

FORMULATION AND EVALUATION OF HERBAL BASED PATCH FOR WOUND HEALING THROUGH IN-VITRO SCRATCH ASSAY

Submitted in partial fulfilment of the requirements for the award of

Bachelor of Technology degree in Biotechnology

by

SHANMUGAPRIYA (38230030)



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A" by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **shanmugapriya.s(38230030)**, who carried out the project entitled **"FORMYLATION AND EVALUATION OF HERBAL BASED PATCH FOR WOUND HEALING THROUGH IN-VITRO SCRATCH ASSAY"** under my / our supervision from **January 2022 to April 2022**.

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DECLARATION

I, **SHANMUGAPRIYA (38230030)**, hereby declare that the Project Report entitled **“FORMULATION AND EVALUATION OF HERBAL BASED PATCH FOR WOUND HEALING THROUGH IN-VITRO SCRATCH ASSAY”** done by me under the guidance of **Dr. Gracy Jenifer and Dr. Jerrine Joseph** at **Sathyabama Institute of Science and Technology, Chennai** is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

Wounds have a serious negative impact on the healthcare economy of a country, especially on the economy of developing countries where resources are poor and funding is very limited. It is presumed that about 80% of people living in developing countries use traditional medicines which are majorly prepared from medicinal plants to meet their primary healthcare needs. Due to the large reservoir of medicinal plants and adequate traditional knowledge on wound healing, many people in Africa and other developing countries use medicinal plants in the treatment of diabetic wounds and related complications. Wound healing in the external and internal biological organs involves a series of complex overlapping processes which demand excellent communication between cells. It is an orderly and highly controlled process characterized by hemostasis, inflammation, proliferation, and remodeling. Diabetes is a global health problem predicted to rise to over 642 million by 2040. Different approaches have been adopted in the treatment of diabetic wounds, and medicinal plants are certainly one of those approaches that have drawn global attention. *Mimosa pudica* L. (Mimosaceae) also referred to as touch me not, live and die, shame plant and the humble plant is a prostrate or semi-erect subshrub of tropical America and Australia, also found in India heavily armed with recurved thorns and having sensitive grey-green leaflets that fold and droop at night or when touched and cooled. Senna flowers extract used in the treatment of ulcers. In this project, we intend to develop an herbal-based healing gel for the foot ulcer.

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Chapter 1

INTRODUCTION

1.1 Diabetes

The term “diabetes mellitus” is defined to be metabolic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or sometimes both, (WHO,1999). In 2020, according to IDF 88 million people have diabetes in southeast Asia. Of these 88 million people, 77 million people belong to India. To overcome diabetes, the patients require early diagnosis, treatment, and changes in their lifestyle. There is different treatment for controlling diabetes, like insulin therapy, pharmacotherapy, and diet therapy. But there is no effective treatment. Available treatments have some disadvantages in treating diabetes such as drug resistance, side effects, and even toxicity (Wesam Kooti,2016). So, there is a need for medication to treat diabetes effectively without any side effects. So, the use of medicinal plants is recommended for treating disease.

1.2 Diabetic Wounds

- WHO (2015) to 463 million people in 2020 India - **69.2 million** people living with diabetes in and it is expected to rise by more than 5th from 406 million in 2018 to 511 million in 2030.
- Diabetes currently affects over 425 million people worldwide. Peripheral neuropathy is survey nerve damage caused by chronic blood sugar and diabetes.
- Foot ulcer is a wound that occurs in approximately 15 % of patients with diabetes and is commonly located on the bottom of the foot.
- Diabetic foot is one of the most significant and devastating complications of diabetes, and is defined as a foot affected by ulceration that is associated with neuropathy and/or peripheral arterial disease of the lower limb in a patient with

diabetes. The prevalence of diabetic foot ulceration in the diabetic population is 4–10%; the condition is more frequent in older patients.

- It is estimated that about 5% of all patients with diabetes present with a history of foot ulceration, while the lifetime risk of diabetic patients developing this complication is 15%.

The majority (60–80%) of foot ulcers will heal, while 10–15% of them will remain active, and 5–24% of them will finally lead to limb amputation within a period of 6–18 months after the first evaluation.

- In this project we intend to develop an herbal-based healing gel for the foot ulcer.
- The main purpose of herbals that are used in wound healing potential, assessment of revascularization, and predictability of amputation level.
- *Mimosa pudica* is majorly possesses antibacterial, antiviral, antivenom, antifertility, anticonvulsant, antidepressant, aphrodisiac.
- It prevents black spots, treats uneven skin tone, and improves skin complexion. Senna flowers extract is used in the treatment of ulcers.

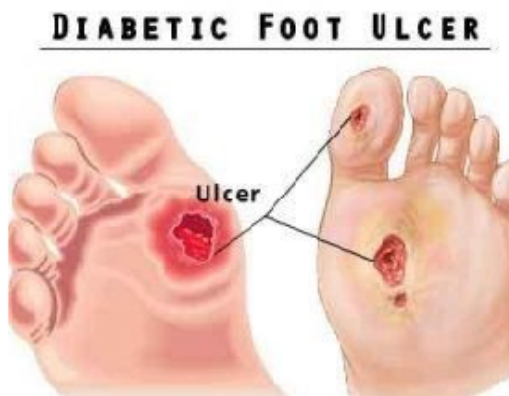


Fig 1.1: Diabetic foot ulcer

1.3 Wound healing

Wound healing is a multifaceted and overlapping process that follows a systematic sequence. There are three main stages involved in the process of wound healing: inflammation, proliferation, and remodeling. Inflammation follows hemostasis (blood clotting) and can last for up to 6 days³. During the proliferative phase, new blood vessels are formed by angiogenesis, collagen is synthesized and deposited. This further leads to the formation of granulation tissue, epithelialization, and wound contraction⁴. Tissue remodeling phase starts at the end to replace the granulation tissue with a scar tissue, which takes approximately 3 to 6 weeks. In the case of diabetic wounds, the inflammation phase is prolonged and the proliferative phase fails to begin. This leads to chronic unhealed wounds⁵. Thus, diabetic wound healing is a serious challenge in clinical practice⁴. Pathophysiology of impaired diabetic wound healing is still unclear. It is presumed that delayed healing is due to the persistence of prolonged inflammation, and inadequate angiogenic response.

1.3.1 Reasons diabetic wounds are slow to heal

- High blood sugar
- Neuropathy
- Reduced immune system
- Poor circulation
- Infection

1.4 Medicinal plants

Many medicinal plants have been reported to possess wound healing activity and found useful in the treatment of wounds. Medicinal plants are an important source of new chemical substances that have beneficial therapeutic effects. Extensive research has been carried out in the area of wound healing management through medicinal plants.

1.5 *Senna Auriculata*

Senna auriculata is a leguminous tree in the subfamily Caesalpinioideae. It is commonly known by its local names matura tea tree, avaram or ranawara and the English version **avaram Senna**. It occurs in the dry regions of India and Sri Lanka. It is common along the sea coast and the dry zone in Sri Lanka.



Fig 1.2 *Senna auriculata* (Avaram)

People use *Cassia auriculata* for diabetes, eye infections (conjunctivitis), joint and muscle pain (rheumatism), constipation, jaundice, liver disease, and urinary tract disorders.

The dried powder of flowers of *Senna auriculata* (L.) Roxb. is used to clean the hair, and taken by *diabetic* patients and in wounds.

Flowers: Flowers are bright yellow and irregular and large (50 mm) and have axillary raceme inflorescence, 2–8 flowered. Flowers are bisexual, zygomorphic, pentamerous, 4–5 cm; sepals are rounded at apex, imbricate; petals free, imbricate, unequal, 1.5–3 cm long; stamens 10, the 3 lower ones large and stand fertile, others usually sterile; ovary superior, falcate, with 1.5 cm long, stalked, style (fruit a flattened) cylindrical pod 5–18 × 1–2 cm, transversely undulate between the 10–20 seeds, indehiscent, green turning to brown when mature (Kirtikar and Basu, 1918; Evans, 1996).

1.6 *Mimosa pudica*

It majorly possesses antibacterial, antivenom, antifertility, anticonvulsant, antidepressant, aphrodisiac, and various other pharmacological activities. The herb has been used traditionally for ages, in the treatment of urogenital disorders, piles, dysentery, sinus, and also applied on wounds.

Mimosa pudica Linn. (Fabaceae) is mainly known as a sensitive plant, humble plant sleeps sleeping, honteuse, dormidera, morivivi, touch-me-not, marie-honte, mayhont, grass, timawi and having many other sleep names. It is a small and transient, prostrate or growing bush. It can reach a maximum height of 1 m together with another support. It has curved spines. The plant has a dummy system with large fibrous radical nodules. The color of the flower is pink with globose heads. The pods have groups and have from two to four brown seeds.

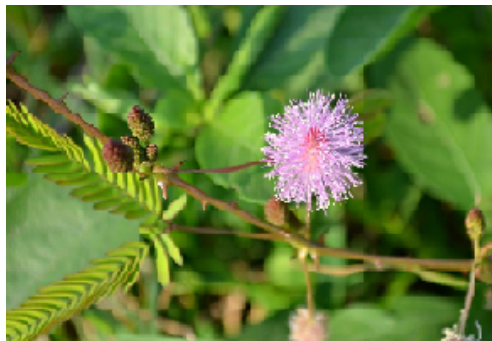


Fig 1.3 *Mimosa pudica*

1.7 Transdermal drug delivery system

Transdermal therapeutic systems defined as patches which helps in delivering an accurate amount of drug into the systematic circulation of particular area through skin. (Ash Sedef can et al, 2013).



Fig 1.4 Image of transdermal patch

Transdermal patch works simply. A drug is loaded inside the patch, which is worn on the skin for extended period of time. Through a diffusion process, the drug enters the blood stream directly through skin barrier. Due to high concentration on the patch and low concentration in the blood, the drug will keep diffusing into the blood for a long period of time, at the same time maintain the constant concentration of drug in the blood flow.

1.7.1 Advantages of TDDS

- It acts as alternative for the oral route.
- The patch permits constant dosing rather than the peaks and valleys in medication level.
- It can be easily worn and removed after the usage.
- Self medication is possible.

1.8 Gel formation

Gel represents an important therapeutic advance for diabetic neuropathic foot ulcers with adequate blood supply. Randomized controlled trials have shown that it is effective in increasing healing rates.

Wound dressing materials are used as a protective barrier against pathogens during the wound healing process. These materials should be biocompatible. They should also help in cell attachment, proliferation, migration, and differentiation.

Chapter 2

LITERATURE SURVERY

- ❖ Abubakar Amali Muhammad *et al.*, (2020) explored *M. Oleifera* for in vitro wound healing potential. Crude extract of different solvent was preliminary screened and active crude extract are chosen HPLC and LC-MS analysis were performed to the crude extract to confirm the presence of bioactive compounds. Among all the extract aqueous fraction showed good proliferation and cell viability in HDF cell. It helps to analyse that *M. Oleifera* have Vicenin-2 compound as phytochemical which helps in faster compound healing.
- ❖ Akter. R *et al.*, (2012) analysed cytotoxic present in the medicinal plants like ethanolic extract of *Curcuma longa*, *Curcuma zedoaria*, *Streblus asper*, *Enhydra fluctunas* and *Scoparia dulcis* using brine shrimp larvae. The lethal concentration and IC50 values were calculated among the 5 plants, *Curcuma longa*, *Curcuma zedoaria*, *Streblus asper* has less than 250µg/ml and showed good source of bio-active compounds.
- ❖ Atul Tripathi *et al.*, (2017) prepared transdermal patch for antiemetic therapy by solvent evaporation technique and evaluated for organoleptic characteristics and physicochemical parameters. Also, patch is studied for in vitro permeation study by using barrier membrane, Kesarychein diffusion cell and result showed that time dependent drug release. The drug release percentage were found to be 76.25% within 24 hours.
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Chapter 3

AIMS AND SCOPE OF PRESENT INVESTIGATION

Aim:

To formulate and to develop the diabetic herbal based wound treatment.

Objectives:

- Collection and prepare the plant extracts
- Antimicrobial assays to confirm their activity
- Formulate the gel composition, standardize it and evaluate its physical-chemical properties
- *In vitro* studies to determine the efficacy of the gel.

Chapter 4

Experimental or Material and Methods Used

4.1 COLLECTION OF PLANT MATERIAL

Fresh tubers of *Mimosa pudica*, which were collected from Shollinganallur, Chennai, Tamil Nadu were used in this study.

Dried powder of *Senna auriculata* were collected from Amazon an online market.

4.2 Preparation of plant extract

The collected tubers were cleaned with running tap water and rinsed in distilled water. The tubers were cut into fine pieces, shade dried for 4 days and grinded into fine powder by using mechanical method.

4.2.1 *Senna auriculata* extraction

- *Senna auriculata* extracts with solvent (ethyl acetate) using soxhlet method.AA



Fig 4.1 *Senna auriculata* extraction

4.2.2 *Mimosa pudica* extraction

- *Mimosa pudica* extract with solvent(ethyl acetate) using soxhlet method.



Fig 4.2 *Mimosa pudica* extraction

4.3 In-vitro antioxidant assay

In vitro (Latin *in glass*; often not italicized in English usage studies are conducted using components of an organism that have been isolated from their usual biological surroundings, such as microorganisms, cells, or biological molecules. For example, microorganisms or cells can be studied in artificial culture media, and proteins can be examined in solutions. Colloquially called "test-tube experiments", these studies in biology, medicine, and their subdisciplines are traditionally done in test tubes, flasks, Petri dishes, etc. They now involve the full range of techniques used in molecular biology, such as the omics.

4.4 Work flow

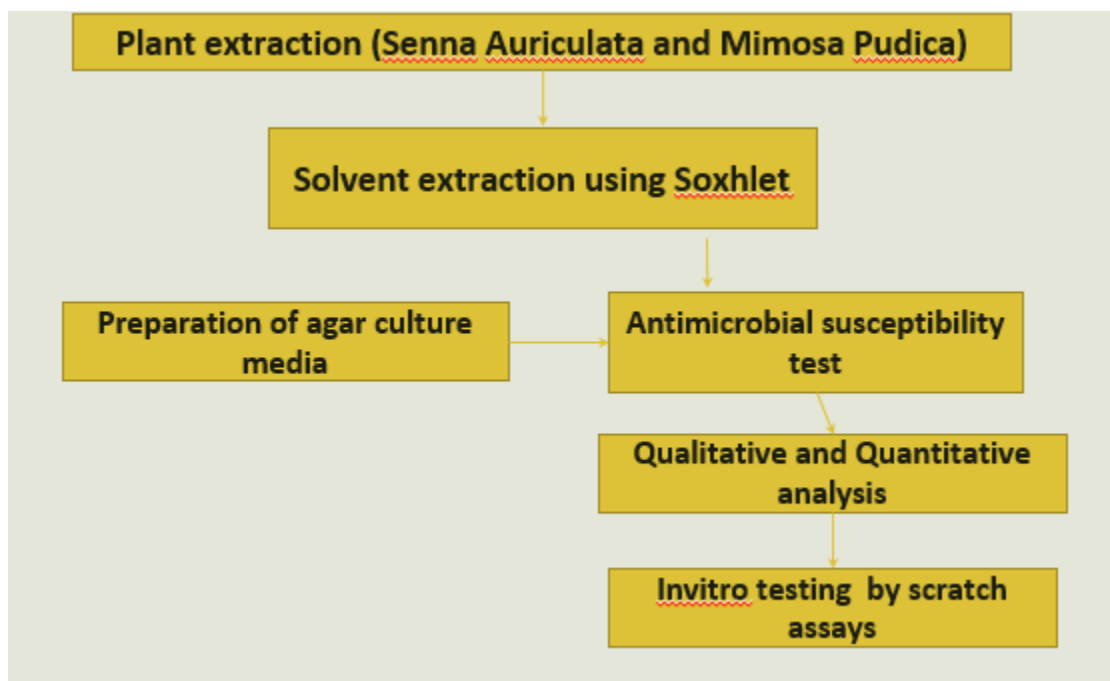


Fig 4.3 Work flow of gel formation

Materials

- *Senna auriculata*
- *Mimosa pudica*



Fig 4.4 Sample collection

Plant extraction

- Materials extracts with solvent (ethyl acetate) using soxhlet method.





Fig 4.5 Extraction using soxhlet method

- *Mimosa pudica* extracts with solvent (ethyl acetate) using soxhlet method.



Fig 4.6: *Mimosa pudica* extraction

- *Senna auriculata* extracts with solvent (ethyl acetate) using soxhlet method.

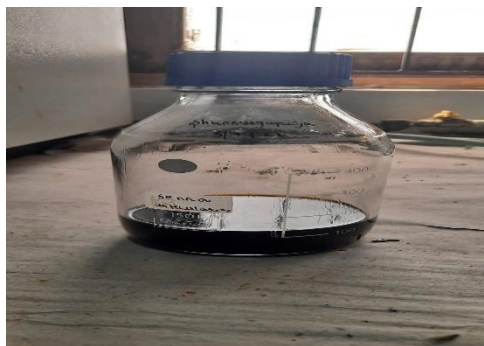


Fig 4.6 *Senna auriculata* extraction

4.5 Qualitative analysis

Qualitative Analysis is the determination of non-numerical information about a chemical species, a reaction.

4.5.1 Materials and methods

- **Alkaloids**

0.5ml of sample was taken to which few drops of petroleum ether and 2-3 drops of Dragendorff's reagent (potassium bismuth iodide solution) was added in a test tube. Appearance of pale-yellow colour indicates that absence of alkaloids in this extract.

- **Tannins**

0.5ml of sample was taken to which few drops of petroleum ether and few drops of ferric chloride solution was added in it, appearance of brownish black colour indicates the presence of tannins in all these extracts.

- **Flavonoids**

0.5ml of sample was taken to which few drops of petroleum ether, few fragments of magnesium ribbon and concentrated hydrochloric acid was added drop wise, absence of colour means absence of flavonoids in this extract, same test is repeated.

- **Sterols and Triterpenoids**

0.5ml of sample was taken to which few drops of petroleum ether, chloroform extract, few drops of conc. sulphuric acid was added and mixed well, no colour appears in these two indicates disappearance of sterols and triterpenoid.

- **Carbohydrates**

All four extracts were treated with Benedict's reagent (alkaline solution of cupric citrate complex) absence of red precipitates boiling on water bath indicates the absence of carbohydrates.

- **Glycosides**

1ml of sample was taken to which 0.5ml of glacial acetic acid was added along with 1% of ferric chloride. Brown ring formation indicates the presence of glycosides.

- **Saponins**

All four extracts were taken separately in test tubes and water was added and it was shaken well, no persistent foam is formed which indicates absence of saponins.

- **Phenols**

0.5ml of sample was taken and 5% of ferric chloride was added. Appearance of blue color indicated the presence of phenol



Fig 4.7 Qualitative analysis of *Mimosa pudica*

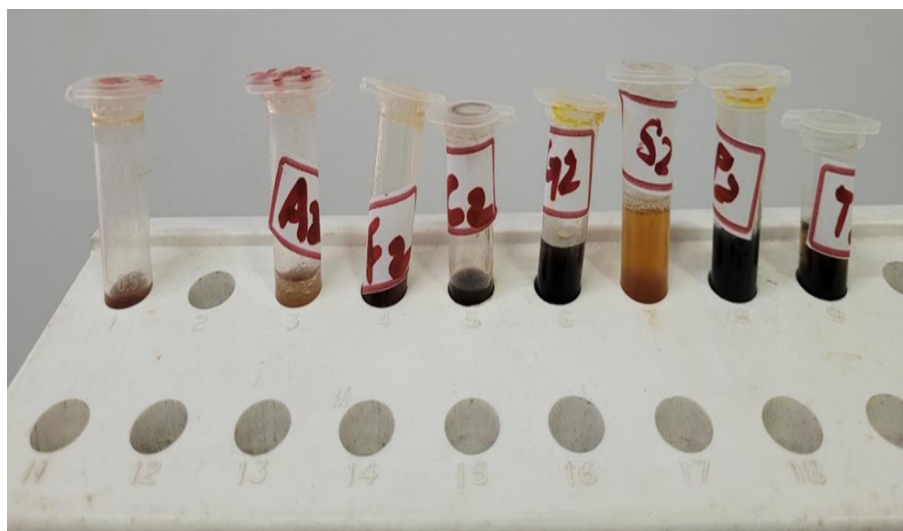


Fig 4.8 Qualitative analysis of *Senna auriculata*

Qualitative analysis results

Phytochemical analysis	<i>Minimosa pudica</i>	<i>Senna auriculata</i>
Alkaloids	++	-
Flavonoids	-	-
Sterols and Triterpenoids	-	-
Carbohydrates	-	++
Glycosides	-	++
Saponins	-	++
Phenol	++	++
Tannins	++	++

Table 1: Qualitative analysis results

4.6 Quantitative Analysis

Phenol

The total phenolic content in plant extract was determined with The Folin-reagent Ciocalteu's reagent. In the procedure, sample extract, was mixed with 0.75ml of Folin Ciocalteu reagent and 0.75ml of 0.06% Na_2CO_3 and incubated at room temperature for 1.5 hours. Using a spectrophotometer, absorbance was determined at 750 nm. The calibration curve was produced using Gallic acid solution as a standard and the total phenolic content of the extract was expressed in milligrams of Gallic acid per gram of dry weight and the standard graph was constructed.

The total phenolic content of *Mimosa pudica* and *Senna auriculata* was found to be 53.33 mg/ml and 93.85mg/ml respectively

Senna auriculata showed higher phenolic content as compared to *Mimosa pudica*.

Sample name	Phenolic content mg/ml
(<i>Minimosa pudica</i>)	53.44
(<i>Senna auriculata</i>)	93.85

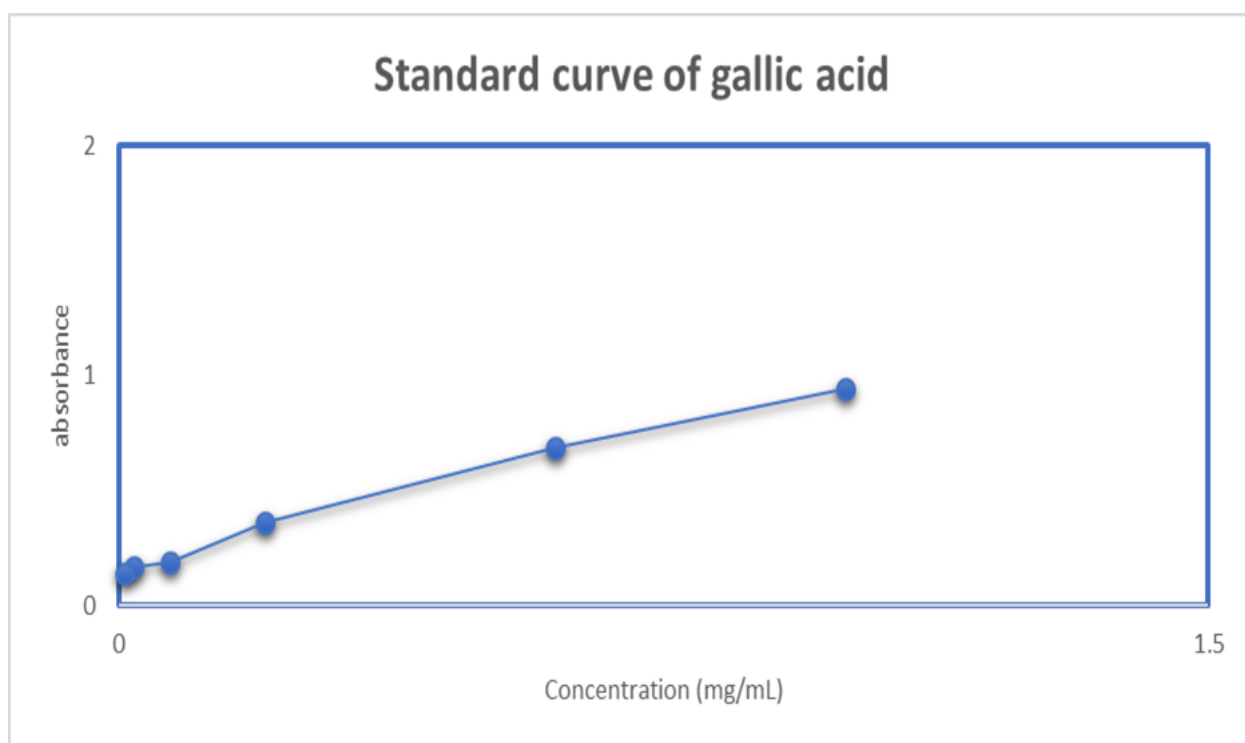


Fig 4.9: Graphical representation

4.7 Culture media

- *Pseudomonas*
- *Staphylococcus*

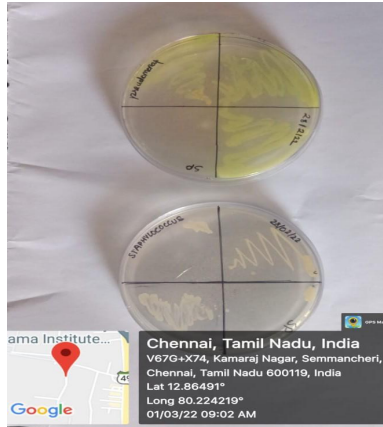


Fig 4.10 Culture media

4.8 Antibacterial activity

- Antibacterial activity by using agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 μ L) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

- Microorganism used
 - Staphylococcus*
 - Pseudomonas*
 - E.coli*
 - Pneumonia*

- The highest zone in *Senna auriculata*. solvent (a) ethyle acetate diameter in 13mm(staphylococcus)
- Highest zone in *Senna auriculata*. solvent (a) ethyle acetate diameter in 12mm (pseudomonas).

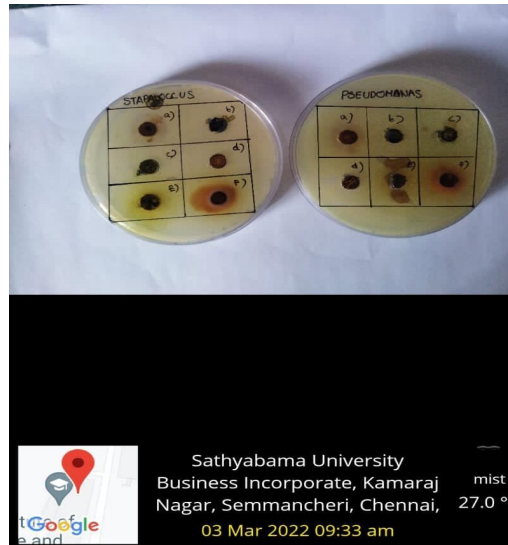


Fig 4.11 Zone activity

- Zone in *Senna auriculata* solvent (a) ethyl acetate diameter in 6mm(e.coli)
- Zone in *Senna auriculata* solvent (a) ethyl acetate diameter in 8mm(pneumonia).



Fig 4.12: Zone activity range of *senna* and *mimosa*

4.9 Gel formation

4.9.1 Carbopol 940

It is an extremely efficient rheology modifier capable of providing high viscosity and forms sparkling clear gels or hydro-alcoholic gels and creams. Its short flow, non-drip properties are ideal for applications such as clear gels, hydroalcoholic gels, and creams.

Senna auriculata 0.86 gm was taken and added to *Mimosa pudica* ethyl acetate extraction Carbopol 940 was added and gel was formed.

pH

pH is a measure of how acidic/basic water is. The range goes from 0 to 14, with 7 being neutral. pHs of less than 7 indicate acidity, whereas a pH of greater than 7 indicates a base. pH is really a measure of the relative amount of free hydrogen and hydroxyl ions in the water.

pH of the formed gel was 5.

4.9.2 Gel formed method

The extraction of the *Senna auriculata* 0.86g and diluted *Mimosa pudica* with ethyl acetate 1 ml has been taken and mixed with distilled water then 0.25g of Carbopol are mixed. Then formed accordingly.

Chapter 5

RESULTS, DISCUSSION and PERFORMANCE ANALYSIS

5.1 Antioxidant activity of *Senna auriculata*

- DPPH assay revealed that leaf extract had the highest antioxidant activity comparable with Vitamin C (leaf $IC_{50}=16.02\pm0.94$ $\mu\text{g/mL}$; Vitamin C= 6.21 ± 0.04 $\mu\text{g/mL}$). Total phenolics content of leaf extract also highest (245.50 ± 3.54 mg gallic acid/g) which attributed to the antioxidant activity of L.
- *Senna singueana*: Antioxidant, Hepatoprotective, Antiapoptotic Properties and Phytochemical Profiling of a methanol bark extract.
- The antioxidant potential of the extract was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free

radical. IC sub(50) of the methanol extract of *Senna tora* was 109.65 $\mu\text{g mL}^{-1}$ which indicated the strong antioxidant activity of the plant extract.

- Methanol extract of *Senna tora* possess strong antioxidant activity. However the aqueous extract showed mild antioxidant activity. In case of antibacterial activities test, the extract was subjected for its effectiveness against both Gram-positive and Gram-negative bacteria in agar diffusion method.
- The zones of inhibition produced by the crude methanol and aqueous extract against few sensitive strains was measured and compared with those of standard antibiotic Gentamycin. It is evident that both extracts are active against the bacteria at low concentrations. The obtained results provide a support for the use of this plant in traditional medicine and suggest its further investigation.

5.2 Cytotoxic studies:

- Methanolic, diethyl ether and *n*-hexane extracts of leaves of four species of *Senna* (*S. gardneri*, *S. macranthera*, *S. splendida* and *S. trachypus*) were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Using linear retention indices and mass spectral data, 34 compounds were identified, including fatty acids, flavonoids, terpenoids and steroids that were not reported previously for these species.
- Additionally, the cytotoxicity of the extracts against different tumor cell lines was determined. The cytotoxicity was then correlated with the chemical composition of the extracts by partial least squares-discriminant analysis (PLS-DA). The *n*-hexane extract of *Senna* and the ethyl ether extract of *Senna* were the most active against human colon (59.75 and 31.37%, respectively) and human glioblastoma (52.85 and 48.28%, respectively) cell lines.

5.2.1 Invitro antioxidant evaluation of *Senna auriculata*:

- This study was aimed to determine total phytochemical components as well as *in vitro* antibacterial, antioxidant, anti-inflammatory, anti-diabetic, and wound healing attributes of *Senna auriculata* (L.) Roxb. leaves extract. Among different solvent extracts tested, methanolic extract revealed

maximum alkaloid, flavonoids, phenolic, and tannin contents of 90.41 ± 0.15 mg atropine equivalent/g, 156.64 ± 0.97 mg quercetin equivalent/g, 155.44 ± 1.65 mg gallic acid equivalent/g, and 121.31 ± 2.34 mg gallic acid equivalent/g of extract, respectively.

- Antibacterial activity of methanolic extract was determined against indicator pathogens using agar well diffusion assay which exhibited potential activity against *Bacillus subtilis*. Antioxidant, anti-inflammatory, anti-diabetic, and wound healing attributes of methanolic extract were analyzed using standard methodologies. The extract exhibited potential antioxidant traits by scavenging ABTS (IC₅₀ value – 224.2 µg/mL) and DPPH (IC₅₀ value – 76.24 µg/mL) in a concentration dependent manner. Further, the extract demonstrated significant anti-inflammatory activities at varied doses with IC₅₀ value of 55.7 µg/mL.
- The methanolic extract exhibited promising rates of α-amylase and α-glucosidase inhibition with IC₅₀ values of 49.45 and 38.72 µg/mL, respectively. Further, the extract showed no toxicity on L929 mouse fibroblast cell lines ($99.66 \pm 0.14\%$ cell viability). Most importantly, the treatment of cell monolayer of L929 mouse fibroblast cell lines with methanolic extract showed high rate (100%) of wound healing trait. GC–MS analysis of methanolic extract revealed the presence of Mome inositol, 13-Docosenamide, (Z)-, Cycloheptasiloxane, tetradecamethyl-, and Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester as predominant compounds.
- In conclusion, the biological properties of *S. auriculata* leaves suggested its vast role as ideal antibacterial, antioxidant, anti-inflammatory, anti-diabetic, and wound healing agents in future.

5.3 Cytotoxicity, Antimicrobial and Antioxidant Studies of the Mimosa Pudica:

- The petroleum ether, chloroform and methanol crude extracts of the two different plant parts (aerial part and root) of *Mimosa pudica* (Mimosaceae) were screened *in vitro* for cytotoxicity studies by brine shrimp lethality bioassay and antimicrobial screening by disc diffusion method.
- The methanol crude extract of the aerial part was screened *in vitro* for antioxidant activity using the 1, 1-diphenyl-2-picrylhydrazyl-hydrate (DPPH) free radical scavenging assay. The petroleum ether and methanol crude extracts of the root showed potential cytotoxic activities (LC_{50} 0.05 $\mu\text{g/ml}$ and 0.035 $\mu\text{g/ml}$ respectively) whereas the other extractives showed poor cytotoxicity.
- All the crude extracts showed poor activity or inactivity against the test microorganisms. On the other hand, the methanol crude extract of the aerial part showed moderate antioxidant activity (IC_{50} 296.92 $\mu\text{g/ml}$) compared to ascorbic acid (IC_{50} 131.29 $\mu\text{g/ml}$).
- The overall experimental results suggest the biologically active constituents present in the methanolic extract of *Mimosa pudica* and justify its use in folkloric remedies.

5.3.1 *Invitro antioxidant evaluation of mimosa pudica:*

- The study of free radicals and antioxidants in biology is producing medical revolution that promises a new age of health and disease management. The present study was performed to evaluate the *in vitro* antioxidant effect of the ethanolic extract of *Mimosa pudica* (Mimosaceae) against free radical damage by different standard methods such as DPPH (1,1-diphenyl-2-picrylhydrazyl), Nitric Oxide (NO), ABTS (2, 2e present study was performed to evaluate the (IC₅₀) and Hydrogen peroxide free radical model.

- The test extract exhibited significant inhibition in Nitric oxide and DPPH free radical formation with IC₅₀ values of 78.1 µg/ml for Nitric Oxide (NO), ABTS (2, 2, 2-triphenyl-1-picrylhydrazyl) and Hydrogen peroxide radicals IC₅₀ values of 81.00±3.85 and 449.60±2.55 µg/ml. Out of these four free radicals the extract showed potent activity on Nitric oxide and DPPH, which is compared to that of ascorbic acid and rutin taken as standards.

5.4 Scratch wound healing assay_ MG-63

- The *in vitro* scratch assay is an easy, low-cost and well-developed method to measure cell migration *in vitro*. The basic steps involve creating a “scratch” in a cell monolayer, capturing the images at the beginning and at regular intervals during cell migration to close the scratch, and comparing the images to quantify the migration rate of the cells. Compared to other methods, the *in vitro* scratch assay is particularly suitable for studies on the effects of cell–matrix and cell–cell interactions on cell migration, mimic cell migration during wound healing *in vivo* and are compatible with imaging of live cells during migration to monitor intracellular events if desired.
- Besides monitoring migration of homogenous cell populations, this method has also been adopted to measure migration of individual cells in the leading edge of the scratch. Not taking into account the time for transfection of cells, *in vitro* scratch assay *per se* usually takes from several hours to overnight.
- The scratch assay is an *in vitro* technique used to assess the contribution of molecular and cellular mechanisms to cell migration. The assay can also be used to evaluate therapeutic compounds before clinical use.

- The wound healing assay is a standard *in vitro* technique for probing collective cell migration¹⁻⁶ in two dimensions. In this assay, a cell-free area is created in a confluent monolayer by physical exclusion or by removing the cells from the area through mechanical, thermal or chemical damage.
- The *in vitro* scratch assay is a straightforward and economical method to study cell migration *in vitro*¹. This method is based on the observation that, upon creation of a new artificial gap, so called “scratch”, on a confluent cell monolayer, the cells on the edge of the newly created gap will move toward the opening to close the “scratch” until new cell–cell contacts are established again. The basic steps involve creation of a “scratch” on monolayer cells, capture of images at the beginning and regular intervals during cell migration to close the scratch, and comparison of the images to determine the rate of cell migration.

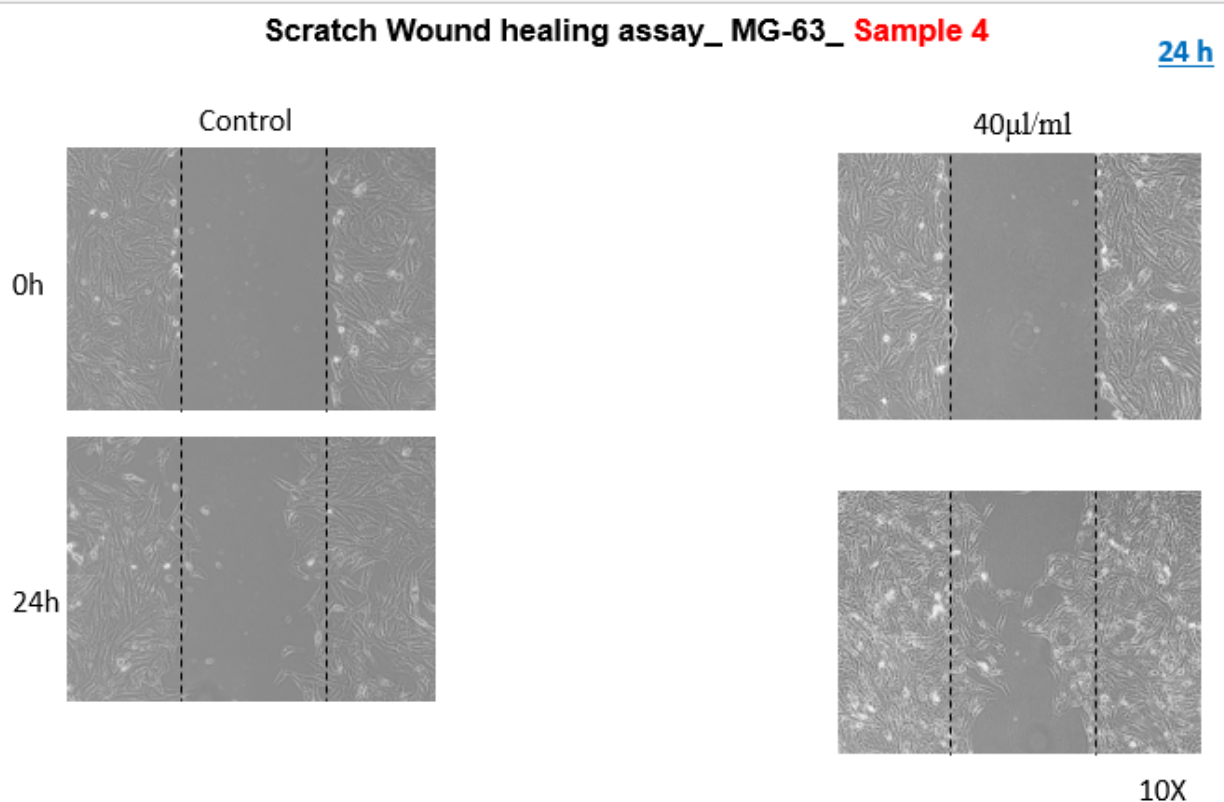


Fig 4.13: Scratch assay of sample

5.4 MTT Assay:

- The MTT assay involves the conversion of the water-soluble yellow dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to an insoluble purple formazan by the action of mitochondrial reductase. Formazan is then solubilized and the concentration determined by optical density at 570 nm.

5.4.1 Assay protocol

- Discard media from cell cultures. ...
- Add 50 μ L of serum-free media and 50 μ L of MTT solution into each well.
- Incubate the plate at 37°C for 3 hours.
- After incubation, add 150 μ L of MTT solvent into each well.
- Wrap plate in foil and shake on an orbital shaker for 15 minutes.
- Read absorbance at OD=590 nm.

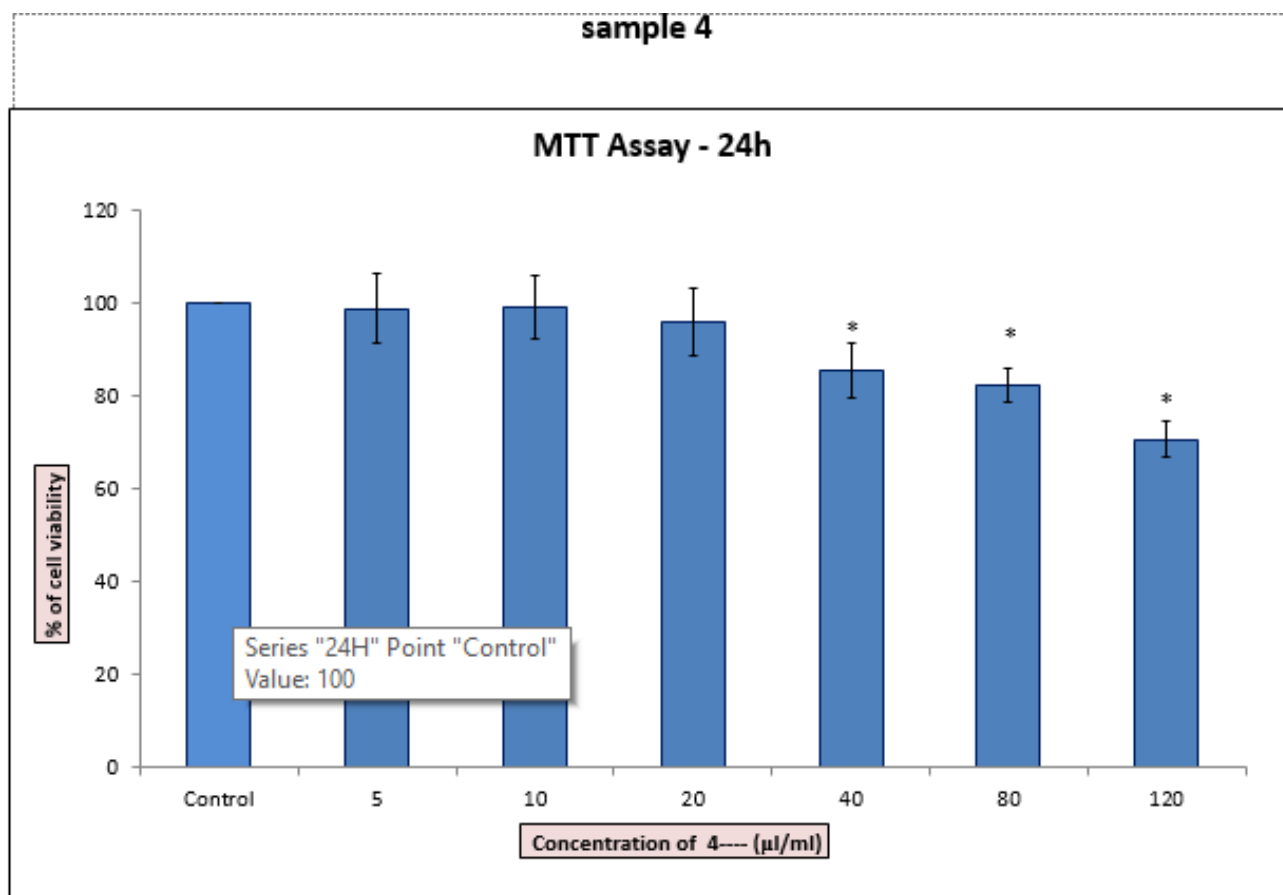


Fig 4.14: Graphical representation of sample

5.5 Formulation of herbal wound healing transdermal gel

- Herbal drug like *senna auriculata* leaf extract and *Mimosa pudica* leaf extract and carbopol 940 were used in gel formation. The extraction of *senna auriculata* and *mimosa pudica* were mixed with distilled water.
- The mix of the samples then mixed with carbopol 940. The formation of gel formed after mixing of carbopol 940.

Table 1: Optimized formulation for wound healing gel:

S.no	Ingredients	Amount (g/ml)
1	<i>Senna auriculata</i>	0.85
2	<i>Mimosa pudica</i>	1
3	Carbopol 940	1

5.6 Image of wound healing gel



Fig 4.15 Prepared of gel before centrifuge

5.6.1 Centrifuge:

- A centrifuge is a device that uses centrifugal force to separate various components of a fluid. This is achieved by spinning the fluid at high speed within a container, thereby separating fluids of different densities (e.g. cream from milk) or liquids from solids.

- The centrifuge technique used here for the separation of water, dust and the gel. The sample is placed in the centrifuge apparatus at 4000 rpm for 20 minutes.
- After the process, the gel form was clear and the dust particles settled down at the bottom of the centrifuge tube.



Fig 4.16 Prepared gel after centrifuge.

- The clear sample of gel obtained after the centrifuge technique.

Table 2: Parameters

S.no	Physical appearance	Result
1	Appearance	Jellified preparation
2	Colour	Light brown
3	Clarity	Clear

4	Flexibility	Yes
5	Smoothness	Good



Fig 4.17 Result of the prepared gel.

Chapter 6

SUMMARY and CONCLUSION

Summary

In this project our intention is to develop an herbal based healing gel for the foot ulcer. The main purpose of herbals that used in wound healing potential, assessment of revascularization and predictability of amputation level. *Mimosa pudica* is majorly possesses antibacterial, antiviral, antivenom, antifertility, anticonvovulsant, antidepressant, aphrodisiac. It prevents black spots, treats uneven skin tone and improves skin complexion. *Senna* flowers extract used in the treatment ulcers. Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. In this method, the finely ground crude drug is placed in a porous bag or “thimble” made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. Dichloromethane, ethyl acetate and methanol are the solvents used in this process. The sample with ethyl acetate has extract after 4-5 hours. The qualitative and quantitative analysis for the samples has been done. Qualitative Analysis is the determination of non-numerical information about a chemical species, a reaction, etc. Examples would be observing that a reaction is creating gas that is bubbling out of solution or observing that a reaction results in a color change.

Quantitative analysis (QA) is a technique uses mathematical and statistical modeling, measurement, and research that to understand behavior. Determination of the amount, as well as the nature, of each of the elements composing a substance. The results are tabulated.

Antibacterial test was done. The activity zone has been tested. Solvent ethyl acetate has more reactivity than other 2 solvents. The *senna* has more reactive to ethyl acetate than *mimosa*. Zone in *Senna auriculata* solvent (a) ethyl acetate diameter in 6mm (e.coli). Zone in *Senna auriculata* solvent (a) ethyl acetate diameter in 8mm

(pneumonia). The highest zone in *Senna auriculata* solvent (a) ethyl acetate diameter in 13mm (staphylococcus). Highest zone in *Senna auriculata* solvent (a) ethyl acetate diameter in 12mm (pseudomonas). The extraction of the *Senna auriculata* 0.86g and diluted *Mimosa pudica* with ethyl acetate 1 ml has been taken and mixed with distilled water then 0.25g of Carbopol are mixed. The mix of the samples then mixed with carbopol 940. The formation of gel formed after mixing of carbopol 940.

The centrifuge technique used here for the separation of water, dust and the gel. The sample is placed in the centrifuge apparatus at 4000 rpm for 20 minutes.

After the process, the gel form was clear and the dust particles settled down at the bottom of the centrifuge tube.

The formed gel is light brown in color and clarity is clear. The smoothness is good.

Conclusion

Wound healing is a biological process that begins with trauma and ends with scar formation. The goals of wound care includes reducing risk factors that inhibit wound healing enhancing the healing process and lowering the incidence of wound infections. Many medical plants provide leads to find therapeutically useful compounds, thus more efforts should be made towards isolation and characterization of the active principles and elucidation of the relationship between structure and activity. Plant extract like *senna* and *mimosa* has wound healing properties not been commercially available in wound healing creams. So that here we taken this as to replace chemical with herbal ingredients. The *senna* and *mimosa* were the two herbs used for the preparation of gel. The *senna* has more reactive properties to the microbes. The extraction of both *senna* and *mimosa* were mixed with carbopol 940 for the gel formation. The invitro scratch has tested for the cell lines. The clear gel was obtained after centrifuge technique. The formed gel packed. The prepared gel tested invitro testing. This wound gel is less side effects. The prepared gel show reliable physical appearance and has good flexibility. This product can also be developed as a Band-Aid. The in vivo test can done for this and research can also done in the future. The combination of traditional and modern knowledge can produce better products.

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