CHAPTER I

1. INTRODUCTION

Plants have been used for health and medical purposes for several thousands of years. The number of higher plant species on earth is about 2,50,000. It is estimated that 35,000 to 70,000 species have, at one time or another, been used in some cultures for medicinal purposes. A majority of the world's population in developing countries still relies on herbal medicines to meet its health needs. Herbal medicines are often used to provide first-line and basic health service, both to people living in remote areas where it is the only available health service, and to people living in poor areas where it offers the only affordable remedy. Even in areas where modern medicine is available, the interest on herbal medicines and their utilization have been increasing rapidly in recent years. According to World Health Organization (WHO), about 80% of the world’s population relies on medicinal plants for their primary health care needs both in the developing countries and developed countries where modern medicines are predominantly used.1-3

Since time immemorial man has used various parts of plants in the treatment and prevention of many ailments.4 Historically all medicinal preparations were derived from plants, whether in the simple form of plant parts or in more complex form of crude extracts, mixtures, etc. Today, a substantial number of drugs are developed from plants which are active against a number of diseases.5 The majority of these involve isolation of active ingredient (chemical compound) found in a particular medicinal plant and its subsequent modification. In the developed countries, 25 percent of drugs are based on plants and their derivatives and the use of medicinal
plants is well known among the indigenous people in rural areas of many developing
countries. In the past, our ancestors made new discoveries of the healing power of
plants through trial and error. Although some of the therapeutic properties attributed
to plants have proven to be erroneous, medicinal plant therapy is based on the
empirical findings of hundreds and thousands of years.

Though herbal products have become increasingly popular throughout the
world, one of the impediments in its acceptance is the lack of standard quality control
profile. The quality of herbal medicine that is, the profile of the constituents in the
final product has implications in efficacy and safety. However, due to the complex
nature and inherent variability of the constituents of plant-based drugs, it is difficult to
establish quality control parameter through modern analytical technique which are
expected to help in circumventing this problem. Furthermore, the constituents
responsible for the claimed therapeutic effects are frequently unknown or only partly
explained. This is further complicated by the use of combination of herbal ingredients
as being used in traditional practice. It is common to have as many as herbal
ingredients in one product. Thus, batch to batch variation starts from the collection of
raw material itself in the absence of any reference standard for identification. These
variations multiply during storage and further processing. Hence for herbal drugs and
products, standardization should encompass the entire field of study from cultivation
of medicinal plant to its clinical application. Plant materials and herbal remedies
derived from them represent substantial portion of global market and in this respect
internationally recognized guidelines for their quality assessment and quality control
are necessary.
According to WHO, standardization and quality control of herbal is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. Attention is normally paid to quality indices such as macro and microscopic examination, foreign organic matter, ash values, moisture content, extractive values, crude fibre, qualitative chemical evaluation, chromatographic examination, quantitative chemical evaluation, toxicological studies.8-10

Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions.11 Current estimates indicate that nearly 6 million people suffer from chronic wounds worldwide. Community based epidemiological study of wounds in India revealed the prevalence of acute and chronic wounds as 10.55 and 4.48 per thousand population’s respectively.12 Healing of chronic lower extremity wounds is a global problem.13

In Ayurveda, 70 % of the wound healing drugs are of plant origin, 20 % of mineral origin and the remaining 10 % consisting of animal products. These drugs are stated to be effective in different conditions such as Vrana (wounds or ulcers), Nadivrana (sinuses), Vidradhi (abscess), Visarpa (erysipelas), Upadamsha (syphilitic ulcers), Vranajakrimi (maggots in wounds), Dustavrana (septic wounds), Vranashotha (inflammatory changes of wounds), Vranavisha (cellulitis), Ugravrana (purulative ulcer), Netavrana (hordeolum or stye sepsis), Pramehapidaka (diabetic carbuncle) and Bhagandara (fistula-in-ano).14
Research on wound healing agents is one of the developing areas in modern biomedical sciences and many traditional practitioners across the world particularly in countries like India and China have valuable information of many lesser-known hitherto unknown wild plants for treating wounds and burns.\(^5\) Traditional forms of medicine practiced for centuries in Africa and Asia are being scientifically investigated for their potential in the treatment of wound related disorders.\(^6\) Use of various herbs and traditional medicine be economical also in present situation of escalating health care cost hence an abreast is required to dig out as much as possible from the treasure of nature in order to shore up the good health among the advance techniques of wound care.\(^7\)

### 1.1. WOUNDS

Wound is defined as the disruption of cellular and anatomic continuity of a tissue. According to the Wound Healing Society (WHS), wounds are physical injuries that result in an opening or break of skin that causes disturbance in the normal skin anatomy and function. They result in the loss of continuity of epithelium with or without the loss of underlying connective tissue.\(^8,9\) This includes injury of underlying tissues / organs caused by surgery, a blow, a cut, chemicals, heat/cold, friction / shear force, pressure or as a result of disease. Wound may arise due to physical, chemical, microbial agents, thermal or immunological damage to the tissue.

### 1.2. CLASSIFICATION OF WOUNDS

There is no definite method of classifying wounds. There are many different types of wounds ranging from mild to severe to potentially fatal.
1. Based on anatomical site

Wounds can be referred by their anatomical site, e.g. abdominal or axillary wound.

2. Based on underlying cause of wound creation

Wounds are classified as open and closed wounds.\textsuperscript{20}

i) Open wound

In this case, blood escapes the body and bleeding is clearly visible. It is further classified as incised wound, laceration or tear wound, abrasions or superficial wounds, puncture wounds, penetration wounds and gunshot wounds.

Incised Wound

It is an injury with no tissue loss and minimal tissue damage. It is caused by a sharp object such as knife. Bleeding in such cases can be profuse, so immediate action should be taken.

Abrasions or Superficial Wound

It is caused by sliding fall onto a rough surface. During abrasion, the topmost layer of the skin i.e. epidermis is scraped off that exposes nerve ending resulting in a painful injury. Blood loss similar to a burn can result from serious abrasions.

Laceration Wound or Tear Wound

This is the nonsurgical injury in conjunction with some type of trauma, resulting in tissue injury and damage.
**Puncture Wound**

They are caused by some object puncturing the skin, such as needle or nail. Chances of injection in them are common because dirt can enter into the depth of wound.

**Gunshot Wound**

They are caused by a bullet or similar driving into or through the body.

**Penetration Wound**

Penetration wounds are caused by an object such as a knife entering and coming out from the skin.

**ii) Closed Wound**

In closed wounds blood escapes the circulating system but remains in the body. It includes contusion or bruises, hematomas or blood tumor, crush injury etc.

**Contusion or bruises**

Bruises are caused by a blunt force trauma that damage tissue under the skin.

**Hematomas or blood tumor**

They are caused by damage to a blood vessel that consequently causes blood to collect under the skin.

**Crush injury**

Crush injury is caused when great or extreme amount of force is applied on the skin over long period of time.
3. Based on the basis of physiology of wound healing

Wounds are popularly categorized by their level of chronicity as either an acute or a chronic wound.

Acute Wounds

Acute wound is a tissue injury that normally precedes through an orderly and timely reparative process that result in sustained restoration of anatomic and functional integrity. Acute wounds are usually caused by cuts or surgical incisions and complete the wound healing process within the expected time frame.\textsuperscript{21}

Chronic Wounds

Chronic wounds are wounds that have failed to progress through the normal stages of healing and therefore enter a state of pathologic inflammation. Chronic wounds either require a prolonged time to heal or recur frequently. Local infection, hypoxia, trauma, foreign bodies and systemic problems such as diabetes mellitus, malnutrition, immune deficiency or medications are the most frequent causes of chronic wounds.\textsuperscript{22, 23}

4. Based on wounds with or without tissue loss

Wounds are generally classified as wounds without tissue loss (eg: in surgery), and wounds with tissue loss (eg: burn wounds)

Avulsion

This term describes a wound where there is tissue loss, preventing the closure of the wound edges. An avulsion may be caused by gouging or tearing of tissue.
**Strains**

Strains are injuries to muscles, fascia or tendons caused by stretching forces. Patients complain of pain and stiffness and there may be some associated swelling. It is usually important to exclude other injuries such as fractures. Strain injuries usually resolve with rest followed by progressive mobilization.

**Sprains**

A sprain describes an injury to the fibrous tissues surrounding a joint. Fibrous ligaments around the joint are injured, usually as a result of excessive movement of the joint. A mild sprain may involve tearing a few of the fibers in a ligament, in more serious cases there will be associated hematoma formation. In severe cases there may be complete tearing and disruption of a ligament. Patients usually present with local heat, pain, swelling, disability and possible discoloration over the area. Ankles are commonly sprained; if the ankle is turned inwards there will be injury to the lateral ligaments. Sprains usually take longer to recover than strains.

### 1.3. FACTORS AFFECTING WOUND HEALING

Wound healing is a normal biological process in the human body. Many factors can adversely affect this process and lead to improper and impaired wound healing. Understanding of these systemic and local factors and their influence on wound healing is essential for better therapeutic opportunity for wound treatment.\(^{24}\)

**Systemic factors**

1. **Nutrition**

Several macro and micro nutrients play a vital role in wound healing.
Macronutrients

Relevant macronutrients include proteins, carbohydrates, fats and water. Protein is essential for collagen and protein synthesis on wound site. A state of malnutrition may provide an inadequate amount of protein and this can decrease the rate of collagen synthesis, wound tensile strength or increased chance of infection. Carbohydrate aids cell proliferation and phagocytic activity of leucocytes to prepare wounds for fibroplasia and its deficiency decreases resistance to infection and impairs collagen synthesis.

Micronutrients

Relevant micronutrients include vitamins A, B-complex, C, E and K and minerals such as copper, iron and zinc.

2. Medication

Many drugs are known to impair wound healing. Chemotherapeutic agents used in cancer are the largest group well known to delay wound repair. Systemic glucocorticoids interfere with normal healing process by reducing collagen synthesis and fibroblast proliferation.

3. Old Age

Elderly age is found to associate with delayed wound healing. It is reported that the fibroblast growth and activity diminishes. Collagen production and wound contraction is slow in older individuals.

4. Body type

Body type may also affect wound healing. An obese patient may experience a compromise in wound healing due to poor blood supply to adipose tissue. In addition,
some obese patients suffer from protein malnutrition, which further impedes the healing.

5. **Diabetes and other disease conditions**

   Diabetic patients are more susceptible to wound healing. In a study, wound infection rate was found to be 11% higher in diabetic patients than in general patient’s population. Acute and chronic liver diseases are also associated with delay in wound healing. Patients with altered immune function have an increased susceptibility to wound infection.

6. **Venous sufficiency**

   Adequate blood supply and tissue perfusion is extremely important for wound healing. Excessive pain, cold and anxiety can cause local vasoconstriction and increased healing time. Smoking and use of tobacco decreases tissue perfusion and oxygen tension in wound.

7. **Inherited disorders**

   Inherited disorders such as Pseudoxanthoma elasticum, Ehlers-Danlos syndrome, cutis laxa, progeria, Werner’s syndrome and epidermolysis bullosa impairs wound healing.

**Local Factors**

1. **Desiccation**

   The inflammatory process is accelerated in a moist environment, leading to faster healing. Conversely, a dry environment will lead to dehydration and cell death.
2. **Infection**

   Wound infection is probably the most common reason of impaired wound healing. *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are the primary causes of delayed healing and infection in both acute and chronic wounds.\(^{21}\)

3. **Skin maceration**

   If the peri-wound area is exposed to excess moisture from exudates, perspiration or incontinence, maceration and damage to the surrounding tissue can occur. This may predispose to infection, skin sensitivities, irradiation, further skin breakdown and impede wound healing.

4. **Pressure, friction and shear**

   Mechanical forces such as pressure, friction and shear significantly impair wound healing by prolonging tissue damage. When pressure at the wound site is excessive or sustained, blood supply to the capillary network may be disrupted. This impedes blood flow to the surrounding tissue and delays healing.

5. **Trauma and oedema**

   Wounds heal slowly and may not heal at all in an environment in which they are repeatedly traumatized or deprived of local blood supply by oedema. Oedema interferes with the transportation of oxygen and cellular nutrition to the wound.

6. **Oxygen tension**

   Inadequate oxygen perfusion results in the formation of unstable collagen with low tensile strength and lower tissue resistance to infection by decreasing the phagocytic activity of leucocytes.

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7. Foreign body

Unnecessary sutures, fragments of steel, glass, even bone can impede the healing.

8. Size, location and type of wound

Wound in richly vascularised area heals faster than those in poorly vascularised area. A small incision wound heals faster than larger ones caused by trauma.

9. Radiotherapy

Local irradiation impairs wound healing by depleting dermal fibroblasts and decreasing the proliferative potential of endothelium. High dose may lead to vessel narrowing and reduced blood flow causing delay in wound healing.

1.4 PHASES OF WOUND HEALING

Wound healing involves a complex interaction between epidermal and dermal cells, the extra cellular matrix, controlled angiogenesis and plasma-derived proteins, all coordinated by an array of cytokines and growth factors. This dynamic process is classically divided into four overlapping phases such as Hemostasis, Inflammation, Proliferation and Remodeling (Fig.1.1).32

1. Hemostasis

Hemostasis occurs immediately after initial injury. Platelet is the key cell responsible for this function, in which body forms a clot to prevent further bleeding. The coagulation cascade is activated through extrinsic and intrinsic pathways, leading to platelet aggregation and clot formation in order to limit blood loss.33,34 As blood spills into the site of injury, the blood components and platelets come in contact with...
exposed collagen and other extracellular matrix components. This contact, triggers the release of clotting factors from the platelets and the formation of a blood clot composed of fibronectin, fibrin, vitronectin and thrombospondin. The blood clot and platelets trapped within it are not only important for haemostasis, as the clot also provides a provisional matrix for cell migration in the subsequent phases of haemostatic and inflammatory phases. The cytoplasm of platelets contains α-granules filled with growth factors and cytokines, such as Platelet Derived Growth Factor (PDGF), Transforming Growth Factor-β (TGF-β), Epidermal Growth Factor (EGF) and Insulin-Like Growth Factor (IGF). These molecules act as promoters in wound healing cascade by activating and attracting neutrophils and later macrophages, endothelial cells and fibroblasts. Platelets also contain vasoactive amines, such as serotonin, that are stored in dense bodies and cause vasodilation and increased vascular permeability, leading to fluid extravasations in the tissue that result in oedema which in turn potentiates itself during the following inflammatory phase.

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**Fig. 1.1: Phases of wound healing**
2. The Inflammatory Phase

The humoral and cellular inflammatory phase follows two separate phases, an early inflammatory phase and a late inflammatory phase.⁴⁰

Early inflammatory phase

It activates the complement cascade and initiates molecular events, leading to infiltration of the wound site by neutrophils, whose main function is phagocytosis in order to destroy and remove bacteria, foreign particles and damaged tissue.

The neutrophils begin to be attracted to the wound site within 24 - 36 h of injury by various chemoattractive agents including TGF-β, complement components such as C3a and C5a, formyl methionyl peptides produced by bacteria and platelet products.

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.⁴¹-⁴⁶ These cells are originally blood monocytes that undergo phenotypic changes on arrival into the wound to become tissue macrophages. Attracted to the wound site by a myriad of chemoattractive agents, including clotting factors, complement components, cytokines such as PDGF, TGF-β, leukotriene B₄ and platelet factor IV as well as elastin and collagen breakdown products. Macrophages have longer lifespan than neutrophils and continue to work at a lower pH.⁴⁷,⁴⁸ These cells are fundamental for the late stages of the inflammatory response, acting as key regulatory cells and providing an abundant reservoir of potent tissue growth factors, particularly TGF-β, as well as other mediator activating keratinocytes (TGF-α, heparin binding epidermal growth factor, Fibroblast Growth Factor [FGF], collagenase), fibroblasts and endothelial cells.⁴⁹-⁵⁰
The last cells to enter 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. IL-1 plays an important role in collagenase regulation, which is later needed for collagen remodelling, production of extracellular matrix components and their degradation.

### 3. Proliferative Phase

The proliferative phase starts on the third day after wounding and lasts for about 2 weeks thereafter. It is characterized by fibroblast migration, deposition of newly synthesized extracellular matrix and abundant formation of granulation tissue.

**Fibroblast migration**

Following injury, fibroblasts and myofibroblasts in the surrounding tissue are stimulated to proliferate for the first 3 days. Then they migrate into the wound, being attracted by factors such as TGF-β and PDGF which are released by inflammatory cells and platelets. Once in the wound, they proliferate profusely and produce matrix proteins hyaluronan, fibronectin, proteoglycans, type 1 and type 3 procollagen. All of their products are deposited in the local *milieu*.

By the end of the first week, abundant extracellular matrix accumulates which further supports cell migration and is essential for the repair process. Now, fibroblasts change to their myofibroblast phenotype. At this stage, they contain thick actin bundles below the plasma membrane and actively extend pseudopodia, attaching to fibronectin and collagen in the extracellular matrix. Wound contraction, which is an important event in the reparative process helps to approximate the wound edges and then takes place as these cell extensions retract. Having accomplished this task, redundant fibroblasts are eliminated by apoptosis.
**Collagen synthesis**

Collagens are an important component in all phases of wound healing. Synthesized by fibroblasts, they impart integrity and strength to all tissues and play a key role, especially in the proliferative and remodelling phases of repair.\(^{55}\) Collagens act as a foundation for the intracellular matrix formation within the wound. Unwounded dermis contains 80% type 1 and 25% type 3 collagen whereas wound granulation tissue expresses 40% type 3 collagen.

**Angiogenesis and granulation tissue formation**

Modelling and establishment of new blood vessels is critical in wound healing and takes place concurrently during all phases of the reparative process. Resident endothelial cells are responsive to a number of angiogenic factors, including FGF, Vascular Endothelial Growth Factor (VEGF), PDGF, angiogenin, TGF-α and TGF-β. A fine balance is kept by the action of inhibitory factors, such as angiostatin and steroids. Inhibitory and stimulatory agents act on proliferating endothelial cells directly as well as indirectly by activating mitosis, promoting locomotion and by stimulating host cells to release endothelial growth factors.\(^{56, 57}\) Under hypoxic conditions, molecules are secreted from the surrounding tissue, promoting proliferation and growth of endothelial cells. In response, a four-step process takes place: (i) production of proteases by endothelial cells for degradation of the basal lamina in the parent vessel in order to crawl through the extracellular matrix (ii) chemotaxis (iii) proliferation (iv) remodeling and differentiation. FGF and VEGF play central regulatory roles in all of the processes.\(^{58-60}\) Initially, there is no vascular supply in the wound centre so, the viable tissue which is limited to the wound margins is perfused by uninjured vessels and by diffusion through undamaged interstitium.
Capillary sprouts from the surrounding edges invade the wound clot and within a few days, microvascular networks composed of many new capillaries are formed.

**Epithelialization**

Migration of epithelial cells starts from the wound edges within a few hours of wounding. A single layer of cell 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 start to form.

**4. Remodeling Phase**

As the final phase of wound healing, the remodeling phase is responsible for the development of new epithelium and final scar tissue formation. Synthesis of the extracellular matrix in the proliferative and remodeling phase is initiated contemporarily with granulation tissue development. This phase may last up to 1 or 2 years or sometimes for an even more prolonged period of time. Remodeling of an acute wound is tightly controlled by regulatory mechanisms with the aim of maintaining a delicate balance between degradation and synthesis, leading to normal healing. Along with intracellular matrix maturation, collagen bundles increase in diameter and hyaluronic acid and fibronectin are degraded. The tensile strength of the wound increases progressively in parallel with collagen collection. Collagen fibres may regain approximately 80% of the original strength compared with unwounded tissue. The acquired final strength depends on the localization of repair and its duration, but the original strength of the tissue can never be regained.
Synthesis and breakdown of collagen as well as extracellular matrix remodeling takes place continuously and both tend to equilibrate to a steady state about 3 weeks after injury. Matrix metalloproteinase enzymes produced by neutrophils, macrophages and fibroblasts in the wound are responsible for the degradation of collagen. Their activity is tightly regulated and synchronized by inhibitory factors. Gradually, the activity of tissue inhibitors of metalloproteinase increases, culminating in a drop in activity of metalloproteinase enzymes, thereby promoting new matrix accumulation.\(^61\)

Although, the initial deposition of collagen bundles are highly disorganized, new collagen matrix becomes more oriented and cross-linked over time. Its subsequent organization is achieved during final stages of the remodeling phase, to a greater extent by the wound contraction that has already begun in the proliferative phase. The underlying connective tissue shrinks in size and brings the wound margins closer together, owing to fibroblast interactions with the extracellular matrix. The process is regulated by a number of factors in which PDGF, TGF-β and FGF being the most important. As wound heals, the density of fibroblasts and macrophages are further reduced by apoptosis. With time, the growth of capillaries stops, blood flow to the area declines and metabolic activity at the wound site decreases. The end result is a fully matured scar with a decreased number of cells and blood vessels and a high tensile strength.\(^62-63\)

**1.5 GROWTH FACTORS AND CYTOKINES IN WOUND HEALING**

The repair process is initiated immediately after injury by the release of various growth factors and cytokines.\(^64-66\) It is not exactly known how all growth factors and cytokines influence each other.
Platelet Derived Growth Factor (PDGF)

PDGF is released in large amounts from platelets, immediately on wounding. Macrophages, fibroblasts and endothelial cells are also able to secrete PDGF. This cytokine is chemotactic to a variety of cells such as, neutrophils, monocytes, macrophages and fibroblasts. Furthermore, it enhances proliferation of fibroblasts in an early stage of wound healing and it stimulates these cells to produce extracellular matrix. It can activate monocytes to mature into macrophages that secrete a number of other growth factors and cytokines. In a later stage, PDGF stimulates fibroblasts to contract collagen matrices and induces its transition into myofibroblasts.\textsuperscript{67}

Transforming Growth Factor \(\alpha\) (TGF-\(\alpha\))

This growth factor belongs to the same family as EGF and has some overlapping functions.\textsuperscript{68} It stimulates mitosis of keratinocytes and fibroblasts, therefore suspected to be involved in the early re-epithelialization and granulation tissue formation. Both keratinocytes and hair follicle epithelial cells are identified as a source for TGF-\(\alpha\).\textsuperscript{69}

Transforming Growth Factor \(\beta\) (TGF-\(\beta\))

TGF-\(\beta\) is released from platelets in large amount immediately after injury, resulting in a fast infiltration of neutrophils, macrophages and fibroblasts because of the chemoattractant properties of TGF-\(\beta\). This growth factor is also synthesized by macrophages, lymphocytes, fibroblasts, bone cells and keratinocytes.\textsuperscript{70} MCP-1 induces TGF-\(\beta\) release from fibroblasts. Three different isoforms of TGF-\(\beta\) exist which have overlapping functions. They are mitogenic for fibroblasts and inhibit proliferation of most other cells, including keratinocytes. In addition, TGF-\(\beta\) are potent stimulators of the expression of extracellular matrix proteins and integrins. It is
suggested that TGF-β stimulates re-epithelialization and the formation of granulation tissue, since it was shown to stimulate angiogenesis, fibroblast proliferation, myofibroblast differentiation and matrix deposition. It has a double-edged effect on fibroblast growth by inhibitory together with EGF and stimulating together with PDGF.

**Fibroblast Growth Factor (FGF)**

Upregulation of FGF after injury is found in keratinocytes, macrophages, endothelial cells and fibroblasts. Some functions of FGF are angiogenesis stimulation and migration regulation and differentiation of the target cells. Most types of FGF like FGF1 and FGF2 have a broad mitogenic function. They stimulate proliferation of various cells including keratinocytes and fibroblasts. FGF7, also known as Keratinocyte Growth Factor (KGF) is unique among the FGF family in that it is strongly mitogenic for epithelial cells including keratinocytes, but not stimulatory for fibroblasts or endothelial cells. The most potent stimulator of KGF expression was PDGF, but KGF induction is also found by IL-1β, TNF-α and IL-6. KGF also causes strong upregulation in acute human wounds. KGF not only stimulates proliferation and migration of epithelial cells but also affects their differentiation. It is suggested that reduced FGF expression can be correlated to impaired wound healing.

**Vascular Endothelial Growth Factor (VEGF)**

Expression of the VEGF gene is strongly induced after cutaneous injury with keratinocytes and macrophages as the main producers. Several growth factors including KGF, EGF, TGF-α and HGF have been shown to stimulate the production of VEGF by keratinocytes in culture. It is suggested that VEGF stimulates angiogenesis, since its receptors are found on blood vessels in granulation tissue.
Impaired wound healing is associated with a reduced expression of VEGF and over expression of VEGF accelerated healing. Treatment of ischemic wounds with VEGF improves wound healing.\textsuperscript{73}

**Epidermal Growth Factor (EGF)**

EGF is produced by epithelial cells and lymphocytes. It stimulates mitosis of keratinocytes and fibroblasts and is therefore suspected to be involved in re-epithelialization and granulation tissue formation. It is an attractant for fibroblasts and also stimulates migration and division of epithelial cells. The cells should be exposed for more than 4 hours to EGF to complete division. Topical application of EGF accelerated wound closure of full-thickness wounds.\textsuperscript{74}

**Insulin-like Growth Factor (IGF)**

Two different forms of IGF are produced by wound fibroblasts and also found in platelets from which it is released during clotting. It is a potent stimulator of mitogenesis and survival of many different cell types. It alone has minimal effects on wound healing but dermal and epidermal synthesis is increased significantly when IGF is applied together with PDGF.\textsuperscript{75}

**Connective Tissue Growth Factor (CTGF)**

Vascular endothelial cells express CTGF which stimulates proliferation and chemotaxis of fibroblasts. Furthermore, it is a potent inducer of extracellular matrix production and in these processes it acts as a mediator of TGF-β. Expression of CTGF during wound repair is found together with the growth of granulation tissue.\textsuperscript{76}
**Tumor Necrosis Factor - α (TNF-α)**

This growth factor is released primarily by mononuclear cells after stimulation by bacterial and matrix products, shortly after release of MCP-1. It upregulates its own synthesis by macrophages and stimulates macrophages to express other cytokines like IL-6, IL-8, Granulocyte Macrophage - Colony Stimulating Factor (GM-CSF), Granulocyte - Colony Stimulating Factor (G-CSF), MCP-1 and IL-1. TNF-α is implicated as a possible mediator of angiogenesis and can activate monocytes to mature into macrophages. Furthermore, it triggers the activation of metalloproteinases and affects collagen synthesis. The average level of TNF-α in chronic wound is higher than in acute wounds.  

**Interleukin - 1 (IL-1)**

IL-1 is stored in large amounts in the epidermis of intact skin and is released during post wounding. It is also released from disrupted endothelial cells, shortly after the release of MCP-1. This cytokine is involved in many processes that are associated with inflammation and tissue repair such as activation and chemotaxis of neutrophils and macrophages, proliferation of keratinocytes and fibroblasts, angiogenesis, matrix synthesis and collagen production. IL-1 can stimulate macrophages to express other cytokines like IL-6, IL-8, GM-CSF, G-CSF, MCP-1 and auto secretion of IL-1.

**Interleukin 4 (IL-4)**

IL-4 is secreted by mast cells, which are believed to stimulate a number of fibroblast activities such as migration, proliferation and synthesis of the extracellular matrix via this secretion. A late increase of IL-4 is found at the wound site, correlating with the downregulate expression of several other cytokines.
Interleukin - 6 (IL-6)

It appears that IL-6 is crucial for the quick start of the healing response, both via its mitogenic effects on wound edge keratinocytes and via its chemoattractive effect on neutrophils. IL-6 is also known as a potent stimulator of fibroblast proliferation, it is important in inhibiting extracellular matrix breakdown during proliferation. Levels of IL-6 are two to four times higher in chronic wounds than the late acute wounds.

Interleukin - 8 (IL-8)

It is expected that IL-8 is released from a preformed pool in keratinocytes or produced by wound cells like endothelial cells and fibroblasts. The expression of IL-8 is triggered by IL-1 and TNF-α, also correlates strongly with neutrophil infiltration in the wound site. IL-8 was found to be a major chemoattractant for polymorphonuclear leukocytes.78

Interleukin - 10 (IL-10)

The anti-inflammatory cytokine IL-10 is thought to be involved in the limitation and termination of the inflammatory process. IL-10 regulates growth and differentiation of immune cells, keratinocytes and endothelial cells. It inhibits infiltration of neutrophils as well as the expression of several cytokines. An increased expression of IL-10 was shown to correlate with impaired wound healing.79

Macrophage Chemoattractant Protein (MCP)

MCP-1 is released on skin disruption from resident keratinocytes at the wound edge. Keratinocytes of the hyperproliferative wound epidermis are pinpointed as the major source of MCP, but some endothelial cells and inflammatory cells in the granulation tissue also express MCP. Several cytokines, including TNF-α, IFN-γ, IL-1
and PDGF induce MCP production in fibroblasts. The expression of MCP in keratinocytes in the wound edge can be inhibited by nitric oxide. It is found that MCP-1 is a dominant monocyte chemoattractant during wound healing and is also responsible for the lymphocyte recruitment in the initial period of healing.\textsuperscript{80}

**Macrophage Inflammatory Protein - \( \alpha \) (MIP-\( \alpha \))**

MIP-\( \alpha \) is produced by macrophages and has been shown to play a critical role in macrophage recruitment. It is not a direct angiogenic factor but act as one by the attraction of macrophages which are a source of angiogenic cytokines.\textsuperscript{81}

**Interferon-\( \gamma \)-induced Protein - 10 (IP-10)**

The expression of IP-10 after the initial wound healing can be correlated to the lymphocyte infiltration in the wound site. Also an inhibitory effect of IP-10 was observed in angiogenesis.\textsuperscript{82}

### 1.6 ROLE OF HERBAL MEDICINE IN WOUND HEALING

The main effects of the active constituents of the plant extracts towards wound healing are given below:

- Phyto-chemical constituents contributing to antimicrobial activity
- Phyto-chemical constituents working as free radical scavengers (antioxidants)
- Active components having enhanced mitogenic activity (contributing to increased cell proliferation), angiogenesis, enhanced collagen production and increased DNA synthesis.

Ideally, active substances present in plant extracts are anticipated to interfere with one or more phases of wound healing process in a positive manner in proper sequence and at the right time frame to show improved efficacy. Soluble compounds
in the plant extracts such as the flavonoids, quinones, phenolic acids and phenyl propanoids have been found to possess considerable anti-microbial as well as anti-oxidant properties.

Flavonoids are strong scavengers of reactive oxygen species. In wound, there is a tendency for sharp rise in the concentration of reactive oxygen species due to the activation of platelets, neutrophils, macrophages, lymphocytes and fibroblasts at different time points of the healing process. Infection from microbes also adds to the woes. In such situations, plant flavonoids would benefit the healing process by modulating the concentrations of reactive oxygen species. However, quantitative information and correlations are yet inadequate.\(^{83}\)

1.6.1 Role of plants with antimicrobial potential in wound healing

Open wounds are particularly prone to infection, especially by bacteria and also provide an entry point for systemic infections. Infected wounds heal less rapidly and also often result in the formation of unpleasant exudates and toxins that will be produced with concomitant killing of regenerating cells.\(^{84}\) Wound infections are most common because of poor hygienic conditions. The common wound pathogens includes bacteria, fungi, protozoa and viruses among which the most common are beta-hemolytic *Streptococci* (*Streptococcus pyogenes*), *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella* species and *Coliforms*.\(^{85}\) A wide range of antibiotics are being used at present for treating wound infections, but they are proved to have adverse effects in human. Also these pathogens develop resistance to the antibiotics targeted against them. Emergence of multidrug resistant microorganisms and a decrease in effective antibiotics has driven health / wound care professionals and practitioners to revisit the old ancient healing
methods of traditional and alternative medicines involved in wound management since eternity. Side effects associated with allopathic drugs and synthetic compounds have prompted research into herbal and natural products. Consequently, there is a need to stimulate healing and restore the normal functions of the affected part of the body to ease the discomfort and pain associated with wounds, preventing infection and activating tissue repair processes. Antibacterial and healing compounds in a traditional remedy can provoke this occurrence and may be beneficial in treating wounds.  

1.6.2 Role of plants with antioxidant potential in wound healing

Many facets of wound healing under redox control require a delicate balance between oxidative stress and antioxidants. While, the normal physiology of wound healing depends on low levels of reactive oxygen species and oxidative stress, an overexposure to oxidative stress leads to impaired wound healing. Antioxidants are postulated to control wound oxidative stress and thereby accelerate wound healing. Natural products and naturally derived antioxidants are becoming more popular. Antioxidant therapy for wound healing is promising but only few animal studies and even fewer clinical studies are available. As a field of science, the use of antioxidants for wound healing is in its infancy and future studies will better elucidate the role of antioxidants in wound healing.  

When wound occurs, it is generally accompanied by classical symptoms of inflammation, i.e., pain, reddening and edema. The inflammation stage begins immediately after injury; first with vasoconstriction and platelet aggregation at the injury site and then infiltration of leukocytes and T-lymphocytes into the wound area. The cicatrisation process proceeds naturally, since the damaged tissue attempts to
reestablish hemostasis. In the inflammatory stage, the main aim is the removal of debris, damaged tissue and bacteria by neutrophils and macrophages which have a role in anti-microbial defense and debridement of devitalized tissue by production of proteolytic enzymes and Reactive Oxygen Species (ROS). ROS is produced in high amounts at the site of wound as a defense mechanism against invading bacteria. However, the presence of increased number of neutrophils and ROS overwhelm the antiprotease substances that normally protect the tissue cells and extracellular matrix. At high concentrations, ROS can induce severe tissue damage and even lead to neoplastic transformation decreasing the healing process by damages in cellular membranes, DNA, proteins and lipids. Fibroblasts may be killed and skin lipids will be made less flexible by excess ROS. Because of these, the overall role of antioxidants appears to be significant in the successful treatment and management of wounds. Antioxidants reduce these adverse effects of wounds by removing products of inflammation. They counter excess proteases and ROS often formed by neutrophil accumulation in the injured site and protect protease inhibitors from oxidative damage. The most likely mechanism of antioxidant protection is direct interaction of extracts (or compounds) and hydrogen peroxide rather than altering the cell membranes and limiting damage. Compounds with high radical-scavenging capacity have been shown to facilitate wound healing.

1.6.3 Role of plants with angiogenic potential in wound healing

Plants promote wound healing by multiple mechanisms, usually by promoting angiogenesis. Numerous study indicate that plants such as Aloe vera, Hippophae rhamnoides L., Angelica sinensis, Cinnamomum cassia, Astragalus membranaceus, Stewartia koreana, Uncaria rhynophylla, Salvia miltiorrhiza, Patrinia villosa, Rehmannia glutinosa and four ginsengs: Panax ginseng, P. schinsen, P. notoginseng
and *P. quinquefolium* have noteworthy proangiogenic potential. During wound healing process most of these plants promote angiogenesis predominantly via upregulation of VEGF or FGF expression and/or activation of the mitogen-activated protein kinases pathway.\(^92\) The chemical constituents responsible for proangiogenic activity are often polyphenols, sterols and saponins.\(^93\)

### 1.7 NEED FOR THE STUDY

Phytomedicines for wound healing are not only cheap and affordable but are also purportedly safe as hypersensitive reactions are rarely encountered with the use of these agents. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. However, there is a need for scientific validation, standardization and safety evaluation of plants of traditional medicine before these could be recommended for healing of the wounds. Herbal or phytoconstituents derived from plants need to be identified and formulated for the treatment and management of wounds. In this direction, a number of herbal products are being investigated at present. Various herbal products have been used in the management and treatment of wounds over the years.\(^94-98\)

Research on wound healing drug is a developing area in modern biomedical sciences which helps to develop newer drugs from natural resources. There are number of reports that natural products such as flavonoids, tannins, stilbenes and triterpenes have wound healing activity.\(^99\) However “Mother nature” has been kind to us by creating various herbs which assist in the healing process. The growing popularity of natural and herbal medications, easy availability of raw materials, cost-effectiveness and paucity of reported adverse reaction prompted us to formulate an herbal topical preparation and to assess its wound healing ability.
Several indigenous drugs have been described in an ancient text of Ayurveda for the management of wounds, cuts, bruises and burns. Based on the traditional claim and phytoconstituents present, the plant *Dodonaea viscosa* was selected for the study. This plant is rich in flavonoid and traditionally used to heal different types of wounds. Since, there is no scientific evidence has been proved in the flavonoid rich fraction of *Dodonaea viscosa*, the present work is focused on the same.

1.8 REFERENCES


71. Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM. Transforming growth factor type β: rapid induction of fibrosis and angiogenesis


