



# FORMULATION AND EVALUATION OF TOPICAL HERBAL WOUND HEALING GEL

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## Abstract

The current study's objective was to manufacture and assess the herbal gel's potential for wound healing and antibacterial activity using *Tinospora cardifolia* and Tridax. The preparation uses Tridax and *Tinospora* species, both of which have significant anti-inflammatory and therapeutic properties. Carbapol was the gelling agent employed in this study. In the current investigation, five formulations with various carbapol concentrations as well as *Tinospora cardifolia* and Tridax extracts were created and optimised. The optimised formulation was chosen for additional research. Initial physicochemical measures, similarly surface pH, spreadability, viscosity, homogeneity, together with antimicrobial tests, be used to characterise the formulation. The outcome showed that the surface pH fell inside the skin pH range. For optimised formulations in Trials 1 and 2, the gel's viscosity and spreadability were appropriate, and the zone of inhibition was likewise acceptable. Under typical storage settings, the preparation was stable and did not cause skin irritation.

**Keywords:** Plant extract, *Tinospora cardifolia*, *Tridax procumbens*, *Stepalococas*, *E coli*

## Introduction to Quality aspects of Herbals<sup>1</sup>:

### Herbal Medicine :

The parts of numerous plants known to have therapeutic characteristics, such as their roots, stems, leaves, bark, fruits, seeds, or flowers, are used to make herbal medicines. Additionally, a lot of commonly used medications come from plants. The word "drug" actually derives from the French word "drogue," which means "dry herb."

### The raw material:

Raw material means crude drugs. Unless otherwise stated, this phrase refers to primarily entire, fragmented, or cut plants, plants' components, algae, and fungus in an undisturbed state. Typically, herbs come in dried form. In some circumstances, exudates that have not undergone additional processing fall under the category of herbs.

**Herbal preparation:**

The term "herbal formulation" refers to a dosage form that contains one or more herbs or processed herbs in specific amounts to provide specific nutritional, cosmetic, and other benefits. These benefits may be used to diagnose, treat, or mitigate disease in humans or animals, or to change the physical characteristics of humans or animals.

**Quality in the context of herbal drugs<sup>2</sup> :**

The effectiveness and safety of herbal products are crucial considerations. A drug's status as determined by its identity, purity, content, and other chemical, physical, or biological features, as well as by the manufacturing methods, is referred to as its quality. Accepted Good Agricultural Practices (GAP) can regulate this. The quality of a plant product is affected by the environmental conditions present during growing. These include picking the right seeds, fostering healthy plant growth, applying fertiliser, and harvesting, drying, and storing. In actuality, GAP procedures are and always will be a crucial component of quality assurance.

**Factors affecting its quality about herbs<sup>3</sup>:**

1. Drug Adulteration
2. Faulty collection
3. Imperfect preparation
4. Incorrect storage
5. Gross replacement of plant matter
6. Using previously used medications in place

**Its need for quality evaluation of herbal drugs:**

1. According to the WHO, "quality control" refers to the collection of actions done to guarantee the identification and purity of a certain medicine. An crucial function of the pharmaceutical sector is quality control<sup>4</sup>.
2. Herbal medications are subject to quality control to guarantee their consistency, security, and effectiveness. Chemical fingerprinting has been shown to be an effective method for ensuring the quality of herbal medications<sup>5</sup>.
3. Consider the hue, texture, flavour, and aroma. Herbs that have been dried need to resemble fresh herbs in terms of appearance, flavour, and aroma. The flavour and potency of the dried herbs have also been diminished along with their colour and fragrance<sup>6</sup>.
4. Sensory examination (macroscopic and microscopic) is typically one of the procedures for quality control of herbal medicines<sup>7</sup>.

**• Herbs have its following characteristics<sup>7</sup>:**

- A. They are small plants with soft and delicate petals.
- B. They have a tender, soft, and delicate green exterior.
- C. They have a short lifespan, which means they can only live one or two years.
- D. They are shorter in size, and they may grow between 2 and 3 metres.

**The Limitations of Herbal Medicine<sup>3</sup>:**

1. Restrictions relating to the care of medicinal plants Indiscriminate harvesting and subpar post-harvest handling procedures

2. Lack of investigation into domestication, the creation of high-yielding cultivars, etc.
3. Ineffective farming and reproduction techniques.
4. Low yields and subpar goods are the result of ineffective processing methods.
5. Inadequate quality control measures.
6. Lack of R&D on the development of products and processes. challenges in marketing.
7. Insufficient staff and equipment.
8. a lack of local equipment manufacturing facilities.
9. lack of access to the newest market and technology information.

Distribution restrictions for herbal medications A complex mixture of organic compounds, including fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, lingams, and terpenes as well as other tiny molecules like peptides and oligosaccharides, can be found in both the raw plant and the extract.

### **Introduction of Plants**

In both traditional and ayurvedic medical practises, *Tinospora cordifolia* (also known as guduchi) is a common herb. Alternative names: *Tinospora cardifolia*, Its stem extract can be used to treat skin conditions and burning feelings. As a remedy for a snakebite or a scorpion sting, *Tinospora cordifolia* root and stem are used in combination with other medications. The aqueous extract of *Tinospora cordifolia* significantly reduced inflammation in both arthritis caused by formalin and cotton pellet granuloma.

*Tridax procumbens* The leaves of *Mandonia wedd* and *Ptilostephium Kunth* (L.) are traditionally used by tribal people to treat wounds. The mature leaves are ground into a paste and applied to the wound's surface. Dermal wounds have historically been treated with the juice of *Tridax procumbens* leaves. A medical herb called *Tridax procumbens* has been used for a variety of ailments for centuries, most notably cuts, wounds, and burns. A wound may be an inevitable occurrence in an organism's life. The healing process starts in the early stages of inflammation but typically ends once the harmful impact has been removed<sup>9</sup>. There, both plants are demonstrating or testing. *E. coli* and *Staphylococcus* bacteria and wound healing.

## Morphology<sup>10</sup> : Tinospora Cardifolia :

### Aerial Roots:



**Fig 1: Aerial Root**

The young aerial roots are long and filiform, like a string, squarish. The mature roots are fleshy and resemble the structure of a young aerial stem. The dried aerial roots are 3-6 cm in diameter, light grey-brown or creamy white in colour, odourless and have a bitter taste.

### Stem:



**Fig 2.Stem**

The stem's morphology revealed a greyish green colour with smooth surfaces and node swelling. Older specimens have a light brown surface covered in round lenticels that resemble warts. The dried stem has a rough surface due to longitudinal fissures of fractures running along the rows of lenticels and is cylindrical, slender, somewhat twisted, and 6 to 12 cm in diameter. The outer bark is paper-thin and ranges in hue from brown to grey. Circular and noticeable lenticels are present.

### Leaves:



**Fig 3. Leaves**

The leaves are straightforward, according to morphology. The leaf blade measures 5 to 12 cm wide, ovate like a circle, cordate, and has smooth regions. Shiny and light brown in colour, the underside. When the leaves are

seen in huge numbers, they appear to be very green, however the over-mature leaves are actually a yellowish-yellow tint. The leaves have an odd odour and a harsh flavour.

## **Tridax<sup>11</sup> :**

### **Appearance**

A perennial herb known as *Tridax procumbens* has a creeping stem that can grow to be 8 to 30 inches (20 to 75 cm) long.

### **Foliage**

The opposite, pinnate, oblong to ovate, 1-2 inches (2.55 cm) long leaves of *Tridax procumbens* have connate bases, coarsely serrated margins, and sharp apices.

### **Flowers**

White rays and yellow disc blooms are present in *Tridax procumbens* flowers. On a 4 to 12 inch (10 to 30 cm) long stalk, they measure about 0.4 to 0.6 inches (1.5-2 cm) in width. Spring is the time for flowering.

### **Fruits**

Achenes, which are dark brown to black in colour, rectangular, and 0.08 inches (2 mm) long, are the fruits. Each one has a head of pappus bristles that varies in length from 0.12-0.24 inches (3-6 mm).



**Fig 4: Tridax**

### **The objectives of this study were:**

1. To carry out extraction plant.
2. Evaluation of the prepared extract.
3. Formulation of gel by using green chemistry approach.
4. Physicochemical evaluation of formulated antimicrobial gel.
5. To study the antimicrobial activity of developed formulation.
6. To carry out research on short-term stability in accordance with ICH recommendations.

## Extract and Excipient Profile

### Mode of Action of *Tinospora Cardifolia* <sup>16</sup>

The granulation tissue of the wound is predominantly made up of fibroblasts, collagen, edoema, and tiny new blood vessels when the extract is applied to an animal wound. The wound margin's undifferentiated mesenchymal cells change into fibroblasts and begin to migrate into the wound gap alongside the fibrin strands. Extracellular tissue's main building block is collagen, which provides stability and strength. Collagen is made of the amino acid hydroxyproline, which has been utilised as a biochemical indicator for tissue integrity.

### Tridax Procumbens Action Mode <sup>17</sup>

Lysyl oxidase activity, protein and nucleic acid content, and tensile strength were all significantly increased in animals treated with extract.

### 1) Carbapol

Structure :

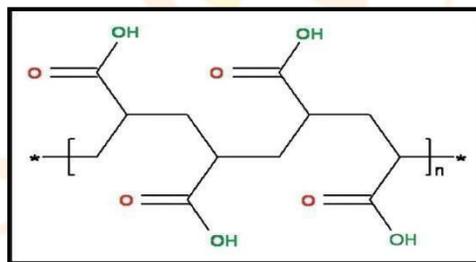


Fig 5. Structure of Carbapol

**IUPAC Name:** Poly(acrylic acid)

**Other Names:** PAA, PAAC, Acrysol, Acumer.

**Chemical Formula:** (C<sub>3</sub>H<sub>4</sub>O<sub>2</sub>)

**Molar Mass:** variable

Uses :

- disposable agents.
- 1) Ion exchange resins and adhesives are made with polyacrylic acid and its derivatives for use in diapers.
  - 2) They are frequently used in medicines as thickening, dispersing, suspending, and emulsifying agents.

### 2) Propylene Glycol

Structure :

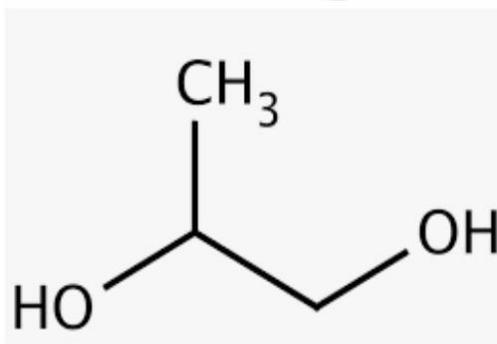


Fig 6: Structure of Propylene Glycol

**IUPAC Name:** Propane-1,2-diol

**Other name :** 1,2-dihydroxypropane,methylglycol

**Chemical Formula :** C<sub>3</sub>H<sub>8</sub>O<sub>2</sub>

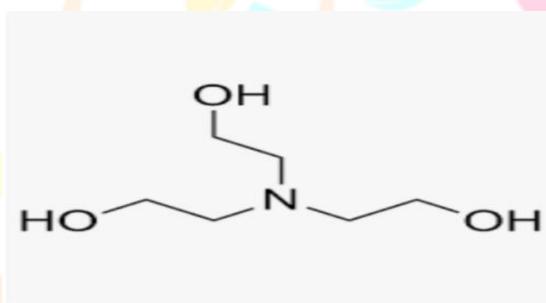
**Molar Mass :** 76.09 g/mol

**Uses :**

1. To make polyester compound and as a base for deicing solution.
2. It is used in gel formulation.
3. It helps presence moisture as well as dissolve colour and flavours.

### 3) Triethanolamine

**Structure :**



**Fig 7 : Triethanolamine**

**IUPAC Name:** 2-[bis(2-hydroxyethyl)amino]ethanol

**Other Name :** Trolamine

**Chemical Formula :** C<sub>6</sub>H<sub>15</sub>NO<sub>3</sub>

**Molar Mass :** 149.19 g/mol

**Uses :**

1. It is used in making surfactant such as for emulsifier.
2. It is used in sunscreen lotion.

#### Materials and Methods:

**Materials:** The Herbal Wound Healing Gel of *Tinospora Cardifolia* and Tridax was prepared using following natural plant ,chemical, apparatus and instrument.

**Natural Plant :** *Tinospora Cardifolia*, Tridax

**Chemicals:** Carbapol, Polyethylene glycol, Triethanolamine,

**Apparatus:** Apparatus such as beaker, glass slide, measuring cylinder, test tube, mortar volumetric flask,sonicator apparatus, Soxhlet apparatus.

**Instruments:** pH meter, Mechanical stirrer, Viscometer, Incubator,Auto-clave,Hot Air Oven.

Sr.No	Material	Function
1	Carbopol 934	Gelling Agent
2	Propylene glycol	Co-Solvent
3	Triethanolamine	Adjust pH
4	Distilled Water	Vehicle
5	Extraction of <i>Tinospora Cardifolia</i>	Active ingredients
6	Extraction of <i>Tridax</i>	Active ingredients

**i) Extraction method of *Tinospora Cardifolia*<sup>18</sup>:**

1. *Tinospora Cardifolia* stems cleaned with 70% ethanol.
2. Then *Tinospora Cardifolia* stem dried in shade and converts it into powder.
3. The powdered stem was ultrasonically extracted with chloroform for three hours.
4. Then it was filtered and filtrate was collected.
5. Collected filtrate was stored in well closed container and further used for preparation purpose.



**Fig 8: Extraction of *Tinospora Cardifolia***

**Extraction *Tridax Procumbens*<sup>19</sup>:**

1. A Soxhlet extractor was used to pack the complete plant powder, and it was extracted at 50°C with 95% ethanol.
2. The extraction process lasted 72 hours.
3. The filtrate from the extract-filtering process was collected.
4. The recovered filtrate was placed in a tightly sealed container and used for further preparation.



**Fig 9: Extraction of *Tridax procumbens***

**Preformulation Study :****II) Phytochemical Screening of leaf and stem extracts of *Tinosporacordifolia*<sup>20,21,22</sup>****Preliminary Phytochemical Screening of the *Tinospora Cordifolia* extracts:**

The Preliminary phytochemical analysis gives primary idea about presence of phytochemical of the Extract.

Sr.No	Test	Observation
1	<b>Analyze for Alkaloids (Wagner;s reagent)</b> Wagner's reagent, which contains 1.27 grammes of iodine and 2 grammes of potassium iodide in 100 millilitres of water, was used to test the extract for alkaloids.	rusty brown precipitation (or coloration).
2	<b>Analyze for carbohydrates (Benedict's test):</b> A 2 ml amount of the various extracts was mixed with a few drops of Benedict's reagent, heated in a water bath for five minutes, and then cooled.	Brownish Reddish Precipitate

3	<p><b>Analyze for flavonoids (Alkaline reagent test)</b></p> <p>To 2mL of extracts, a few drops of a 20% sodium hydroxide solution were applied. formation of a bright yellow colour that fades to colorlessness when diluted hydrochloric acid is added.</p>	Flavonoids
4	<p><b>Analyze for Amino Acid and Proteins (1% Ninhydrin solution)</b></p> <p>2 ml of filtrate were treated with 2–5 drops of 1% ninhydrin solution and then placed in a boiling water bath for 1–2 minutes to test for amino acids and proteins.</p>	Purple Colour
5	<p><b>Analyze for saponin (foam test):</b></p> <p>In a test tube, 12ml of water was added to 2ml of extract. The mixture was vigorously shaken.</p>	creation of enduring
6	<p><b>Analyze for Tannin (Braymer;s test )</b></p> <p>2 mls of the extract were treated with a 10% ferric chloride solution to test for tannins (Braymer's test).</p>	bluish or green Solution in Color
7	<p><b>Analyze for terpenoids (Salkowki's test)</b></p> <p>Involved treating 2 mL of each extract with 1 ml of chloroform and a few drops of strong sulfuric acid.</p>	Reddish Brown ppt

**Phytochemical Screening of Tridax Procumbens<sup>23</sup> :**

Sr.No	Test	Observation
1	<b>Analyze for Alkaloids ( Wagner;s reagent)</b> Wagner's reagent was used to test a little amount of extract for the presence of alkaloids (1.27g of iodine and 2g of potassium iodide in 100ml of water)	Reddish Brown ppt
2	<b>Analyze for Carbohydrate ( Benedict test )</b> To a 2 ml sample of each of the different extracts, a few drops of Benedict's reagent were added. The mixture was then heated in a water bath for 5 minutes, cooled, and looked for a reddish brown precipitate.	Reddish Brown ppt
3	<b>Analyze for Flavonoids ( Alkaline reagent test )</b> To 2mL of extracts, a few drops of a 20% sodium hydroxide solution were applied. formation of a bright yellow colour that fades to colorlessness when diluted hydrochloric acid is added.	Flavonoids
4	<b>Analyze for Phenols ( Ferric Chloride test )</b> A portion of the extracts were given an aqueous 5% ferric chloride treatment.	Deep blue or black colour
5	<b>Analyze for Saponin ( Foam test )</b> In a test tube, 12ml of water was added to 2ml of extract. The liquid was forcefully shaken before being observed.	Continuous Form
6	<b>Analyze for Tannins ( Braymer's test )</b> 10% ferric chloride solution was applied to 2 ml of the extract.	Greenish Colour

7	<b>Analyze for Terpenoids ( Salkowki's test )</b> Two millilitres of each extract were treated with one millilitre of chloroform and a few drops of strong sulfuric acid.	A reddish brown ppt
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### **Method of Preparation :**

### **Formulation Gel:**

### **Procedure:**

1. Carbopol 940 was measured and distributed in half the amount of water. It was permitted to enlarge.
2. Accurately weighed plant extract was added to Propylene Glycol & Sonicated.
3. Sonicated solution added in carbopol base.
4. The PH was changed by adding triethanolamine to make it 7. The remaining water was added to the mixture to correct the weight.

Chemicals	(Trial) 1	2	3	4	5
Carbopol	0.5%	1.0%	1.0%	1.0%	1.0%
Propylene glycol	10%	10%	10%	10%	10%
Triethalamine	To Calibrate pH 7				
Water	QS	QS	QS	QS	QS
Extract of TinosporaCardifolia	0.5%	0.5%	0.75%	1%	1.25%
Extract of Tridax	0.5%	0.5%	0.75%	1%	1.25%

### **Evaluation of Gel<sup>24,25</sup> :**

Different factors, including appearance, colour, pH, viscosity, homogeneity, Spreadability, extrudability, extract content, and extract content uniformity, were assessed for formulations.

### **Appearance:**

The Appearance of formulation is lustrous.

**Colour:**

The colour of formulation was pale yellow.

**Homogeneity:**

Visual inspection was used to check the homogeneity of the gel formulations for the presence of any aggregates.

**Spreadability:**

When two slides are placed in between them and a specific force is applied, spreadability is measured in terms of the number of seconds it takes for the slides to separate from the gel. In order to compress the glass slides into uniform thickness, the extra sample was sandwiched between the two glass slides and a set amount of weight was applied to them. The process of separating the two slides was timed while a weight of 70 g was added. The formula  $S = ML/T$ , where M is the weight attached to the top slide, L is the length of the glass slides, and T is the time it takes to separate the slides, was used to calculate spreadability.

**pH:**

With the aid of a digital pH metre, gel compositions' pH levels were determined. A precise 2.5gm of gel was used. 25ml of pure water was used to weigh, dissolve, and store the mixture for two hours. The formulation's pH was measured in triplicate, and the average results range from 6.8 to 7.2.

**Microbial growth:**

Agar media with nutrients was used to research microbial growth. This approach involved using blank and sample petriplates, aseptically transferring the gel sample onto the sample plates in a cross pattern, and then observing the microbial growth. *Staphylococcus aureus* strain antimicrobial activity was evaluated and found to display strong antibacterial activity. No signs of microbial development were seen.

**II) Anti-Microbial Studies:**

The formulation was evaluated for its anti-microbial properties by the zone of inhibition method; the micro-organisms used in the study were *Staphylococcus aureus*, *E coli*.

**II) Stability:**

A stability protocol was created, and in accordance with it, stability tests of the gel formulation were done for three months at 25°C and 60% relative humidity and 40°C and 75% relative humidity. To determine the stability of the produced formulation, the impact of temperature and time on the physiochemical properties of the gel was examined. The several physical characteristics that were investigated include pH, colour, odour, viscosity, homogeneity, and grittine.

**Result And Discussion :****Preformulation Study :****Phytochemical Screening of Extraction<sup>20,21,22</sup> :**

Sr.No	Test	Inference
1	Analyze the Alkaloid ( <b>Wagner's reagent</b> )	Present
2	Analyze the Carbohydrate ( <b>Benedict Test</b> )	Present
3	Analyze the Flavonoids ( <b>Alkaline Reagent Test</b> )	Present
4	Analyze the Amino acid and Protein ( <b>1% ninhydrin solution</b> )	Present
5	Analyze the Saponin ( <b>Foam Test</b> )	Present
6	Analyze the Tannins ( <b>Braymer's test</b> )	Present
7	Analyze the Terpenoids ( <b>Salkowki's Test</b> )	Present
8	Analyze the Cardiac Glycosides ( <b>Keller Kelliani's Test</b> )	Present

**Phytochemical Screening Of Tridax Procumbens<sup>23</sup> :**

Sr.No	Test	Observation
1	Analise the Alkaloid ( <b>Wagner's reagent</b> )	Present
2	Analise the Cardiac glycosides ( <b>Keller Kelliani's Test</b> )	Present
3	Analise the Flavonoids ( <b>Alkaline Reagent Test</b> )	Present
4	Analise the Phenols ( <b>Ferric chloride test</b> )	Present
5	Analise the Saponin ( <b>Foam Test</b> )	Present
6	Analise the Tannins ( <b>Braymer's test</b> )	Present

7	Analyse the Terpenoids ( <b>Salkowki's Test</b> )	Present
8	Analyse the Carbohydrate ( <b>Benedict's Test</b> )	Present

**Phytochemical Screening of Tinospora Cardifolia :**



**Fig 11: Leaf Extraction Test**



**Fig 12: Stem Extraction Test**

**Extraction Test of Tridax:**

**Fig 13: Tridax Extraction Test**



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### Formulation Trial:



Fig 14: Wound Healing Gel

### Antimicrobial Studies

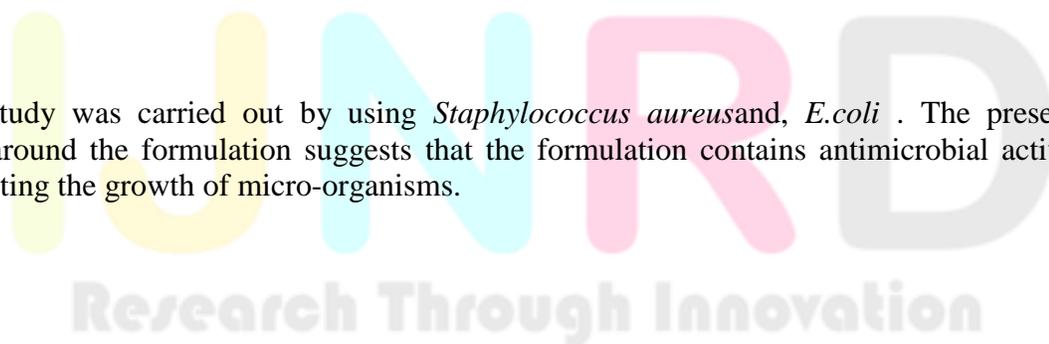


Fig 15 *E.coli* Culture



Fig 15 *E.coli* Culture

Antimicrobial Study was carried out by using *Staphylococcus aureus* and, *E.coli* . The presence of a clear zone around the formulation suggests that the formulation contains antimicrobial activity capable of inhibiting the growth of micro-organisms.



**Stability:**

TEST	STATION						
	INITIAL	ONE MONTH		TWO MONTH		THREE MONTH	
		25°C/60 %RH	40°C/75 %RH	25°C/60 %RH	40°C/75 %RH	25°C/75 %RH	40°C/75 %RH
Appearance	Lustrous	Lustrous	Lustrous	Lustrous	Lustrous	Less Lustrous	Less Lustrous
pH	7	7	7	7	7	7	7
Colour	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Light Yellow	Light Yellow
Odour	Attribute	Attribute	Attribute	Attribute	Attribute	Attribute	Attribute
Viscosity	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous
Spreadability	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent

Fig 17 :Stability Study

**Conclusion :**

There is a tonne of evidence supporting the importance of plant extracts in wound healing. Regarding Tridax and *Tinospora cardifolia* identification, the current study offers useful information. In accordance with the Green Chemist method, we created a polyherbal gel. Fresh extract from *Tinospora Cardifolia* and Tridax plants was gathered and evaluated in that study, demonstrating the phytochemical content of the extract. Additionally to the creation of polyherbal gel with enhanced formulation and prepared antimicrobial activity The formulation's performance against *E. coli* and *Staphylococcus* clearly demonstrated that it had an impact on a number of infections. Therefore, additional research could be done to isolate, purify, and standardise the plant. The scientific community will be able to use the plant in a herbal formulation after the wound healing study is done using an

animal investigation that reveals the pharmacological activity of the formulation. Thus, this offers a wide range of inquiry potential into potential future developments.

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