

## Development of a Poly Herbal Ointment for promoting Wound healing with Antimicrobial, Antioxidant and Anti-Inflammatory Benefits

Gomathi A R\*<sup>1</sup>, Deepika V<sup>2</sup>, Kavipriya Loganathan<sup>3</sup>, Bharathi M<sup>1</sup>, Saravanakumar A<sup>4</sup>, Mohan P<sup>1</sup>

<sup>1</sup>\*Department of Pharmaceutics, Vellalar College of Pharmacy, Maruthi Nagar, Thindal, Erode, Tamilnadu – 638012, India.

<sup>2</sup>Department of Pharmacognosy, Vellalar College of Pharmacy, Maruthi Nagar, Thindal, Erode, Tamilnadu – 638012, India.

<sup>3</sup>Department of Pharmacology, Vellalar College of Pharmacy, Maruthi Nagar, Thindal, Erode, Tamilnadu – 638012, India.

<sup>4</sup>Department of Pharmaceutical Biotechnology, Vellalar College of Pharmacy, Maruthi Nagar, Thindal, Erode, Tamilnadu – 638012, India.

**Abstract:** Herbal medicine is among the oldest and most significant contributions to humanity. A variety of plants and herbs have gained recognition in the medical field for their therapeutic properties. For over a thousand years, these plants have been employed to address different health issues. The healing properties of these plants have been transmitted through generations in human cultures. Wound healing is a multifaceted and continuous process that begins right after an injury takes place. It includes several phases: homeostasis, blood clotting, inflammation, proliferation, and remodeling. Each of these phases can either promote or impede healing, affected by a range of internal and external factors like infections, hormone levels, and nutritional status. A delay in healing can increase the risk of infections, insufficient recovery, and the development of unsightly scars. Research suggests that an extract from a polyherbal ointment notably accelerates the healing of wounds, probably because it contains phytochemicals such as flavonoids and phenolics that boost healing capabilities. This reinforces the traditional uses of polyherbal remedies. The research revealed that the extract, derived from the dried powders of *Sida acuta*, *Tridax procumbens*, and *Leucas aspera*, exhibits antimicrobial, anti-inflammatory, and antioxidant properties. When incorporated into an ointment base, this extract showed favorable physicochemical characteristics. Overall, the results suggest that plant extracts and their formulations have great potential for applications in wound healing and skincare.

**Keywords:** *Leucas aspera*, *Tridax procumbens* and *Sida acuta*, antioxidant, FRAP assay, Poly Herbal Ointment, Anti-microbial activity, Phytochemical and anti-inflammatory.

## Introduction

Herbal medicine involves utilizing medicinal plants to both prevent and treat illnesses. This practice encompasses a wide range of approaches, from traditional and folk remedies found in various cultures to the application of standardized herbal extracts [1]. The World Health Organization defines traditional herbal medicines as plant-based substances that are minimally processed and have been used for healing in local or regional traditions [2]. Indigenous herbal medicines refer to those that have been historically employed within a specific community or region, and are well-recognized by the local population for their composition, therapeutic uses, and dosages due to their long-standing use. These remedies are readily available to the local community or in the surrounding area. However, if these medicines are marketed or distributed outside their original community, they are required to adhere to national regulations regarding the safety and effectiveness of herbal products. Herbal medicine systems boast a long-standing history, backed by documented theories and principles, and are recognized by numerous countries. For example, traditional practices like Ayurveda, Unani, and Siddha have played a significant role in shaping the evolution of herbal medicines. These modified herbal products have undergone changes in their shape or form, which includes adjustments in dosage, administration methods, herbal components, preparation techniques, and medical uses. They are required to adhere to national laws regarding the safety and effectiveness of herbal remedies. Imported herbal products include all herbal medicines sourced from abroad, which consist of both raw ingredients and completed products. These imported herbal medicines need to be registered and sold in their countries of origin. Furthermore, data on safety and efficacy must be provided to the appropriate authorities in the importing nation, in accordance with the safety and efficacy standards established by herbal regulations [3]. The Ayurvedic text 'Sarangdhar Samhita,' dating back to 1300 AD, introduced the idea of Polyherbalism. This concept defines a Polyherbal formulation as one that includes between three to thirty different plants, where one or two are the primary active ingredients, while the others serve supportive roles. These supporting herbs act as catalysts, enhancing absorption, facilitating transportation, and minimizing toxicity. Recent studies indicate that combining plants with different potencies can create a synergistic effect, functioning through either pharmacokinetic or pharmacodynamic synergy. The advantages of these formulations include high effectiveness, a broad therapeutic range, fewer side effects, improved affordability, and greater accessibility [4, 5]. Wound healing

primarily refers to the recovery of the skin, which starts right after an injury occurs to the epidermis and can take years to complete. This intricate process involves well-coordinated cellular, humoral, and molecular mechanisms. Wound healing is often divided into two categories: primary and secondary. Primary healing occurs when a non-infected wound that is properly aligned heals without complications, with surgical wounds being a typical example. However, if the healing process is disrupted by issues such as infection, wound separation, insufficient oxygen, or immune system problems, the process transitions to secondary healing. In this stage, granulation tissue develops, and epithelial cells cover this new tissue, which makes these wounds more susceptible to infections and can lead to slower healing. The wound healing process includes four overlapping phases: inflammation, proliferation, and remodeling [6]. Several factors can affect wound healing, which can be divided into local and systemic categories, including oxygen availability, infection, poor blood circulation, wound contamination, and inadequate wound care [7]. A polyherbal formulation consists of herbs that possess potential therapeutic properties, along with the following polyherbal combinations that include effective therapeutic agents: *Tridax procumbens* is a well-regarded plant known for its production of secondary metabolites with various medicinal applications, including anti-anemic, anti-inflammatory, anti-diabetic, and anesthetic effects. This species has a rich history of traditional use among different cultures. Belonging to the Asteraceae family, it originates from Central and South America and is recognized for its notable medicinal properties, biological activities, and phytochemical constituents [8]. Researchers evaluated the antioxidant capabilities of *Tridax procumbens* using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and assessed its total phenolic content through the Folin-Ciocalteu method. Results showed that *T. procumbens* achieved an antioxidant activity percentage (AA %) of 96.70, exceeding that of the standard compounds gallic acid (92.92%) and ascorbic acid (94.81%). The plant's reductive potential was also significant, measuring 0.89 nm for *T. procumbens*, compared to 0.99 nm for gallic acid at the same concentration. Additionally, the total phenolic content of *T. procumbens* was found to be 12 mg/g GAE [17]. This plant is rich in various phytochemical compounds, including alkaloids, carotenoids, flavonoids, saponins, acids,  $\beta$ -Sitosterol, and tannins, with a notable abundance of oleanolic acid and a significant presence of potassium (atomic number 19). Furthermore, bioactive compounds such as luteolin, glucoluteolin, quercetin, and isoquercetin have been identified in its flowers, while chlorophyll and carotenoids have been analyzed in its leaves [26]. Oxidative stress and a compromised antioxidant system have been associated with the onset of various diseases, and polyphenols and flavonoids are often used to

treat conditions related to oxidative stress. *Tridax procumbens* has been traditionally utilized to address a variety of health concerns [18]. Additionally, the plant possesses medicinal attributes such as antimicrobial, larvicidal, antinociceptive, cytotoxic antioxidant, and hepatoprotective effects. The leaves of *Leucas* are specifically used to treat rheumatism. Various parts of the plant contain numerous phytochemical compounds, primarily including terpenoids, fatty acids, nicotine, ursolic acid, glucosides, beta-sitosterol, sterols, diterpenes, and phenolic compounds. *L. aspera* is abundant in active phytochemicals that demonstrate a wide range of biological and pharmacological effects, indicating its potential as a source of new therapeutic agents [9]. The crude methanol extract derived from the leaves of *Leucas aspera* showed notable scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DDPH) and superoxide radicals, outperforming other extracts with different polarities. This extract was selected for the purification of its bioactive constituents. Using activity-guided repeated fractionation through silica gel column chromatography, a compound with significant antioxidant capabilities was isolated [19]. Free radicals can induce oxidative stress, which damages healthy cells and is associated with aging, cancer, cardiovascular diseases, and inflammatory disorders. The antioxidant activity is closely linked to the presence of phenolic compounds, flavonoids, and tannins. The research examines the phytochemicals present in various solvent extracts from the leaves of *Leucas aspera* (Willd) Link (*L. aspera*) and evaluates their antioxidant effects using multiple in-vitro models [20]. Research conducted in indigenous regions has revealed that the plant is utilized in diverse traditional practices, which vary from one area to another. Commonly reported health issues treated with this plant include fever, headaches, and infectious diseases. Numerous laboratory studies have been conducted to support these traditional uses scientifically, leading to the identification of several compounds from the plant. This review highlights the plant's applications in folk medicine across different regions and discusses the validated in vitro activities observed in laboratory tests [10]. Extracts from *S. acuta* demonstrated significant pharmacological properties and intriguing chemical compositions. These extracts contain various bioactive compounds that are essential in combating oxidative stress, a key factor associated with the development of chronic illnesses like diabetes, cardiovascular conditions, and neurodegenerative disorders [21]. The preliminary phytochemical assessment identified the presence of alkaloids, steroids, flavonoids, phenols, terpenoids, and cardiac glycosides. The greatest zone of inhibition was observed against *Bacillus subtilis* and *Escherichia coli* at the maximum concentration tested [22]. The bioactive compounds found in this plant; including diterpenes, lignans, flavonoids, and squalene, possess numerous therapeutic properties. Extracts

from *Leucas aspera* showcase a variety of effects, including antimicrobial, antioxidant, anticancer, phytotoxic, antivenom, thrombolytic, hepatoprotective, anti-inflammatory, antiulcer, analgesic, antinociceptive, antimalarial, antipyretic, and antidiabetic properties. Different parts of the plant such as the roots, leave, flowers, and stems exhibit distinct activities. The phytochemical components of *Leucas aspera* can be applied in therapeutic contexts for humans due to their pharmacological benefits [23].

## Methodology

- **Gathering and Verifying Plant Specimens**

The above-ground portions of *Leucas aspera*, *Tridax procumbens*, and *Sida acuta* were gathered from the Thindal region by ABS HERBAL GARDENS in SALEM. The authentication certificate is numbered AUT/VCP/235. After the aerial parts were collected, they were thoroughly cleaned, dried in a shaded area, and ground into a powder, which was then kept in an airtight container for future use.

- **Sample Preparation Method**

Ten grams of *Leucas aspera* is heated with 100 ml of double distilled water for 5 hours using a reflux condenser in a water bath. After cooling, the mixture is filtered. The resulting filtrate is then evaporated under vacuum to obtain the aqueous extract. Similarly, ten grams of *Sida acuta* is heated with 100 ml of ethanol for 5 hours under the same conditions, cooled, and filtered. The filtrate is also evaporated under vacuum to produce the ethanolic extract. Finally, ten grams of *Tridax procumbens* is treated with 100 ml of methanol for 5 hours using the reflux condenser in a water bath, cooled, and filtered, with the filtrate evaporated under vacuum to yield the methanolic extract.

## Pharmacological Assessment

### 1. Phytochemical Analysis Test

Phytochemical screening is the method used to identify the diverse components found in plant extracts. These extracts contain numerous chemical constituents that can elicit various physiological and therapeutic responses. Consequently, they are often analyzed for biologically active and medicinally significant phytochemicals that play a role in specific biological functions. Notable examples of these phytochemicals include alkaloids, steroids, carbohydrates, saponins, tannins, and flavonoids, among others [24, 25].

- **Total Flavonoids**

The total flavonoid content was assessed using the aluminum chloride colorimetric method. In the analysis, 1 ml of each extract at concentrations of 10, 20, 40, 60, 80, and 100 µg/ml in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. This mixture was incubated at room temperature for 30 minutes. The absorbance of the resulting solution was then measured at 415 nm, with three readings taken to ensure accuracy. A sample blank was prepared in the same way, replacing aluminum chloride with distilled water. The flavonoid concentration in the extracts was expressed as quercetin equivalent (mg of QE/g of tissue) [14].

S. No	Ingredients	F1
1.	Each plant Extracts	1g
2.	Wool fat	0.5g
3.	Hard paraffin	0.5g
4.	Ceto stearyl alcohol	0.5g
5.	White soft paraffin	8.5g

- **Total Phenolics Content**

For each concentration, 1 mL was mixed with 5 mL of 10% Folin–Ciocalteu reagent (FCR) and 4 mL of 7% Na<sub>2</sub>CO<sub>3</sub>, leading to a final volume of 10 mL. The resulting blue solution was shaken well and incubated in a water bath at 40°C for 30 minutes. After the incubation period, absorbance was measured at 760 nm, using a blank as a reference. The FCR reagent interacts with the phenolic compounds in the plant extracts, resulting in a dark blue color that can be measured using a UV-visible spectrophotometer. The absorbance values obtained at different concentrations of gallic acid were used to generate a calibration curve. The total phenolic content of the extracts was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry sample weight (mg/g). The total phenolic content for all samples was calculated using the provided formula [14, 15]

$$C = C \left( \frac{V}{M} \right)$$

## 2. Development of Poly Herbal Formulation

Preparation Type: Absorption Ointment Base

### Procedure

In a china dish placed in a water bath at 70°C, combine hard paraffin and cetostearyl alcohol. Add wool fat and white soft paraffin to the mixture, stirring until all components are fully melted. If necessary, strain or decant the mixture, then continue stirring until it cools down before transferring it into an appropriate container.

### Formula for the ointment

#### Preparation of poly herbal ointment

##### Procedure

In a china dish placed in a water bath heated to 70°C, hard paraffin and cetostearyl alcohol are combined. Wool fat and white soft paraffin are then incorporated into this mixture and stirred until everything has melted. Next, the extracts of *Leucas aspera*, *Tridax procumbens*, and *Sida acuta* are added while continuously stirring. The ointment is prepared using these specific extracts in appropriate proportions.

##### Evaluation Test

##### Color and Smell

The physical characteristics, such as color and smell, were assessed through visual inspection.

##### Texture

The texture was found to be smooth without any greasiness.

##### pH

The pH of the formulated herbal ointment was measured using a digital pH meter. A solution of the ointment was created with 100 ml of distilled water and allowed to sit for 2 hours. The pH was measured three times for the solution, and the average value was calculated.

### **Spreadability**

The spreadability was assessed by placing an ample amount of the sample between two slides, which were then compressed to achieve a uniform thickness using a specific weight for a set duration. The time taken to separate the two slides was recorded as a measure of spreadability. The calculation for spreadability was done using the following formula.

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

Where, S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

### **Extrudability**

The formulation was placed in a collapsible tube. To assess extrudability, the amount of ointment needed to push out a 0.5 cm ribbon in 10 seconds was measured.

### **LOD**

The limit of detection (LOD) was determined by drying the formulation in a Petri dish over a water bath at 105°C. Regarding solubility, the formulation dissolves in boiling water and mixes effectively with alcohol, ether, and chloroform. For wash ability, the formulation was applied to the skin, and the ease of removal with water was evaluated. A non-irritancy test was conducted by applying the herbal ointment to human skin and observing for any adverse reactions.

## **3. Anti-Microbial Activity**

### **Agar Well Diffusion Method**

#### **Testing Microbial Strains**

All extracts were evaluated for their effects on both gram-positive strains (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa*) using the cup plate method.

### **Cultivation of Test Microbes**

To culture the bacterial strains, a Nutrient Broth Medium (NBM) was created by mixing 8% nutrient broth with double-distilled water and agar-agar. This mixture was autoclaved at 15 psi for 25 to 30 minutes. Agar test plates were made by pouring 15 ml of NBM into petri dishes under sterile conditions and allowed to sit at room temperature to stabilize. Bacterial cell cultures were preserved in a peptone saline solution through regular subculturing and were incubated at 37°C for 24 hours [16].

### **Preparation of agar plates and sampling of test substances**

Agar plates were prepared by streaking a swab of bacterial strains across the entire sterile agar surface 2 to 3 times, rotating the plate at a 60° angle to ensure even distribution of the inoculum. Organic solvent extracts from plant parts, with a concentration of 1 mg/ml, were utilized to assess antimicrobial activity using the agar well diffusion method. Three wells, each 10 mm in diameter, were created in the previously inoculated nutrient agar plates. A volume of 100 µl of each extract was placed directly into the wells and allowed to diffuse before being incubated at 37°C for 24 hours. The antimicrobial activity was evaluated by measuring the zones of inhibition (in mm) after the incubation period was completed.

## **4. Anti-inflammatory Activity**

### **Egg Albumin Denaturation Method**

The reaction mixture consisted of 5 mL total volume, comprising 0.2 mL of egg albumin (sourced from a fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), and 2 mL of the drug at varying concentrations (100, 250, and 500 µg/mL). An equal volume of double-distilled water was utilized as a control. The mixtures were incubated at a temperature of 37 ± 2°C in a BOD incubator for 15 minutes, followed by a heating step at 70°C for five minutes. After allowing the mixtures to cool, the absorbance was measured at a wavelength of 660 nm, with the vehicle serving as a blank. Diclofenac sodium was used as the reference drug at concentrations of 100, 250, and 500 µg/mL. The inhibition percentage of protein denaturation was determined using a specific formula.

$$\% \text{ inhibition} = 100 \times [V_t / V_c - 1]$$

## 5. Antioxidant Activity

### FRAP Assay

The FRAP assay was performed using plant extracts dissolved in their respective solvents at concentrations of 50, 100, 150, 200, 250, and 300 µg/ml. For each concentration, 1 ml of the extract was mixed with 2.5 ml of phosphate buffer and 2.5 ml of 1% ferric cyanide. This mixture was incubated in a water bath at 50°C for 20 minutes. After allowing it to cool, 2.5 ml of 10% trichloroacetic acid was introduced, and the solution was centrifuged for 5 to 6 minutes at 3000 rpm. Following this, 2.5 ml of the supernatant was combined with 2.5 ml of distilled water and 0.5 ml of a freshly made 0.1% ferric chloride solution. The absorbance was then measured at a wavelength of 700 nm.

## Result and Discussion

### Preparation of Extracts:

Extracts of polyherbs, including *Leucas aspera*, *Tridax procumbens*, and *Sida acuta*, were obtained using maceration with water, methanol, and ethanol. The resulting extracts exhibited a green color and a semi-solid, greasy texture.

### Phytochemical analysis

The extract underwent a qualitative chemical test for the purpose of phytochemical evaluation.

### Quantification of Total Phenol and Flavonoids

#### Total Phenol Content

Phenolic compounds can be found in both edible and inedible plants and their components. These compounds are recognized for their diverse biological effects, particularly their antioxidant capabilities. In a study measuring the total phenol content of extracts from *Tridax procumbens*, *Sida acuta*, and *Leucus aspara*, the readings at 760 nm indicated values of 68.60 mg/ml, 63.76 mg/ml, and 59.71 mg/ml, respectively, all expressed in terms of gallic acid equivalents.

#### Total Flavonoid Content

Research has indicated that flavonoids can have beneficial effects on diseases associated with lipid peroxidation. Both phenolic compounds and flavonoids are classified as polyphenols, recognized for their capacity to neutralize free radicals. These substances act as antioxidants by providing hydrogen molecules to free radicals. Our research shows that the methanol extract of *Tridax procumbens* contains a higher concentration of flavonoids than the aqueous and ethanol

extracts of *Leucas aspera* and *Sida acuta*, although the latter two also have notable flavonoid levels. Specifically, the flavonoid concentration in the methanol extract of *Tridax procumbens* is 57.16 mg/ml, compared to 47.74 mg/ml in the ethanol extract of *Sida acuta* and 35.05 mg/ml in the aqueous extract of *Leucas aspera*, all quantified as quercetin equivalents.

### **Physicochemical Evaluation of Formulated Ointment**

The polyherbal ointment that was formulated was assessed according to the Indian Pharmacopoeia for its physicochemical properties. The properties evaluated included irritancy, spreadability, viscosity, pH, and loss on drying (LOD), and the findings are detailed in the results.

### **Antimicrobial Activity**

Due to the considerable increase in antibiotic resistance, it is essential to evaluate the antimicrobial effects of herbal remedies as viable options for treating skin and wound infections in humans. This research examined the antimicrobial activity using the Agar well diffusion technique with a range of microorganisms, measuring the zones of inhibition. The evaluation included both single extracts and a combination of herbs against gram-positive and gram-negative bacteria. The findings related to the zones of inhibition are detailed in Table 8.

The greatest activity was observed in the gram-positive bacterium *Staphylococcus aureus* when treated with a 1 mg/ml concentration of the crude extract of *Tridax procumbens*. This was followed by the gram-negative bacteria, which exhibited similar results with the crude extract of *Sida acuta* at the same concentration. Additionally, the study revealed that a combination of these extracts (in a polyherbal formulation) resulted in increased activity.

### **Anti-Inflammatory Activity**

#### **Egg Albumin Denaturation Method**

The formation of auto-antigens in certain rheumatic conditions may stem from the denaturation of proteins occurring in the body. This process of denaturation likely involves alterations in electrostatic, hydrogen, hydrophobic, and disulfide bonds. Various anti-inflammatory drugs have shown a dose-dependent ability to prevent protein denaturation caused by heat. Research on anti-arthritis effects using the egg albumin denaturation technique was performed with different concentrations of *Sida acuta*, *Leucas aspera*, and *Tridax procumbens*. Among these, *Leucas aspera* demonstrated the most significant inhibition of egg albumin denaturation,

followed by *Tridax procumbens* and *Sida acuta*. Furthermore, the polyherbal formulation displayed considerable activity, nearly matching that of the standard reference.

### Antioxidant Activity

#### FRAP Assay

The FRAP assay was performed, measuring absorbance at 700 nm for the aqueous extract of *Leucas aspera*, the ethanolic extract of *Sida acuta*, and the methanolic extract of *Tridax procumbens*. This assay is based on the concept that compounds with reducing potential can react with potassium ferricyanide ( $\text{Fe}^{3+}$ ) to form potassium ferrocyanide ( $\text{Fe}^{2+}$ ), which then interacts with ferric chloride to create a ferric-ferrous complex that absorbs maximally at 700 nm. The results indicate that the reducing power of the extracts and standards increases with higher concentrations, as detailed in Table 10. A strong linear correlation is observed in the reducing power for both the standard and the formulation, as shown in Figures 8 and 9. Among the extracts tested, the ethanolic extract of *Sida acuta* exhibited the highest FRAP value, although it was still lower than that of the standard ascorbic acid.

**Table 1. List of plant extracts Percentage yield obtained**

Plant	Quantity used	% Yield
<i>Sida acuta</i>	100 g	10.6 g
<i>Leucus aspara</i>	50 g	6.2 g
<i>Tridax procumbens</i>	50 g	5.8 g

**Table 2. Preliminary Phytochemical Screening of Extract**

Phytochemical constituents	Aqueous extract of <i>Leucas aspera</i>	Methanol extract of <i>Tridax procumbens</i>	Ethanol extract of <i>Sida acuta</i>
Carbohydrates	+	+	+
Alkaloids	+	+	+

Steroids	+	+	+
Terpenoids	-	+	+
Glycosides	+	+	+
Phenolic compounds	+	+	+
Tannins	+	+	+
Saponins	-	+	-
Flavonoids	+	+	+
Proteins and amino acids	+	+	-

**Table 3. Estimation of total phenol content of standard**

Concentration( $\mu\text{g/ml}$ )	Absorbance
0	0
10	0.072
15	0.089
25	0.124
50	0.224
75	0.357
100	0.486

**Table 4. Estimation of total phenol content of plant extracts**

Sample	Concentration ( $\mu\text{g/ml}$ )	Absorbance	TFC
SA	100	0.524	47.74
LA	100	0.384	35.05
TP	100	0.628	57.16

**Table 5. Estimation of total flavonoid content of standard**

Concentration( $\mu\text{g/ml}$ )	Absorbance
0	0
10	0.072
15	0.089
25	0.124
50	0.224
75	0.357
100	0.486

**Table 6. Estimation of total flavonoid content of plant extracts**

Sample	Concentration ( $\mu\text{g/ml}$ )	Absorbance	TFC (mg/ml)
SA	100	0.524	47.74
LA	100	0.384	35.05
TP	100	0.628	57.16

**Table 7. List of physicochemical parameters**

Physicochemical parameters	Observation
Colour	Greenish
Odour	Characteristic
Consistency	Smooth
PH	6.5
Extrudability	0.26gm
Loss on drying	30%
Solubility	Soluble in boiling water, Miscible with alcohol, Ether & chloroform

Washability	Good
Non irritancy	Non irritant
Spreadability (seconds)	6

**Table 8. Zone of inhibition of various plant extracts and polyherbal ointment**

Organism			Zone of inhibition in mm		
Aqueous Extract of leucas aspera (1 mg / ml)	Ethanol extract of sida acuta (1mg /ml)	Methanol extract of Tridax procumbens (1mg / ml)	Polyherbal ointment	Standard Amoxycillin (50 µg /ml)	
E. coli	21	26	24	26	34
Pseudomonas aeruginosa	15	17	20	23	28
Staphylococcus aureus	21	20	22	24	30
Bacillus subtilis	18	17	20	22	31

**Table 9. %inhibition of plant extracts and ointment formulation**

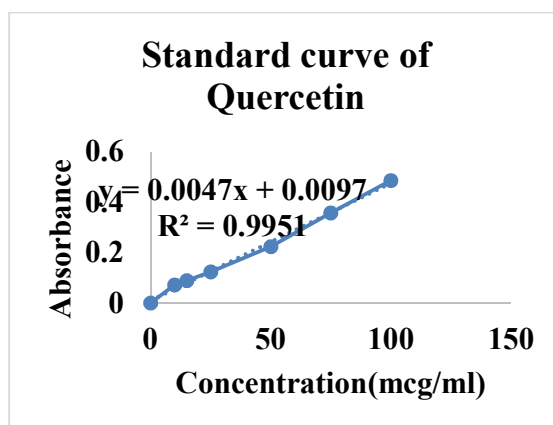
Concentration (mcg/ml)			%Inhibition		
Diclofenac sodium	Sida Acuta	Leucus Aspara	Tridax procumbens	Formulation	
50	10.25	2.56	7.69	5.12	7.69
100	28.20	5.12	20.51	15.38	20.51
150	46.15	17.94	30.76	25.64	25.64
200	64.10	25.64	35.89	33.33	30.76
250	74.35	38.46	46.15	38.46	66.66

300	87.17	56.41	76.92	66.66	82.05
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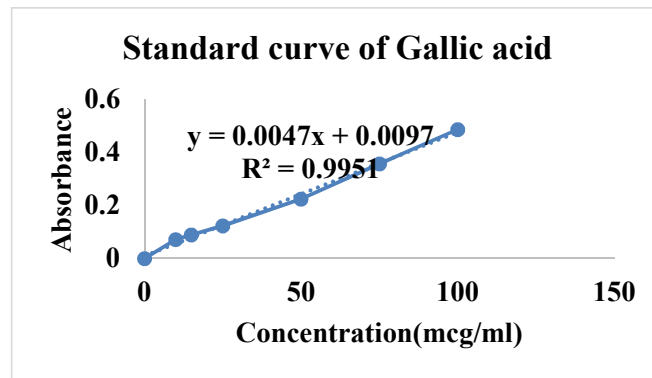
**Table 10. Reducing power of plant extracts and polyherbal ointment**

CONC (mcg/ml)	Abs of AA	Abs of SA	Abs of LA	Abs of TP	Abs of PF
0	0	0	0	0	0
50	0.074	0.052	0.032	0.043	0.065
100	0.116	0.105	0.087	0.097	0.11
150	0.163	0.15	0.138	0.145	0.151
200	0.202	0.182	0.178	0.172	0.198
250	0.251	0.195	0.182	0.185	0.232
300	0.298	0.245	0.192	0.217	0.283

**Fig 1. Estimation of total phenol content of standard and plant extracts**



**Fig 2. Estimation of total flavonoid content of plant extracts**



**Fig 3. E.coli**



**Fig 4. Staphylococcus aureus**



**Fig 5. Antimicrobial activity of plant extracts**



**Fig 6. Bacillus subtilis**



**Fig 7. Pseudomonas aeruginosa**



**Fig 8. Ferric reducing power determinations of ascorbic acid and Polyherbal formulation**

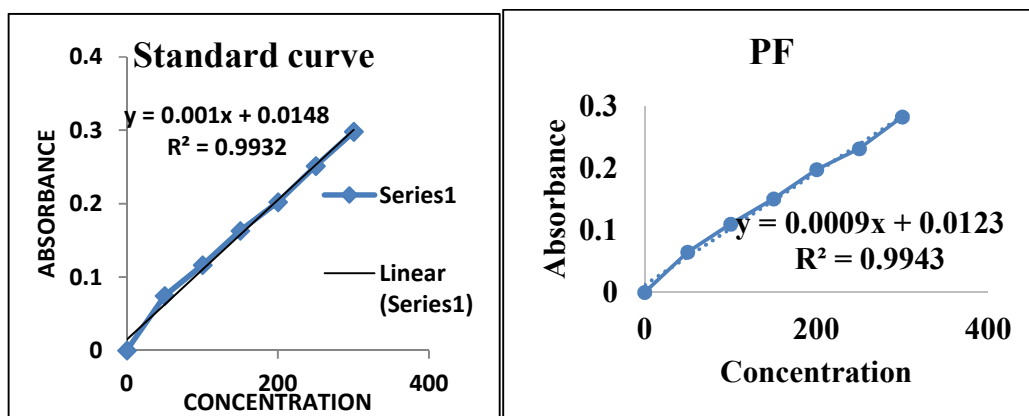
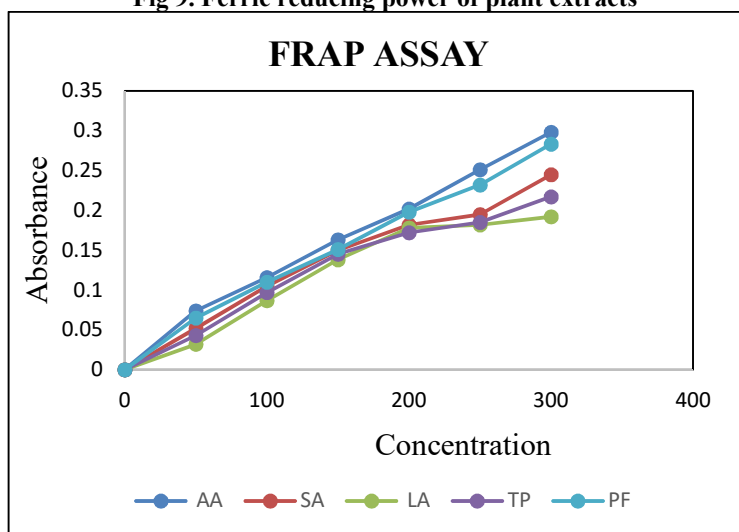


Fig 9. Ferric reducing power of plant extracts



**Conclusion**

Wound healing is a complex and continuous process that begins immediately after an injury happens. It involves several stages, including homeostasis, blood clotting, inflammation, proliferation, and remodeling. Each of these stages can either help or hinder the healing, depending on various factors like infections, hormones, and nutrition. If healing takes too long, it can lead to a higher risk of infection, improper recovery, and unattractive scars. This research found that a poly herbal ointment extract is effective in promoting wound healing. The positive outcomes are attributed to the presence of phytoconstituents such as flavonoids and phenolics, which are recognized for their healing properties and back the traditional use of poly herbal

remedies. The study concludes that the extract made from dried powders of *sida acuta*, *Tridax procumbens*, and *leucas aspera* has antimicrobial, anti-inflammatory, and antioxidant effects. When this poly herbal extract is mixed into an ointment base, it demonstrates excellent physical and chemical characteristics. Overall, the results indicate that plant extracts and their formulations hold great promise for wound healing and skincare.

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