

# Formulation and Evaluation of Bryophyllum Pinnatum [Lam.] Kurz.Gel for Wound Healing Topical Application

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## CHAPTER - 1

### INTRODUCTION

skin is the largest organ in the human body in terms of surface area. Internal tissues are protected by it from mechanical damage, microbial infection, UV light, and severe temperatures. This renders it extremely vulnerable to harm, with serious consequences for both individual patients and the healthcare system as a whole [1][2]. Patients with diabetes, the elderly, and those with genetic abnormalities like sickle cell disease are all at risk for irregular wound healing, which can lead to long-term complications. Surprisingly, the existing interventions have had little influence on the issue. Although there are various wound healing methods available, they are only modestly effective. As a result, more effective wound healing therapies are required. The precise coordination of multiple different cell types in sequential phases is required for skin restoration. The epidermis is the outer, impermeable layer of the skin that protects it from the hostile external environment in healthy skin. Sebaceous glands and hair follicles are all found in the epidermis. Extracellular matrix (ECM), vasculature, and mechanoreceptors are abundant in the dermis, which serves as an energy reserve for the skin. It also provides the dermis with a steady supply of growth factors. Aside from these cell types, each layer has resident immune cells that constantly scan the skin for harm. When the skin is injured, multiple cell types in these three layers must work together at specific times to heal the wound. Hemostasis, inflammation, angiogenesis, growth, re-epithelialization, and re-modelling take place in a chronological order, but they also overlap [3]. As a result, skin restoration is one of the most difficult processes in the human body. Constriction of the wounded blood arteries and activation of platelets to produce a fibrin clot are the first responses to a lesion [4].

The fibrin clot stops blood flow and acts as a scaffold for inflammatory cells to enter. As an initial line of defense against germs, neutrophils are quickly drawn to the clot [5]. Within 48–96 hours of damage, monocytes are recruited and convert into tissue-activated macrophages at the wound site [6]. To combat self and foreign antigens, the adaptive immune system, which includes Langerhans cells, dermal dendritic cells, and T cells, is also activated. Understanding the variation within these immune cell populations, particularly how distinct subsets are involved in cellular debris clearance vs infection resolution, is becoming increasingly important [7, 8].

Angiogenesis happens after the inflammatory phase has ended. Angiogenesis is the formation of new blood vessels by endothelial cells proliferating, migrating, and branching. Pericytes inside the basal lamina are stimulated concurrently with endothelial cell growth

[9] and scaffold and provide structural integrity to the endothelial cells [10]. Some researchers believe that these activated pericytes are more plastic mesenchymal stromal cells [11]. In addition to local cells, circulating progenitor cells from the bone marrow have been discovered to assist in the development of new blood vessels during wound healing [9–12]. Several cell types are involved in the development of new blood vessels, with the majority of cellular variety occurring in the perivascular region. Local fibroblasts multiply and penetrate the clot to generate contractile granulation tissue when new blood vessels develop. Some fibroblasts transform into myofibroblasts in this area, pulling the wound borders closer [13]. The microenvironment of the wound shifts from an inflammatory to a growth state as dividing fibroblasts deposit ECM [14]. Re-epithelialization happens at the same time as unipotent epidermal stem cells proliferate from the basement membrane and terminally differentiated epidermal cells de-differentiate [15]. Stromal vascular cells and their subgroups have been widely studied in subcutaneous adipose tissue [16]. These cells secrete growth factors and cytokines that aid in wound healing and neovascularization. Increased inflammation can influence the result of wound recovery, so inflammatory cells in the subcutaneous tissue have a lot of attention, especially in obesity and type 2 diabetes. In most circumstances, healing restores the skin's barrier function and tensile strength to near-normal levels. Adult wound healing, in contrast to prenatal wound healing, which is a regenerative process that recreates the original skin architecture, results in a fibrotic scar that acts as a quick patch for the wound [3]. Excessive scarring tips the scales in the direction of fibrotic hypertrophic scarring and keloid development [17].

Cutaneous wound healing is a vital physiological process that involves the cooperation of a variety of cell types and their products [18]. Early in the inflammatory stage, attempts to restore the damage caused by local aggression begin. Finally, they result in repair, which is the replacement of specialized structures caused by collagen deposition, and regeneration, which is the process of cell proliferation and posterior differentiation by pre-existing cells in the tissue and/or stem cells [19]. These methods are not mutually exclusive, which means that after a skin lesion, regeneration and repair can occur in the same tissue, depending on the cell strains affected by the injury. Following the commencement of the injury, tissue regeneration and healing ensue. Whether as a result of trauma or a specific clinical condition. All of the stimuli that disrupt the physical continuity of functioning tissues combine to form a single lesion. External or internal stressors, as well as physical, chemical, electromagnetic, or thermal stimulation, can produce lesions. Furthermore, the lesions may cause harm to individual organelles or entire cells [18]. Tissue repair is a straightforward linear process in which growth factors drive cell proliferation, resulting in the integration of dynamic changes involving soluble mediators, blood cells, extracellular matrix synthesis, and parenchymal cell proliferation. Inflammatory reaction, cell proliferation, and production of the extracellular matrix elements, as well as the post-healing period, known as remodeling, are the stages of cell and biochemical events in wound repair. These stages do not exclude one another; rather, they overlap over time. The goal of this literature review is to emphasize the biological processes involved in wound healing, with a focus on the cells, growth factors, and cytokines involved in tissue repair.

### **Wounds and wound types**

Damage or disturbance to the normal anatomical structure and function is classified as a wound [20]. This can be as simple as a rupture in the skin's epithelial integrity, or it can be more serious, extending into subcutaneous tissue and causing injury to tendons, muscles, arteries, nerves, parenchymal organs, and even bone [25]. Wounds can develop as a result of pathological processes that originate either outside or

internally within the organ in question. They can be the product of a disease process or have an unintentional or purposeful etiology. Wounding affects tissue and disturbs the immediate environment within it, regardless of the source or the form. Bleeding, vascular constriction with coagulation, complement activation, and an inflammatory response are all physiological responses to the noxious factor [21–23].

Normal wound healing is a dynamic and complex process involving a series of coordinated events, including bleeding, coagulation, initiation of an acute inflammatory response to the initial injury, regeneration, migration and proliferation of connective tissue and parenchyma cells, as well as synthesis of extracellular matrix proteins, remodeling of new parenchyma and connective tissue and collagen deposition [24, 25]. Wound healing starts right after an injury and involves resident and migratory cell populations, extracellular matrix, and soluble mediators. The following mechanisms are involved in the processes described above: (i) inflammatory mediators and growth factors; (ii) cell–cell and cell– extracellular matrix interactions that regulate cell proliferation, migration, and differentiation; (iii) epithelialization, fibroplasia, and angiogenesis events; (iv) wound contraction; and (v) remodeling. These processes are activated when a physical damage occurs and continue throughout the healing process [26–29].

## CLASSIFICATION OF WOUNDS

Wounds can be classified according to various criteria [20]. In the treatment of injuries and wounds, time is crucial. According to the length of time it takes for a wound to heal, it can be classified as acute or chronic [21, 22].

### Acute wounds

Acute wounds are wounds that mend themselves and heal normally by following a rapid and ordered healing pathway, resulting in both functional and anatomical restoration. Healing takes between 5 to 10 days, or less than 30 days in most cases. Acute wounds can develop as a result of a surgical procedure or traumatic tissue loss [20, 21]. Because of the huge defect within the tissue, an operation to remove a soft tissue tumor located in the skin and underlying parenchyma can often result in a massive, albeit noncontaminated lesion that cannot be repaired by primary intention. Traumatic wounds are also a common occurrence. They can affect just the soft tissue or they can be linked to bone fractures. The AO Foundation's (Arbeitsgemeinschaft für Osteosynthesefragen/Association for the Study of Internal Fixation) classification system, which is one of the most thorough and extensively used, was utilized to classify these combined injuries. Closed and open fractures, as well as skin, muscle, tendon, and neurovascular injuries, are all included in this classification scheme.

### Chronic wounds

Chronic wounds are ones that do not heal normally and cannot be mended in a timely and orderly manner [20, 30]. Various variables disrupt the healing process by prolonging one or more stages in the phases of hemostasis, inflammation, proliferation, or remodeling. Infection, tissue hypoxia, necrosis, exudate, and high levels of inflammatory cytokines are among these variables [31]. A persistent state of inflammation in the wound triggers a cascade of tissue responses, all of which contribute to the wound's non-healing status. Functional and anatomical outcomes are poor as a result of the uncoordinated healing, and these wounds frequently reoccur [20, 32].

### Complicated wounds

A complex wound is a unique type of wound that has both an infection and a tissue defect [22]. The

wound is always at risk of infection. In contrast, the defect develops as a result of a traumatic or post-infectious etiology, or a large tissue excision (e.g., in tumor management). Regardless of the cause, size, location, or care, every wound is polluted. The virulence, amount, and kind of microorganisms, as well as the local blood supply and the patient's natural resistance, all play a role in whether or not a manifest infection develops. The five well-documented signs and symptoms of infection are redness, heat, discomfort, oedema, and loss or limited function in the affected region. The prevalence of wound infections is determined by the surgical technique used and the wound's location. Depending on the level of pollution, Aseptic wounds (bone and joint operations), contaminated wounds (abdominal and lung operations), and septic wounds (abdominal and lung operations) are the three types of wounds (abscesses, bowel operations, etc.). Closed wounds are those in which the underlying tissue has been traumatized but the skin layer has not been severed, and open wounds are those in which the skin layer has been injured but the underlying tissue has been exposed [22, 32].

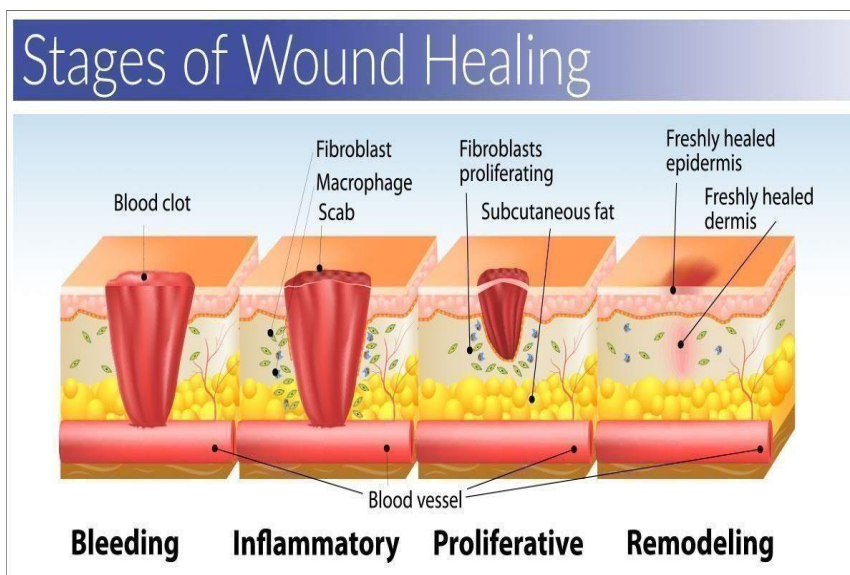
**The wound healing processes.**

Wounding and wound healing occur in all of the body's tissues and organs. Many of these repair mechanisms are found in many types of tissues. Although healing is a continuous process, it is arbitrarily split into several phases to help comprehension of the physiological processes that occur in the wound and surrounding tissue [33]. Healing is a multi-step process that involves coordinated interactions between many immune and biological systems. It entails a series of meticulously timed and regulated procedures and occurrences that correspond to the presence of different cell types in the wound bed at different stages of the healing process [34–36].

Acute and chronic wounds have different timing and interactions between the components involved in wound healing, but the main phases remain the same [20, 37]. The numerous steps of acute tissue repair that are initiated by tissue injury can be grouped into a four-phase sequence: (i) coagulation and hemostasis, which occur immediately after injury;

(ii) inflammation, which occurs soon after; (iii) proliferation, which occurs within days of the injury and includes the major healing processes; and (iv) wound remodeling, which occurs after scar tissue formation and can last up to a year or more [26, 27].

**STAGES OF WOUND HEALING**



**Figure 1: Visualization of the four different stages of wound healing.**

### Coagulation and haemostasis phase

Immediately after injury, coagulation and hemostasis take place in the wound [27, 37]. These systems are primarily designed to avoid exsanguination. It is a method of protecting the circulatory system and keeping it intact so that the important organs' functions are not impaired despite the injury. A second goal is to provide a long-term matrix for invading cells that are required in the later stages of healing [20, 27–29, 37]. The amount of fibrin deposited at the wound site is determined by a dynamic balance between endothelial cells, thrombocytes, coagulation, and fibrinolysis, which influences the course of the reparative processes [28, 29].

Noxious insult causes microvascular injury and extravasation of blood into the wound [38]. Injured arteries constrict rapidly due to the neural reflex mechanism, which causes vascular smooth muscle cells in the circular muscle layer to contract. The contraction is strong enough to stop bleeding from a 0.5-cm-diameter arteriole. However, the procedure is only efficient in transversally disrupted veins and may result in full blood leakage cessation. In longitudinally separated arterioles, on the other hand, it widens the gap [28, 29].

Reflex vasoconstriction can lessen or even stop bleeding for a short time. However, the vascular smooth muscle tone is only effective for a few minutes until hypoxia and acidosis in the wound wall promote passive relaxation and bleeding restarts. The hemostatic mechanisms would be useless in the long run if it weren't for the creation of an insoluble fibrin plug [20, 38]. Together with hemostatic events, the coagulation cascade is activated through extrinsic and intrinsic pathways, leading to platelet aggregation and clot formation in order to limit blood loss [28]. Blood components and platelets come into touch with exposed collagen and other extracellular matrix components as blood flows into the injury site. This interaction causes platelets to release clotting factors, resulting in the development of a blood clot made up of fibronectin, fibrin, vitronectin, and thrombospondin [20, 28, 29, 39]. The blood clot and platelets trapped within it are vital for more than just haemostasis; the clot also acts as a temporary matrix for cell migration during the haemostatic and inflammatory phases. Platelets have granules in their cytoplasm that carry growth factors and cytokines include platelet derived growth factor (PDGF), transforming growth factor (TGF), epidermal growth factor, and insulin-like growth factors [28]. These molecules act as promoters in the wound healing cascade by activating and attracting neutrophils and, later, macrophages, endothelial cells and fibroblasts [29, 35]. Platelets also contain vasoactive amines, such as serotonin, which are stored in dense bodies and promote vasodilation and increased vascular permeability, resulting in fluid extravasation in the tissue and oedema, which then potentiates itself during the inflammatory phase that follows [29, 33]. Eicosanoids and other products of arachidonic acid metabolism are released after injury to cell membranes and have potent biological functions in the immediate inflammatory response [20, 29, 38, 39].

### INFLAMMATORY PHASE

Following that is the humoral and cellular inflammatory phase, which aims to form an immunological barrier against invading microbes. It has two distinct phases: an early inflammatory phase and a late inflammatory phase [40]. Phase 1 of the inflammatory process is the early inflammatory response, which begins during the late phase of coagulation and ends shortly after, serves a variety of purposes. It triggers the complement cascade and sets in motion molecular mechanisms that result in neutrophils infiltrating the wound site and preventing infection [26]. The neutrophils begin by performing phagocytosis, which involves destroying and removing bacteria, foreign particles, and injured tissue. Because acute wounds

with a bacterial imbalance will not heal, phagocytotic activity is critical for the subsequent stages [20, 35, 40]. Various chemo attractive substances, including as TGF-, complement components such as C3a and C5a, and formyl methionyl peptides produced by bacteria and platelet products, begin to attract neutrophils to the wound site within 24 –36 hours of injury. Neutrophils become sticky as a result of changes in the control of surface adhesion molecules, and begin to adhere to the endothelial cells in the post- capillary venules surrounding the wound in a process known as margination [29, 40]. The neutrophils then roll along the endothelium's surface, propelled ahead by blood flow. Selectin-dependent interactions mediate these adhesions and rolling processes, which are categorised as weak attachments [40, 41]. Chemokines secreted by endothelial cells rapidly activate a stronger adhesion system, which is mediated by integrins [36]. Cells stop rolling and migrate out of the venules, squeezing between the endothelial cells by a process known as diapedesis [40, 41]. The subsequent migration now depends on chemokines and other chemotactic agents. Once in the wound environment, neutrophils phagocytose foreign material and bacteria, destroying them by releasing proteolytic enzymes and oxygen- derived free radical species [26, 33, 41]. Neutrophil activity gradually changes within a few days of wounding, once all the contaminating bacteria have been removed [40, 41]. After performing the assignment, the neutrophils must be removed from the wound before moving on to the next step in the healing process. Extrusion of redundant cells to the wound surface as slough and apoptosis allow the entire neutrophil population to be eliminated without causing tissue injury or amplifying the inflammatory response [40, 42].

### **Late inflammatory phase**

As part of the late inflammatory phase, 48 – 72 h after injury, macrophages appear in the wound and continue the process of phagocytosis [40]. These cells begin as blood monocytes, but when they enter the wound, they undergo phenotypic modifications to become tissue macrophages. Macrophages have a longer lifespan than neutrophils and work at a lower pH. They are attracted to the wound site by a variety of chemo attractive agents such as clotting factors, complement components, cytokines such as PDGF, TGF-, leukotriene B4 and platelet factor IV, as well as elastin and collagen breakdown products [43, 44]. The last cells to enter the wound site in the late inflammatory phase are lymphocytes, attracted 72 h after injury by the action of interleukin-1 (IL-1), complement components and immunoglobulin G (IgG) breakdown products [26, 40, 42]. Collagenase regulation, which is later required for collagen remodeling, the formation of extracellular matrix components, and their breakdown, is aided by IL-1 [40, 42, 45].

### **Proliferative phase**

When ongoing injury has ceased, hemostasis has been achieved and an immune response successfully set in place, the acute wound shifts toward tissue repair [33, 40, 41]. The proliferative phase begins the third day after injury and lasts around two weeks. It's marked by fibroblast migration and the deposition of newly produced extracellular matrix, which acts as a substitute for the fibrin and fibronectin-based provisional network. This stage of wound healing is characterized by an abundance of granulation tissue production at the macroscopic level. Below is a summary of the several processes that occur during the proliferative phase [37, 42].

### **Fibroblast migration**

Following injury, fibroblasts and myofibroblasts in the surrounding tissue are stimulated to proliferate for the first 3 days [46]. They then migrate into the wound, drawn by substances secreted by inflammatory cells and platelets such as TGF- and PDGF. On the third day following damage,

fibroblasts develop in the wound, and their accumulation necessitates phenotypic modulation. They proliferate rapidly once within the wound, producing matrix proteins such as hyaluronan, fibronectin, proteoglycans, and type 1 and type 3 procollagen. All of their products end up in the local environment [20, 45, 46]. By the end of the first week, abundant extracellular matrix accumulates, which further supports cell migration and is essential for the repair process [24, 29]. The phenotype of fibroblasts now shifts to that of myofibroblasts. They have large actin bundles beneath the plasma membrane and are actively extending pseudopodia to connect to fibronectin and collagen in the extracellular matrix at this stage [43, 47]. As these cell extensions retract, wound contraction occurs, which is a crucial event in the reparative process that helps to approach the wound borders. Apoptosis is used to destroy excess fibroblasts after they have completed this task [48, 49].

### **Collagen synthesis**

Collagens have a crucial role in wound healing at all stages. Fibroblasts produce the proteins that give all tissues their integrity and strength, and they play an important role in the repair process, particularly during the proliferative and remodeling phases [49–51]. Collagens serve as a basis for the wound's intracellular matrix development. The dermis of an unwounded person includes 80% types 1 and 25% type 3 collagen, whereas wound granulation tissue contains 40% type 3 collagen [20].

### **Angiogenesis and granulation tissue formation**

In wound healing, the modelling and development of new blood vessels is crucial, and it occurs at all stages of the healing process. Numerous angiogenic factors released during the hemostatic phase stimulate angiogenesis in addition to recruiting neutrophils and macrophages [48, 52, 53]. FGF, vascular endothelial growth factor (VEGF), PDGF, angiogenin, TGF- $\beta$ , and TGF- $\alpha$  are all angiogenic agents that local endothelial cells respond to. Inhibitory agents such as angiostatin and steroids maintain a delicate equilibrium [54–57]. Inhibitory and stimulatory agents act on proliferating endothelial cells directly as well as indirectly, by activating mitosis, promoting locomotion and by stimulating the host cells to release endothelial growth factors [58, 59]. Under hypoxic conditions, chemicals from the surrounding tissue are released, promoting endothelial cell proliferation and growth. In response, a four-step process occurs: (i) endothelial cells produce proteases in order to degrade the parent vessel's basal lamina and crawl through the extracellular matrix; (ii) chemotaxis; (iii) proliferation; and (iv) remodeling and differentiation. FGF and VEGF are important regulators in all of these processes [53, 55, 58–60]. Initially, there is no vascular supply in the wound Centre, so viable tissue, which is limited to the wound margins, is perfused by uninjured vessels and by diffusion through undamaged interstitial [20, 42, 61]. Capillary sprouts from the wound's borders infiltrate the clot, and a microvascular network made of many new capillaries' forms within a few days.

### **Protrusion**

With three types of interconnected filaments, the cytoskeleton is anchored at cell–cell junctions and cell–extracellular matrix adhesions, providing mechanical support for the cell [62]. The action network is well-known for its dynamic reconfiguration, as well as its role as a mechano-effector and in cell migration coordination. Actin polymerization occurs at the leading edge of motility, determined by the maximum concentration of chemoattractive material, pushing the plasma membrane outward. Filopodia, or projecting structures, arise in endothelial cells and are packed with filamentous actin [63]. Unidirectional movement of the cell is maintained through the action of a cyclic assembly and disassembly of actin filaments in front of and well behind the leading edge, respectively [64, 65].

### **Adhesion**

Adhesion to a solid substratum is a particularly important step in cell migration. Integrins, which act as major receptors for extracellular matrix proteins and are hence essential for cell motility, are involved [66, 67]. In addition, these molecules are also involved in signal transduction, and in regulating and stimulating migration [67, 68]. Adhesion and migration are inversely related; greater adhesion leads to a faster rate of migration, but additional attachment reduces mobility [66, 67]. Endothelial cells can change the strength of their adhesion, with weakly adherent cells moving quicker than highly adherent cells. The cell's morphology changes from an oval or spindle shape to an uneven, flattened one once it attaches to the extracellular matrix.

### **Traction**

The cell can drag the cytoplasm forward by creating traction to the substratum using contractile forces communicated through the integrin–cytoskeletal connections [62]. Myosin motor proteins, which are coupled to contractile actin bundles along the cell, generate the force for movement. Myosin and actin fibre interactions move the cell body forward. The extracellular matrix-binding proteins on the trailing edge of the migrating cell must also relinquish their connections at the same moment [67, 69].

### **Epithelialization**

Migration of epithelial cells starts from the wound edges within a few hours of wounding. A single layer of cells initially forms over the defect, accompanied by a marked increase in epithelial cell mitotic activity around the wound edges. Cells migrating across them attach to the provisional matrix below. When the advancing epithelial cells meet, migration stops and the basement membrane starts to form [37, 42, 50].

## **REMODELLING PHASE**

The remodeling phase of wound healing is responsible for the growth of new epithelium and the generation of ultimate scar tissue. Extracellular matrix synthesis begins concurrently with granulation tissue formation in the proliferative and remodeling stages. This stage can continue anywhere from a year to two years, or even longer in certain cases [47,50]. Regulatory systems carefully govern the remodeling of an acute wound in order to maintain a precise balance between degradation and synthesis, which leads to proper healing. Collagen bundles grow in diameter as the intracellular matrix matures, and hyaluronic acid and fibronectin are destroyed [46,54]. The wound's tensile strength rises in lockstep with the amount of collagen collected [54,55]. When compared to unwounded tissue, collagen fibers can restore up to 80% of their original strength.

The acquired ultimate strength is determined by the repair's location and duration, but the tissue's initial strength can never be recovered. Collagen synthesis and degradation, as well as extracellular matrix remodeling, are ongoing processes that equilibrate to a stable state around 3 weeks following injury [49,55]. Collagen breakdown is caused by matrix metalloproteinase enzymes generated by neutrophils, macrophages, and fibroblasts in the wound. Inhibitory factors carefully control and coordinate their action. The activity of tissue inhibitors of metalloproteinases gradually rise, ending in a decrease in the activity of metalloproteinase enzymes, encouraging the build-up of new matrix [43,55,74].

A variety of factors influence the process, the most essential of which being PDGF, TGF, and FGF [55,74]. Apoptosis reduces the density of fibroblasts and macrophages as the wound heals [31,65]. Capillary development slows, blood flow to the region diminishes, and metabolic activity at the wound site reduces with time.



**WOUND HEALING:** [70]**Wound:**

In simple terms, there will be a break in the continuity of tissue that can be caused by the injury or trauma.

**Healing:**

Healing is the process for restoring the damage caused by the break of the tissue.

Two main processes:

- Regeneration
- Repair

**CLASSIFICATION:** [71, 72]

Wounds can be classified according to various criteria. Time is an important factor in injury management and wound repair. Thus, wounds can be clinically categorized as acute and chronic according to their time frame of healing.

**COMPLICATIONS OF WOUNDS:** [81–84]

- Oxygenation
- Infection
- Foreign body
- Venous insufficiency
- Nutrition
- Medications: glucocorticoid steroids, non-steroidal anti-inflammatory drugs, chemotherapy
- Alcoholism and smoking
- Immunocompromised conditions: cancer, radiation therapy, AIDS
- Age and gender
- Sex hormones
- Stress
- Ischemia

Here the project is done *Bryophyllum pinnatum*

**BRYOPHYLLUM PINNATUM** - *Kalanchoe pinnata*, formerly known as *Bryophyllum pinnatum*, also known as the air plant, cathedral bells, life plant, miracle leaf, and Goethe plant is a succulent plant native to Madagascar, which is a popular houseplant and has become naturalized in tropical and subtropical areas.

*Bryophyllum pinnata* (*B. pinnata*) is a common medicinal plant used in traditional medicine of India and of other countries for curing various infections, bowel diseases, healing wounds and other ailments.

In worldwide plants which as medicinal uses for the treatment of various disease condition [78]. Medicinal plants are safe and economical compared to the allopathic medicines [79]. In this article, topic will be discussed on the wound healing, the plant of *Bryophyllum pinnata*.

The physical constituents of the *Bryophyllum pinnata* have various therapeutic agents [80]. It is a succulent herb (or) glabrous 0.3 - 1.2m height [81].

**VERNACULAR NAMES:**

- English Name: Air plant
- Tamil Name: Ranakalli
- Hindi: Zakhm-hayat
- Sanskrit: Parna – beeja, Asthi-bhaksha
- Other names: Miracle Plants, Divine Herb.

**SYNONMYS:**

- *Bryophyllum germians*,
- *Bryophyllum calycinum*,
- *Cotyledon pinnata*
- *Cotyledon calyculata*,
- *Sedum madagascariense*,
- *Verea pinnata*

In the Last few years, researchers are being focused and monitoring the various herbal drugs for the prospective therapy effect [82]. The various traditional use of *Bryophyllum pinnata* in India, the leaf decoction is applied on open cut to stop the bleeding. Extract of leaves is consumed in empty stomach for the treatment of urinary bladder[83] . And some other users are like abdominal discomfort, scabies, wounds etc.,[84].

**ETHNOPHARMACOLOGY**

In Indochina and the Philippines Islands, different species of *Bryophyllum pinnatum* are utilized medicinally. It has been naturalized in India's hot and humid regions. Bitter tonic, astringent to the intestines, analgesic, carminative, and beneficial in diarrhea and vomiting, the leaves and bark [85]. It is used to treat a variety of pains and inflammations, as well as bacterial, viral, and fungal infections, as well as leishmaniasis, earaches, upper respiratory infections, stomach ulcers, flu, and fever [86]. The leaves of this plant exhibit antibacterial [87,88], antifungal, antiulcer [89], anti-inflammatory, analgesic [89], antihypertensive [90], powerful anti-histamine, and anti-allergic action in traditional medicine [91]. The lightly roasted leaves are used by the Creoles to treat cancer, inflammation, and fevers with a leaf infusion. For migraines and headaches, Palikir mixes the leaf juice with coconut oil or andiroba oil and rubs it on the forehead. Heat the leaves and use them topically on boils and skin ulcers, according to the Siona indigenous people.

Natives around Ecuador's Rio Pastaza utilize a leaf infusion to treat fractured bones and internal injuries. In Peru, indigenous tribes combine the leaf with aguardiente (sugar cane rum) and apply it to the temples for headaches; they soak the leaves and stems in cold water overnight and drink it for heartburn, urethritis, fevers, and a variety of respiratory ailments. In addition, the root infusion is employed. For earaches, some Amazon tribes strain the juice from fresh leaves and combine it with mother's milk. It's also used to help with delivery and encourage menstruation in Mexico and Nicaragua. Its fleshy leaves are commonly used as a herbal medicine for a variety of human ailments in Nigeria and other West African nations, including hypertension, diabetes mellitus, bruises, wounds, boils, abscesses, insect bites, arthritis, rheumatism, joint problems, migraines, and body pains. The plant's leaves have a lot of therapeutic potential and may be used both orally and topically. Hemostatic, refrigerant, emollient, mucilaginous, vulnerary, depurative, anti-inflammatory, disinfectant, and tonic are some of the qualities

of the leaves. They can help with vata and pitta imbalances, cuts, wounds, haemorrhoids, menorrhagia, skindiscoloration, and more [76, 77].

## PHARMACOLOGICAL ACTIVITIES [94-100]

### Antimicrobial activity

The researchers discovered that *Bryophyllum pinnatum* leaf extracts (aqueous, methanol, palm wine, Omidun, local gin, and fresh leaf juice) at dilutions of 256, 128, 64, 32, 16, 8, 4 mg/ml exhibited different antibacterial activity against the Gram-positive and Gram-negative microorganisms examined. With the control drug, methanol extract exhibited significant antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* (Ciprofloxacin). Even the extract from *Bryophyllum pinnatum*'s crushed leaves had a considerable effect on several Gram positive and Gram-negative bacteria. Other extracts had poor to moderate efficacy against the organisms tested [173].

Two isolated flavonoid compounds were found to have antibacterial action in another in-vitro research Compound 1(5I Methyl 4I, 5, 7 trihydroxy flavone) and compound 2(4I, 3, 5, 7 tetrahydroxy 5-methyl 5I -propenamine anthocyanidines) from *Bryophyllum pinnatum* leaf were tested in bacteria (three gram-negative organisms, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, and a gram-positive organism (*Candida albicans* and *Aspergillus Niger*). *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Aspergillus niger*, and *Candida albicans* were all successfully inhibited by the chemicals. However, compound 1 was unable to suppress *E. coli*.

### Anticancer property

The existence of growth inhibitory activity in human cervical cancer cells was discovered in vitro research of *Bryophyllum pinnatum* leaves crude extracts and their particular chromatographic fractions. The cytotoxic activity of the leaf extract and its fraction F4 (Petroleum Ether: Ethyl Acetate: 50:50) is dosage dependent, and the suppression of viral transcription of HPV18 in cells treated with fraction F4 was lower, indicating a higher concentration of active principles [127].

### Anti-hypertensive activity

The study found that *Bryophyllum pinnatum* aqueous and methanolic leaf extracts (50- 800mg/kg i.v. or i.p.) had antihypertensive action on arterial blood pressure and heart rates in normotensive and spontaneously hypertensive rats. In hypertensive rats, the hypotensive impact was stronger than in normotensive rats. Even leaf extracts (0.25-5.0 mg/ml) reduced the rate and force of contractions in isolated guinea-pig atria in a dose- dependent manner.

### Anti- Diabetic activity

The study found that *Bryophyllum pinnatum* aqueous leaf extract had anti- diabetic effect in diabetes caused rats (Glucose D-3g/kg) at four different doses (200, 400, 800mg/kg, and 800mg/kg Plus glibenclamide 2mg/kg). When compared to the other dose, the 200mg/kg aqueous extract resulted in a considerable reduction in blood sugar levels. However, the combination of 800mg/kg aqueous extract + 2mg/kg glibenclamide was more effective and efficient than the 200mg/kg and other single doses [128].

### Wound healing property

The wound healing effect of *Bryophyllum pinnatum* leaf extracts (petroleum ether, water, and alcohol) in the dose of 400mg/ kg orally on healing of excision wound, re- sutured incision, and dead space wound models in Albino rats for 10 days was discovered. And water extract was applied topically to an excision wound model for 21 days, till eschar formed. When

compared to the control group, all three extracts (petroleum ether, alcohol, and water) showed a substantial increase in the breaking strength of the incision wound. In a dead space wound model, granuloma breaking strength and hydroxyproline concentration of granulation tissue were considerably higher than in the control group. In an excision wound model, water extract exhibited a substantial increase in wound contraction and scar formation on the 17th after wounding day. In an excision wound model, even topical administration of water extract accelerated the healing process [121].

#### **Ant lithogenic activity**

The ant lithogenic effect of fresh leaf juice of *Bryophyllum pinnatum* was discovered in 23 clinically diagnosed lithiasis patients with stone sizes ranging from (>5mm to demonstrate considerable liver protection against DENA-induced liver damage [129].

#### **Anti-inflammatory activity**

In Formaldehyde-induced hind paw oedema in rats, leaf extracts of *Bryophyllum pinnatum* (pet-ether, chloroform, acetone, methanol, aqueous, alkaloidal fraction, flavonoids fraction, phenol and phenolic acid, alkaloidal anhydride) were found to have anti-inflammatory activity in doses of 500mg/kg orally once a day for two days. When compared to the conventional medicine Indomethacin, the methanolic fraction demonstrated more or less substantial suppression of formaldehyde-induced oedema in early phases and considerable inhibition in later phases [130].

#### **Neuropharmacological activity**

In mice, the effects of *Bryophyllum pinnatum* aqueous leaf extracts on several neuropharmacological activities were investigated. In doses of 50, 100, and 200 mg/kg, the extract was observed to cause a significant reduction in exploratory activity in a dose-dependent manner. It also had a strong sedative effect, as demonstrated by a reduction in coarse behavior and a potentiation of pentobarbitone-induced sleep time. It delayed the onset of strychnine- and picrotoxin-induced convulsions (seizures), with the preventive effect being larger in picrotoxin-induced convulsions than strychnine-induced convulsions. Picrotoxin-induced mortality is also reduced in mice with an LD50 of 641mg/kg. All of these results indicate that the extract has a depressive effect on the central nervous system [108].

## **CHAPTER - 2 REVIEW OF LITERATURE**

### **PHARMACOGNOSY**

1. *Bryophyllum pinnatum* belongs to the family Crassulaceae. It's used for Ethnomedical practices. Leaf gathered from south-eastern Nigeria in mice [137].
2. *Bryophyllum pinnatum* is generally known as Panphuti which belong to family Crassulaceae growing extensively in tropical Africa, tropical America, India, China, and Australia. It's a imperishable condiment grows 3-5 bases altitudinous, fleshy dark green leaves that are distinctively scalloped and trimmed in red, and bell-suchlike pendulous flowers [138].
3. The species *Bryophyllum pinnatum* (Lam) Pers. are native from Brazil and Madagascar, belonging to the Crassulaceae family and being extensively used by population as a natural anti-inflammatory agent. These species have analogous splint morphology and for this reason, they're known by the same popular name as "saião" or "coirama" [139].
4. *Bryophyllum pinnatum* (Lank.) Oken (Crassulaceae) is a imperishable succulent condiment extensively used in traditional drug to treat numerous affections. Its wide range of uses in folk drug justifies its being called "life factory" or "rejuvenation factory", egging experimenters' interest [140].

5. *Bryophyllum pinnatum* is a imperishable condiment, extensively used in the treatment of several conditions in myth drug [141].
6. *Bryophyllum pinnatum* ( family Crassulaceae) was extensively used in traditional drug. They're plant in especially in tropical Africa, India, China, Tropical America [142].
7. *Bryophyllum pinnatum* (Lam.) Kurz (Crassulaceae) is a imperishable condiment growing extensively and used in folkloric drug in tropical Africa, tropical America, India,China, and Australia[143].
8. Garugapinnata Roxb. (Burseraceae) is a medium-sized tree extensively available each over the tropical regions of Asia. *Bryophyllum pinnatum* (Lam) Oken. (Crassulaceae) is an indigenous and fantastic factory grown in tropical regions [144].

### PHARMACOLOGY\

9. *Bryophyllum pinnatum* is extensively used in ayurvedic system of drug as tangy, analgesic, carminative and also useful in diarrhea and vomiting. It's naturalized throughout the hot and wettish corridor of India. The leaves of *Bryophyllum pinnatum* have a variety of uses in the traditional system of drug in India. They're eaten for diabetes, diuresis, dissolving order monuments, respiratory tract infections, as well as applied to injuries, boils, and nonentity mouthfuls. It's useful for precluding alcoholic, viral and poisonous liver damages [145].
10. This study investigates the antioxidant exertion, carbohydrate digesting enzymes exertion and inhibitory exertion of cholinergic enzyme of waterless excerpt and fragments (n-hexane, ethyl acetate, n-butanol, residual waterless bit) of *B. pinnatum* leaves were delved [146].
11. It's extensively used in treatment of hemostatic and crack mending. It's also used in treatment of immunomodulatory, CNS depressant, analgesic, anti- inflammatory, antidiabetic, anticonvulsant, anticancer, antiallergic, nephroprotective, hepatoprotective, antileishmanial, antiulcer exertion. Fromthis review of factory, it highlights the chemical element and medicinal uses of factory [142].
12. Antioxidant, anticancer, antidiabetic, anti-inflammatory, anesthetics, crack mending and hepatoprotective conduct which are incorporated [147].
13. Anti-inflammatory, antioxidant, anticancer, crack mending, antidiabetic conditioning. [144].

### PHYTOCHEMICAL

14. The godly condiment contains a wide range of active composites, including alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, lipids and organic acids, have been insulated from this species (148).
15. *Bryophyllum pinnatum* contains precious phytochemicals similar as polyphenols, tannins, glycosaponins, flavonoids, steroidal glycosides and numerous other important chemical ingredients[149].
16. The qualitative phytochemical result showed that factory samples contains alkaloids, tannins, saponin, terpenoid, glycoside, phenols and flavonoid [150].
17. Alkaloids, tannins, saponins and flavonoids were present in all shops and all but alkaloids increased with increase in intervals of water deficiency. Alkaloid content dropped by 1.3 to 10.5 while the other phytochemicals increased by 12 to 206 in response to water deficiency stress. This exploration has revealed that water stress increases the tannin, saponin and flavonoid contents of *Bryophyllum* but decreases the alkaloid content[151].
18. The 9 phytochemicals linked, oleic acid constituted the loftiest percent 26.60, followed by nascence-

- D-Glucopyranoside, methyl,24.83. Other composites include n-Hexadecanoic acid,17.83, Octadecanoic acid,14.45,-Dihydroxy-6-methyl,6.19, Benzaldehyde,etc[152].
19. Phytochemicals plant present were reducing sugars, saponins, steroids, tannins, alkaloids, flavonoids, and phenol.[153]
  20. Phytochemical analysis results revealed the presence of primary metabolites (w/ w); carbohydrates (48.540.6)> proteins (21.060.6)> lipids (1.320.001). Secondary metabolites (mg/ g) were also insulated; methanol excerpt has advanced attention of flavonoids (95.810.026) and glycosaponins (39.90.103) while in water excerpt polysaccharide (139.40.026) and polyphenols (114.930.0234) were plant to be in maximum amounts as compared to the other excerpts [149].
  21. This analysis revealed the presence of alkanes, alkene, carboxylic acids, dicarboxylic acids, tricarboxylic acids, sugars, sugar acids, alcohols, sugar alcohols, adipose acids, monoaryl phenolics, steroids, vitamin and cyclic composites. A number of active composites, including phenols, steroids, and organic acids, have been linked in BP excerpts [154].
  22. The physiochemical study shows presence of alkaloid, flavonoid, lipids, organic acids, phenols, triterpene, glycoside and bufadienolides [142].
  23. Flavonoids, terpenoids, glycosides, steroids and these secondary metabolites [147].

### CHAPTER - 3

#### AIM AND SCOPE

##### AIM:

The present study was undertaken for the formulation and standardization of gel using *Bryophyllum pinnatum*.

##### SCOPE:

Hemostasis, inflammation, proliferation, and remodeling are the four exact and highly planned phases of wound healing as a natural biological process in the human body. All four phases must occur in the correct order and time period for a wound to heal properly.

A wide range of plant-derived active principles representing a variety of phytochemicals have shown consistent wound healing activity and may be useful in wound treatment. With the foregoing facts in mind, the *Bryophyllum pinnatum* formulation was created, and it was chosen to test wound healing activity and the likely underlying mechanism of action.

### CHAPTER - 4 PLAN OF WORK

1. Collection, identification and authentication of plant specimen.
2. Preparation of extract
  - Ethanol extract
3. Preliminary phytochemical screening
4. Formulation of gel
5. Evaluation of gel
6. In-vitro studies

**CHAPTER - 5 PLANT PROFILE****PLANT PROFILE OF *BRYOPHYLLUM PINNATUM* [136]**

- BIOLOGICAL NAME : *Bryophyllum pinnatum*
- COMMON NAME : cathedral bells

**CLASSIFICATION:[6]**

- Kingdom: plantae
- Division: magnoliophyte
- Order: rosales(saxifragales)
- Family: crassulaceae-stonecrap
- Genus: Bryophyllum
- Species: pinnata

**PART USED:**

- Leaf

**PHYTOCHEMISTRY:**

Major bioactive constituents of the leaves are polyphenol, tannins, glycosaponins, flavonoids, steroidal glycosides, analgesic, anti-septic, sedative, Antioxidant, anti-pyretic, anti-inflammatory, anti-arthritic, anti-allergic.

**MACROSCOPIC CHARACTERS:****Colour and appearance:**

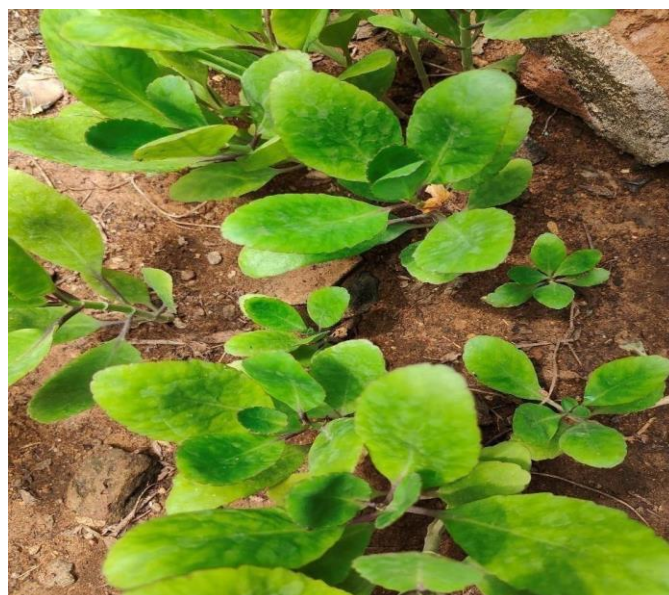
The leaves are green in colour, and the dried leaves are brown in colour.

**Odour:**

Aromatic

**Taste:**

Sour taste



**Figure 2: Plants of *Bryophyllum pinnatum***

## CHAPTER - 6 MATERIALS AND METHODS

### 6.1 PHARMACOGNOSTICAL STUDY [148-152]

Fresh leaves are used for macroscopically and microscopically evaluation, while the coarse drug is used to determine the physiological parameters including loss on drying, extractive value, ash value and phytochemical analysis.

### COLLECTION, IDENTIFICATION AND AUTHENTICATION

The plant specimen such as fresh leaves was collected and dried. The leaf of *Bryophyllum pinnatum* is to be collected from the local area and the leaf was authenticated by a Botanist. **Macroscopy:**

External feature of test sample was documented using Nikon COOLPIX 5400 digital camera.

#### Microscopy:

Sample was preserved in fixative FAA for more than 48 hr. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with safranin. Transverse sections were photographed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss Axiom Cam Erc5s digital camera under bright field light. Magnifications were indicated by scale bar.

### DETERMINATION OF PHYSICO-CHEMICAL STANDARDS [152,153,154]

#### Ash Value

The ash composition of the synthetic drug is usually considered to be the residue that persists during incineration. It simply contains and adheres to inorganic salts that occur naturally in the product and may contain inorganic matter added for adulteration purposes. The amount of ash varies with limited limits for a specific drug that ranges greatly between different drugs.

#### 6.2.1 (A) Determination of Total Ash

Approximately 2 g of powdered content was determined precisely in a silica crucible that was already activated and weighed. The concentrated product was placed as a fine coating on the bottom of the crucible. The crucible was burned at temperature, not above 450°C, unless it was carbon-free. The crucible was cooled and weighed for constant weight. The percentage of total ash was measured using air-dried drugs.

#### (B) Determination of Acid-Insoluble Ash

The ash collected as stated in the total ash determination has been boiled for 5 minutes with 25 ml of hydrochloric acid. The insoluble ash was deposited on an ash less filter paper and cleaned with hot water; the insoluble ash was moved to a silica crucible which is pre-weighted. Referring to the air-dried drug the amount of acid-insoluble ash was measured.

#### Loss on drying

Five grams of the granulated synthetic drug is accurately measured in a tarred dish and dried in the oven at 100-105°C. It was cooled in the desiccators and weighed again. The loss of drying was calculated in terms of the volume of dry powder obtained.

#### Extractive Values

##### (A) Water soluble extractive value

1 g of coarse powder was macerated for 24 hours, followed by decanting and drying the marc. Solvents were obtained from 75°C to 80°C by distillation of the extracts. The extracts were allowed to dry under the desiccator and the percentage of yield was evaluated.



### 6.23 (B) Ethanol Soluble Extractive

1 g Air-dried coarsely compressed drug has been macerated in a closed flask of 100 ml of 90 percent ethanol for 24 hours, periodically shaking for the first 6 hours and allowing it to rest for 18 hours. It was then cleaned easily, taking solvent loss precautions. Drying to 105°C, 25 ml of filtrate in a shallow dish was evaporated and weighted to dryness. The ethanol-soluble extractive quantity was calculated in comparison to the air-dried drug.

### PRELIMINARY PHYTOCHEMICAL SCREENING: [85–87]

The leaf will be subjected to qualitative test for the identification of various active constituents' viz. flavonoids, glycosides, tannins, Saponin. According to standard procedure.

### TEST FOR CARBOHYDRATES AND GLYCOSIDES

Small quantity of the sample was dissolved separately in 4 ml distilled water and filter. The filtrate was subjected to the following test to detect the presence of carbohydrates and glycosides.

#### 6.3.1 (A) MOLISH'S TEST:

The filtrate was treated with 2 to 3 drops of 1% alcoholic  $\alpha$ -naphthol solution and 2 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

#### 6.3.1 (B) FEHLING'S TEST:

The filtrate was treated with 1ml of Fehling's solution A and B and heated on the water bath. A reddish precipitate was obtained shows the presence of carbohydrates.

#### (C) LEGAL'S TEST:

To the hydro lysate 1 ml of pyridine and few drops of sodium nitroprusside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

#### 6.3.1(D) BORNTRAGER'S TEST:

Hydro lysate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink colour, showing the presence of glycosides.

### TEST FOR FIXED OILS AND FATS:

#### 6.3.2 (A) SPOT TEST:

Small quantity of sample pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

#### SAPONIFICATION TEST:

Few drops of 0.5% alcoholic potassium hydroxide were added to a small quantity of various extract along with a drop of phenolphthalein. The mixture was heated on the water bath for 1 to 2 hrs. Formation of a soap partial neutralization of alkali indicates the presence of fixed oils and fats.

### TEST FOR PROTEINS AND FREE AMINO ACIDS

Small quantity of the sample was dissolved in few ml of distilled water and treated with following reagents.

### 6.3.3 (A) MILLON'S TEST

Appearance of red color shows the presence of proteins and free amino acids.

### 6.3.3 (B) NINHYDRIN REAGENT

Appearance of purple color shows the presence of proteins and free amino acids.

### (C) BIURET TEST

Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate were added appearance of pink or purple colour shows the presence of proteins and free amino acids.

### TEST FOR SAPONINS

#### (A) FOAM TEST

- The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 min.

**La** – Lamina; **Scl** – Sclereids; **Ep** – Epidermis; **MT** – Mesophyll Tissue

- The formation of 1cm layer of foam shows the presence of saponins.

### TEST FOR PHENOLIC COMPOUNDS

Small quantity of extract was taken in distilled water and test for the presence of phenolic compounds and tannins was carried out with the following reagents:

- Dilute ferric chloride solution (5% W/V) – Violet colour
- B.1% solution of gelatin containing 10% sodium chloride – White precipitate

### TEST FOR PHYTOSTEROL:

Small quantity of the sample was dissolved in 5ml of chloroform separately. Then this chloroform solution was subjected to the following tests to detect the presence of phytosterols.

#### 6.3.6 (A) LIBERMANN –BURCHAD'S TEST:

The above prepared chloroform solution was treated with drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3ml of acetic anhydride. A bluish green color appeared indicates the presence of phytosterol.

#### (B) SALKOWSKI REACTION

To 1ml of the above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown colour produced shows the presence of phytosterols.

### TEST FOR ALKALOIDS

Small quantity of the extract was treated with few drops of diluted hydrochloric acid and filtered. The filtrate was used for the following tests.

#### 6.3.7 (A) MAYER'S REAGENT (POTASSIUM MERCURIC IODIDE SOLUTION)

Alkaloids give cream colour precipitate with Mayer's reagent.

#### 6.3.7 (B). DRAGENDORFF'S REAGENT (POTASSIUM BISMUTH IODIDE SOLUTION)

Alkaloids give reddish brown precipitate with Dragendorff's reagent.

6.3.7 (C) HAGER'S TEST (SATURATED SOLUTION OF PICRIC ACID) Some alkaloids give yellow-coloured precipitates with Hager's reagent.

(D) WAGNER'S TEST (SOLUTION OF IODINE IN POTASSIUM IODIDE) Alkaloids yielding reddish brown precipitate with Wagner's reagent.

**TEST FOR FLAVONOIDS**

**6.3.8 (A) WITH AQUEOUS NaOH SOLUTION:**

Small quantity of the sample was dissolved in aqueous sodium hydroxide and appearance of yellow colour indicates the presence of flavonoids.

**(B) SHINODA’S TEST**

Small quantity was dissolved in alcohol; to those pieces of magnesium followed by concentrated hydrochloric acid was added drop by wise and heated. Appearance of magenta colour shows the presence of flavonoids.

**TEST FOR MUCILAGE AND RESINS**

Small quantities of extract were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in oil and examined for its swelling property for the presence of gum and mucilage

**TEST FOR STARCH**

To the aqueous extract add weak aqueous iodine solution, blue colour indicates presence of starch, which disappears on heating and reappears on cooling.

**TEST FOR WAXES**

To the test solution add alcoholic alkali solution, waxes get saponified.

**FORMULATION OF GEL: [88]**

**CRBOPOL 934P GELS:**

Weighed quantity of CARBOPOL 934P (for ease in discussion CARBOPOL 934P is considered as CARBOPOL 934P throughout the remaining text) was taken and added to the distilled water. Extract was solubilized in an appropriate amount of ethanol and this ethanolic dispersion of extract was transferred to aqueous dispersion of CARBOPOL 934. The mixture was stirred gradually by means of a stirrer and CARBOPOL 934 was allowed to soak for 2 h. Triethanolamine was added to neutralize the CARBPOL 934 solution and to form the gel. The pH was adjusted 6.8.

**Table 1: Composition of topical gel formulations of Extract (% w/w)**

Materials	C	C1	C2	C3	C4	C5
<b>Extract</b>	1.00	1.00	1.00	1.00	1.00	1.00
<b>CRB</b>	1.00	1.00	1.00	1.00	1.00	1.00
<b>Triethanolamine</b>	0.30	0.30	0.30	0.30	0.30	0.30
<b>Menthol</b>	-	1.25	2.5	3.75	5	6.25
<b>Ethanol</b>	15.00	15.00	15.00	15.00	15.00	15.00
<b>Distilled water</b> <b>Q.S. to make</b>	q.s	q.s	q.s	q.s	q.s	q.s

<b>Total</b>	100.00	100.00	100.00	100.00	100.00	100.00
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**Evaluation of Herbal Gel: [157,158]**

**6.4.2 (A) Physical Appearance:**

Physical parameters like coloured and appearance of gel were checked.

**6.4.2 (B) Measurement of pH:**

The pH of herbal gel formulations was determined by using digital pH meter. 1 gm of gel dispersed in 10 ml of water Keep aside for 2 hours The measurement of pH of formulation was administered in 3 times.

**6.4.2 (C) Homogeneity:**

All developed gel formulations were tested for homogeneity by visual inspection after the gels are set into the container. They were tested for his or her presence and appearance of any aggregates (Gupta, 2010).

**6.4.2 (D) Spreadability:**

Spreadability decided by glass slide and wooden block apparatus. Weights about 20 gm were added to the pan. The time were noted for upper slide to maneuver Separate completely from the fixed slide (Shivhare, 2009). An excess amount of gel 2 gm under study was placed on this ground slide. The gel was then sandwiched between these slides. Another glass slide having the fixed ground slide. There is provided with the hook. A 1 kg weighted was placed on the highest of the slides for five minutes to supply a consistent film of the gel and take away air between the slides. Excess of the gel was removed far away from the sides. The top plate was then subjected to tug with the assistance of string attached to the hook and therefore the time in seconds required by the highest slide to hide a distance of 7.5 cm be noted. A shorter or less interval indicates better Spreadability. Spreadability of gel was calculated using the subsequent formula (Pawar, 2013).

Spreadability =  $M \times L / T$  Where, M = Weight in the pan which is tied to the upper slide, L = Length moved by the glass slide T = Time in second taken to separate the slide completely each other.

**6.4.2 (E) Viscosity:**

Viscosity decided by using Brookfield viscometer (DV-III programmable Rheometer). Formulated gels were tested for his or her rheological behaviours at 250C. The measurement was remodelled range of speed from 10rpm to 100rpm with 30seconds between 2 successive speeds then during a reverse order (Bhramaramba, 2015).

**6.4.2 (F) Extrudability:**

Gel formulations were filled in standard capped collapsible aluminium tubes and sealed to the absolute best. The extrudability decided by pressing of the thumb.

**6.4.2 (G) Clarity:**

The clarity of all the three batches decided by visual inspection (Pandey, 2011).

**6.4.2 (H) Gel strength:**

Gel strength decided by the time in seconds required by the load to penetrate within the gel. A Sample amount of 5 gm of every of the optimize batches was taken and three 5 gm weight was placed on the surface of gel. The time in seconds required by the load to penetrate 0.5 cm within the gel.

#### 6.4.2 (I) Bio adhesive Strength (Velraj M, 2009)

Bio adhesive strength was determined by using glass slide and wooden block apparatus. Bio adhesive strength used to measure the force required to detach the formulation from cellophane membrane. Specified amount that's 1 gm of prepared gel was taken on glass slide wrapped with cellophane membrane. Intimate contact was provided by the movable glass slide was placed on fixed slide. Two-minute contact time was given to make sure intimate contact between formulation and membrane. The weight was added within the pan which is provided to apparatus until slides got detached. The bio-adhesive force, expressed because the detachment stress in dyne/cm<sup>2</sup> decided by the formula (Jaiswal, 2012). Detachment stress =  $m \cdot g / A$  Where, m = Weight required to detach two glass slides from one another (gm). g = Acceleration thanks to gravity i.e., 980 cm/s<sup>2</sup>. A = Area of membrane exposed (cm<sup>2</sup>).

#### 6.4.2 (J) Stability study:

Stability studies were through with open and shut container. Here, by subjecting the product to room temperature for 1 month (Kaur, 2013) & (Allen L).

### INVITRO STUDIES:

#### Assay of Antibacterial Activity (159):

Antibacterial activity of *Bryophyllum pinnatum* extract was examined against several species of gram positive and gram-negative bacteria by using cup plate method. Late exponential phase of the test bacteria was prepared by inoculating 1% (v/v) of the cultures into the fresh Muller-Hinton broth and incubating on an orbital shaker at 37°C and 100 rpm overnight. Before using the cultures, they were standardized with a final cell density of roughly 10<sup>8</sup> cfu ml<sup>-1</sup>. Muller Hinton agar were prepared and inoculated from the standardized cultures of the test organisms then spread as uniformly as possible throughout the whole media. Agar well was made with sterile borer, test proteins introduced into the well and incubated at 37°C for 24 hrs. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (mm) on the surface of plates and the results were reported as Mean after three repeats.

### Anti-Inflammatory Activity

#### 6.5.2 (A) Membrane Stabilization

##### 6.5.2 (a) Red blood cells (RBCs) suspension preparation

Fresh human blood was collected and taken in a centrifuge tube with heparin. The test tubes were centrifuged for 10 min at 3000 rpm. It was washed for 3 times with normal saline, and the volume of blood was measured. They are made up to 10% suspension using normal saline.

##### (b) Heat-induced Hemolysis

Heat-induced hemolysis was performed using 1 ml of test extracts along with 10% prepared RBC suspension. Aspirin was used a standard. Incubation was done at 56°C for a period of 30 min. Absorbance was read for the supernatant at 560 nm. Percentage inhibition of Hemolysis calculated using: % inhibition =  $\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of control}} \times 100$

##### Antioxidant Activity

#### 6.5.3 (A) DPPH Assay

##### 6.5.3 (a) Preparation of DPPH:

0.024 g of DPPH was taken and 50 ml of ethanol was added. Then transfer the solution in volumetric flask (100 ml). Make up to 100 ml using ethanol. After the DPPH Solution was prepared, the solution

was kept in spectrometer at 517nm. if the spectra come between 1.08 – 1.12, the DPPH solution is ready for test. If it less than 1.08, add DPPH and if it is greater than 1.12, add ethanol.

**6.5.3 (b) Preparation of Standard:**

Take 10mg of Ascorbic Acid and add 10ml of ethanol. Then five different concentrations were taken from the prepared standard (50µg, 100µg, 150µg, 200µg,250µg) using ethanol make up the volume of 10ml.

**Table 2: Concentration of Antioxidant Activity**

Concentration	50µg	100µg	150µg	200µg	250µg
100ml of DPPH Solution	3ml	3ml	3ml	3ml	3ml
Ascorbic Acid (10mg/ml)	5ml	5ml	5ml	5ml	5ml
Extract (10mg/ml)	5ml	5ml	5ml	5ml	5ml

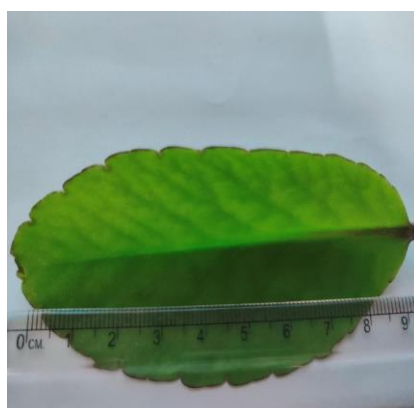


**CHAPTER - 7**

**RESULTS AND DISCUSSION**

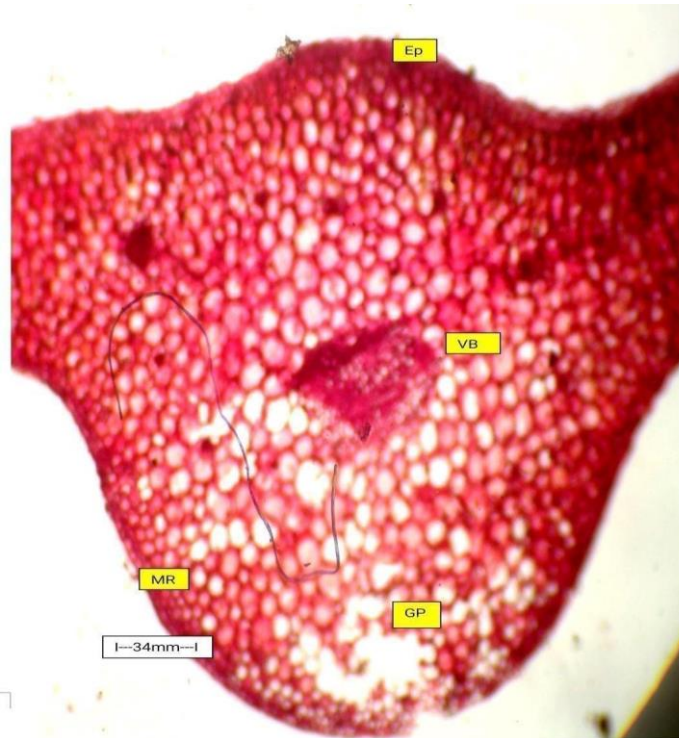
A gel dosage form for the plant *Bryophyllum pinnatum* was formulated using its ethanolic leaf extract. An antibacterial assay was performed on agar plates and other media using the formulated gel. Further studies to be done include gel evaluation and anti- inflammatory activity and anti-oxidant activity is done.

**7.1 (A) Macroscopy:**



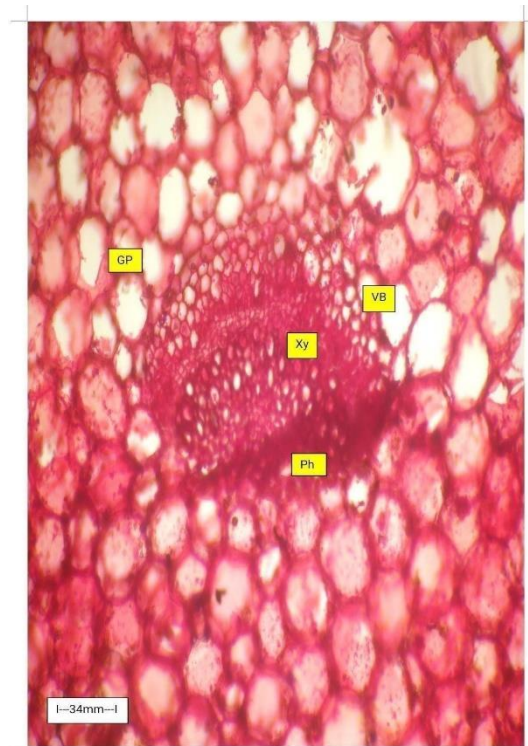
**Figure 3: Leaf of *Bryophyllum pinnatum***

**(B) Microscopy:**



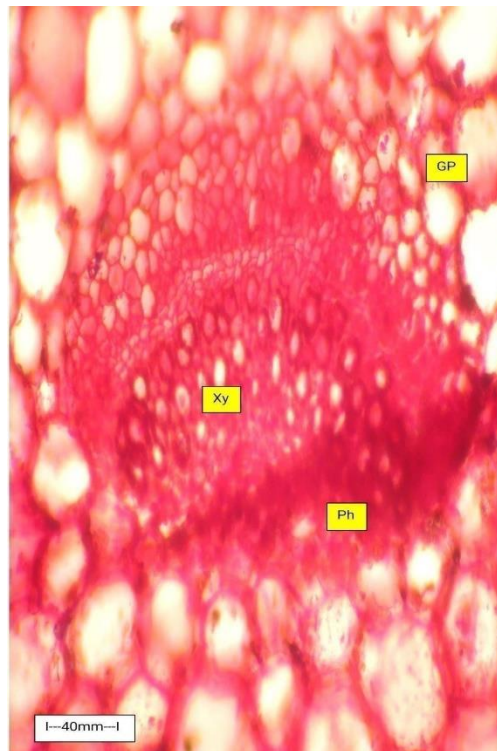
**Figure 4: TS of *Bryophyllum pinnatum* Leaf (Midrib Region)**

**Ep** – Epidermis; **VB** – Vascular Bundle; **MR** – Midrib;  
**GP** – Ground parenchyma



**Figure 5: TS of *Bryophyllum pinnatum* Leaf (Vascular Bundle Region)**

**GP** – Ground Parenchyma; **VB** – Vascular Bundle; **Xy** – Xylem;  
**Ph** – Phloem



**Figure 6: TS of *Bryophyllum pinnatum* Leaf (Vascular Bundle Region)** GP – Ground Parenchyma; Xy – Xylem; Ph – Phloem



**Figure 7: TS of *Bryophyllum pinnatum* Leaf (Lamina Region)**  
La – Lamina; Scl – Sclereids; Ep – Epidermis; MT – Mesophyll Tissue



**DETERMINATION OF PHYSICO-CHEMICAL STANDARDS**

**Table 3: Physio-Chemical evaluation table**

S.NO		Bryophyllum pinnata
Ash value	Total ash	13.76 %
	Acid Insoluble ash	1.73%
Loss on drying		91.46%
Extractive value	Water extractive value	2.13%
	Alcohol extractive value	10.18%

**PRELIMINARY PHYTOCHEMICAL ANALYSIS**

**Table 4: Preliminary Phytochemical Analysis**

S.NO	Phytoconstituents	Ethanol extract
1	Alkaloids	+
2	Carbohydrate	+
3	Glycoside	+
4	Flavonoid	+
5	Steroid	-
6	Tannin	-
7	Saponin	+
8	Oil and Fats	-
9	Protein	+
10	Amino acid	+
11	Phenolic compounds	+
12	Gums and Mucilage	-

**GEL PARAMETER**

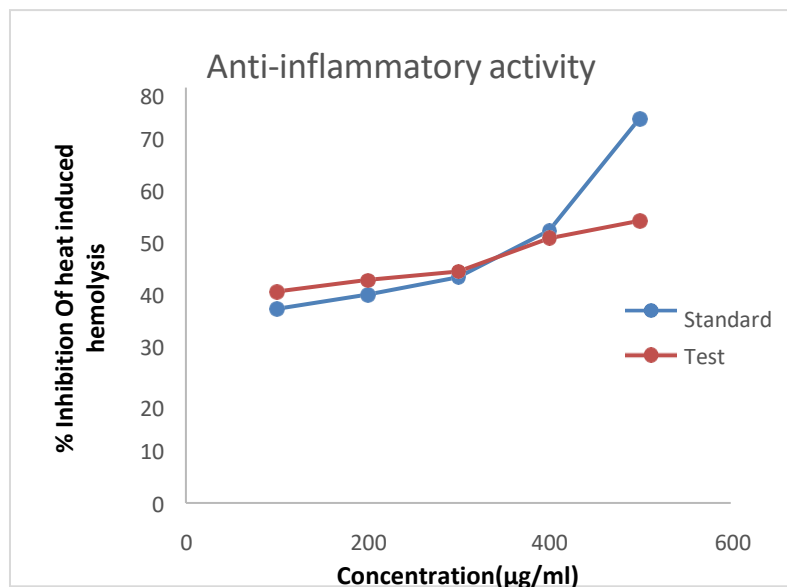
**Table 5: Evaluation Parameter of Gel**

Concentration	pH	viscosity	Spreadability	Net content	extrudability	Physical Appearance
C	6.8	0.3850	32.19	67 gm	Good	Dark green, homogenous
C1	6.8	0.3862	45.05	68 gm	Excellent	Greenish, homogenous
C2	6.8	0.3873	56.39	69 gm	Excellent	Greenish, homogenous
C3	6.8	0.3882	64.00	70 gm	Excellent	Greenish, homogenous
C4	6.8	0.3891	71.38	75 gm	Excellent	Light green, homogenous
C5	6.8	0.3906	75.74	76 gm	Excellent	Light green, homogenous

**IN VITRO STUDIES**

**Table 6: Anti-Inflammatory Activity**

Concentration(µg/ml)	% Inhibition Of heat induced hemolysis	
	Standard	Test
100	37.37604	40.71866
200	40.16156	42.94708
300	43.50418	44.61838
400	52.41783	51.02507
500	74.14485	54.36769
IC50	305.5594	392.0056



**Figure 8: % Inhibition of heat induced hemolysis.**

**Table 7: One-way Anova for Anti-inflammatory Activity**

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	5	247.6045	49.52089	221.5067
Column 2	5	233.6769	46.73538	32.92186

ANOVA						
Source of Variation	SS	df	MS	F	P-Value	F crit
Between Groups	19.397	1	19.397	0.1524	0.7063	5.3176
Within Groups	1017.7	8	127.21			
Total	1037.1	9				

**Table 8: Antioxidant Activity**

Concentration (µg)	DPPH ASSAY	
	Standard	Test

50	55.32164	46.19883
100	66.49123	69.00585
150	76.78363	84.79532
200	87.36842	87.1345
250	97.95322	90.93567
IC50	23.8247	30.9804

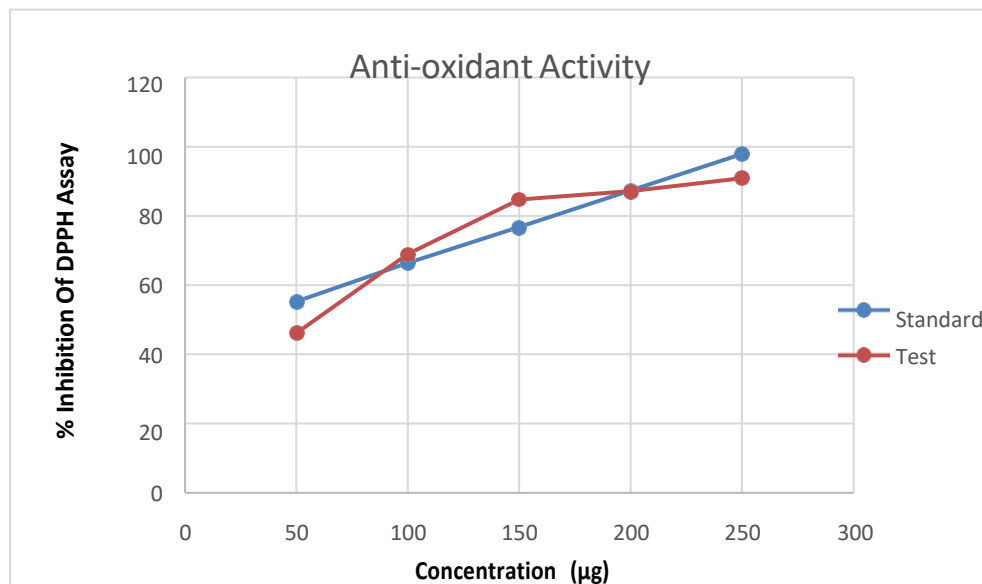


Figure 9: % Inhibition of DPPH Assay.

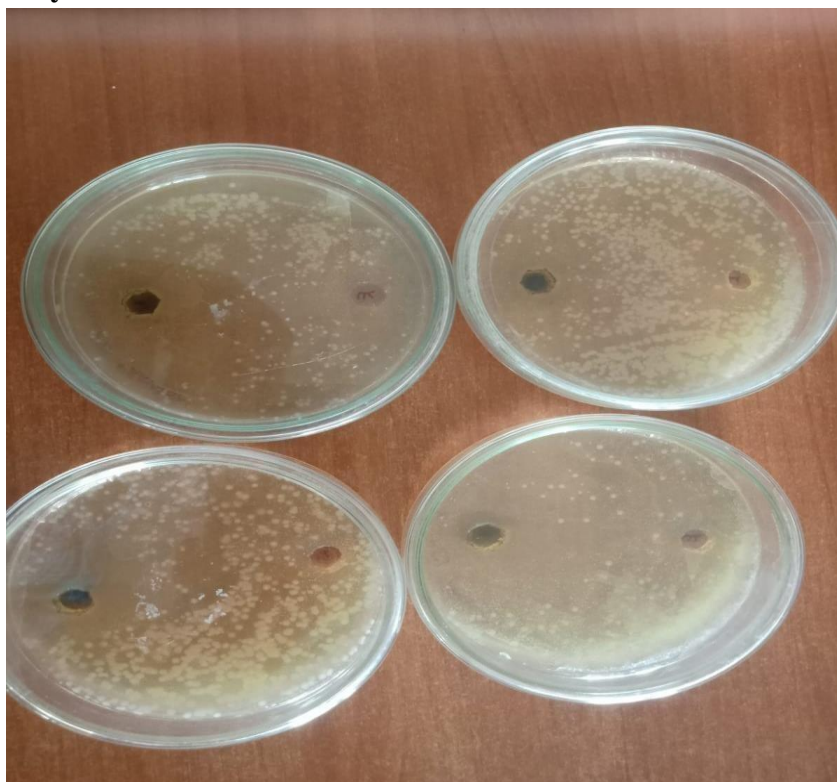
Table 9: One Way Anova for Anti-Oxidant Activity.

SUMMARY				
Groups	Count	Sum	Average	Variance
Column 1	5	383.9181	76.78363	281.685
Column 2	5	378.0702	75.61403	340.173

ANOVA

Source of Variation	SS	df	MS	F	P-value	Fcrit
Between Groups	3.419875	1	3.419875	0.01099	0.91905	5.31
Within Groups	2487.432	8	310.929			
Total	2490.852	9				

**Anti-Microbial activity:**



**Figure 10: Anti-Bacterial Activity of Protein from *Bryophyllum pinnatum* against *Staphylococcus aureus*-5021, *Bacillus subtilis*-2717, *Pseudomonas aeruginosa*-2492, *Klebsiella pneumonia*-2957**

From the crude sample characterization of Protein by SDS-Page was carried out and the mixture of protein was observed. The effectiveness of the extracted protein was checked by conducting antimicrobial studies on the following gram-positive bacteria and gram negative bacteria using Mueller Hinton agar as medium and the extracted protein was found to be effective against gram positive and gram negative Bacteria.

**Table 10: Antibacterial activity of isolated proteins**

Sample	<i>Staphylococcus aureus</i> -5021	<i>Bacillus subtilis</i> -2717	<i>Pseudomonas aeruginosa</i> -2492	<i>Klebsiella pneumonia</i> -2957
8 hrs	3mm	2mm	5mm	4mm
12 hrs	10mm	10mm	15mm	8mm
16 hrs	15mm	13mm	17mm	16mm
24 hrs	19mm	18mm	23mm	21mm

**CHAPTER - 8**

**SUMMARY AND CONCLUSION**

The dissertation entitled “Formulation and Evaluation of gel using *Bryophyllum pinnatum* for wound healing” deals with macroscopical and microscopical character including phytochemical and pharmacological aspects of *Bryophyllum pinnatum*. Therapeutically *Bryophyllum pinnatum* beneficial plant in traditional claim and the in Indian medicine system, has been selected in the present work. The

research survey shows that no investigation on the aspects of wound healing activity was performed on herbal extract containing *Bryophyllum pinnatum*.

The Pharmacognostical part of the research can be widely used for the determination of the crude drug from the plant. The qualitative study or physiochemical analysis was performed, and potentiality of the drug was noted.

The antioxidant activity is carried out by free radical scavenging activity by DPPH and total antioxidant phomolybdenum assay methods. The IC<sub>50</sub> value of the ethanol extract of leaves in each method were found to be nearly equal to the standard Ascorbic acid drug and demonstrated the strongest antioxidant activity. As the ethanolic extract showed highest antioxidant activity hence it is chosen for In-vitro wound healing activity.

Similarly, the wound healing activity is performed by anti-inflammatory activity and anti-microbial activity. The gel was prepared and evaluated by several methods. The extract was prepared by maceration and introduced for the study. In all the in-vitro assays the ethanol extract was found to be more effective. Having said that, *Bryophyllum pinnatum* can be widely used against wound healing, it highly full fills the healing property. Hence it can be suggested in the field of Wound medicaments.

The future work of this study presents the structural elucidation of protein components by using NMR, Mass spectroscopy, etc. Then this study undergoes animal study in future.

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