

## SYNERGISTIC WOUND HEALING POTENTIAL OF A POLYHERBAL OINTMENT ENRICHED WITH MIMOSA PUDICA AND MORINGA OLEIFERA

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### ABSTRACT

Healing of cutaneous wounds is a vital physiological processes made up of numerous cell strains working together and their goods. The processes of tissue repair and regeneration are made up of a series of cellular and molecular events that follow the beginning of a tissue lesion in order to repair the injured tissue. An alternative is herbal medicine therapy that makes use of various plants and plant extracts. Herbal remedies work with the body's immune system to establish a uniform detoxification procedure. Herbal remedies have produced many of the most effective medications in the last ten years to the extensive collection of medications that modern medicine has access to, both in unrefined form and as a pure chemical that serves as the foundation for contemporary medications. Despite the fact that herbal remedies provide numerous benefits, they also have

drawbacks. Therefore, the new treatment for illness requires which are highly effective and have few adverse effects. The mechanism underlying the reason for using natural remedies is that nature has all the components needed to cure illness. Because of their anti-inflammatory and antioxidant properties, Mimosa pudica and Moringa oleifera exhibit wound healing activity. The mechanical evaluation metrics such as spreadability, viscosity, and pH, Tests for homogeneity are crucial for assessing pharmaceutical topical mixtures. The purpose of this study was to compare various herbal formulations used for topical therapeutic agent delivery

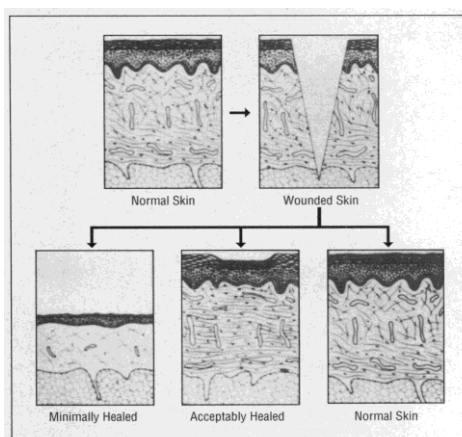
at the time of injury to speed up skin healing as quickly as possible while causing the patient as little pain, discomfort, or scarring as possible healing procedure.

**KEYWORDS:** Cutaneous wound healing, Herbal formulations, *Mimosa pudica*, *Moringa oleifera*, Anti-inflammatory activity, Antioxidant activity, Collagen synthesis, Topical drug delivery.

## INTRODUCTION

A wound is characterized by harm or disturbance to the typical anatomical structure and operate.<sup>[1]</sup> Whatever the cause, the majority of wounds heal easily. However, some wounds are susceptible to obstacles. healing, even though these don't stop the wounds from healing are properly handled. A small percentage of injuries will turn into persistent and non-recoverable. In these situations, the ultimate objective is to manage the symptoms and avoid complications instead of mending the injury.<sup>[2]</sup> Pathological conditions can cause wounds. procedures that start either internally or externally inside the affected organ. They are able to have an unintentional or deliberate aetiology, or they may be the outcome of an illness process, injuries, regardless of the reason and damages the tissue in any way and disturbs the surrounding environment. A physiological reaction to the harmful element causes bleeding, constriction of the vessels with coagulation, complement activation, and an inflammatory reaction.<sup>[3],[4],[5]</sup>

Damage to biological tissues, like the skin, results in a wound. organs and mucous membranes. Numerous injuries can cause wounds; there are Therefore, in order to avoid infections and additional damage, the wounds should be cleaned and appropriately attired.<sup>[6]</sup>



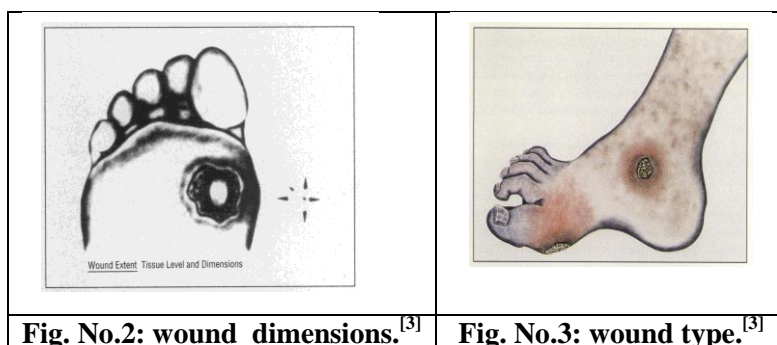
**Fig. No. 1: forms of wound healing.**<sup>[3]</sup>

## Classification of Wound

Classification of wounds is crucial from the perspective of management, diagnosis, selecting the best course of action, required time for wound healing and foreseeing the dangers and infections that could develop while the wound heals procedure.<sup>[7],[8],[9]</sup> Healthcare systems are severely harmed by skin wounds, and the global economy. According to a recent report, almost one billion people have both acute and chronic injuries worldwide.<sup>[10]</sup> The pathogenesis of skin wounds determines whether they are acute or chronic and repercussions.<sup>[11]</sup> They are typically categorized as chronic wounds such as acute injuries like knife cuts and skin ulcers; chronic wounds are those brought on by metabolic disorders. It takes a long time for these wounds to heal In contrast, acute wounds heal in a balanced and brief duration. Chronic wounds are unbalanced. in the synthesis and breakdown of ECM and cells, For instance, collagen.<sup>[12]</sup>

## Chronic Wounds

Chronic wounds are those brought on by metabolic conditions. These injuries require a lot of Unlike acute wounds, which heal quickly, in a reasonable amount of time. Prolonged There is an imbalance in the production and deterioration of cells and extracellular matrix, such as collagen.<sup>[13][14]</sup> In persistent infections, a cruel There is a cycle whereby persistent inflammation brought on by Persistent biofilm causes ongoing and excessive creation of NETS, which damages tissue and increases the formation of biofilms.<sup>[15]</sup> The prolonged presence of myeloid cells is a characteristic of chronic wound healing. populations in the final stages of inflammation, including neutrophils, monocytes, and macrophages mation.<sup>[16]</sup> The Additionally, mi RNAs control the epigenetic regulation of the chronic wound formation process. inflammatory reactions through signa ling pathway modification.<sup>[17]</sup> Microbiological analysis of wounds usually entails a qualitative evaluation (i.e., microorganism types) and a semi-quantitative evaluation of the microbiological load (For example, an estimate of the microbial load shown as growth that is light, medium, or heavy.<sup>[18]</sup>

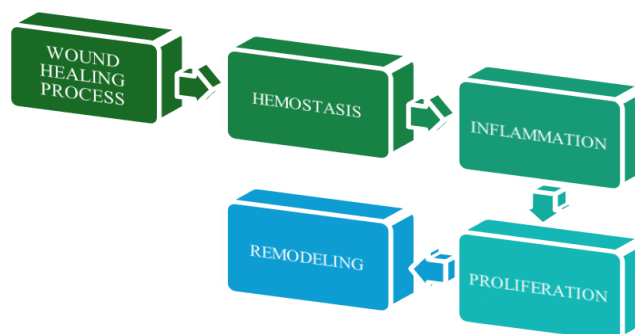


## Acute Wounds

Environmentally induced wounds Acute wounds are those that result from a traumatic injury. The precise and accurate balance of these wounds creation and breakdown of cells and extracellular matrix, and Consequently, healing occurs in a systematic manner. Acute injuries are divided into numerous groups according to the kind of environmental elements that contribute to harm. In general, There are two types of acute wounds: (1)(2) Surgical Wounds and Traumatic Wounds.<sup>[19],[20],[21]</sup> The healing of cutaneous wounds is a complicated process that involves numerous immune and structural cells, whose release of growth factors, chemokines, and cytokines coordinates the stages of recovery. There are four sequential stages of typical wound healing. phases: acute inflammation, hemostasis, which occurs minutes to hours after skin damage, mation, which takes one to three days, and proliferation, which typically takes a few days to a month, and lastly, the development of scars or skin remodeling.<sup>[22],[23],[24]</sup>

## Wound Healing Concept

The dynamic process of wound healing consists of four ongoing, overlapping, and carefully planned stages. Each phase's events must take place in a exact and controlled way. disruptions, anomalies, or extensions in the process may result in a chronic wound that doesn't heal or in delayed wound healing. The following processes are necessary for the best possible wound healing in adult humans:(1) quick hemostasis; (2) suitable inflammation; (3) differentiation of mesenchymal cells ferentiation, migration, and proliferation to the site of injury; (4) appropriate angio-genesis; (5) stimulate the regrowth of epithelial tissue over the wound surface); and (6) appropriate collagen synthesis, cross-linking, and alignment lagen to give the healing tissue strength.<sup>[25]</sup>



**Fig. No. 4: Wound Healing Process.**<sup>[25]</sup>

## Mechanism of Wound Healing

### 1] Collagen Formation

An essential part of the extracellular matrix, collagen plays vital functions in controlling the stages of wound healing in either its natural, fibrillar form or as substances that dissolve in the wound environment. Any of these phases' deficiencies cause the wound to stall in a persistent, non-healing condition that usually calls for some kind of intervention to steer the process back to the end. Persistent inflammation is one of the main elements of a chronic wound's hostile environment increased ECM component degradation brought on by elevated metallo proteinases and other enzymes and inappropriate activation of soluble mediators involved in wound healing. Collagen, which is essential has been used as an additional wound treatment to regulate a number of these processes encourage recovery. The importance of collagen in various biological processes that are pertinent to reviewed, along with an overview of the most recent research on the application of collagen-based Products for wound care are offered.<sup>[26]</sup>

### 2] Anti-inflammatory

The inflammatory phase of wound healing is crucial, but too much inflammation can impede healing. Inflammatory mediators like prostaglandins, cytokines (IL-1, TNF- $\alpha$ ), and enzymes like cyclooxygenase (COX) are inhibited by anti-inflammatory drugs. Flavonoids and phenolic compounds are examples of phytochemicals that minimize tissue damage by reducing oxidative stress and leukocyte migration. This regulated decrease in inflammation facilitates a quicker transition to the proliferative phase, improving tissue regeneration and repair.<sup>[27,28]</sup>

### 3] Tissue Regeneration

In wound healing, fibroblast activity, cell proliferation, and re-epithelialization all contribute to tissue regeneration, which creates new tissue. Growth factors promote angiogenesis and collagen synthesis, while phytochemicals improve cell division and repair, hastening the healing of injured tissue.<sup>[27, 28]</sup>

## Need for Herbal formulation in modern Therapy

Mother Nature has given us many ways to stay healthy. Among them is the herbal medications. For thousands of years, herbal medicines have been used to treat a variety of illnesses. People are becoming more interested in using herbal medicines to treat a variety of diseases. The plant medication rasayana has always been used to treat a number of human

ailments played a significant part. The World Health Organization (WHO) reports that over 80% of the majority of people on the planet rely on traditional medicine to meet their basic medical needs.<sup>[29]</sup> A typical method for determining which herb might be best for a particular ailment would be to classify herbs based on the types of issues they can address assist. But before using herbs independently, a thorough understanding of them should be acquired utilize.<sup>[30]</sup> The cost of herbal products is one benefit. They are comparatively less expensive than the synthetic medications that doctors now frequently prescribe. In actuality, consumers on a tight budget are choosing this remedy for their health issues in order to obtain as much savings as possible to take care of their health-related issues.<sup>[31]</sup> Occasionally, the Traditional synthetic medications used to treat patients with long-term illnesses can be substituted with herbal remedies. This is essential because their synthetic equivalents frequently have significant adverse effects. Herbal remedies ease the shift from acute, transient to long-term, chronic therapy.<sup>[32]</sup>

Because they interact with biological tissues without causing irritation or toxicity, herbal formulations are regarded as biocompatible. They are appropriate for topical applications such as wound healing because of their natural origin.<sup>[33],[34]</sup> Because of their mild and natural composition, herbal medicines typically have fewer side effects than synthetic ones. For long-term therapeutic use, this makes them safer.<sup>[35],[36]</sup> Because they come from readily accessible plant sources and require less expensive processing, herbal formulations are economical. As a result, a larger population can access them.<sup>[37],[38]</sup>

## OVERVIEW OF MEDICINAL PLANTS

### **Mimosa pudica plant**

*Mimosa pudica* L. is an annual or perennial herb that creeps. It has identified as Lajjalu in Ayurveda and discovered to have antidepressant, analgesic, aphrodisiac, and antiasthmatic qualities. *M. pudica* is recognized for its tonic, emetic, and sedative qualities and has long been utilized to treat a variety of illnesses including tumors, insomnia, diarrhea, dysentery, alopecia, and different infections of the urogenitalia. Research on *M. pudica*'s phytochemistry have demonstrated the existence of non-protein amino acids and alkaloids. (mimosine), flavonoids, tannins, terpenoids, sterols, and C-glycosides and fatty acids.<sup>[39]</sup>



**Fig. No. 5: Mimosa Pudica Plant.**<sup>[39]</sup>

**Taxonomy**<sup>[40]</sup>

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Fabales

Family : Fabaceae/ Mimosaceae

Sub – family : Mimosoideae

Genus : Mimosa

Species : Pudica

**M. pudica's botanical description**<sup>[41]</sup>

**Characters - M.Pudica**

Plant - Glandular hairs and short, prickly branches

Leaves - Bipinnate, touch-sensitive

Flowers - Axillary, globose head, and lilac pink in Color

Stem - Erect, thin, prickly, and well-branched

Calyxes - Companulate

Petals - Petals Crenate in the direction of the base

Pods - 1.5–2.5 cm long, closely prickly on the falcate and sutures

Blooming and Time for fruiting in between August to October in Indian condition

**Vernacular Names**<sup>[42]</sup>

Sanskrit – Lajja

English – Sensitive plant

Hindi – Laajvanti and Chhui-mui

Bengali – Lajjabati

Telugu – Attapatti and Peddanidrakanni Usesss:

Tamil – Tottaaladi and Thottalchnungi

Kannada – Lajja, Nachika and Mudugu-davare

Malayalam – Tintarmani.

## Uses

### Traditional Uses<sup>[41],[43]</sup>

1. Used to treat dysentery and diarrhea
2. Used in piles (hemorrhoids)
3. Used to treat urinary issues
4. Helps with insect and snake bites (traditional use)

### Medicinal Uses<sup>[44],[43]</sup>

1. Demonstrates hepato protective (liver protective) activity.
2. Used in the treatment of diabetes
3. Demonstrates CNS and antidepressant activity.
4. Used to treat gynecological conditions.

## MACROSCOPY

### Root

Tapered, cylindrical pendant with secondary and tertiary branches, varying in length up to 2-cm thick, surface more or less rough or longitudinally wrinkled; cut, grayish-brown to brown pale yellow, wood-like, fracture-hard, and bark-fibrous; Taste: slightly astringent; smell: distinct.

### Stem

Cylindrical, up to 2.5 cm in diameter; sparsely prickly, covered with long, weak bristles longitudinally grooved, external surface light brown, internal surface grey, bark fibrous; easily separable from wood.

**Leaf**

Digitately compound with one or two pairs of sessile, hairy pinnae, alternate, petiolate, stipulate, linear lanceolate; leaflets 10–20 pairs, 0.6–1.2-cm long, 0.3–0.4-cm broad, sessile, obliquely narrow or linear oblong; obliquely rounded at base, acute, nearly glabrous; yellowish green.

**Fruit**

Lomentum, simple, dry, 1–1.6-cm long, 0.4–0.5-cm broad, with indehiscent segments and persistent sutures having —two to five seeds with yellowish spreading bristle at sutures, 0.3-cm long, glabrous, and straw colored.

**Seed**

Compressed, oval-elliptic, brown to gray, 0–0.3-cm long, 2.5-mm broad, having a central ring on each surface.

**MICROSCOPY****The root**

Mature roots exhibit tangentially elongated cells with five to twelve layers a few exfoliated or crushed outer layers; secondary cortex made up of thin-walled, tangentially elongated cells with six to ten layers; secondary phloem made up of fibers, crystal fibers, sieve elements, and Phloem rays, phloem fibers, and phloem parenchyma single or in clusters, arranged in bands that are tangential; thick crystal fibers walled, with three to twenty-five chambers and one, two, or four prismatic calcium oxalate crystals; uni-to-multi-seriate phloem rays—two more prevalent; secondary xylem is made up of typical components that xylem rays pass through; vessels dispersed throughout secondary xylem with reticulate thickenings and bordered pits; crystal fibers with one or, in rare cases, two or four prismatic crystals of calcium-oxalate in every chamber; thick-walled parenchyma, dispersed throughout the secondary xylem; uni-to-bi-seriate xylem rays seldom multi-seriate, narrow, and broader toward secondary phloem toward the center; calcium oxalate prismatic crystals and starch grains and tannin found in xylem rays, phloem, and secondary cortex and parenchyma; simple and complex starch grains with two to three parts, ranging in size from 6 to 20 mm, rounded to oval and 16–28 mm in diameter, in that order.

**The Stem**

A mature stem displays an exfoliated, four to eight layered cork of tangentially extended cell containing reddish-brown material; wide secondary cortex made up of big, somewhat thick walled, parenchymatous cells that are tangentially elongated to oval, filled with reddish-brown material, some cells have prismatic calcium oxalate crystals, several lignified, single or in clusters, dispersed throughout; secondary phloem made up of standard components, two to five strips of Fibers alternate with thin sieve element strips and parenchyma, long, thick-walled crystal fibers that contain Each chamber contains a single calcium oxalate crystal; phloem rays radially elongated secondary xylem with thick walls made of typical components that xylem rays pass through, such as vessels and drum-shaped spiral thickenings, pointed-end-pitted tracheids, fibers of two varieties: longer with a narrow lumen and shorter with a wide lumen; xylem rays that are thick-walled, radially elongated, 1–6 cells wide, and 3–30 cells high; polygonal, parenchymatous pith cells that have spaces inside of them.

**The leaf**

The cortex has four to seven layers of thin-walled, parenchymatous tissue, while the petiole has a single layer of epidermis covered in a thin cuticle cells; a ring of pericycles; four central vascular bundles exhibit two laterally positioned, smaller vascular bundles, one in every wing.

**The Fruit**

Displays a single-layered epidermis with a small number of nonglandular shaggy, branched hair; five to six layers of thin-walled mesocarp amphicribal vascular bundles and parenchymatous cells dispersed throughout this area; endocarp of lignified cells with thick walls Parenchymatous cells with a single layer and thin walls came next.

**The seed**

Displays radially elongated, single-layered cells, followed by five to angular, six-layered cells with dark brown contents; Endosperm is made up of elongated or angular cells, a few comprising calcium oxalate prismatic crystals; cotyledons comprise a few cells with rosette crystals and thin-walled cells of calcium oxalate; a straight embryo with a thick, short radical.<sup>[45]</sup>

## Phytochemistry

### 1] Mimosine and Alkaloids

The most widely studied and characteristic compound in *M. pudica* is mimosine, a non-protein amino acid (also classified as an alkaloid)<sup>[46]</sup> Mimosine is structurally similar to tyrosine and is known for its antimitotic properties, which it achieves by chelating metal ions that are necessary for enzymes such as DNA polymerase.<sup>[47]</sup> The cell cycle is specifically stopped in the late G1 stage by this antimitotic action phase, stopping the replication of DNA. Although this characteristic is investigated for anticancer studies, it is also in charge of the plant's toxicity. Mimosine can be broken down by rumen microbes in ruminants, but it is a significant toxin in non-ruminants that can cause depressed growth, goitrogenic effects, and alopecia (hair loss).

### 2] Phenolics and Flavonoids

The strong antioxidant activity of *M. pudica* is primarily due to its abundance of flavonoids and phenolic compounds. Important C-glycosyl flavonoids Vitexin, isovitexin, orientin, and isoorientin are examples of derivatives of apigenin and luteolin. The direct bond between C and C between these compounds' sugar and aglycone (flavonoid nucleus) provides substantial stability against both enzymatic and acidic hydrolysis in contrast to the more prevalent O-glycosides. Their bioavailability and persistence may be improved by this increased stability. *in vivo*, supporting the plant's long-lasting hepatoprotective and anti-inflammatory properties.<sup>[48]</sup>

### 3] Tannins and Terpenoids

The plant's traditional use is explained by the presence of tannins, including hydrolyzable and condensed (proanthocyanidins) as an astringent and to treat bleeding, dysentery, and diarrhea.<sup>[49]</sup> The astringent characteristic is caused by the precipitation of mucosal and salivary proteins. Its ability to bind proteins is also essential to its antimicrobial and wound-healing properties, since tannins can combine with microbial enzymes to inhibit their activity and create a "leathery" layer of protection over wounds. Furthermore, Numerous sterols and terpenoids, including campesterol, stigmasterol, and  $\beta$ -sitosterol, have been found and are recognized for their cholesterol-lowering, anti-inflammatory, and other various biological actions.<sup>[50]</sup>

## Pharmacological Activities

### 1] Wound Healing Activity

By accelerating wound contraction and promoting epithelialization, the *Mimosa pudica* extract demonstrated notable wound healing activity.<sup>[51]</sup> *Mimosa pudica*'s flavonoids and tannins aid in the production of collagen and quicker wound healing. *Mimosa pudica* ethanolic extract accelerates wound healing and improves epithelialization.<sup>[51]</sup> *Mimosa pudica*'s antimicrobial activity promotes efficient wound healing by preventing infection.<sup>[52]</sup> *Mimosa pudica*'s phytochemicals encourage the production of collagen, which raises the healed tissue's tensile strength. *Mimosa pudica* speeds up the transition to the proliferative phase by reducing inflammation at the wound site. In experimental models, the *Mimosa pudica* extract dramatically accelerated the rate of wound contraction and shortened the healing period.<sup>[51]</sup>

### 2] Anti-inflammatory Activity

The production of inflammatory mediators like prostaglandins and cytokines is decreased by *mimosa pudica* extract. In experimental models, the plant significantly reduces edema, suggesting a potent anti-inflammatory potential.<sup>[53]</sup> By scavenging free radicals and lowering inflammation, flavonoids found in *Mimosa pudica* contribute to its anti-inflammatory effect.<sup>[54]</sup> By creating a barrier and lessening tissue irritation, tannins aid in the reduction of inflammation.<sup>[55]</sup> *Mimosa pudica* reduces inflammation by inhibiting inflammatory enzymes like cyclooxygenase (COX).<sup>[70]</sup> The plant's antioxidant qualities lessen oxidative stress, which is strongly associated with inflammation. To reduce inflammation, the extract modifies the activity of immune cells, particularly macrophages.<sup>[54]</sup> *Mimosa pudica* has long been used to treat inflammatory diseases like pain and swelling. Research indicates that *Mimosa pudica*'s anti-inflammatory properties increase with dosage. According to certain studies, *Mimosa pudica* generally shows strong anti-inflammatory activity on par with conventional medications.<sup>[53]</sup>

### 3] Antimicrobial Activity

*Staphylococcus aureus* and other Gram-positive bacteria are strongly inhibited by *Mimosa pudica* extract. Additionally, the plant has inhibitory effects on *Escherichia coli* and other Gram-negative bacteria. By preventing microbial growth, alkaloids like mimosine contribute to antimicrobial action. *Mimosa pudica* contains flavonoids that damage microbial cell membranes and prevent growth. *Mimosa pudica* contains flavonoids that damage microbial

cell membranes and prevent growth. *Mimosa pudica* contains flavonoids that damage microbial cell membranes and prevent growth. By precipitating microbial proteins and creating protective barriers, tannins have antimicrobial properties. *Mimosa pudica* extracts exhibit quantifiable zones of inhibition against various microbes.<sup>[56],[57],[58]</sup>

#### **4] Antioxidant Activity**

Because *Mimosa pudica* contains flavonoids and phenolic compounds, it has a strong ability to scavenge free radicals.<sup>[59]</sup> *Mimosa pudica*'s antioxidant potential is largely attributed to its flavonoids and phenolic compounds. By neutralizing reactive oxygen species (ROS), the extract lessens oxidative stress. In DPPH radical scavenging tests, *Mimosa pudica* exhibits strong antioxidant activity.<sup>[60]</sup> The concentration of *Mimosa pudica* extract increases antioxidant activity.<sup>[61]</sup> *Mimosa pudica* contains antioxidants that aid in tissue repair and shield cells from oxidative damage. By lowering oxidative damage at the wound site, antioxidant activity promotes quicker wound healing.<sup>[60]</sup>

#### **5] Analgesic Activity**

*Mimosa pudica* extract shows significant analgesic activity by reducing pain response in experimental models. The plant's analgesic effect is attributed to its reduction of prostaglandins and other pain mediators. Higher extract concentrations increase *Mimosa pudica*'s analgesic activity.<sup>[62]</sup>

#### **6] Immunomodulatory activity**

By activating immune cells like macrophages and lymphocytes, *mimosa pudica* extract improves immune response. The plant balances pro-inflammatory and anti-inflammatory reactions by controlling cytokine levels.<sup>[63]</sup> *Mimosa pudica* modulates immune functions, strengthening the body's defense against infections.<sup>[64]</sup>

#### **Moringa Oleifera Plant**

The tree *Moringa oleifera* Lam. is widely distributed in numerous nations that are tropical or subtropical. It has grown commercially in India, Africa, South and Central America, Mexico, Hawaii, and all of Asia and Southeast Asia. It's called the drumstick tree-based when its young seed pods first appeared, the horse-radish tree according to the flavor of prepared ground root as well as the ben oil tree from oils extracted from seeds. Within Immature seed pods are consumed in some places, but the Leaves are frequently utilized as a staple food due

to their high nutritional value. No clinical human Studies have been carried out to examine the effectiveness of *M. oleifera* to treat malnutrition.<sup>[65]</sup>



**Fig. No. 6: Moringa Oleifera Plant.**<sup>[66]</sup>

### **Taxonomy**<sup>[66]</sup>

Kingdom: Plantae

Superkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Dilleniidae

Order: Capparales

Family: Moringaceae

### **Biological Source**

*Moringa oleifera*, a member of the Moringaceae family, is a successful treatment for malnutrition. *Moringa* has a lot of nutrients. Due to the existence of numerous vital phytochemicals, calcs found in its seeds, pods, and leaves. Actually, moringa is claimed to offer ten times more vitamin C than oranges. 17 times more calcium than milk, more vitamin A than carrots, 15 times more potassium and 9 times more protein than yogurt 25 times more iron than spinach and more than bananas.<sup>[67]</sup>

### **M. Oleifera botanical description**

*Moringa oleifera* is a small to medium-sized deciduous tree in the Moringaceae family that grows quickly. The plant has delicate, drooping branches and soft, corky bark. Its leaves are light green, feathery, and tripinnate. Usually carried in clusters, the fragrant flowers are either creamy white or yellowish-white. The plant yields drumsticks, which are long, thin, pendulous fruits.<sup>[68]</sup> Because of its nutritional and therapeutic value, the plant, which has a deep root system, is extensively grown in tropical and subtropical areas. The leaves are

compound and arranged in an alternating pattern, and the stem is soft and woody. The seeds are dark brown and contained in triangular seed pods, while the flowers are aromatic and bisexual. Traditional medicine uses nearly every part of the plant, including the leaves, bark, roots, flowers, and seeds.<sup>[69]</sup>

## **DISTRIBUTION**

*Moringa oleifera*, a member of the Moringaceae family, is also referred to as the drumstick tree, horseradish tree, or miracle tree. It is widely found in tropical and subtropical areas and is prized for its therapeutic and nutritional qualities. The plant's numerous pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and wound-healing qualities, are attributed to its abundance of vitamins, minerals, flavonoids, phenolic compounds, and antioxidants.<sup>[67]</sup> In traditional medicine, the plant is widely used to treat skin conditions, wounds, inflammation, infections, and anemia. The plant's leaves, roots, bark, flowers, and seeds are among the various parts that have medicinal value. *Moringa oleifera* has drawn a lot of interest in herbal research and pharmaceutical applications because of its bioactive components.

## **Vernacular Names<sup>[70]</sup>**

Latin - *Moringa oleifera*

Sanskrit - Subhanjana

Hindi - Saguna, Sainjna

Gujarati - Suragavo

Tamil - Morigkai

Telugu - Mulaga, Munaga

Malayalam - Murinna, Sigru

Punjabi - Sainjna, Soanjna

Unani - Sahajan

Ayurvedic - Akshiva, Haritashaaka, Raktaka, Tikshnagandhaa

Arabian - Rawag

French - Moringe à graine ailée, Morungue

Spanish - Ángela, Ben, Moringa

Portuguese - Moringa, Moringueiro

Chinese - La ken

English - Drumstick tree, Horseradish tree, Ben tree.

### **Morphology**

A small, quick-growing evergreen or deciduous tree, *Moringa oleifera* typically reaches a height of as nine meters, with corky, gummy bark and soft, white wood. Roots taste like horses. radish. The leaves have longitudinal cracks, a main axis that is 30 to 75 cm long, and jointed branches. Glandular at joints, the leaflets are whole and glabrous. The leaflets are green, hairy, and nearly red-tinged mid-veins, hairless on top, and paler and hairless below, with full (not toothed) edges that are short-pointed at the base and rounded or blunt-pointed at the top. The twigs are green and finely hairy. Large axillary down panicles of fragrant, white flowers The seeds are three-angled, and the pods are pendulous and ribbed.<sup>[71,72,73]</sup>

### **Medicinal Uses**

*Moringa oleifera* possesses antimicrobial, antioxidant, anti-inflammatory, analgesic, antidiabetic, hepatoprotective, and wound healing activities. The plant is also used in the treatment of skin infections, fever, inflammation, and digestive disorders.<sup>[74]</sup>

### **Conventional Use**

*Moringa oleifera* is widely used as a vegetable, animal feed, nutritional supplement, and natural water purifier. Various parts of the plant are also utilized in agricultural applications and traditional home remedies.<sup>[75]</sup>

### **Phytochemistry**

*Moringa oleifera* is abundant in substances that include the rhamnose, simple sugar, and a rather unusual collection of substances known as isothiocyanates and glucosinolates. The bark of the stem has been found to include two alkaloids, specifically Vanillin and moringine,  $\beta$ -sitosterol.<sup>[76]</sup>

### **Flavonoids**

The high flavonoid content of the *Moringa* genus is the primary cause of its strong antioxidant activity. The majority of The genus contains flavonoids in the forms of flavanol and glycoside. The most prevalent flavonoids in the Rutin, quercetin, rhamnetin, kaempferol, apigenin, and myricetin belong to this genus.<sup>[77]</sup>

### **Glucosinolate**

Many glucosinolates, including 4-O-( $\alpha$ -L-rhamnopyranosyloxy)-benzyl, are found in *moringa* species. Depending on the physiological characteristics and maturity of glucosinolate, also referred to as glucomoringin (GMG).

### Acid Phenolic

Gallic acid is the primary phenolic acid found in *M. oleifera* leaves. Caffeic acid, ferulic acid, ellagic acid, and The leaves also contain gentisic acid, syringic acid,  $\rho$ -coumaric acid, coumaric acid, and chlorogenic acid traces of sinapic acid and acid were found.<sup>[78]</sup>

### Terpenes

According to reports, *M. oleifera* leaves contain lutein as a major carotenoid, including -E-luteoxanthin, 13-Z-lutein, all-E-zeaxanthin, and 15-Z- $\beta$ -carotene.  $\beta$ -amyrin,  $\alpha$ -amyrin, and lupeol acetate.<sup>[77]</sup>

### Alkaloids

Marumoside A and Marumoside B are two novel pyrrole alkaloid glycosides found in *M. oleifera* leaves.

### Pharmacological Activity

#### 1] Wound Healing Activity

Because it contains bioactive substances like flavonoids, tannins, phenolic compounds, and antioxidants, *Moringa oleifera* shows notable wound healing activity. These phytoconstituents speed up the healing process by encouraging the production of collagen, tissue regeneration, epithelialization, and a decrease in inflammation at the wound site. The plant's antimicrobial and antioxidant qualities also aid in preventing oxidative damage and wound infection during the healing process.<sup>[79]</sup>

#### 2] Antimicrobial Activity

Because *Moringa oleifera* contains phytochemicals like flavonoids, tannins, alkaloids, and phenolic compounds, it has strong antimicrobial activity against a variety of pathogenic microorganisms. By preventing the growth of bacteria and fungi, the plant extracts aid in the healing process and help prevent wound infections. The plant is helpful in herbal remedies for wound and skin care because of its antimicrobial properties.<sup>[80]</sup>

#### 3] Anti Inflammatory Activity

Because *Moringa oleifera* contains flavonoids, phenolic compounds, and other bioactive components, it has strong anti-inflammatory properties. By limiting tissue damage and blocking inflammatory mediators, these phytochemicals aid in the reduction of inflammation.

The plant's anti-inflammatory qualities are crucial for accelerating wound healing and minimizing swelling at the wound site.<sup>[81]</sup>

#### 4] Antioxidant Activity

Because it contains flavonoids, phenolic acids, vitamins, and other phytoconstituents, *Moringa oleifera* has potent antioxidant activity. These antioxidants lessen oxidative stress, counteract free radicals, and shield tissues from cellular damage. The plant's antioxidant qualities aid in tissue repair and speed up the healing of wounds.<sup>[82]</sup>

#### 5] Analgesic Activity

Because *Moringa oleifera* contains flavonoids, alkaloids, and other bioactive substances, it has strong analgesic properties. By inhibiting inflammatory mediators, the plant extract relieves painful conditions related to inflammation and wounds.<sup>[83]</sup>

#### 6] Immunomodulatory Activity

Because *moringa oleifera* contains flavonoids, vitamins, and phenolic compounds that help control and improve the immune response, it has immunomodulatory activity. The plant extract enhances the body's defense system and increases immune cell activity, which may promote quicker wound healing and infection prevention.<sup>[84]</sup>

### PHARMACOGNOSTIC EVALUATION

#### ➤ Test for alkaloids

**Table No. 1: Test For Alkaloids.**

SR. NO	TEST	OBSERVATION OF MIMOSA PUDICA	OBSERVATION OF MORINGA OLIFERA	INFERENCE OF MIMOSA PUDICA	INFERENCE OF MORINGA OLEFIRA
1	<b>Dragendorff's Test:</b> 2 mL extract + dilute HCl + Zragendorff's reagent	Orange reddish-brown precipitate	Orange precipitate	Alkaloids present	Alkaloids present



**Fig. No. 7: Dragendroff's Test For Mimosa Pudica.**



**Fig. No. 8: Dragendroff's Test For Moringa Olefira.**

➤ **Test for flavonoids**

**Table No. 2: Test For Flavonoids.**

SR. NO	TEST	OBSERVATION OF MIMOSA PUDICA	OBSERVATION OF MORINGA OLEFIRA	INFERENCE OF MIMOSA PUDICA	INFERENCE OF MORINGA OLEFIRA
	<b>Shinoda Test:</b> 2 mL extract + magnesium ribbon + conc. HCl	Pink/red colour	Reddish pink colour	Flavonoids present	Flavonoids present



**Fig. No. 9: Shinoda Test For Mimosa Pudica.**



**Fig. No. 10: Shinoda Test For Moringa Olefira.**

## ➤ Test for saponin

Table No. 3: Test For Saponin.

SR. NO	TEST	OBSERVATION OF MIMOSA PUDICA	OBSERVATION OF MORINGA OLEFIRA	INFERENCE OF MIMOSA PUDICA	INFERENCE OF MORINGA OLEFIRA
1.	<b>Foam Test:</b> 2 mL extract + distilled water, shake vigorously	Stable persistent foam	Stable froth formation	Saponins present	Saponins present



Fig. No. 11: Foam Test For Mimosa Pudica.



Fig. No. 12: Foam Test For Moringa Olefira.

## ➤ Test for Tannins

➤ Table No. 4: Test For Tannins.

SR. NO	TEST	OBSERVATION OF MIMOSA PUDICA	OBSERVATION OF MORINGA OLEFIRA	INFERENCE OF MIMOSA PUDICA	INFERENCE OF MORINGA OLEFIRA
1.	<b>Ferric Chloride Test:</b> 2 mL extract + 5% FeCl <sub>3</sub> solution	Blue-black colour	Greenish-black colour	Tannins present	Tannins present



Fig. No. 13: Ferric Chloride Test For Mimo.



Fig. No. 14: Ferric Chloride Test For Moringa Olefira.

➤ Test for Glycosides

Table No. 5: Test For Glycosides.

SR. NO	TEST	OBSERVATION OF MIMOSA PUDICA	OBSERVATION OF MORINGA OLEFIRA	INFERENCE OF MIMOSA PUDICA	INFERENCE OF MORINGA OLEFIRA
1.	<b>Keller–Killiani Test:</b> 2 mL extract + glacial acetic acid + FeCl <sub>3</sub> + conc. H <sub>2</sub> SO <sub>4</sub>	Brown ring at interface	Brown-violet ring at interface	Glycoside Present	Glycoside Present



Fig. No. 15: Keller–Killiani Test For Mimosa Pudica.



Fig. No. 16: Keller–Killiani Test For Moringa Olefira.

## EXTRACTION

### Mimosa pudica by Maceration

For 72 hours at room temperature with sporadic shaking, 100 g of shade-dried powdered Mimosa pudica leaves were macerated with 70% ethanol in a ratio of 1:10 (100 g powder in 1000 mL solvent). Following the maceration process, the extract was filtered through Whatman filter paper and concentrated by low-temperature evaporation to produce a semisolid mass that was kept in an airtight container for later analysis.<sup>[85]</sup>



**Fig. No. 17: Extraction Of Mimosa Pudica.**

### Moringa oleifera by Maceration

After being air-dried and ground up, 500 g of the leaves were macerated in one liter of 50% ethanol for seventy-two hours. The ethanol was decanted, filtered multiple times using Whatman's No. 1 filter and cotton wool paper and concentrated. The yield as a percentage was 15.61%, which 500 g of dried, ground leaves yielded an extraction of 78.05 g. The dark tan-colored extract produced on each day of the experiment was recently dissolved in a vehicle containing 5% Tween 80, which acted as automobile.<sup>[86]</sup>



**Fig. No. 18: Extraction of moringa oleifera.**

## Formulation Development of Ointment

### Type W/O

Helps keep moisture at the wound site and keeps the skin from drying out by creating an occlusive effect. The protective layer that the oily phase creates over the wound speeds up healing and improves the absorption of the herbal components from *Moringa oleifera* and *Mimosa pudica*. W/O bases are appropriate for wound-healing ointments because they enhance the emollient properties, lessen irritation, and prolong the medication's skin contact time.<sup>[87]</sup>

## INSTRUMENTS AND GLASSWARES

Table No. 6: Instruments And Glassware.

Instrument	Glassware's
Beaker	Weighing balance
Measuring cylinder	Refrigerator
Petri plate	Magnetic Stirrer
Pipette	PH meter

## MASTER FORMULA

Table No. 7: Master Formula.

Name of Ingredient	F1	F2	F3	F4	F5	Role
<b>Active Pharmaceutical Ingredient (API)</b>						
Mimosa Pudica Extract	0.4	0.4	0.4	0.4	0.4	Wound Healing
Moringa Oleifera Extract	1	1	1	1	1	Anti-Oxidant, Anti-Inflammatory
<b>Oil Phase</b>						
Beeswax	1.2	1.3	1.5	1.8	2.0	Stiffening Agent
Emulsifying Wax	0.8	0.9	1	1.1	1.2	Emulsifying Agent
Liquid Paraffin	2.8	2.5	2.3	2.1	2.0	Emollient
<b>Aqueous Phase</b>						
Glycerin	1.8	1.7	1.6	1.5	1.5	Humectant
Purified Water	q.s	q.s	q.s	q.s	q.s	Vehicle
Methyl Paraben	0.04	0.04	0.04	0.04	0.04	Preservative

## Method of Preparation of Herbal Ointment

- To create a homogenous molten mixture, precisely weighed amounts of white soft paraffin and beeswax were combined in a clean beaker and heated to approximately 70°C in a water bath.
- To create a smooth ointment base, liquid paraffin was then gradually added to the molten mixture while being constantly stirred.

- To ensure even mixing, the prepared ethanolic extracts of *Moringa oleifera* and *Mimosa pudica* were gradually added to the ointment base while being constantly stirred.
- To prevent lump formation, the mixture was continuously stirred until a homogenous ointment formed. After that, it was allowed to cool gradually at room temperature.
- Lastly, the prepared herbal ointment was put into hygienic, airtight containers and kept dry and cool for additional testing.<sup>[88]</sup>



**Fig. No. 19: Herbal Ointment Preparation On Ointment Slab.**

## EVALUATION PARAMETER

The evaluations were carried out on the ointment by using the following parameters.

### 1] Organoleptic Properties

**a. Colour:** The color of the prepared herbal ointment was observed visually under normal daylight conditions.

**b. Odour:** The odor of the formulation was evaluated manually by smelling the ointment.

**c. Appearance:** The appearance of the ointment was examined for smoothness and uniformity by visual inspection.

**d. Texture:** The texture of the ointment was determined by rubbing a small quantity between the fingers.

**e. Consistency:** The consistency of the formulation was checked manually to determine its softness and spreadability.

### 2] pH Determination

A calibrated digital pH meter was used to measure the pH after 1 g of ointment was dissolved in 100 mL of distilled water.

### 3] Spreadability

For the determination of spreadability the amount of sample is placed in between two slides and which compressed to uniform thickness by placing definite weight for definite time. Time required for the separation of two slides was known as spreadability. Spreadability is better when time taken for the separation of two slides is less. Spreadability was calculated by the

#### Formula

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

Where,

S = Spreadability

M = Tide weight on upper slide L = Length of glass slide

T = Time taken for the separation of slides

### 4] Extrudability

In order to measure the amount of ointment extruded in a specific amount of time, extrudability is assessed by filling a collapsible tube with the ointment and applying pressure to the tube. The ease of extrusion from the tube indicates good formulation consistency.

### 5] Washability

By applying a small amount of ointment to the skin or hand, washing it with tap water or a mild soap solution, and then observing how easily the ointment is removed, one can determine the formulation's water solubility and cleansing behavior.

### 6] Viscosity

A Brookfield viscometer is used to measure viscosity. The ointment sample is placed in the sample holder, the spindle is rotated at various speeds, and resistance to flow—a measure of the ointment's thickness and consistency—is recorded.

### 7] Test for Irritability

An irritancy test involves applying a small amount of ointment to a designated area of skin (typically the forearm), watching for redness, swelling, or irritation for up to 24 hours, and recording any negative skin reactions.

### 8] Grittiness

A small amount of ointment is applied to the skin or a glass slide, and it is gently rubbed to check for coarse particles or undissolved material, which should not be present in a good ointment formulation. This is how grittiness is assessed.

### RESULT

The present study was done for the preparation and evaluation of herbal ointment. Herbal extract was prepared by using simple maceration process for obtaining better yield of extract. The chemical constituents and their activity does not harm to the body.

### PHYSICAL APPEARANCE

On the basis of organoleptic properties.

**Table No. 8: Result For Organoleptic Properties.**

Sr. No	Formulation	Colour	Odour	Consistency	Greasiness	Homogeneity
1	F1	Light brown	Mild	Soft	Non-greasy	Good
2	F2	Brown	Herbal	Smooth Semisolid	Non-greasy	Good
3	<b>F3</b>	<b>Dark Brown</b>	<b>Characteristics odour</b>	<b>Highly viscous</b>	<b>Non-greasy</b>	<b>Homogenous</b>
4	F4	Greenish Brown	Strong Herbal	Thick semisolid	Non-greasy	Very good
5	F5	Darkish Brown	Characteristics Herbal	Moderately thick	Slightly non-greasy	Very Good

### pH MEASUREMENT OBSERVATION

The pH of the prepared formulations of herbal ointment was found to be in the range of  $6.2 \pm 0.2$  to  $6.8 \pm 0.2$ . The obtained pH values ensure compatibility with the skin and reduce the possibility of irritation because they are within the acceptable skin pH range (5.0–7.0). The formulation's suitability for topical application is confirmed by the stability of the herbal constituents in this slightly acidic to neutral range.

**Table No. 9: Result For Ph. Measurement.**

Parameter	F1	F2	<b>F3</b>	F4	F5
pH value	6.2	6.3	<b>6.5</b>	6.6	6.8



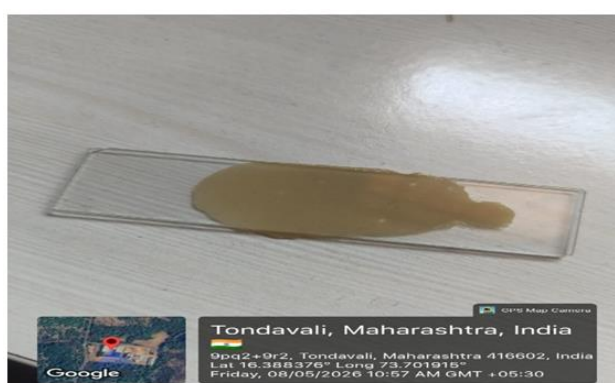
**Fig. No. 20: Measurement of ph.**

### SPREADABILITY TEST

With a spreading diameter of  $4 \pm 0.1$  cm under standard weight, the herbal ointment formulations demonstrated good spreadability. The spreadability values were found in the range of 9.2 to 13.1 gm.cm/sec. Good spreadability indicates ideal consistency and ease of application, which helps the formulation to spread uniformly on the skin surface and enhances therapeutic efficacy. Among all formulations, F3 showed optimum spreadability value (11.8 gm.cm/sec) with suitable consistency and ease of application; therefore, it was considered as the standard batch for further evaluation studies.

**Table No. 10: Result For Spreadability.**

Parameter	F1	F2	F3	F4	F5
Spreadability value	9.2gm.cm/sec	10.4gm.cm/sec	<b>11.8gm.cm/sec</b>	12.5gm.cm/sec	13.1gm.cm/sec



**Fig. No. 21: Spreadability.**

### VISCOSITY

A semisolid and stable formulation was indicated by the viscosity of the herbal ointment formulations, which was found in the range of  $2600 \pm 2.0$  cP to  $4500 \pm 2.0$  cP. The viscosity

values were within the acceptable range for topical ointments, providing sufficient thickness for better retention at the application site while maintaining suitable spreadability. Among all formulations, F3 showed optimum viscosity (3800 cP), which provided a proper balance between consistency and ease of application; therefore, it was considered as the standard batch for further evaluation studies.

**Table No. 11: Result For Viscosity.**

Parameter	F1	F2	F3	F4	F5
Viscosity Value	2600 cP	3200 cP	<b>3800 cP</b>	4200 cP	4500 cP



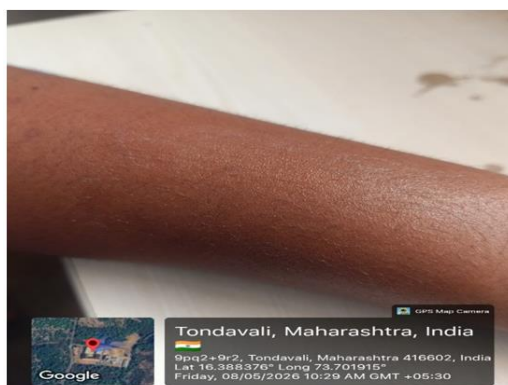
**Fig. No. 22: Viscosity.**

## SKIN IRRITATION

In the skin irritation study carried out on volunteers, all formulations showed no signs of erythema, edema, redness, or itching after topical application. The ointment formulations were found to be safe and non-irritating to the skin. Among all batches, F3 showed better skin compatibility and was considered as the standard batch due to its good spreadability, stability, and absence of irritation.

**Table No. 12: Result For Skin Irritation.**

Parameter	F1	F2	F3	F4	F5
Result	<b>No irritation</b>	<b>No irritation</b>	No irritation	<b>No irritation</b>	<b>No irritation</b>



**Fig. No. 23: Skin irritation test.**

**WASHABILITY**

The washability of all ointment formulations was evaluated by applying the preparation on the skin and washing with water. All formulations were found to be washable without leaving significant residue on the skin surface. Among all batches, F3 showed optimum washability with easy removal and smooth skin feel, therefore it was considered as the standard batch.

**Table No. 13: Result For Washability.**

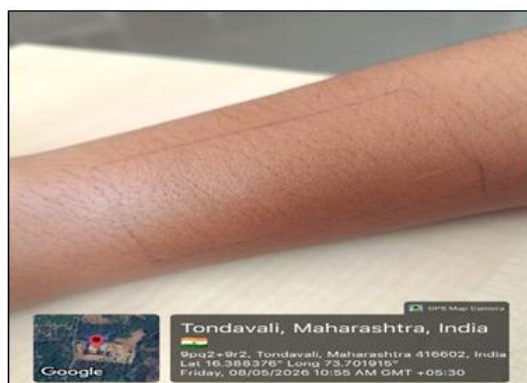
Parameter	F1	F2	F3	F4	F5
Result	Easily Washed	Easily Washed	<b>Easily Washed</b>	Easily Washed	Easily Washed

**Before**



**Fig. No. 24: After Washability.**

After

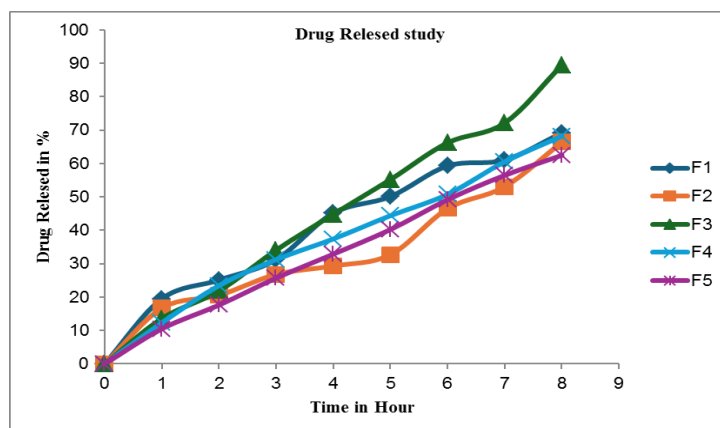


## Drug released study

### Drug Release Study Using Franz Diffusion Cell

The *in vitro* drug release study of the formulated *Mimosa pudica* and *Moringa oleifera* herbal ointment was carried out using a **Franz diffusion cell**. The diffusion cell consisted of a donor compartment and a receptor compartment separated by a suitable semipermeable membrane. The receptor compartment was filled with phosphate buffer (pH 7.4) and maintained at  $37 \pm 0.5^\circ\text{C}$  under continuous magnetic stirring to simulate physiological conditions. A known quantity of the ointment formulation was placed in the donor compartment. At predetermined time intervals (1, 2, 3, 4, 5, 6, 7 and 8 hours), aliquots were withdrawn from the receptor compartment and replaced with an equal volume of fresh dissolution medium to maintain sink conditions. The collected samples were analyzed using an appropriate analytical method UV-Visible spectrophotometry to determine the amount of active constituents released. The cumulative percentage drug release was calculated and plotted against time to evaluate the release profile of the formulation. The Franz diffusion cell study demonstrated the diffusion behavior and sustained release characteristics of the *Mimosa* and *Moringa* ointment, indicating its potential effectiveness for topical wound-healing applications.

Time in (hours)	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	19.49	16.7	13.58	12.34	10.5
2	25.12	20.54	21.87	23.44	17.62
3	31.24	26.8	34.06	31.24	25.71
4	45.16	29.32	44.81	37.43	32.87
5	50.13	32.7	55.21	44.39	40.24
6	59.3	46.5	67.22	50.78	49.17
7	61.08	53.08	72.15	60.54	56.47
8	69.15	66.37	89.45	68.21	62.47



Among all the formulations (F1–F5), formulation F3 shows the highest cumulative percentage drug release (89.45%) at the end of 8 hours. Therefore, F3 was selected as the optimized formulation due to its superior drug release characteristics compared to the other batches.



**Fig. No. 25: franz diffusion cell.**

## OVERALL DISCUSSION

The prepared herbal ointment demonstrated significant wound healing and antimicrobial activity due to the presence of herbal extracts. The formulation effectively showed good physicochemical properties, stability, and absence of skin irritation. Among all formulations, F3 exhibited optimum performance with better spreadability, consistency, washability, and stability. These findings suggest that the herbal ointment is safe, effective, and suitable for topical application in wound healing management.

## CONCLUSION

The present study was aimed at formulating and evaluating a herbal ointment for wound healing activity using herbal plant extracts. The formulations were prepared successfully and evaluated for various parameters such as colour, odour, consistency, homogeneity, pH,

spreadability, viscosity, washability, extrudability, stability, and skin irritation test. The results obtained from the evaluation studies showed that all formulations possessed satisfactory physicochemical properties and were suitable for topical application. Among all the batches, formulation F3 showed optimum performance with good consistency, better spreadability, suitable viscosity, easy washability, and no signs of skin irritation. The herbal ointment also exhibited good stability during the study period. Based on the overall evaluation results, it can be concluded that the prepared herbal ointment is safe, stable, and effective for wound healing application.

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