

## Natural wound healing and bioactive natural products

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### Abstract

A wound is a disruption of the normal anatomical structure and function of a tissue. Wounds cure in an orderly and timely repair process which is characterized by three dynamic and interactive phases: inflammation, proliferation and the remodeling. The present review was designed to elaborate the cellular and molecular targets for plant secondary metabolites that target the various aspects of wound repair process. The common mechanism of action of natural products established through *in vitro* and animal studies include direct action on skin cells regeneration, increase in connective tissue deposition, antioxidant activity, inflammatory cells activity and modulation of cytokine and growth factor production and/or function. All these demonstrated pharmacological effects could be exploited to overcome an acute or pathological wound healing conditions. The therapeutic potential of various chemical classes of natural products that act through one or multiple targets are discussed.

**Keywords:** Natural products, secondary metabolites, wound healing, inflammation, proliferation, remodeling.

### Introduction

A wound is defined as the disruption of the normal anatomical structure and function of a tissue (Maklebust and Sieggren, 1996). In general, wounds cure in an orderly and timely repair process which is characterized by dynamic, interactive events described in 3 phases: inflammation, proliferation and remodeling (Singer and Clark, 1999). In order to assess the healing effects of natural products, *in vivo* and *in vitro* assay models may be employed. Based on these assessments, many new therapies that target various aspects of wound repair are emerging in recent years. Among them, plant extracts from folklore medicine have been shown to be beneficial for treatment of wounds. The term natural products here is to be unde-

stood as the whole organism, crude extract, fraction or chemical constituents of plants, animals, microorganisms, or mineral origin. The most active compounds isolated from these plants (primary and secondary metabolites) may contribute to one or more potential mechanisms contributing to improve wound healing. These include immune cells and epithelial cells activity during acute wound healing, as well as extracellular matrix (ECM), cytokines, growth factors, reactive oxygen species (ROS) and various inflammatory mediators.

The present work was aimed to outline the cellular and molecular targets of wound healing drugs and review natural compounds which have demonstrated beneficial effects through various experimental models. Animal studies are extensively used for our critical review while clinical studies, where available, are used to further substantiate the therapeutic potential of natural wound healing drugs. For clearer understanding of proposed mechanism of actions, the general wound healing process and various available experimental models are also described.

### **The wound healing process**

The wound healing process is characterized by dynamic, interactive events involving soluble mediators, blood cells, ECM, and parenchymal cells that results in permanent restoration of anatomic and functional integrity (Singer and Clark, 1999). Chronic wounds therefore suggest a failure in some aspects of the repair process (Maklebust and Sieggren, 1996). The term “orderly” refers to the sequence of wound healing phases that include inflammation, tissue formation and tissue remodeling (Maklebust and Sieggren, 1996; Singer and Clark, 1999).

### **Wound healing phases**

#### ***The inflammatory phase***

The inflammatory phase occurs 1-5 days after injury and initiates the wound healing cascade. The main function of this phase is to remove the debris and prepare the wound for the regeneration of the new tissue (Maklebust and Sieggren, 1996). Clinically, the inflammatory phase is characterized by local erythema, oedema, and tenderness of the affected tissue remodeling (Maklebust and Sieggren, 1996). The body's first response to wounding is naturally local vasoconstriction that last 5-10 minutes (Maklebust and Sieggren, 1996). Platelets aggregate at the site of injury and a fibrin clot forms via the activation of the coagulation cascade. Thrombin induces platelet degranulation, leading to the release of growth factors such as platelet derived growth factor (PDGF), transforming growth factor beta (TGF- $\beta$ ), epidermal growth factor (EGF) and transforming growth factor-alpha (TGF- $\alpha$ ), as well as adhesive glycoproteins including fibronectin (Karukonda *et al.*, 2000b). In addition to providing haemostasis, the fibrin clot acts as a matrix for colonization by inflammatory cells which are attracted to the wound site via chemotaxis from PDGF and TGF- $\alpha$  (Karukonda *et al.*, 2000b). As the surrounding tissues become ischemic, the brief period of vasoconstriction is followed by sustained vasodilatation leading to an increase in vascular permeability and leakage of neutrophils into the wound space. Enzymes, fluids, and proteins enter and are trapped in the extracellular space, where they cause inflammation (Maklebust and Sieggren, 1996).

As the concentrations of inflammatory cytokines and growth factors released from platelets decrease in an acute wound area, major cells of the immune system (the neutrophils, basophils, mast cells, T-cells, B-cells, etc.), but predominantly neutrophils, migrate and accumulate during the early hours of inflammation (Karukonda *et al.*, 2000b; Li *et al.*, 2007). The nature of cells and mediators involved during this phase depends on a wide range of factors including the type of pathogen, auto-immune, chemical or physical injury, the tissue or organ involved (Karukonda *et al.*, 2000b).

The other characteristic feature of the inflammatory phase is oxidative burst. While ROS at high concentrations have pronounced bacteriostatic effects, at low concentrations they function as second messengers. If the inflammatory phase is not resolved in time and the concentration of ROS exceeds the antioxidant capacity of the cell, the condition called oxidative stress results. This status can cause excisional dermal wound retardation, breaking reduction of the incision wounds, collagen decrease and increase glycosaminoglycan synthesis (Kanta, 2011).

After 24 hours of wounding injury, neutrophils start to recede as monocytes enter the wound. Monocytes are soon differentiated to key wound healing players, macrophages (Maklebust and Sieggren, 1996), which outnumber neutrophils by the third day (Karukonda *et al.*, 2000b). The recruitment of neutrophils and monocytes to the wound site is orchestrated by mast cells and chemotactic factors released during haemostasis. The monocyte-specific chemotactic signals include monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 while other chemotactic factors generated during the coagulation process, such as kallikrein, fibrinopeptides released from fibrinogen, and fibrin degradation products do also play a significant role. Substances released by mast cells, such as tumor necrosis factor, histamine, proteases, leukotrienes, and cytokines (interleukins), represent additional sources of inflammatory mediators for the recruitment of leukocytes. They serve to up-regulate the expression of important intercellular adhesion molecules both on leucocyte and endothelial cell surface thereby mediating inflammatory cells immigration. The resulting coordinated cell-cell and cell-matrix interactions allow neutrophils to perform their function of microbial killing and phagocytosis including damaged matrix proteins within the wound bed (Li *et al.*, 2007).

Leucocytes involved in phagocytosis further release proteases, including neutrophil elastase and neutrophil collagenase, also known as matrix metalloproteinase-8 (MMP-8) (Majewska and Gendaszewska, 2011). Hence, leucocytes are responsible for debriding the wound, regulating fibroplasias, and degrading collagen in the wound healing process (Maklebust and Sieggren, 1996). Proteases also initiate wound healing by removing damaged ECM components, which must be replaced by new, intact ECM molecules (Schultz and Mast, 1998). Activated macrophages further secrete tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin one beta (IL-1 $\beta$ ), which have a variety of effects on different cells. TNF- $\alpha$  and IL-1 $\beta$  stimulate the endothelial cells of the capillaries to express cell adhesion molecules and also induce the production of a chemotactic cytokine, interleukin-8 (IL-8). This enables the inflammatory cells to bind to vascular endothelial cells, traverse the capillary basement membrane and enter the surrounding tissues. TNF- $\alpha$  also induces macrophages to produce IL-1 $\beta$ , which is mitogenic for fibroblasts and up-regulates MMP expression. Both TNF- $\alpha$  and IL-1 $\beta$  directly influence the deposition of collagen in the wound by inducing synthesis of

collagen by fibroblasts and by up-regulating the expression of MMPs. In addition, these cytokines downregulate the expression of tissue inhibitors of metalloproteinases (TIMPs) (Schultz and Mast, 1998). Interferon gamma (IFN- $\gamma$ ), produced by lymphocytes, the last leucocyte population entering the wound site during the inflammatory phase, inhibits fibroblast migration and down-regulates collagen synthesis (Schultz and Mast, 1998; Majewska and Gendaszewska, 2011). Inflammatory cells also secrete growth factors; including TGF- $\beta$ , TGF- $\alpha$ , heparin-binding epidermal growth factor (HB-EGF) and basic fibroblast growth factor (bFGF). The growth factors secreted by macrophages continue stimulating migration of fibroblasts, epithelial cells and vascular endothelial cells into the wound (Schultz and Mast, 1998). Moreover, macrophages release an angiogenic factor, and a growth factor which stimulates fibroblast production and promotes collagen synthesis in the second phase of wound healing (Maklebust and Sieggren, 1996).

### ***The Proliferative Phase***

The proliferative phase, also called the granulation tissue formation phase, occurs between 3-12 days (karukonda *et al.*, 2000a). Granulation tissue formation involves proliferation of fibroblasts, deposition of collagens and other ECMs, and development of new blood vessels (Li *et al.*, 2007). This phase is characterized by deposition of connective tissue and collagen cross-linking and epithelial cells migration across the wound surface. Cellular migration is guided by the wound matrix and anatomical tissue planes. Collagen is responsible for filling the wound and providing strength (Maklebust and Sieggren, 1996). As the Proliferative phase progresses, TGF- $\beta$  released by platelets, macrophages and T lymphocytes becomes a critical signal. TGF- $\beta$  is considered to be a master control signal that regulates a host of fibroblast functions. TGF- $\beta$  has a three-pronged effect on ECM deposition. First, it increases transcription of the genes for collagen, proteoglycans and fibronectin thus increasing the overall production of matrix proteins. At the same time, TGF- $\beta$  decreases the secretion of proteases and also stimulates the protease inhibitor, the TIMP. Other cytokines considered to be important are interleukins, fibroblast growth factors and TNF- $\alpha$  (Diegelman and Evans, 2004).

### ***Fibroblast proliferation and collagen production***

The process of cellular migration and proliferation occurs under the control of various cytokines. Some are derived from inflammatory cells and others from the epithelial cells themselves (Monaco and Lawrence, 2003). Fibroblasts secrete insulin-like growth factor-1 (IGF-1), bFGF, TGF- $\beta$ , PDGF, keratinocyte growth factor (KGF); endothelial cells produce vascular endothelial growth factor (VEGF), bFGF and PDGF; and keratinocytes synthesise TGF- $\beta$ , TGF- $\alpha$ , and IL-1 $\beta$  (Schultz and Mast, 1998). These mediators continue to stimulate cell proliferation, synthesis of ECM proteins and capillary formation. As the fibroblasts and other cells migrate to the site of injury, they begin to proliferate and the cellularity of the wound increases. If the wound is not infected, the number of inflammatory cells in it begins to decrease after a few days (Schultz and Mast, 1998).

As the proliferative phase progresses, the predominant cells in the wound site are the fibroblasts (Li *et al.*, 2007). Fibroblasts attach to the cables of the provisional fibrin matrix and begin to produce collagen. The biosynthesis of collagen includes formation of procollagen chains and successive proline and lysine hydroxylation. In the extra-cellular spaces, fur-

ther modifications occur that ultimately lead to deposition and cross-linking required for normal ECM formation (Mariggio *et al.*, 2009). This important cross-linking step gives collagen its strength and stability over time. Dermal collagen in normal tissue is a strong and highly organized molecule. In contrast, collagen fibers formed in scar tissue are much smaller and have a random appearance. The regained tensile strength in a wound will never approach normal. In fact the maximum tensile strength that a wound can ever achieve is approximately 80% of the normal skin. Finally, in the process of collagen remodeling, collagen degradation also occurs. Specific collagenase enzymes in fibroblasts, neutrophils and macrophages clip the molecule at a specific site through all three chains, and break it down to characteristic three-quarter and one-quarter pieces. These collagen fragments undergo further denaturation and digestion by other proteases (Diegelmann and Evans, 2004)

### ***Re-epithelialization***

The replacement of dead or damaged tissue by new and healthy cells begins by a process called epithelialization (Maklebust and Sieggren, 1996). The process of epithelialization is stimulated by the presence of EGF and TGF- $\alpha$  that are produced by activated wound macrophages, platelets and keratinocytes (Diegelman and Evans, 2004). Re-epithelialization begins at the wound edges as early as 24 h post-injury and granulation of the wound starts around post-injury day 5 (Karukonda *et al.*, 2000a). Within the first 24 hours of injury, undamaged epithelial cells at the wound margin begin to reproduce. Epithelial mitosis causes accelerated reproduction and leads to a ridge forming around the periphery of the wound. These cells migrate across the wounded area essentially as a monolayer. The migration of epithelial cells continues until overlap is achieved with other epithelial cells migrating from different directions. At that point, “contact inhibition” results in cessation of cellular migration (Monaco and Lawrence, 2003). Once the epithelial bridge is complete, enzymes are released to dissolve the attachment at the base of the scab resulting in removal. Cellular migration may also require the secretion of MMPs to penetrate eschar or scab (Diegelman and Evans, 2004). Epithelial cell migration requires the development of actin filaments within the cytoplasm of migratory cells and the disappearance of desmosomes and hemidesmosomes that link them to one another and to the basement membrane, respectively. At least some of these processes are dependent on changes in integrins expressed on the cell membranes. It is thought that decreased calcium or increased magnesium concentrations stimulate the downregulation of the critical integrins. If the epidermal basement membrane is intact, cells simply migrate over it. In wounds in which the epidermal basement membrane is destroyed, the cells initially begin to migrate over the fibrin–fibronectin provisional matrix. As they migrate across the matrix, however, epithelial cells regenerate a new basement membrane (Monaco and Lawrence, 2003).

Re-establishment of a basement membrane under the migrating cells involves the secretion of tenascin, vitronectin, and type I and V collagens. When contact inhibition is achieved, hemidesmosomes re-form between the cells and basement membrane, and tenascin and vitronectin secretion diminishes. The cells become more basaloid, and further cellular proliferation generates a multilaminated neoepidermis covered by keratin. The neoepidermis is similar to the native epidermis, although it is slightly thinner, the basement membrane is flatter, and rete pegs that normally penetrate the dermis are absent (Monaco and Lawrence, 2003).

## Neoangiogenesis

Due to the high metabolic activity at the wound site, there is an increasing demand for oxygen and nutrients. Local factors in the wound microenvironment such as low pH, reduced oxygen tension and increased lactate actually initiate the release of factors needed to bring in a new blood supply (Diegelman and Evans, 2004). Angiogenesis refers to the formation of new capillaries, formed as bud-like structures from preexisting vessels adjacent to the wound (Maklebust and Sieggren, 1996; Schultz and Mast, 1998). New capillaries grow into red loops of blood vessels that impart a granular appearance to the wound surface. This occurs in 5 steps (Schultz and Mast, 1998; Diegelman and Evans, 2004):

(1) Tissue damage leads to the release of bFGF normally sequestered within intact cells and ECM. Thrombin in the clot upregulates cellular receptors for VEGF and potentiates its effects. Endothelial cells exposed to thrombin also release gelatinase A, which promotes the local dissolution of basement membrane. Platelets–released growth factors including angiopoietin-1 (Ang-1) stimulate endothelial proliferation, migration, and tube formation.

(2): Angiogenesis is amplified by inflammation. Macrophages and monocytes release myriad angiogenic factors as they marginate into the wound bed, including PDGF, VEGF, Ang-1, TGF- $\alpha$ , bFGF, interleukin-8 (IL-8), and TNF- $\alpha$ . Several growth factors (PDGF, VEGF, and bFGF) synergize in their ability to vascularize tissues. Proteases that break down damaged tissues further release matrix-bound angiogenic stimulators. Enzymatic cleavage of fibrin also yields fibrin fragment E (FnE). This fragment stimulates angiogenesis directly, and also enhances the effects of VEGF and bFGF. Expression of the inducible COX-2 enzyme during the inflammatory stage of healing also leads to VEGF production and other promoters of angiogenesis.

(3) Vascular Proliferation. Wound granulation becomes clinically evident as angiogenesis is sustained. Hypoxia is an important driving force for wound angiogenesis. The hypoxic gradient that exists between injured and healthy tissue leads to gene expression of HIF-1 $\alpha$  that triggers VEGF production. The HIF in turn binds to specific sequences of DNA that regulate the expression of VEGF thus stimulating angiogenesis. As new blood vessels enter the wound repair area and the oxygen tension returns to a normal level, oxygen binds to HIF and blocks its activity leading to a decreased synthesis of VEGF. VEGF is present in both wound tissue and wound fluid. One property of VEGF is its ability to induce edema through hyperpermeability, hence its alternate name, vascular permeability factor (VPF). Hypoxia also leads to endothelial cell production of nitric oxide (NO) which promotes vasodilation and angiogenesis to improve local blood flow.

(4) Vascular Stabilization: Newly forming blood vessels must be stabilized or matured. Vascular stabilization is governed by Ang-1, its receptor Tie2, and smooth muscle cells and pericytes. Binding of Ang-1 to Tie2 on activated endothelial cells leads to the production of PDGF and the recruitment of smooth muscle cells and pericytes to the newly forming vasculature. A PDGF deficiency leads to abnormal, poorly-formed immature blood vessels.

(5) At the terminal stages of healing, angiogenesis is suppressed. Growth factor levels decline as tissue normoxia is restored and inflammation subsides. Endogenous angiogenesis inhibitors become the dominant forces. Pericytes that stabilize endothelial cells secrete an inhibitory form of activated TGF- $\beta$  that impedes vascular proliferation. Epidermal production

of interferon- $\beta$  also inhibits angiogenesis. Endostatin, a cleavage product of collagen XVIII, is present in the surrounding vascular basement membrane and inhibits wound vascularity, as does another molecule called vasostatin.

### ***The remodeling phase***

Remodeling constitutes the final stage of wound healing, where the immature collagen matrix is transformed into scar tissue. Although the new collagen increases the tensile strength of the wound, the scar tissue reaches approximately 80-90 % of its eventual strength during the first 3 weeks following injury (Brown, 1988).

Once the initial scar forms, proliferation and neovascularisation cease and the wound enters the remodeling phase, which can last many months. During this last phase, a balance is reached between the synthesis of new components of the scar matrix and their degradation by metalloproteinases such as collagenase, gelatinase and stromelysin. Fibroblasts, the major cell type synthesising the ECM components of collagen, elastin and proteoglycans, are also a major source of MMPs that degrade the matrix as well as the TIMPs (Schultz and Mast, 1998). Fibronectin gradually disappears and hyaluronic acid and other glycosaminoglycans are replaced by proteoglycans (Majewska and Gendaszewska, 2011). Furthermore, they secrete lysyl oxidase, which cross-links components of the ECM. Angiogenesis ceases and the density of capillaries decreases in the wound site as the scar matures. Eventually, remodeling of the scar tissue reaches equilibrium, although the mature scar is never as strong as uninjured skin (Schultz and Mast, 1998).

The gradual disappearance of fibronectin and replacement by hyaluronic acid and other glycosaminoglycans by proteoglycans is regulated by PDGF, TGF- $\beta$ , fibroblast growth factor (FGF) and many other factors. Subsequent wound healing is accompanied by elimination of fibroblasts and macrophages by apoptosis. The growth of capillaries stops with time, the healing area is no longer supplied with blood at an increased rate, and the metabolic activities at the wound site decrease. The wound healing process eventually culminates in a fully matured scar with a decreased number of cells and blood vessels (Majewska and Gendaszewska, 2011).

### **Cellular and molecular targets of wound healing drug therapy**

The main goal of using a target-specific drug is to inhibit a molecular target central to a disease mechanism of interest (Aggarwal *et al.*, 2007). The search for wound healing drugs has traditionally focused on the molecular signaling pathways involved in wound healing retardation, but also on immune cells and epithelial cells activity during acute wound healing or delayed wound. In fact, the important role of fibroblasts, keratinocytes and immune cells function, as well as ECM, cytokines, growth factors, ROS and various inflammatory mediators for the accompanying inflammatory reaction as well as for repair processes during wound healing were reported.

### ***Drugs action on cellular activity***

Cellular activity during wound healing begins during coagulation phase after injury (Schultz *et al.*, 2003). Platelets initiate the release of a number of soluble mediators, including PDGF, IGF-1, EGF, FGF and TGF- $\beta$ . These rapidly diffuse from the wound, and inflam-

matory cells are drawn to the area of the injury. Thus, theoretically, impaired haemostasis will lead to impaired wound healing as demonstrated in haemophilic conditions (Hoffman *et al.*, 2006; Rodriguez-Merchan, 2012). The reduction in bleeding time induced by some drugs suggests their positive effect on the integrity of blood vessel or involvement of platelets forming the haemostatic plug.

### ***Skin cells as drug targets for wound healing***

In general, growth factors are mitogens that stimulate proliferation of wound cells (epithelial cells, fibroblasts, and vascular endothelial cells). Most growth factors are also able to stimulate directed migration of target cells (chemotaxis) and regulate differentiated functions of wound cells, such as expression of ECM proteins (Schultz *et al.*, 2003).

### ***Wound cells***

Several studies have highlighted the wound healing effects of some drugs through stimulating fibroblasts and keratinocytes (Khorshid *et al.*, 2010). Epidermal keratinocytes undergo differentiation in response to several stimuli to form the confined envelope that contributes to the barrier function of skin (Saviniet *et al.*, 2002; Usuiet *et al.*, 2008).

### ***Collagen synthesis***

Some studies suggest that cellular calcium metabolism appears to regulate keratinocytes differentiation and ECM and collagen production as well as wound healing processes (Bhaskar *et al.*, 2004; Huang *et al.*, 2006). Two calcium channel blockers, nifedipine and amlodipine have been shown to increase skin tensile strength, enhanced wound contraction rate and also partially reverse the steroid-induced suppressed wound healing in rats (Bhaskar *et al.*, 2004). Hydroxyproline in collagen is important as it gives the molecule its stable helical conformation. When hydroxyproline is deficient, for example in conditions where collagen is produced under anaerobic or lack of Vitamin C (scurvy), the collagen has an altered structure and undergoes denaturation much more rapidly and at a lower temperature (Diegelman and Evans, 2004). Hence, numerous experimental studies use hydroxyproline content as a marker of collagen production and metabolism (Sasaki *et al.*, 1987; McAnulty, 2005; Lai *et al.*, 2011).

### ***Angiogenesis***

As far as endothelial cells are concerned, inhibition of angiogenesis may lead to inhibition of tumor growth whereas stimulation may improve wound healing (Majewska and Gendaszewska-Darmach, 2011).

### ***Extracellular matrix regulation***

ECM consists of numerous macromolecules classified traditionally into collagens, elastin, and microfibrillar proteins, proteoglycans including hyaluronan, and non collagenous glycoproteins. In addition to being necessary structural components, ECM molecules exhibit important functional roles in the control of key cellular events such as adhesion, migration,



proliferation, differentiation, and survival. Any inherited or acquired structural defect or metabolic disturbance in the ECM may cause cellular and tissue alterations that can lead to the development or progression of a disease. Consequently, ECM molecules are important targets for pharmacotherapy. Specific agents that prevent the excess accumulation of ECM molecules in the vascular system, liver, kidneys, skin, and lungs or alternatively, agents that inhibit the degradation of the ECM in degenerative diseases would be clinically beneficial. Several of today's drugs that act on various primary targets affect the ECM as a byproduct of the drugs actions, and this activity may in part be beneficial to the drugs disease-modifying properties. In the future, agents and compounds targeting directly the ECM will significantly advance the treatment of various human diseases, even those for which efficient therapies are not yet available (Järveläinen *et al.*, 2009).

MMPs are one of the promising targets being explored for designing an array of drug target system. As it is clear from the action of MMPs in natural and pathologic states, MMPs cleave active agents from the ECM by degrading proteins. When specific MMPs are implicated as pharmacologic targets for a particular disease, MMPs can either be inhibited or be used to cleave prodrugs and thus released the active drug selectively in the diseased tissue overexpressing MMPs (Vartak and Gemeinhart, 2007). Jun and Lau (2011) reported that members of the CCN family of matricellular proteins have important roles in inflammation and injury repair via multiple signaling pathways. They elicit cell-specific responses through various mechanisms including expression of biologically active molecules such as growth factors, cytokines, MMPs, other ECM proteins. Consequently, deregulation of CCN protein expression or activities contributes to the pathobiology of various diseases — many of which may arise when inflammation or tissue injury becomes chronic — including fibrosis, atherosclerosis, arthritis and cancer, as well as diabetic nephropathy and retinopathy. Emerging studies indicate that targeting CCN protein expression or signaling pathways holds promise in the development of diagnostics and therapeutics for such diseases.

### ***Modulations of cytokines and growth factors***

Cytokine modulation by local application of therapeutic agents is an extremely appealing modality in wound healing. In a study by Jurjus *et al* (2007), it was clearly demonstrated that various local burns wound care regimens have drastically different effects on the various cellular elements and cytokines involved in the healing process. The increase in the levels of tissue growth factors (e.g. TGF- $\beta$ ), likely to be involved in the healing or fibrotic processes during some pathological conditions, may be a mediator of early collagen deposition and, hence, better healing (Lam *et al.*, 2003; Jurjus *et al.*, 2007).

In another study, oral ingestion of oleic (OLA) and linoleic (LNA) acids accelerated the inflammatory phase of wound healing, through different mechanisms. LNA increased the influx of inflammatory cells, concentration of hydrogen peroxide and cytokine-induced neutrophil chemoattractant-2ab, and the activation of the transcription factor activator protein-1 in the wound at 1 hour post wounding. LNA decreased the number of inflammatory cells and IL-1, IL-6, and macrophage inflammatory protein-3 (MIP-3) concentrations, as well as nuclear factor-kappa B (NF- $\kappa$ B) activation in the wound at 24 hours post wounding. LNA accelerated wound closure over a period of 7 days. OLA increased TNF- $\alpha$  concentration and NF- $\kappa$ B activation at 1 hour post wounding. A reduction of IL-1, IL-6, and MIP-3 concentrations,

as well as NF- $\kappa$ B activation, was observed 24 hours post wounding in the OLA group (Rodrigues *et al.*, 2012).

### **Wound microenvironment**

During the inflammatory phase of wound-healing, neutrophils and macrophages produce large amounts of superoxide radical anions, a phenomenon, which is often described as the “respiratory burst”. Furthermore, other cells such as fibroblasts can be stimulated by pro-inflammatory cytokines to produce ROS. The generation of these reactive molecules is part of the innate immune system and helps to rapidly clean the wound from invading bacteria. Besides their beneficial role in microbial killing, ROS can have a series of negative effects. For example, at low levels, hydrogen peroxide and other ROS inhibit migration and proliferation of various cell types, including keratinocytes. At high levels, ROS can lead to severe tissue damage and even neoplastic transformation (Keller *et al.*, 2006). Therefore, strategies to manipulate the redox environment in the wound are likely to be of outstanding significance in wound healing (Sen *et al.*, 2002; Keller *et al.*, 2006). It seems therefore likely that reduced levels of ROS-detoxifying enzymes result in healing impairments, a hypothesis, which is supported by the observation of reduced activities of SOD, catalase, and GPx in wounds of aged rats compared to young rats; the beneficial effect of antioxidants on wound healing in ischemic rat skin; and the restoration of delayed wound healing seen in diabetic mice by adenoviral delivery of Mn-SOD to the wound site (Keller *et al.*, 2006). A drug which inhibits lipid peroxidation is also believed to increase the viability of collagen fibrils (Senel *et al.*, 1997). Furthermore, fibroblast dysfunctions, such as increased apoptosis, premature senescence, senescence-like phenotype, or poor growth response in the absence of senescence markers, have been documented in chronic wounds. Some of these differential dysfunctions may be secondary to differences in patient age or sex, ulcer size or duration, edge versus base sampling, or culture technique. Nevertheless, the entire spectrum of fibroblast dysfunction may exist and be secondary to, or a response to, different amounts of oxidative stress (Clark, 2008).

The activation of the plasminogen activator system may also be one mechanism by which all-trans retinoic acid exerts beneficial effects in cutaneous wound healing. The compound has been shown to activate the plasminogen activator system in human epidermal keratinocytes by differentially regulating, activating and inhibiting components. A marked induction of cell-associated plasminogen activity after 24 h has also been shown for all-trans retinoic acid. This was associated to an early and short-lived increase of plasminogen activator inhibitor-1 together with a prolonged induction of urokinase-type plasminogen activator and urokinase-type plasminogen activator receptor mRNA levels (Braungart *et al.*, 2001).

### **Experimental models of wound healing**

#### ***In vivo models***

Generally, animal models approximate human wound healing studies better than *in vitro* assays (Rupesh *et al.*, 2011). Although a pig’s skin structure closely resembles that of humans, mice rats and rabbits are preferred for practical reasons. Nevertheless, products whi-

ch have been found to improve wound healing in rats have also done so in humans (Masoko *et al.*, 2010).

In general, there are two main animal models, incisional and excisional, for the determination of the three basic phases of the wound healing process. Dead space and burn wound models are also useful. The incisional wound model is useful for wound tensile strength measurement. An abdominal incision is made in the paravertebral region and sutured. Stripes of equal size (width) are cut for breaking strength measurement or for histopathological examination (Tsala *et al.*, 2013). The excisional model is more appropriate for histological evaluation due to significantly broader morphological changes which occur during the healing process. Typically, a full skin thickness excisional wound is created on the dorsum of the mouse and extends through the panniculus carnosus. Wounds are then photographed regularly and wound closure is calculated based on wound size relative to the original wound dimensions (Wong *et al.*, 2011). Dead space wound can be induced by making a pouch through a small cut in the skin of the rat. A polypropylene material, a polyvinyl alcohol sponge or a steel wire mesh cylinders is implanted subcutaneously beneath the skin. Granulation tissue dry weight, breaking strength and hydroxyproline content are the important parameters to be studied (Udupa *et al.*, 2006; Deskins *et al.*, 2012). Partial thickness burn wound is inflicted upon animals starved overnight and under anaesthesia, by pouring hot molten wax at 80°C into a metal cylinder with circular opening, placed on the back of the animal. Wound contraction and epithelialization period are the two parameters to be studied in this model (Meena *et al.*, 2011)

### ***In vitro* models**

Different *in vitro* assay formats can be used to investigate the behaviour of cell types, which are relevant for human wound and soft-tissue healing. The predominant cell populations in mammalian skin are fibroblasts and keratinocytes. Accordingly, the vast majority of *in vitro* wound healing studies utilize either one or both of these cell types as effective tool to directly visualize cellular interaction (Oberringer *et al.*, 2007). For many years, Boyden chamber based transmembrane assays and scratch wound assays were the only widely available formats to study cell migration and invasion. However, new technologies such as microfluidic chambers and exclusion zone assays have recently emerged as alternative phenotypic screening assays that provide additional or complementary information to researchers (Hulkower and Herber, 2011). The study of cellular behavior in a two-dimensional culture dish offers the ability to investigate specific targets with minimal interference from external factors, but critical *in vivo* cues (paracrine signaling, three dimensional cues, etc.) are missing and thus limit the translational applicability of *in vitro* studies. *In vitro* co-culture experiments partially address the importance of paracrine interactions between different skin cell populations, and therefore serve to evaluate the influence of wound-healing-related factors *in vitro*. However, these models are also limited in their biological relevance to wound healing (Wong *et al.*, 2011).

In the Chorioallantoic membrane (CAM) model, fertilized chicken eggs are kept in a humidified incubator at 37°C with the wide end up. After 3 or 4 days of incubation, the eggs are observed on a self-made lamp and the position of embryo head is circled. 1-3 mL albumin is withdrawn at the narrow end of the eggs with 18 gauge hypodermic needle. The window

is sealed with transparent tape and again incubated. Through the window, a sterile disc treated with the drug of interest is placed inside the eggs at the junction of two blood vessels on the 7-9<sup>th</sup> day of incubation. The window is resealed and the eggs are incubated at 37<sup>o</sup>c for three days. The window is then opened and the growth of new capillary vessels radially converging toward the center is counted under a microscope (Wanga *et al.*, 2004; Barua *et al.*, 2009).

### **Natural products with wound healing activities**

A number of journals have already given considerable level of attention to the review of crude plant extracts as potential wound healing agents. The focus of the present review is therefore on bioactive phytochemical constituents that belong to the various chemical families such as alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds (Rupesh *et al.*, 2011). These bioactive agents usually modulate one or more phases of the healing process. Included to these lists are also multifunctional natural products that act through multiple targets by being anti-inflammatory, antioxidant, etc.

#### ***Natural products acting via modulating cellular activity***

##### *Modulators of the immune cell function*

Throughout the history of medicines, vitamins have been reported for their beneficial effect on wound management. First of all, vitamin A is often required for epithelial and bone tissue development, cellular differentiation, and immune system function (Twining *et al.*, 1997; MacKay and Miller, 2003). Such substantial evidence supports the use of this vitamin as a preoperative nutritional supplement (MacKay and Miller, 2003). Secondly, vitamin C (or ascorbic acid) enhances neutrophil function (MacKay and Miller, 2003) and when supplemented at least at 1 g per day, it is correlated with improved immune cell (cell mediated immunity and phagocytosis) function (Casciari *et al.*, 2003). The action of secondary metabolites on immune system has not been studied extensively but, the anti-inflammatory activity of flavonoids has been shown to be attributed to their ability to inhibit neutrophil degranulation; diminishing the release of arachidonic acid and other mediators from immune cells (Nijveldt *et al.*, 2001). This effect could probably interfere with skin repair.

##### *Modulators of skin cells: fibroblasts and keratinocytes*

To date, numerous crude natural preparations and purified compounds have been tested for their effect on skin cells migration or proliferation.

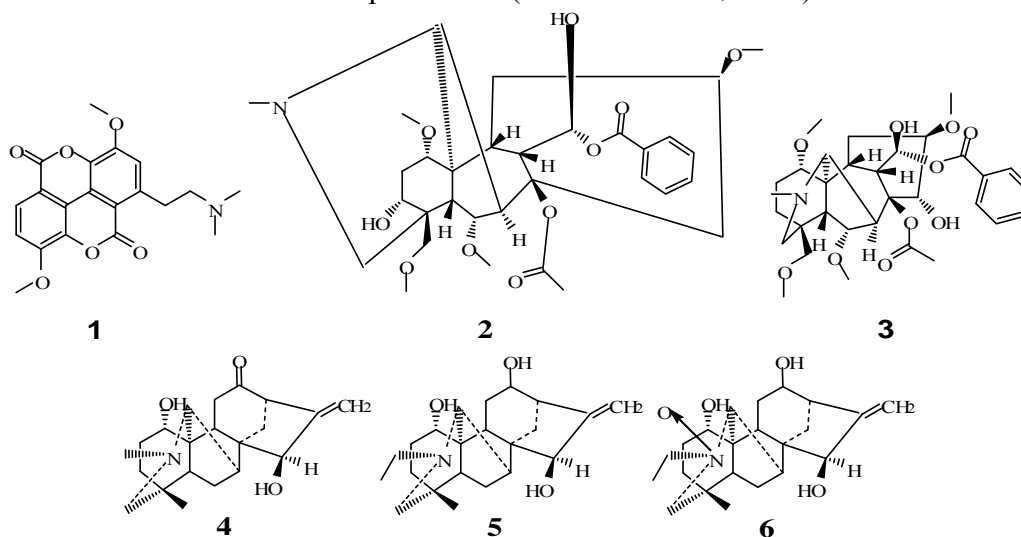
##### *Vitamins*

Products containing alpha-tocopherol (vitamin E), L-ascorbic acid (vitamin C), retinol (vitamin A), and niacinamide (vitamin B3), are effective for the treatment of inflammatory dermatoses and wound healing (Burgess, 2008). Vitamin A and its two important metabolites, retinaldehyde and retinoic acids, are fat-soluble unsaturated isoprenoids necessary for growth, differentiation and maintenance of epithelial tissues. Retinoic acids are major oxidative metabolites of vitamin A and can substitute for it in vitamin A-deficient animals in

growth promotion and epithelial differentiation. The mechanisms of action of natural vitamin A metabolites on human skin are based on the time- and dose-dependent influence of morphogenesis, epithelial cell proliferation and differentiation (Reichrath *et al.*, 2007). Stimulation of keratinocytes growth *in vitro* account for the prohealing effect of vitamin C. It has been shown that vitamin C act through induction of protein-kinase-C-dependent pathway that activates protein-1 DNA binding activity (Savini *et al.*, 2002). The positive effect of vitamin E oral and topical administration on wound contraction has also been documented in aging and diabetic rats (Lin *et al.*, 20012). Various studies have provided links to niacin's role as a potential wound healing agent through nicotinic acid receptors. Mechanistic studies of lauryl nicotinate demonstrated that niacin causes the release of leptin, and downstream signaling of leptin has profound effects on epidermal renewal, wound healing and hair follicle biology in skin (Jacobson *et al.*, 2007; Benavente *et al.*, 2009).

### Alkaloids

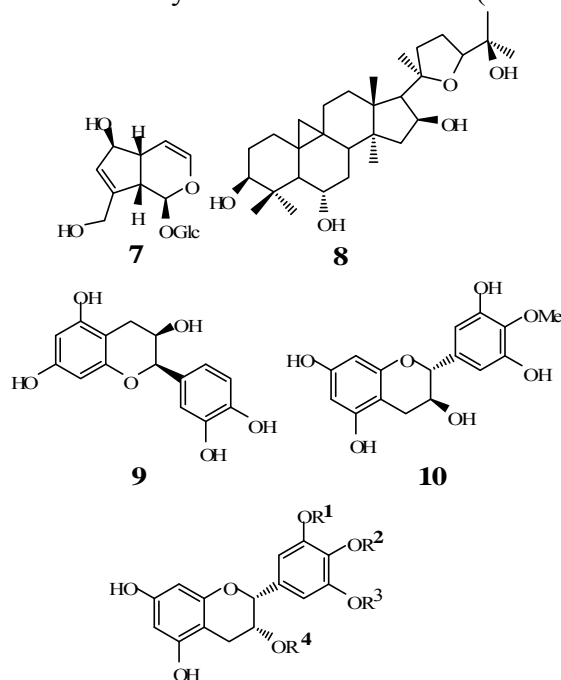
Even though numerous reports highlighted the potential anti-inflammatory effects of alkaloids; their wound healing effects have not been extensively studied. Taspine (**1**), an alkaloid purified from *Croton* (family Euphorbiaceae), has been shown to have wound healing effect both *in vitro* and *in vivo*. The studies revealed that taspine promotes early phases of wound healing ( $\leq 7$  days) in a dose-dependent manner with no substantial modification of later events such as changes in extracellular matrix (Porrás-Reyes *et al.*, 1993). Alkaloids of *Aconitum baikalense* including mesaconitine (**2**), hypaconitine (**3**), songorine (**4**), napelline (**5**), and 12-epinapelline N-oxide (**6**) have also been reported to significantly stimulate the growth of colonies from fibroblast precursors (Nesterova *et al.*, 2012).



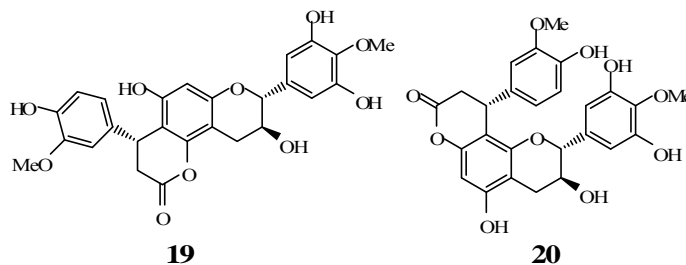
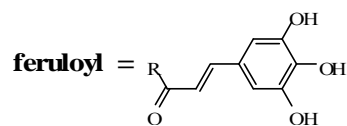
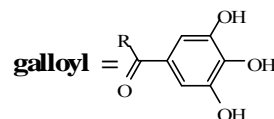
### Terpenoids

Aucubin (**7**), an iridoid glycoside (or monoterpenoid) isolated from *Aucuba japonica* or *Plantago asiatica* with a variety of pharmacological effects, has been examined by Shim *et al.* (2007) on oral wound healing in rats. Re-epithelization and matrix formation of the aucubin-treated group occurred earlier than that of the control group. In addition, the number of inflammatory cells of the aucubin-treated group was demonstrated to be fewer than that of the control group. On the other hand, saponins (triterpenoid glycosides) have been shown to

modulate wound cells function Sevimli-Güret *et al.*, 2011). *In vitro* studies reveal that saponins of *Astragalus* species (cycloastragenol, astragaloside IV, cyclocephaloside I and cyclocanthoside E) can increase both fibroblast proliferation and migration, with cycloastragenol (**8**) showing the highest level of activity when tested at 1 ng/ml concentration. Further *in vivo* study using Sprague–Dawley male rats revealed that cycloastragenol was once again found to be the most remarkable wound healing compound, showing greater cell density, more regularly organized dermis and more newly formed blood vessels (Sevimli-Güret *et al.*, 2011).



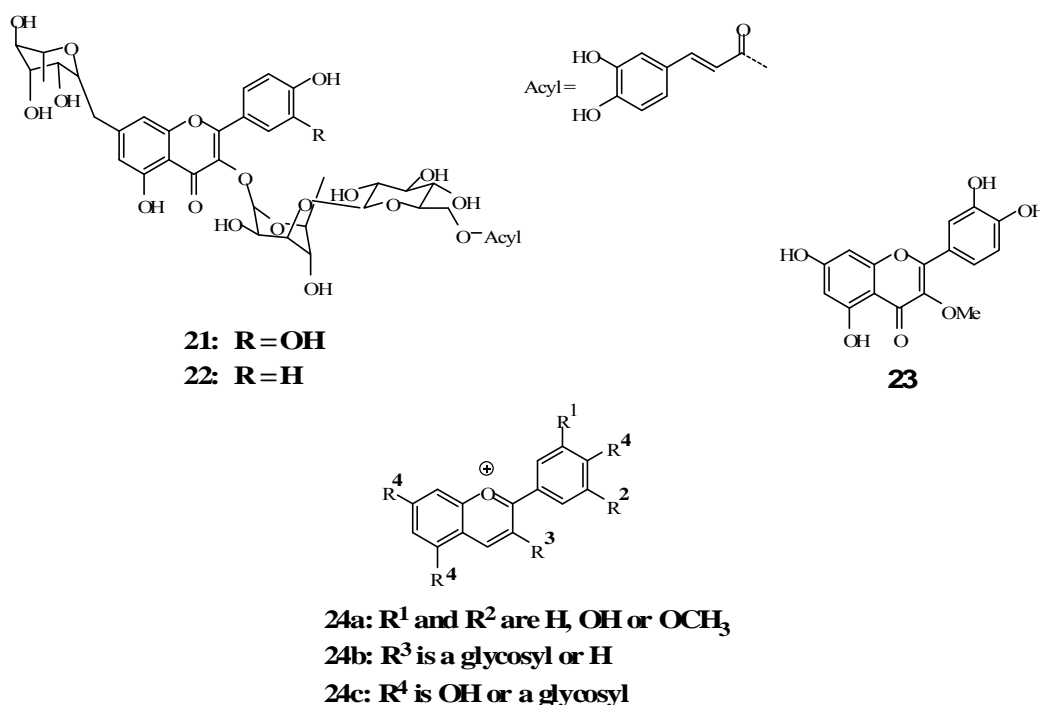
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### Polyphenols

Catechins (**9-18**) are one of the most widely tested classes of flavonoids for their wound healing modulation. The majority of the catechin derivatives isolated from the ethanolic

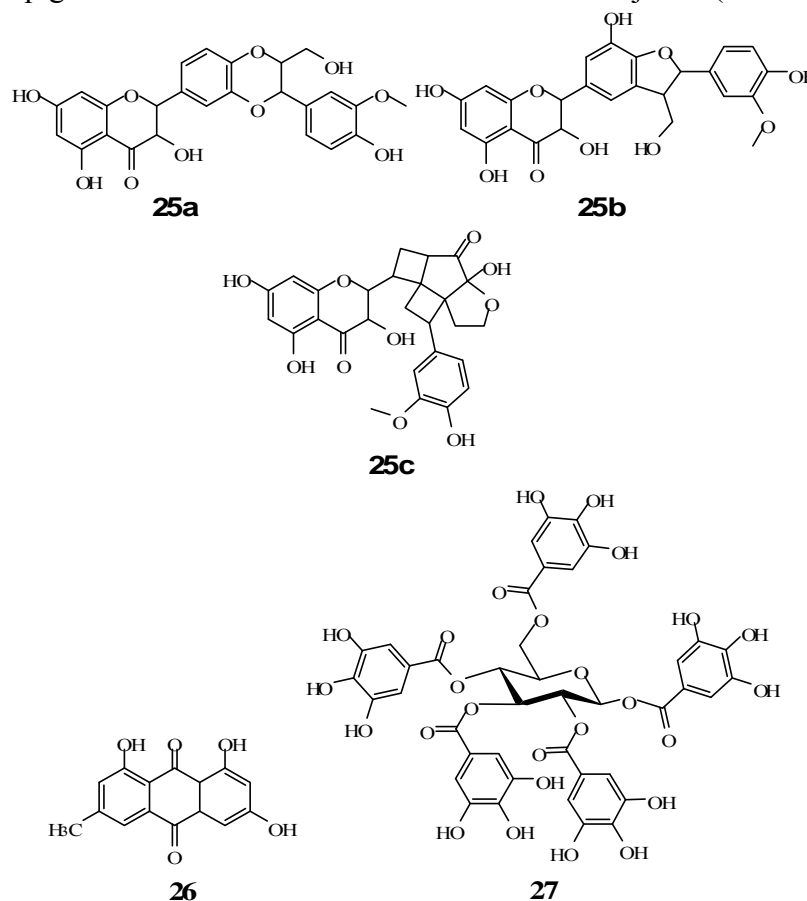
extract of the bark from *Parapiptadenia rigida* showed enhanced fibroblasts proliferation at concentrations of 1 and 10  $\mu\text{M}$  whereas 20  $\mu\text{M}$  concentrations led mostly to a reduced proliferation (Schmidt *et al.*, 2010). Epicatechin-3-O-gallate (**19**) and 4'-O-methylepicatechin-3-O-gallate (**20**) were the most active flavonoids when tested at the concentration of 1  $\mu\text{M}$ , though a reduced activity at a concentration of 10  $\mu\text{M}$  was also noted. According to the authors, the decreased activity at the tested higher concentration could be explained by the possible antiproliferative effect. In contrast to the experimental test agents, the positive control, PDGF, at 2 ng/mL showed an average stimulating effect of 59.5% (Schmidt *et al.*, 2010). Recently, two new glycosylated and acylated flavonols, viz. quercetin-3-O-[(6-caffeoyl)- $\beta$ -glucopyranosyl (1 $\rightarrow$ 3)  $\alpha$ -rhamnopyranoside]-7-O- $\alpha$ -rhamnopyranoside (**21**) and kaempferol-3-O-[(6-caffeoyl)- $\beta$ -glucopyranosyl (1 $\rightarrow$ 3)  $\alpha$ -rhamnopyranoside]-7-O- $\alpha$ -rhamnopyranoside (**22**), together with the known quercetin-3-O-methyl ether (**23**) were isolated from the aerial parts of the fern *Ophioglossum vulgatum* L. The three compounds were all found to be more active than the control (SDS) in scratch-wound healing assays on HaCaT keratinocytes, with quercetin-3-O-methyl ether being the most active with the maximum activity at 20  $\mu\text{M}$  (Clericuzio *et al.*, 2012). Treatment with anthocyanins (**24**), another class of flavonoids, can be considered as a potential therapeutic strategy to promote wound healing and to prevent inflammation under persistent inflammatory condition. Accordingly, both human dermal fibroblasts and keratinocytes showed a significant increase in migration compared to control, after treatment with anthocyanins fractions of various plant extracts (Nizamutdinova *et al.*, 2009; Wang *et al.*, 2013).



#### Other polyphenols

Silymarin, a complex flavolignan mixture of silybine (**25a**), silychristine (**25b**) and silydianine (**25c**) from *Silybum marianum*, has also been shown to have antioxidant properties which would help to prevent oxidative damage and promote the healing process. Sharifi

*et al* (2012) found that silymarin can increase epithelization of wounds and it is able to reduce inflammation in wound. These authors reported that silymarin did not have any effect on wound contraction but other studies using 5-20 % ointment of silymarin documented stimulation of wound contraction in streptozotocin-induced experimental diabetes in rats (Aliabadi *et al.*, 2011). The anthraquinone emodin: 1, 3, 8-trihydroxy-6-methyl-anthraquinone (**26**), isolated from *Rheum officinale*, has been shown to promote excisional wound repair in rats (100-400  $\mu\text{g/mL}$ ) via complex mechanism involving stimulation of tissue regeneration and regulating Smads-mediated TGF- $\beta_1$  signaling pathway (Tang *et al.*, 2007). Recently, 1,2,3,4,6-penta-O-galloyl- $\beta$ -D-glucose (**27**) containing fraction has been shown to enhance cell viability and cellular proliferation of HaCaT keratinocytes at concentration of 100 nM (Wang *et al.*, 2013). Lastly, considering that the delay in wound healing is due to insufficient or excessive fibroblast activity, some authors suggest that inhibition of fibroblast growth by flavonoids such as apigenin is beneficial for the treatment of skin injuries (Khan *et al.*, 2012).



## Modulators of collagen synthesis

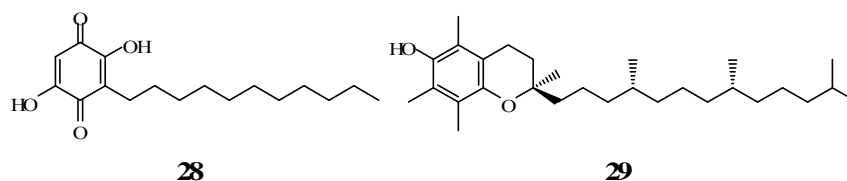
### Vitamins and related compounds

The anti-inflammatory property and the presence of vitamin A and proteins in *Curcuma longa* L. (zingiberaceae) resulted in the early synthesis of collagen fibers by mimicking fibroblastic activity (Raina *et al.*, 2008). Vitamin C is required for the hydroxylation of lysine and proline during the synthesis of collagen, most critically important for tensile strength of a wound (Sudha *et al.*, 2011). Vitamin C is also said to have important functions with respect



to its role in the biosynthesis of connective tissue including in the hydroxylation of proline and lysine residues during collagen biosynthesis (Walingo, 2005).

The quinone compound embelin (**28**) isolated from the leaves of *Embelia ribes* exhibited significant wound healing activity on albino rats (Swamy *et al.*, 2007). In this study, epithelialization of the incision wound was faster with a high rate of wound contraction. The tensile strength of the incision wound has also been shown to significantly increased; granulation tissue displaying increased cross-linking of collagen fibers; and absence of monocytes when compared with the standard skin ointment, Framycetin (**29**) (Swamy *et al.*, 2007). Moreover, embelin is closely related with the well known antioxidant alpha-tocopherol that was shown to increase the abundance of collagen fibers in the scar tissue, when applied topically (Lin *et al.*, 2012; Gupta *et al.*, 2013).

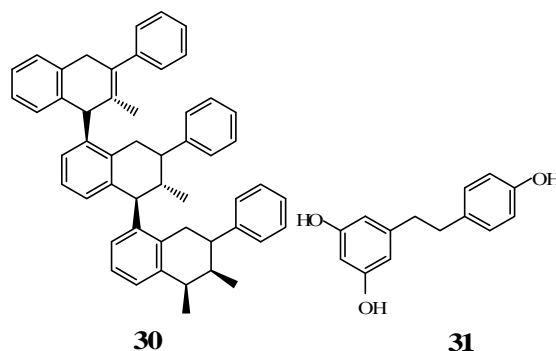


### Flavonoids

Collagen fibers treated with the plant flavonoid, catechin (**9**), have been found to be stable (Madhan *et al.*, 2005). Such stabilization effect has been shown to involve hydrogen bonding and hydrophobic interactions (Madhan *et al.*, 2005).

### Tannins and other phenolic compounds

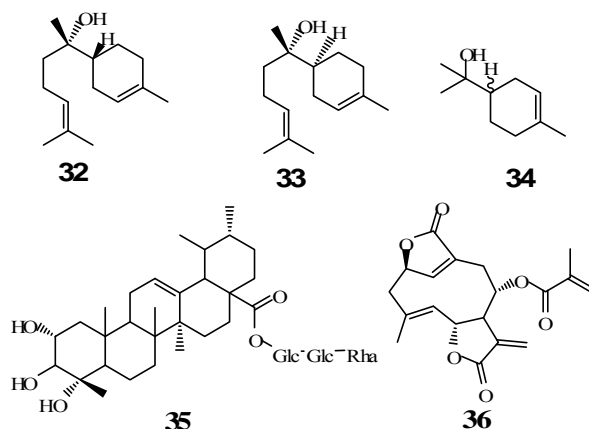
Tannins are phenolic compounds that typically act as astringents and are found in a variety of herbal products used for wound healing. This astringent property is responsible for wound contraction and increased rate of epithelialization at the granulation formation and scar remodeling phases (Chaudhari and Mengi, 2006; Li *et al.*, 2011). Accordingly, topical treatment with a tannin rich fraction of the bark of *Terminalia arjuna* was found to demonstrate significant increase in the tensile strength of the incision wounds. The maximum tensile strength was developed by tannins-fraction treated rats (719 g, compared to the standard reference formulation Betadine (609 g) (Chaudhari and Mengi, 2006). More recently, Natarajan *et al.*, (2012) studied the therapeutic potential of a tannic acid cross-linked collagen scaffolds and demonstrated significant effect in wound closure and wound healing rate. In addition, fir-



m evidence was provided that topical application of a grape seed proanthocyanidin (or condensed tannins, **30**) extract containing 5000 ppm resveratrol (**31**) represents a feasible and productive approach to support dermal wound healing (Khanna *et al.*, 2002). This extract accelerated wound contraction and closure associated with a more well-defined hyperproliferative epithelial region, higher cell density, enhanced deposition of connective tissue, and improved histological reorganization (Khanna *et al.*, 2002).

### Terpenoids

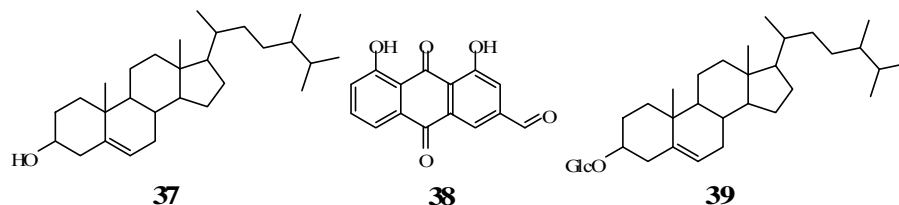
Villegas and co-workers (2001) examined the *in vivo* healing action of commercially available terpenols whose carbon skeletons bear some resemblance to (+)-epi- $\alpha$ -bisabolol (**32**) isolated from *Peperomia galioides*. The results indicated that (+)-epi- $\alpha$ -bisabolol,  $\alpha$ -bisabolol (**33**) and  $\alpha$ -terpineol (**34**) showed significant *in vivo* cicatrizant activity using tensile strength method (ED<sub>50</sub> 155  $\mu$ g/mL, 228 and 240 mg/g mouse, respectively). In comparison with a previous study, these bioactive terpenols are considered less potent than the alkaloid taspine (ED<sub>50</sub> 0.375 mg/kg), but are much less cytotoxic to human foreskin fibroblasts, and more accessible in terms of their chemical synthesis (Vaisberg *et al.*, 1989). The (+)-epi- $\alpha$ -bisabolol (**32**) and (-)-enantiomer (**33**) are the main constituent of chamomile (*Matricaria chamomilla* L.) and has been shown to shorten the healing time in cutaneous burns of guinea pigs exposed to UV light (Isaac, 1979). Other studies revealed that (+)-epi- $\alpha$ -bisabolol (**34**) did not have a significant effect on increasing cell migration (Villegas *et al.*, 2001). Topical or oral application of the triterpenoid, asiaticoside (**35**), in normal as well as in diabetic animals have been demonstrated to significantly enhance the rate of wound healing as increased collagen synthesis and tensile strength of wound tissues were noted (Shukla *et al.*, 1999). The ethanol extract and the isolated sesquiterpene lactone, deoxyelephantopin (**36**), promoted significant wound healing activity by increasing cellular proliferation, formation of granulation tissue, synthesis of collagen and by increase in the rate of wound contraction. This activity was attributed to the presence of active structural moiety, alpha methylene gamma lactone (Singh *et al.*, 2005).



### Modulators of angiogenesis

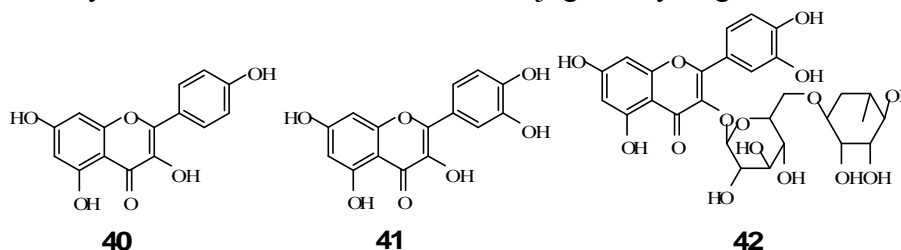
In addition to collagen production, vitamin C enhances angiogenesis (MacKay and Miller, 2003). Being one of the most important wound healing agents, Vitamin C is said to be

essential for both neutrophil and fibroblast function and it strengthens and promotes new blood vessel formation (Sudha *et al.*, 2011).  $\beta$ -sitosterol (**37**) is a plant-derived angiogenic factor which may have potential pharmaceutical applications for the management of chronic wounds. It has been demonstrated that *Aloe vera* gel and its extracts are angiogenic on the CAM of chick embryo.  $\beta$ -sitosterol purified from the *Aloe vera* gel showed a potent angiogenic activity in the CAM assay (64% of the eggs at 10 $\mu$ g/egg, versus 80 % of the eggs at 0.12  $\mu$ g/egg for Phorbol 12- myristate 13- acetate). No angiogenic activity was demonstrated for emodin (**38**) or  $\beta$ -sitosterol glucoside (**39**) isolated from the same extract (Moon *et al.*, 1999). In the presence of heparin,  $\beta$ -sitosterol stimulated neovascularization in the mouse matrigel plug assay and the motility of human umbilical vein endothelial cells in an *in vitro* wound migration assay (Moon *et al.*, 1999). Other reported proangiogenesis molecules are tannins and asiaticosides. Tannin extracts have also a stronger angiogenic effect at the inflammatory phase of the healing process, when compared with erythromycin ointment or Vaseline (Li *et al.*, 2011). Lastly, improved angiogenesis was observed when using asiaticoside (**35**) *in vitro* and *in vivo* (Shukla *et al.*, 1999). Angiogenesis inhibitors can interfere with various steps in angiogenesis, such as the proliferation and migration of endothelial cells and lumen formation (Nijveldt *et al.*, 2001). It has been speculated that flavonoids can inhibit angiogenesis.



### Modulators of the extracellular matrix

The natural compounds that are listed to regulate the ECM are mainly flavonoids. First of all, it is believed that the flavonoids kaempferol (**40**) and quercetin (**41**) in onion extract play a role in reducing scar formation through inhibition of fibroblast activities. In a study using both of onion extract and quercetin, proliferation rates of fibroblasts were shown to be decreased in a dose-dependent manner (Cho *et al.*, 2010). Moreover, conversely to type I collagen, the expression of MMP-1 was markedly increased by both onion extract and quercetin *in vitro* and *in vivo*, indicating that they play a role in the anti-scar effect in skin through up-regulation of MMP-1 expression (Cho *et al.*, 2010). Secondly, Tran and co-workers (2011) reported that quercetin-3-O-rutinoside (rutin, **42**) had been commercialized as an orally administrative drug that supports wound healing. According to these authors, rutin was employed to enhance production and accumulation of ECM. *In vitro* study demonstrated that rutin enhanced cell proliferation while in rat wound *in vivo* model revealed fast contraction of an incision accompanied with facilitated formation of new well-defined ECMs. Histological results after 14 days also demonstrated that rutin-conjugated hydrogel exhibited enhancement



of wound healing as compared with untreated group or a commercialized wound dressing agent, Duoderm. It was also concluded that rutin-conjugated hydrogels-induced better defined formation of neo-epithelium and thicker granulation which were closer to the original epithelial tissue (Tran *et al.*, 2011).

### ***Modulators of cytokines and growth factors***

#### *Alkaloids*

Taspine (**1**), an alkaloid isolated from the sap of *Croton lechleri* L. (Euphorbiaceae) was shown to accelerate the healing process, presumably, by increasing the migration of fibroblasts to the wounded area during the early stages of cicatrization (Villegas *et al.*, 2001). Taspine isolated from *Leontice robustum* has also been shown to enhance wound healing through other mechanisms. Since the effect was comparable to that of bFGF, the authors suggested the possibility of the compound acting as bFGF, through synergism with bFGF or other growth factors by another mechanism (Yalin *et al.*, 2005).

#### *Flavonoids*

In addition to the stimulation of fibroblast and keratinocytes migration, treatment of cells with anthocyanins (**24**) stimulated wound-induced VEGF production in fibroblasts and keratinocytes (Nizamutdinova *et al.*, 2009). However, anthocyanins also inhibited ROS accumulation and VEGF production in TNF- $\alpha$ -stimulated endothelial cells. Furthermore, treatment with anthocyanins reduced, in a dose-dependent manner, the adhesion of inflammatory monocytes to endothelial cells (Nizamutdinova *et al.*, 2009). Anthocyanins also blocked both the translocation of NF- $\kappa$ B p65 into the nucleus and the phosphorylation of the inhibitory factor kappa B alpha inflammatory condition (Nizamutdinova *et al.*, 2009). The influence of some isolated catechins from the ethanolic extract of the bark from *P. rigida* was further evaluated for their effect on TNF- $\alpha$ -induced NF- $\kappa$ B activation in Jurkat cells (Schmidt *et al.*, 2010). In general, concentrations of about 50  $\mu$ M were shown to be necessary to induce 50% inhibition. 4',3''-di-O-methylapocynin-B (**19**) inhibited NF- $\kappa$ B at 10 and 20  $\mu$ M concentrations, whereas higher concentrations led to a decrease of NF- $\kappa$ B inhibition. In the same study, 4'-O-methylepigallocatechin (**11**), epigallocatechin-3-O-gallate (**13**), epicatechin-3-O-gallate (**14**) and 4',3''-di-O-methylapocynin-B (**20**) were investigated for their inhibitory effects on p38MAPK in an ELISA providing the following IC<sub>50</sub> values: 39.16  $\mu$ M; 2.21 $\mu$ M; 1.47 $\mu$ M; and 50.80  $\mu$ M, respectively. Such results lead the authors to speculate that the trihydroxylation of the B ring and esterification by gallic acid increased inhibition of p38 MAPK. Interestingly, introduction of an O-methyl group even led to a slightly increased inhibition. Hence, the observed anti-inflammatory effects of preparations from *P. rigida* in traditional medicine may partly be explained by their influence on NF- $\kappa$ B and phosphorylation of p38 MAPK (Schmidt *et al.*, 2010).

#### *Retinoids*

Anti-inflammatory corticosteroids significantly impair wound healing (Wicke *et al.*, 2000; Durmus, 2003). Retinoids partially, but significantly, reverse this effect by acting on growth factors and collagen deposition in wound healing. For example, oral all-trans- and 9-

cis-retinoic acid partially reversed the decrease in TGF- $\beta$  and IGF-I level induced by methylprednisolone in rats, and significantly increased hydroxyproline content as well as collagen deposition toward normal levels (Wicke *et al.*, 2000).

### Tannins

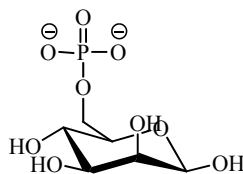
Tannin extracts from *Terminalia chebula Fructus Retz.* was reported to exhibit its powerful angiogenic property by up-regulating VEGF-A expression at the inflammatory phase, but not at the later stages of healing process, thereby resulting in the acceleration of wound maturity (Li *et al.*, 2011). It has been reported that tannin extracts from grape seeds potently upregulates oxidant (hydrogen peroxide) and TNF- $\alpha$  inducible VEGF expression in human keratinocytes (Khanna *et al.*, 2001) and was further shown to drive VEGF transcription. Since VEGF is believed to be the most prevalent, efficacious, and long-term signal for stimulating angiogenesis in wounds, tannin extracts from grape seeds may have beneficial therapeutic effects in promoting dermal wound healing and other related skin disorders (Khanna *et al.*, 2001).

### Terpenoids

Shukla *et al.* (1999) suggested that it is possible that asiaticoside (**35**) may have a growth factor like activity or has the ability to stimulate the expression of growth factors like the b-FGF. Human neutrophil elastase can cause abnormal degradation of healthy tissue resulting in the development of diseases such as rheumatoid arthritis, pulmonary emphysema, adult respiratory distress syndrome, and cystic fibrosis or delayed wound healing. Some sesquiterpene lactones inhibit human neutrophil elastase by modulating many inflammatory processes; for example oxidative phosphorylation, platelet aggregation, histamine and serotonin release. Several sesquiterpene lactones are also known to inhibit the transcription factors NF-kB and NF-AT. These proteins promote the expression of a variety of target genes in response to inflammation, viral and bacterial infections and other stressful situations. Consequently, downstream events, such as release of cytokines IL-1 $\beta$ , IL-6 or TNF- $\alpha$  production and lymphocyte proliferation are also inhibited by sesquiterpene lactones (Siedle *et al.*, 2002).

### Mannose-6-phosphate

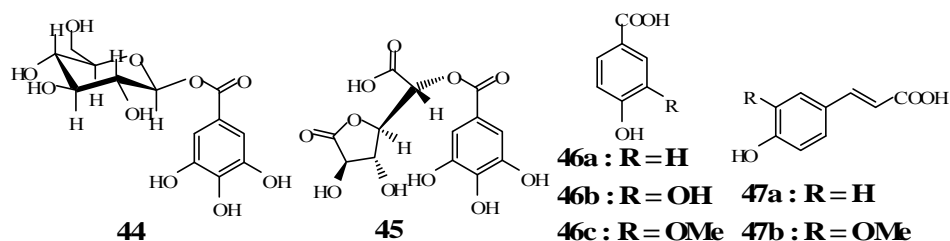
Some scientific evidence indicated that the effectiveness of *Aloe vera* in wound healing and inflammation is attributed to a growth factor-like substance (Davis, 1988; Davis and Maro, 1989; Davis *et al.*, 1991). Subsequently, mannose-6-phosphate (**43**) in *A. vera* was tested as an important factor in the wound healing process, even though it was not possible to ascertain whether the compound functions only to increase insulin-like growth factor II binding or directly stimulates fibroblast activation (Davis *et al.*, 1994).

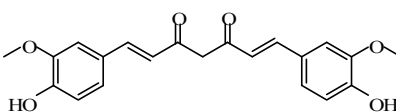
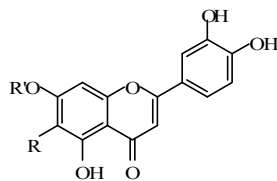
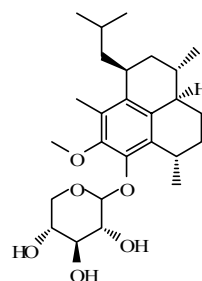


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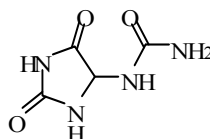
**Modulators of the oxidant-antioxidant balance of the wound microenvironment**

Phenolic compounds have been documented to possess potent antioxidant and free radical scavenging effect, which is believed to be one of the most important components of wound healing. For example, the wound healing effect of tannins may also be attributed to their anti-inflammatory activity due to their antioxidant action (Souza *et al.*, 2007). Ascorbic acid and hydrolyzable tannins, namely emblicanin A (**44**) and emblicanin B (**45**) have been shown to exhibit a very strong antioxidant action (MacKay and Miller, 2003; Majeed *et al.*, 2009). Tissue glutathione oxidation and 4-hydroxynonenal immunostaining results supported that tannin extracts of grape seeds application target the oxidizing environment at the wound site (Khanna *et al.*, 2002). It is generally believed that addition of these antioxidants to the wound microenvironment or in food would support the repair process (Mackay and Miller, 2003). The major and powerful antioxidants present in the extracts of the leaves of *Chromolaena odorata* that protect cultured skin cells (fibroblasts and keratinocytes) against oxidative damage was attributed to phenolic acids [*p*-hydroxybenzoic (**46a**), protocatechuic (**46b**), vanillic (**46c**), *p*-coumaric (**47a**) and ferulic (**47b**) acids] and complex mixtures of lipophilic flavonoid aglycones (flavanones, flavonols, flavones and chalcones) (Phant, 2001). Curcumin (**48**), another well known phenolic acid is able to suppress mechanisms engaged at the onset and progression of inflammation like inhibition of neutrophil oxidative burst. This activity was comparable with that of methotrexate – a drug widely used in the therapy of arthritic patients, which triggers synthesis of the endogenous anti-inflammatory mediator adenosine (Cronstein, 2005). Beside, curcumin could support resolution of inflammation through decreased activity and enhanced apoptosis of neutrophils (Jančinová *et al.*, 2011). Sesquiterpene lactone with alpha methylene gamma lactone isolated from Asteraceae members were reported to possess antibacterial, antifungal and antioxidant properties (Singh *et al.*, 2005). Luteolin (**49a**) and its related glycosides [cynaroside (**49b**), cesioside (**49c**), isoorientin (**49d**) and stereolensin (**49e**)] are active against arachidonic acidsynthesis and hydrogen peroxide scavenging depending on their molecular structures. The presence of ortho-dihydroxy groups at the B ring and OH substitution pattern at C-5 position of the A ring could significantly contribute to the anti-inflammatory (inhibition of leukotriene and thromboxane synthesis) and antioxidant activities of these flavonoids (Odontuya *et al.*, 2005). The long-known anti-inflammatory sponge PLA<sub>2</sub> inhibitors of marine origin, the scalaranes, sesquiterpenes isolated from *Cacospongia mollior*, and the pseudopterosins, diterpene-pentoseglycosides, which also have analgesic properties are used as additives in certain skin-care products. One compound in this family, the semisynthetic methopterosin (OAS1000) (**50**), has entered clinical development for the promotion of wound healing. OAS1000 has also shown anti-inflammatory activity in animal models, which is thought to be due to its inhibition of LTB<sub>4</sub> synthesis in neutrophils (Haefner, 2003).



**48****49a: R, R' = H****49b: R = H, R' = -D-glucose****49c: R = H, R' = -D-primeverose****49d: R = -D-glucose, R' = H****49e: R = O-b-D-glucose, R' = H****50**

Most natural compounds reviewed act through multiple targets such as being anti-inflammatory, antioxidant, etc. This property has also been observed for allantoin (**51**), a constituent of animal and plant origin that has been found to stimulate healing, with abundant growth of healthy granulation tissue in slowly healing suppurative wounds (Robinson W, 1935). Recently, it was suggested that the wound healing mechanism induced by allantoin occurs via the regulation of inflammatory response and stimulus to fibroblastic proliferation and ECM synthesis (Araújo *et al.*, 2010)

**51**

## Conclusion

The wound healing process which includes the inflammation, tissue formation, and tissue remodeling phases is the result of coordinated cellular and biochemical responses. Plant secondary metabolites have been demonstrated as important sources of potential agents that modify the various steps of wound repair. Among the various validated targets for these natural products are modulations of the immune cell function, skin cells (keratinocytes and fibroblasts) proliferation, collagen and other ECM proteins, angiogenesis and cytokines and/or growth factors. Various groups of natural products belonging to the terpenoids, flavonoids and other polyphenols, alkaloids, etc have been identified with potent wound healing effect both *in vitro* and *in vivo*. Some have been shown to act at singular targets while others act at multiple targets through non-specific action. Such medicines from natural sources may lead to better forms of therapy for patients with acute, chronic, and surgical skin wounds. Since, very few clinical trials were carried out to unequivocally confirm the therapeutic potential of the identified natural wound healing compounds; further research should be directed towards achieving this goal.

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## Conflict of interest

The authors declare that they have no competing interests

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