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# Journal of Drug Delivery and Therapeutics

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Research Article

## Preparation and Evaluation of Wound Healing Gel Using Extract of *Psidium guajava* Leaves and *Tridax Daisy* Leaves

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### Article Info:



#### Article History:

Received 21 Oct 2024

Reviewed 04 Dec 2024

Accepted 30 Dec 2024

Published 15 Jan 2025

#### Cite this article as:

Daingade PS, Bhosale KP, Ugale PD, Mundhe VG, Shelke TT, Preparation and Evaluation of Wound Healing Gel Using Extract of *Psidium guajava* Leaves and *Tridax Daisy* Leaves, Journal of Drug Delivery and Therapeutics. 2025; 15(1):117-124  
 DOI: <http://dx.doi.org/10.22270/jddt.v15i1.6960>

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### Abstract

Wounds are physical injuries that results in an opening or breaking of the skin. Proper healing of wounds is very essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. Wound healing is a complex but generally orderly process. Sequential waves of specialized cell types first clear the inciting injury and then progressively build the scaffolding to fill in any resulting defect. The antibacterial potential of the crude leaves extracts of *Psidium guajava* Linn. against some bacteria associated with surgical wound, burns, skin and soft tissue infections were investigated under different conditions. *Tridax procumbens* (L.) is a spreading annual herb found throughout India. The plant is a native of tropical America and naturalized in tropical Africa, Asia, and Australia. Local people known it as "Ghamara," in English popularly called "coat buttons" and is dispensed for "Bhringraj" by some of the practitioners of Ayurveda. The plant has many pharmacological applications such as hepatoprotective activity, anti-inflammatory, wound healing, anti-diabetic activity, hypotensive effect, immunomodulating property, bronchial catarrh, dysentery, and diarrhea and to prevent falling of hair promotes the growth of hair, and antimicrobial activity against both Gram-positive and Gram-negative bacteria. The leaf juice possesses antiseptic, insecticidal and parasiticidal properties, as a remedy against conjunctivitis and is used also to check hemorrhage from cuts, bruises and wounds insect repellent.

**Keywords:** Preparation, Evaluation, pharmacological effect, Wound Healing Gel, Antiseptic.

## 1. INTRODUCTION:

Wound healing, is a common clinical entity, contemporary to mankind. No condition in medicine has been so extensively investigated as wound healing<sup>1</sup>. Several plants and their products are used in folk medicine to treat wounds. Plants like *Tridax procumbens*<sup>2</sup> and *Bryophyllum Pinnatum*<sup>3</sup> have been reported to promote wound healing.

Wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue. It is produced by physical, chemical, thermal, microbial, or immunological damage to the tissue. Wound healing or wound repair is the body's natural process of regenerating dermal and epidermal tissue.

Healing requires the collaborative efforts of various tissues and cell lineages. It involves aggregation of platelets, clotting of blood, fibrin formation, and an inflammatory response to injury, alteration in the ground

substances, angiogenesis and re-epithelialisation. Healing process is not complete until the disrupted surfaces are firmly knit by collagen<sup>4-7</sup>.

### Gels

Gels are semisolid dosage forms which is mostly used for topical application purpose. Gels are also administered for ocular, nasal, vaginal or rectal route. A gel is a semi-solid that can have properties ranging from soft and weak to hard and tough.

### Wound

A wound is a damaged area of the body, such as a cut or hole in the skin or flesh made by a weapon. It can also mean a problem or great unhappiness.

The wound has been described as a result of disruption of the anatomical or useful function of living tissue due to physical, chemical, microbic or electrical shock. Wound

healing is the body's process of making dermal and epidermic tissue.

Cooling is activated when platelets in the blood are found in contact with the exposed albuminoid which leads to platelet aggregation and the absence of natural process factors in the formation of protein clots at the site of injury<sup>8</sup>.

### Open the wounds

In this case the blood flows out of the body and the bleeding is clearly visible. Any classified as; cuts, bruises or cuts of wounds, bruises or external wounds, piercing wounds, penetration wounds and gunshot wounds<sup>9</sup>.

### Bad wounds

It may be tissue damage that generally precedes the systematic and timely recovery that leads to the continuous restoration of anatomic and purposeful integrity. Separate the measurement sometimes caused by cuts or surgical incisions and complete the wound healing process within the expected time 10.

World Health Organization (WHO) as well our country has been promoting traditional medicine because they are less expensive, easily available and comprehensive, especially in developing countries<sup>11-12</sup>.

It is also true that eight percent of the world's population relies on medicinal plants for their primary health care. Whole world including the developed country recognized the importance of traditional medicine and has treatment strategies, guidelines and standard for ethnomedicine<sup>13-14</sup>.

After thorough review of Ayurvedic and Homeopathic system of medicine we have selected following herbs to formulate the cream for wound healing action -

- *Psidium Guajava* leaves extract (Anti-bacterial, Anti-microbial, Anti-oxidant property)
- *Tridax daisy* leaves extract (antiseptic, insecticidal and parasitocidal properties, Anti -Inflammatory)

In this we have made an attempt to formulate most complete herbal cream that contains herbs which will satisfy almost all the mechanism to heal a wound effectively.

### Plant Profile:-

#### 1. *Psidium guajava* leaves:-

- Synonym- Yellow guava leaves, lemon guava leaves
- Biological source: guava trees are native to tropical areas from Southern Mexico to Northern South America.
- Family: Myrtaceae
- Uses: wound healing, stomach ache, intestinal condition, diabetes, etc
- Chemical constituents: Coumarin, Tannins, Saponin, Triterpenoids, Sesquiterpenoids, Flavonoids (Guaijaverin, avicularin)



Figure 1: Guava leaves

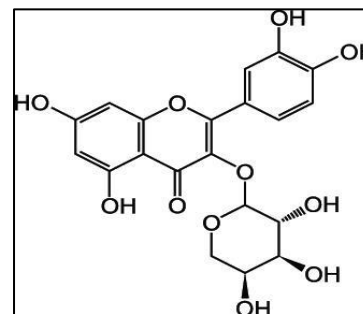


Figure 2: Avicularin

(Flavonoids (Guaijaverin, avicularin) have anti Infectious activity and show potent effect against bacteria that resist in wound healing)

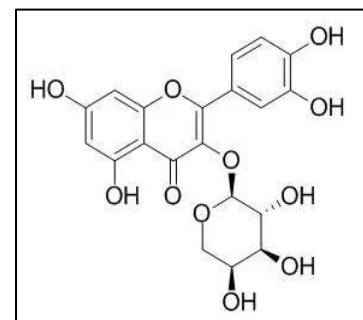


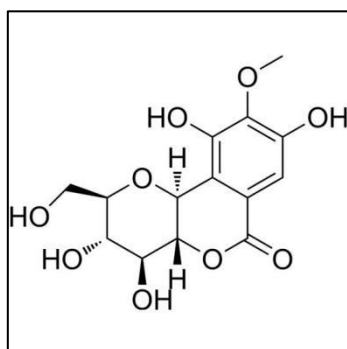
Figure 3: Guaijaverin

#### 2. *Tridax Procumbens* leaves:

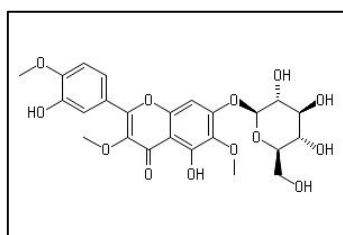
- Synonym: Coatbuttons leaves, tridax daisy leaves, kutkuti leaves, jayanti veda leaves.
- Biological source: widespread weed and pest control plant. Native to the tropical America but it has been introduced to tropical and subtropical and mild temperature regions worldwide.
- Family :- Asteraceae
- Uses: - treat bacterial infection, anticoagulant, antifungal, anti-inflammatory, insects repellent, analgesic activity.
- Chemical constituents:- Alkaloids, Steroids, fatty acids, Saponin, Flavonoids (catechin, centaurein, bergenin)



**Figure 4: Tridax Daisy**



**Figure 5: Centaurein**



**Figure 6: Bergenin**

Helpful in treating bacterial infection Anti-inflammatory activity and help in burns woundhealing.

## 2. MATERIALS AND METHODS

### Material

#### Collection Of Plant Material

Fresh leaves of guava plant and tridax daisy plant were collected from plants they were cleaned and washed with water and allowed to shed dry for 5-6 hours.

#### Chemical Reagents.

The chemicals used during the experiments were of analytical grade.

Carbapol 940 (Cosmo chem), Triethanolamine (Research lab fine chem industries, Mumbai), Methyl Paraben (Research lab fine chem industries, Mumbai), Ethanol (Research lab fine chem industries, Mumbai), Glycerine (Thermosil fine chem industries)

- Carbapol 940 (Gelling agent)

- Triethanolamine (Buffer the pH)
- Methyl Paraben (Preservative)
- Ethanol (Extraction)
- Distilled water (Vehicle)
- Glycerine (Moisturizer)

#### Instruments

- Digital weighing balance
- Beakers
- Conical flasks
- Digital pH meter
- Morter& pestle
- Mechanical stirrer
- Water bath
- Gas burner
- UV- Spectrophotometer

#### Methods

##### Extraction of plant materials

10 g of the powdered sample of the plant was soaked in 100 ml of solution of ethanol and water in concentration of 70 ml and 30 ml respectively in a 250 ml conical flask at room temperature (32 – 35 °C) with shaking after every 4 hrs for 24 hrs.

The extract was filtered using muslin cloth and then Whatman no.1 filter paper. The filtrates was then evaporated to dryness in a rotary evaporator maintained at 45°C to remove residual solvents and then stored in screw capped bottles for further use.

The same experimental procedures were used for the other solvents, namely, acetone and methanol.

##### Qualitative analysis of powdered drug:

UV-Visible spectroscopic method-

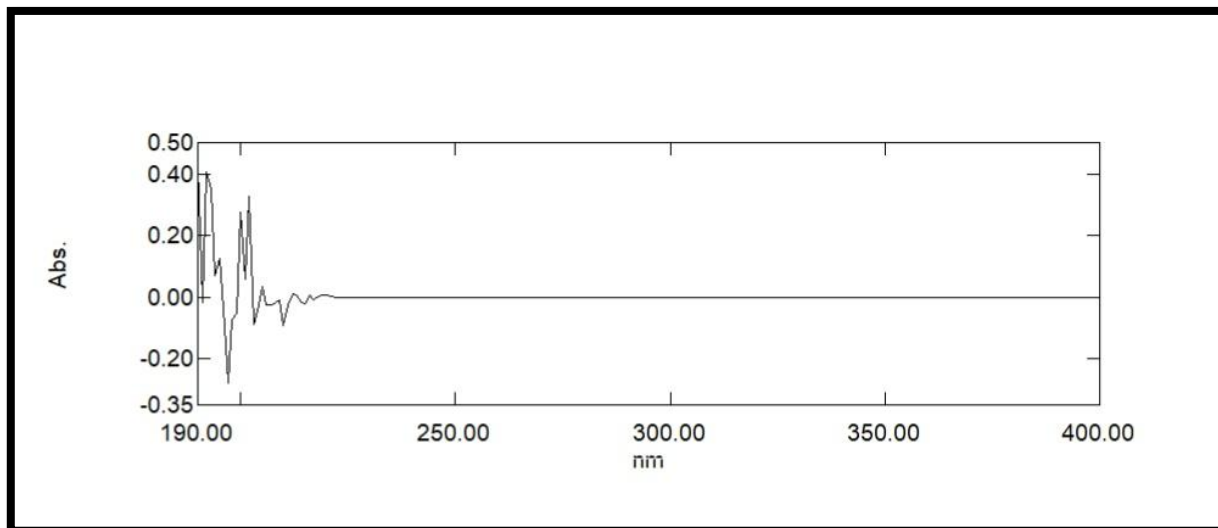
##### A. Psidium guajava leaves

1. Preparation of standard stock solution of Psidium guajava leaves (guava leaves) extract:

An accurately weighed quantity of 100 mg of guava leaves extract was taken in 100 ml of volumetric flask with a part of distilled water and dissolved and then volume was made up to 100 ml.

2. Preparation of working standard:-

The standard stock solution of guava leaves extract was further diluted with distilled water to get the 10 ppm concentration solution and scanned in UV range of 200-400nm.

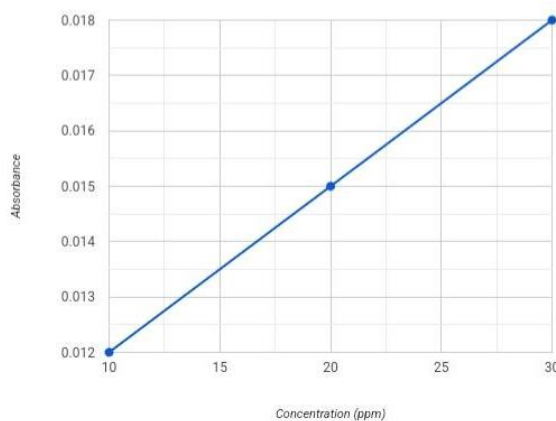


**Figure 7: Overlain UV spectra of Psidium guajava leaves extract in water.**

From the above spectra of guava leaves extract at 10 ppm concentration the  $\lambda_{max}$  is 192nm.

**Table 1: Linearity data of guava leaves extract**

Concentration (ppm)	Absorbance of guava leaves extract at 192 nm
10	0.012
20	0.015
30	0.018



**Figure 8: Linearity data of Psidium guajava leaves extract.**

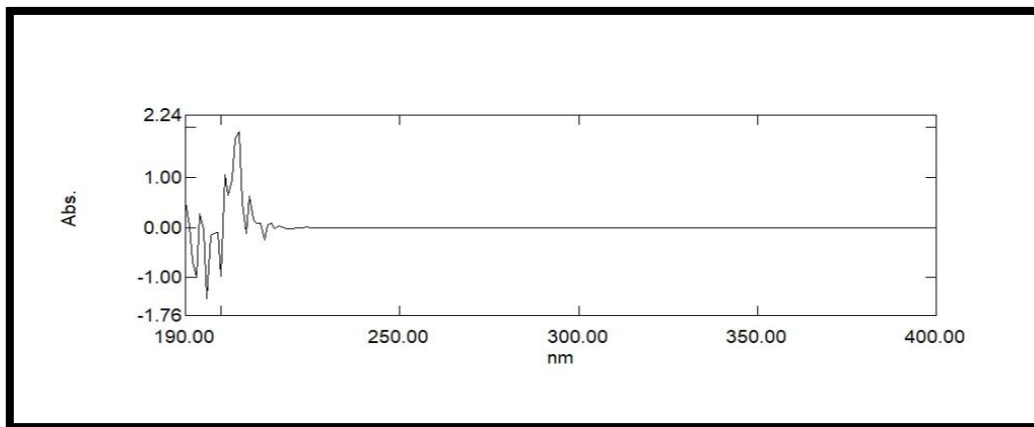
**B. Tridax Procumbens leaves:**

1. Preparation of standard stock solution of Tridax Procumbens leaves( tridax daisy leaves) extract:

An accurately weighed quantity of 100 mg of tridax daisy leaves extract was taken in 100 ml of volumetric flask with a part of distilled water and dissolved and then volume was made up to 100 ml.

2. Preparation of working standard:

The standard stock solution of tridax daisy leaves extract was further diluted with distilled water to get the 10 ppm concentration solution and scanned in UV range of 200-400nm.

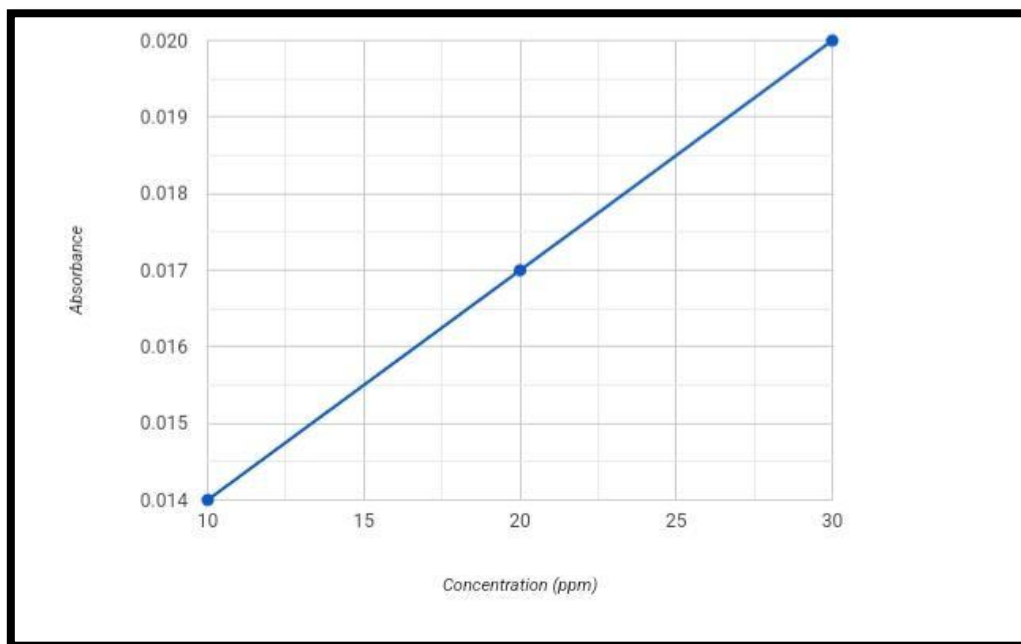


**Figure 9: Overlain spectra of tridax Procumbens leaves extract.**

From the above spectra of tridax daisy leaves leaves extract at 10 ppm concentration the  $\lambda_{max}$  203 nm

**Table 2: Linearity data of Tridax Procumbens leaves extract**

Concentration	Absorbance of tridax daisy leaves extract at 203 nm
10	0.014
20	0.017
30	0.020



**Figure 10: Linearity data of tridax Procumbens leaves extract.**

**Table 3: Phytochemical screening test for Guava & Tridax daisy leaves.**

1.Test for flavonoids	Result	Inference
Extracted Sample +Conc.Hcl+Mg powder	Yellow to yellowish orangecolour	Presence of flavonoids.
2.Test for Saponin	Result	Inference
Extracted Sample +Hotwater + Conc.Hcl	Foam formation occur	Presence of saponin.
3.Test for Tannins	Result	Inference
Extracted Sample + Fecl <sub>3</sub> (1%)	Blue to brownish green colour	Presence of tannins

### 3. FORMULATION OF HERBAL GEL

The required amount of Carbopol 940 was weighed and taken in a 500 ml beaker. Using a mechanical stirrer, the mixture is mixed with 100 ml of de-mineralized water for 1hr to avoid agglomeration. Add required quantity of Triethanolamine and Glycerine to the mixture, it forms a proper gel base and the pH was adjusted to 7 to 8 [15].

Take required quantity of both the extract and triturate with mortar and pestle. Add the rest of the essential oils and triturate them until a homogeneous mixture is formed. Add the mixture to the gel base until it gets uniformly mixed.

#### Formulation

The following ingredients are used for the preparation of herbal under eye derma gel.

**Table 4: Formulation of herbal wound healing gel**

Gel Code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Carbapol 940 (gm)	0.2	0.3	0.4	0.25	0.5	0.55	0.6	0.65	0.8
Triethanolamine (ml)	0.5	0.2	0.6	0.1	0.3	0.4	0.5	0.1	0.5
Methyl paraben (gm)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Extract of Psidium Guajava (gm)	0.3	0.5	0.4	0.2	0.6	0.5	0.4	0.6	0.8
Extract of Tridax Procumbens (gm)	0.3	0.5	0.4	0.2	0.6	0.5	0.4	0.6	0.8
Glycerin (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Water	qs	qs	qs	qs	qs	qs	qs	qs	qs

The following parameters were used for the evaluation of gels.

#### 1. Visual appearance and clarity

The presence of any particulate matter in the in situ formulation is examined for visual appearance and clarity under fluorescent light against both a white and black background.

#### 2. Determination of pH

The gel pH was determined with a digital pH meter, where the glass electrode was fully submerged in the gel to ensure electrode coverage. The measurements were conducted three times, and the average of the readings was documented.

#### 3. Spreadability

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method, spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2 g) under study was placed on the ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with a hook. A 1 kg weight was placed at the top of the two slides for 5 min to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 100 g with the help of string attached to the hook, and the time (in seconds) required by the top slide to cover a distance of 7.5 cm was noted. A shorter interval indicated better spreadability.

Spreadability was calculated using the following formula

$$S = M \times L / T$$

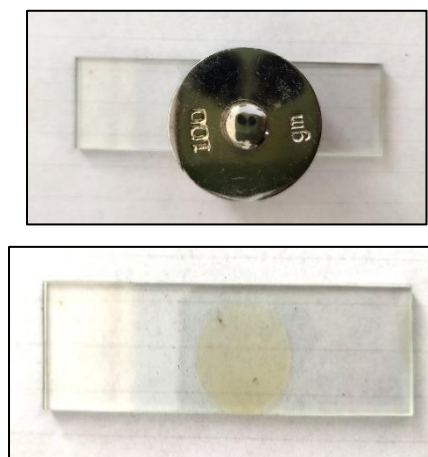
Where,

S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide

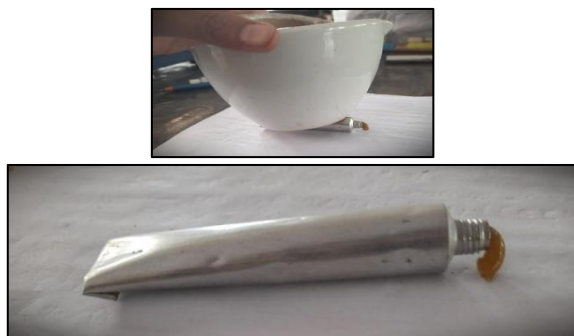
T = Time (in sec.) taken to separate the upper slide from the ground slide.



**Figure 11: Spreadability of Wound Healing Gel**

#### 4. Extrudability

A closed collapsible tube containing about 20g of gel is firmly pressed against the crimped end and a clamp is attached to Reference prevent rewinding. The cap is removed and the gel is extruded. The amount of extruded gel was collected and weighed. The percentage of extruded gel was calculated.



**Figure 12: Extrudability of Wound Healing Gel**

### Photosensitivity

Many pharmaceutical formulations are found to be photosensitive which exhibits instability on exposure to light. To evaluate the photostability of the product, a sun exposure test was carried out. In this test, the herbal gel was dried in the sun from 9 am to 18 pm and the changes in its physical properties were observed.

### 5. Grittiness

All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope. Hence, obviously the gel preparation fulfils the requirement of freedom from particular matter and from grittiness as desired for any topically preparation.



**Figure 13: Grittiness of Formulation**

### 6. Homogeneity

All developed gels were packed in the containers and then tested for homogeneity by visual inspection. They were tested for their appearance and presence of any aggregates.

### 7. Stability study

ICH guidelines were followed for the stability study. The formulated gel was filled in collapsible tubes and stored at different temperatures and humidity conditions, namely 25 ± 2°C / 60± 5% RH, 30± 2°C/ 65± 5% RH, and 40± 2 °C / 75± 5% RH for a period of 3 months and studied for appearance, pH, and spreadability.

## 4. RESULT AND DISCUSSION

Applying gel formulations to wounds is recommended because they have higher viscosities, longer skin residence times, are less greasy, and have a moisturising effect on flaky skin because of their occlusive qualities, increased bioadhesion, reduced irritation, ease of application, and superior release characteristics. The prepared gels were found to be homogeneous and in good appearance and consistency. The pH values of all the formulations were found to be in range of pH (7.2-7.6) and hence it may cause no skin irritation. The spreadability is good and indicates that the gel formulation is easy to apply. The formulated gel is easily extrudable.

The organoleptic evaluation indicated that the powder pack is smooth and has a pleasant fragrance. Physicochemical analysis revealed a low moisture content of just 5%, and the pH was found to be neutral, making it suitable for all skin types. The ash and extractive values were within acceptable limits. Rheological testing confirmed the pack's flow properties, showing it to be free-flowing and non-sticky. These findings affirmed the formulation's overall stability. The pack is rich in key phytoconstituents like carbohydrates, alkaloids, and glycosides, which serve as effective nourishment for the skin. The irritancy test yielded negative results for irritation, redness, and swelling, suggesting that the herbal ingredients, in their natural form and without chemical additives, are compatible with skin proteins. Stability tests conducted at various temperatures over one month showed no significant changes in color, odor, appearance, texture, or pH, confirming the pack's inert nature.

**Table 5: Extrudability study of various gel formulations**

Formulation	Weight of formulation (gm)	Weight of geextruded (gm)	Extrudability amount (%)	Grade
F1	10	9.2	92%	Excellent
F2	10	8.5	85%	Good
F3	10	7.2	72%	Fair

**Table 6: Physical evaluation of various gel formulations.**

Formulation	Concentration of Carbapol 940 (mg)	Appearance	pH	Homogeneity
F1	0.2	Pale brown and smooth	7.3	Homogeneous
F2	0.3	Pale brown and smooth	7.5	Homogeneous
F3	0.4	Pale brown and smooth	6.9	Homogeneous

**Table 7: Spreadability of herbal gel formulations .**

Formulation	Weight on the upper slide	Time taken to separate two slides.	Distance	Spreadability
F1	100 gm	15 sec	7.5 cm	50
F2	100gm	20 sec	7.5 cm	37.5
F3	100gm	30 sec	7.5 cm	25

## 5. CONCLUSION:

Wound healing activity of the developed herbal antiseptic gel formulation is due to the presence of chemical constituents in guava leaves and tridax daisy leaves like centaurein ,guajaverin ,etc . The developed herbal antiseptic gel with the composition of 0.2 gm carbapol 940, guava leaves extract 0.3 gm , tridax daisy leaves extract 0.3 gm,Triethanolamine 0.3 ml ,Glycerine 0.1 ml was found be most suitable gel for healing wounds and this was concluded after performing various evaluation test like stability study, spreadability, extrudability, pH,grittiness. The herbal antiseptic gel is most suitable for treating wounds or for healing the wounds because being herbal it doesn't contain any chemicals that may be allergic to the wounds or worsen them in sensitive cases. Thus in this prepared research work the herbal antiseptic gel has anti-inflammatory, analgesic activity, antifungal and antibacterial activity. Thus it concludes that the prepared antiseptic gel is suitable for treating and healing the wounds.

**Conflict of Interest:** The authors declare no potential conflict of interest with respect to the contents, authorship, and/or publication of this article.

**Author Contributions:** All authors have equal contribution in the preparation of manuscript and compilation.

**Source of Support:** Nil

**Funding:** The authors declared that this study has received no financial support.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Ethics approval:** Not applicable.

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