of evidence supporting polyherbal treatments by integrating traditional knowledge with modern scientific approaches.

MATERIAL AND METHODS

TRIDAX PROCUMBENS

Traditionally in India, the fresh juice from the leaves of *Tridax procumbens* a plant in the genus *Tridax* and family Asteraceae, commonly known as Coat Buttons or Tridax Daisy has been used as one of the most popular remedies for dermal wounds. *Tridax procumbens* is a small, perennial herbaceous plant with a creeping habit. It has slender, hairy stems that can reach up to 1 meter long. The leaves are opposite, ovate-elliptical in shape, and have serrated margins. The flowers are yellow or white and are arranged in heads that are 1-2cm in diameter. This plant is native to tropical America but has been widely distributed throughout the nation, including India, especially in Maharashtra, Madhya Pradesh, and Chhattisgarh where it is considered an invasive species. *T. procumbens* can bloom all year long and is a prolific bloomer. It is a typical weed found in wastelands, lawns, and gardens.



Fig. *Tridax procumbens*.

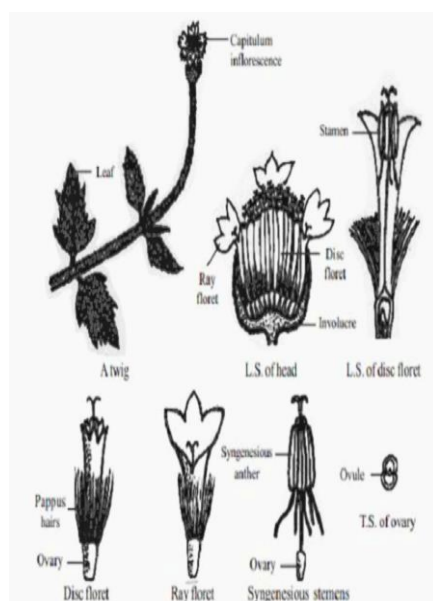


Fig. Schematic diagram of *Tridax procumbens* showing all its parts.

Kingdom	Plantae
Sub kingdom	Tracheobionta
Division	Spermatophyta
Subdivision	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Genus	Tridax
Species	procumbens

CLASSIFICATION

Collection of plant material

Fresh leaves of *Tridax procumbens* were collected in the months of May and June from the areas around the College located in Virgaon, Akole. The leaves of the plant were washed with running water and were kept for drying.



Fig. Collection and washing of leaves of *T. procumbens*.

Preparation of Liquid Extracts

Leaves of *T. procumbens* were first ground into a fine paste from which the obtained paste was filtered twice through filter paper.



Fig. Grinding of *Tridax procumbens* leaves.



Fig. Soxhlet extraction of *Tridax procumbens*

TULSI

Tulsi, moreover known as *Ocimum sanctum* or Sacred Basil, plays a noteworthy part in wound recuperating due to its different phytochemical constituents. Thinkers about have appeared that Tulsi extricates can upgrade wound mending by:

1) Expanding wound compression rate

Tulsi watery extricate has been found to extend the rate of wound withdrawal, subsequently quickening the recuperating prepare.

2) Moving forward wound-breaking quality

The extricate has also been appeared to extend wound-breaking quality in entry point wound models, demonstrating made strides tissue quality and flexibility.

3) Improving epithelization

Tulsi extricate can speed up the epithelization prepare, which is significant for wound closure and tissue recovery.

4) Neutralizing anti-healing impacts

Tulsi extricate has been found to decrease the anti-healing properties of dexamethasone, a steroid known to hinder wound mending.



Fig. Tulsi Plant.

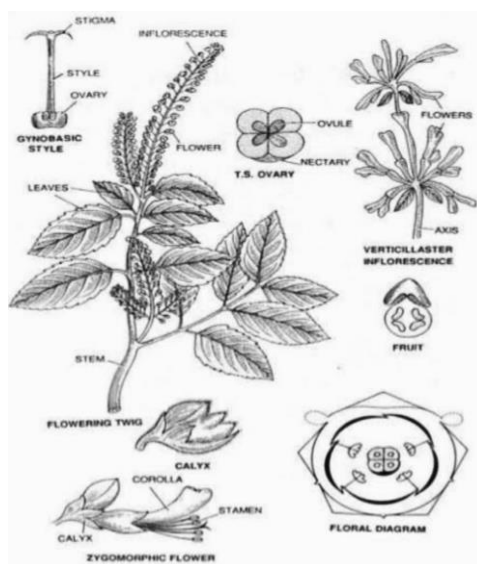


Fig. Schematic diagram of *Tridax procumbens* showing all its parts.

Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	Ocimum
Spices	tenuiflorum or sanctum.
B. Name	Ocimum sanctum

CLASSIFICATION

Collection of plant material

Fresh leaves of Tulsi were collected in the months of May and June from the areas around the College located in Virgaon, Akole. The leaves of the plant were washed with running water and were kept for drying.



Fig. Collection and washing of leaves of Tulsi.

Preparation of Liquid Extracts

Leaves of Tulsi were first ground into a fine paste from which the obtained paste was filtered twice through filter paper.



Fig. Grinding of Tulsi leaves.



Fig. Maceration extraction of Tulsi leaves.



Fig. filtration process of Tulsi leaves.

TITHONIA DIVERSIFOLIA

Tithonia diversifolia, a plant species, has been studied for its potential role in wound healing. Research suggests that *Tithonia diversifolia* extracts possess wound-healing properties, which may be attributed to its:

1. Anti-inflammatory effects: Reducing inflammation and promoting tissue repair.
2. Antimicrobial properties: Preventing infection and promoting a clean wound environment.
3. Antioxidant activity: Reducing oxidative stress and promoting tissue regeneration.

The plant's extracts have been shown to enhance wound contraction, improve tissue strength, and promote epithelization, ultimately accelerating the wound-healing process.



Fig. of *Tithonia diversifolia* plant.



Fig. Schematic diagram of *Tithonia diversifolia* showing all its parts.

CLASSIFICATION

Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Genus	<i>Tithonia</i>
Subject	<i>Tithonia diversifolia</i> (Hemsl.) Gray

Collection of plant material

Fresh leaves of *Tithonia diversifolia* were collected in the months of May and June from the areas around the College located in Virgaon, Akole. The leaves of the plant were washed with running water and were kept for drying.



Fig. Collection and washing of leaves of *Tithonia diversifolia*.

Preparation of Liquid Extracts

Leaves of *Tithonia diversifolia* were first ground into a fine paste from which the obtained paste was filtered twice through filter paper.



Fig. of Grinding of *Tithonia diversifolia* leaves.



Fig. of Soxhlet extraction of *Tithonia diversifolia* leaves.

QUALITATIVE ANALYSIS

A series of phytochemical tests were performed to evaluate and confirm the presence of bioactive compounds present in *Tridax procumbens*, *Tulsi* and *Tithonia diversifolia*.

1. Alkaloid Test

To detect the presence of alkaloids, add a small amount of plant sample paste to a test tube, and then add 2 drops of Mayer's reagent carefully along the sides of the tube. If a white or Smooth precipitate is formed, it indicates the presence of alkaloids.

2. Carbohydrate Test

To test for the presence of carbohydrates in the plant paste, two to three drops of the Molisch reagent were added to the plant paste and mixed in a test tube. Then, roughly 2-3 drops of sulphuric acid were added. The appearance of a violet ring indicates the presence of carbohydrates.

3. Protein Test

Add 4-6 drops of copper sulfate solution and 2 ml of sodium hydroxide to the plant paste, mix the contents well by gently shaking the test tube, and allow the mixture to rest for about 4-5 minutes. A bluish-violet color indicates the presence of protein.

4. Amino Acid Test

After adding plant paste to a test tube, a few drops of ninhydrin reagent were introduced. The test tube was then heated to 40°C and incubated for seven to ten minutes. The appearance of a green or purple color indicates the presence of amino acids in the paste solution.

5. Saponin Test

A plant paste was placed in a test tube, followed by vigorous shaking and the addition of a few drops of double-distilled water. The formation of foam indicates the presence of saponin.

6. Terpenes Test

After adding plant paste to a test tube, a small amount of FeCl solution was introduced. If a deep or dark green color appears, this indicates the presence of tannins.

7. Glycoside Test

To detect the presence of glycosides in a plant paste solution, 1 ml of the paste was mixed with 2 ml of H₂SO₄ and 2 ml of chloroform in a test tube. The solution was thoroughly mixed, and a reddish-brown color ring occasionally appeared, indicating the presence of glycosides.

8. Phenolic Test

To detect the presence of phenols in a plant paste, the paste is first dissolved in water or a mixture of water and ethanol. Then, a few drops of neutral ferric chloride solution (FeCl₃) prepared in deionized water are added to the mixture. Sodium hydroxide is then added until a permanent brown precipitate is formed. The appearance of a red, green, or purple color is indicative of the presence of phenols in the paste.

9. *Flavonoid Test*

When an aqueous plant crude paste was mixed with a 20% NaOH solution, a deep yellow color was observed, which indicated the presence of flavonoids. The addition of a few drops of dilute acid neutralized the color, confirming the existence of a few drops of dilute acid neutralized the color, confirming the existence of flavonoids in the paste.

QUANTITATIVE ANALYSIS

1. *Estimation of Total Alkaloids Content*

10% of acetic acid is added in ethanol, covered, and left to settle for a few hours. To the extract concentrated ammonium hydroxide is added dropwise until the precipitation is complete. The solution is then allowed to settle and the precipitate is collected and washed with dilute ammonium hydroxide followed by filtration, drying, and weighing.

2. *Determination of Tannin Content*

Make aliquots of extract in test tubes, and make up the volume accordingly with distilled water and Folin-Ciocalteu reagent followed by the addition of sodium carbonate solution. Vortex the tubes and record absorbance at 725nm.

3. *Estimation of Total Flavonoid Content*

A standard curve was created by preparing various concentrations of gallic acid in methanol, while quercetin was dissolved in methanol to produce a final concentration of quercetin. Then, Al:Ch was added, and the mixture was incubated for 5 minutes before adding NaOH. The absorbance at 510 nm was measured, and the flavonoid content was estimated in terms of quercetin equivalents.

4. *Estimation of Total Phenolic Content*

A reaction mixture was created by combining extracts with 10% Folin-Ciocalteu's reagent, and 5 ml of 7% Na₂CO₃ solution in 13 ml of deionized water. The mixture was allowed to react for two hours at room temperature in the dark, after which the absorbance was measured at 760 nm using a spectrometer. The mean absorbance value was obtained after three repetitions of the experiment, and the total phenolic content was calculated using a calibration curve created with a gallic acid solution.

5. Estimation of Steroids

1 ml of extract was transferred into volumetric flasks. Sulphuric acid and Iron chloride were added followed by potassium hexacyanoferrate solution. This mixture was heated for 30 minutes and diluted. The absorbance was measured at 780nm.

RAW MATERIALS

- **Beeswax** plays a key role in cold cream as an emulsifying agent that helps the oil penetrate the skin. Beeswax is commonly used in wound healing creams due to its natural anti-inflammatory, anti-bacterial, and moisturizing properties. It creates a protective barrier on the skin, traps moisture, and helps to heal wounds by stimulating collagen generation and cell proliferation.
- **Almond oil**, can help soothe irritated skin, reduce swelling, and potentially expedite the healing of minor wounds and cuts. can be a beneficial ingredient in wound healing creams due to its moisturizing, anti-inflammatory, and antioxidant properties. It's rich in vitamin E, which helps with skin repair and reduces inflammation, making it suitable for soothing irritated skin and promoting healing. Almond oil also helps maintain skin hydration and can contribute to the appearance of reduced scars.
- **Rose water**, It can help speed up healing, reduce inflammation, and prevent infections. Rose water often included in wound healing creams due to its anti-bacterial and antiseptic properties, which help to keep wounds clean and prevent infection. It also has anti-inflammatory properties, which can help reduce redness and swelling around wounds, and its soothing effect can provide comfort to the affected area.
- **Borax** acts as an emulsifier, meaning it helps to mix together the oil and water components of the cream, creating a stable and smooth consistency by reducing surface tension. Boron-containing compounds, like borax, are being investigated for their potential in wound healing due to their anti-inflammatory, antimicrobial, and antioxidant properties. Borax can be used as a crosslinking agent in hydrogels for wound dressings, though its use in this context may be limited by inadequate mechanical properties.

While boric acid, a derivative of borax, is sometimes used in wound treatment, its use is limited due to its potential toxicity.

- **Methanol** is a good solvent for extraction because of its polarity, volatility, and ability to extract a variety of compounds. Methanol (CH₃OH) is a type of alcohol, and it can be present in some wound healing creams, particularly those derived from plant extracts. Methanol is used as a solvent in the extraction process to isolate active compounds from plants, says a research article on methanol extracts and wound healing. In some cases, the methanol solvent might be present in the final cream formulation, while in others, it's removed during the extraction and preparation process.
- **Methyl paraben**, is sometimes used as a preservative in wound healing creams to prevent bacterial and fungal growth, extending the product's shelf life and potentially reducing the risk of infection in the healing wound. Methyl paraben is a synthetic preservative widely used in wound healing creams to prevent microbial growth and extend shelf life, according to Clinikally. It inhibits the growth of harmful bacteria and molds, ensuring the product remains stable and free from contamination.
- **Distilled water**, can be used in wound healing creams and for wound cleansing, but its effectiveness compared to other options like saline is not definitive. While some studies suggest distilled water can be as effective as saline for wound irrigation, other research indicates that isotonic saline may be preferable. Ultimately, the best approach for wound healing involves a moist environment and keeping the wound protected from external factors.

INSTRUMENTS AND EQUIPMENTS

1. Mortar and pestle for grinding the powdered ingredients.
2. Weighing balance and spatulas for ingredient measuring.
3. Water bath for heating the ingredients.
4. Porcelain dish for evaporating liquids or boiling solutions.
5. Measuring cylinders for accurate ingredient proportions.
6. Soxhlet apparatus is a laboratory instrument used for continuous ex-traction of compounds from a solid matrix using a solvent.
7. Reagent bottles, particularly narrow-mouth bottles with screw caps, are commonly used in maceration processes.
8. Pair of tongs for safely handling hot and hazardous objects.
9. Sterilized containers for storing the cream.

FORMULATION OF POLYHERBAL CREAM

The formulation of herbal cream was carried out using all the components mentioned in the Table below. Two different phases (water phase and oil phase) were prepared separately and then mixed. After mixing all the components, homogenization was carried out by giving constant stirring for 20 minutes.

INGREDIENTS	QUANTITY	ROLE
Tridax Proumben	1 ml	Main API
Tulsi	1 ml	Anti bacterial
Tithonia diversifolia	1 ml	Wound healing
Almond oil	8 ml	Moisturizer
White bees wax	3 gm	Thickner
Borax	0.20 gm	Emulsifier
Distilled water	5 ml	Solvent
Rose water	Q.S.	Perfume
Methyl paraben	0.02 gm	Preservative

FORMULA

PROCEDURE

In Oil Phase add: Beeswax, Almond oil, Liquid paraffin Heat gently (around 70–75°C) until beeswax completely melts and blends.



In Aqueous Phase add: Borax, Rose water and methyl paraben Heat to the same temperature (70–75°C) to dissolve borax completely.



Add the herbal extracts to the warm aqueous phase and mix thoroughly.



Slowly pour the aqueous phase into the oil phase with continuous stirring.



Stir until a uniform cream is formed and the temperature drops to room temperature.



add remaining rose water for fragrance.



Fig. Formulation of cream.

RESULT

PHYTOCHEMICAL ANALYSIS

The below table shows the results of the qualitative analysis, carried out from the extract of *Tridax procumbens*, *Tulsi* and *Tithonia diversifolia*.

Tridax procumbens

Table. Qualitative determination of bioactive compounds present in *Tridax procumbens*.

NAME OF TEST	INFERENCE
Alkaloid	+
carbohydrate	-
Protein	-
Amino acid	-
Tannin	+
Saponin	+
Glycoside	-
Phenol	+
Flavonoid	+
Steroid	+



Fig. Phytochemical analysis of *Tridax procumbens*.

Tulsi**Table. Qualitative determination of bioactive compounds present in Tulsi.**

NAME OF TEST	INFERENCE
Alkaloid	+
carbohydrate	-
Protein	-
Amino acid	-
Tannin	+
Saponin	+
Glycoside	-
Phenol	+
Flavonoid	+
Steroid	-

**Fig. Phytochemical analysis of Tulsi.****Tithonia diversifolia****Table. Qualitative determination of bioactive compounds present in Tithonia diversifolia.**

NAME OF TEST	INFERENCE
Alkaloid	-
carbohydrate	+
Protein	+
Anthraquinone	-
Tannin	+
Saponin	+
Glycoside	-
Phenol	-
Flavonoid	+
Steroid	-



Fig. Phytochemical analysis of *Tithonia diversifolia*.

Evaluation of Cream

- **Physical Appearance:** Visual inspection is used to assess physical appearance. The formulated product's color, odor, and condition all are included.
- **pH Determination:** pH is determined on a scale of 10. The pH was determined using litmus paper.



- **Spreadability:** The spreadability of the formulated cream is calculated by measuring the diameter I ml of the product is poured on a glass plate using a pipette and leave it for 5 minutes. The diameter is measured after 5 minutes using a scale.

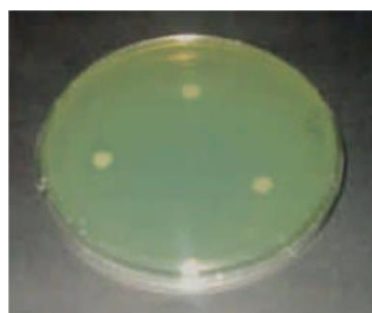


- **Washability:** determines how easily a cream can be removed from the skin using water.



➤ **Antimicrobial Testing**

An antimicrobial susceptibility test was carried out against *E. coli* and *Bacillus* by disc diffusion method. It typically involves evaluating the ability of a substance or product to kill or inhibit the growth of microorganisms that may cause infection in a wound.



➤ **Stability Test**

The formulated cream was kept under two different temperatures (at room temperature and 4°C) for thirty days following the formulation's first day. characteristics including the cream's color, consistency, and homogeneity were noted. The cream samples were stable at both temperatures after 25 days as no alterations in the observation were noticed.

NO. OF DAYS	PARAMETERS	4°C	ROOM TEMP
DAY1	Colour Consistency Homogeneity	Unchanged Smooth Homogenous	Unchanged Smooth Homogenous
DAY 5	Colour Consistency Homogeneity	Unchanged Smooth Homogenous	Unchanged Smooth Homogenous
DAY 10	Colour Consistency Homogeneity	Unchanged Smooth Homogenous	Unchanged Smooth Homogenous
DAY 15	Colour Consistency Homogeneity	Unchanged Smooth Homogenous	Unchanged Smooth Homogenous
DAY 20	Colour	Unchanged	Unchanged

	Consistency Homogeneity	Smooth Homogenous	Smooth Homogenous
DAY 25	Colour Consistency Homogeneity	Unchanged Smooth Homogenous	Unchanged Smooth Homogenous

OBSERVATION

Table. Parameters observed on the day of formulation.

PARAMETERS	OBSERVATIONS
Colour	Green
Odour	Pleasant
Consistency	Smooth
State	Semisolid
pH	7.2
Homogeneity	Homogenous
Spreadability	16 mm
Washability	Washing

DISCUSSION

The results obtained from various evaluations properties indicated that the prepared herbal wound healing cream was smooth and fine in texture with medium greenish and mild, refreshing herbal aroma smell. The prepared cream was easily Spreadable . Irritancy test did not showed any signs of redness, rashes, and swelling. While the pH of the formulation was 6-7. Which is close to skin pH, indicating that the wound healing cream is suitable for application on skin to treat wound. Formulation can be easily washed off from skin with no signs of stains on skin.

CONCLUSION

In conclusion, Tridax procumbens, Tulsi and Tithonia diversifolia are powerful medicinal plant with properties that make them ideal for cream formation their wound healing, anti-inflammatory, antibacterial, and antifungal properties make them effective in treating a variety of illnesses, ailments, and skin conditions, including eczema, psoriasis, and acne. Furthermore, the process of cream formation from Tridax procumbens, Tulsi and Tithonia diversifolia are relatively simple and can be done using readily available materials. This makes them an accessible and affordable treatment option for many people The phytochemical constituents such as alkaloids, flavonoids, tannins, saponins, and other phenolic components are thought to play an important role in the healing of wounds by significantly raising the rate of wound closure and epithelization. Using Tridax procumbens,

Tulsi and *Tithonia diversifolia* cream will not only provide relief from skin conditions but will also promote overall skin health.

Thus, it can be said that herbal formulations are a valuable gift from nature. Herbal medicines are thought to be safer than allopathic ones because allopathic medicines tend to have adverse side effects. In a similar vein, *Tridax procumbens*, Tulsi and *Tithonia diversifolia* cream will be a valuable addition to any medicine cabinet. Their effectiveness, accessibility, and affordability make it a must-have for anyone looking for a natural and safe way to treat their skin conditions.

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