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Research in Phyto-Constituents for Treatment of Wounds

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Abstract

Disruption of normal architecture of skin is referred to as wound. There are different types of wounds like contusion, excision, incision, burn, diabetic, etc. The body has its own mechanism to heal wounds in three major overlapping phases, namely inflammatory, proliferative and remodelling. Any agent that promotes the healing process can be utilized as a wound healing agent. Plants have been a great source of medicines to treat wounds. Elucidation of the mechanism of wound healing helped researchers to investigate plants in detail and find out their active constituents. Various biochemical changes take place during the wound healing process, and these changes served as targets for *in vitro* and *in vivo* models. *In vitro* and *in vivo* models are extensively utilized to evaluate wound healing activity. The present chapter gives an overview of some classes of phyto-constituents having wound healing activity.

Keywords: phyto-constitutents, wound healing, models, phases wound healing, mechanism wound healing, treatments of wounds

1. Introduction

The term wound generally refers to the disruption in the normal architecture of skin, which forms the outer protective layer and the largest organ of the integumentary system for all animals and human beings. The skin plays a critical role in fluid homeostasis and provides sensory functions and thermal regulation. About 15–20% of the total body weight composed of skin, and it receives approximately one-third of body's blood supply at the rate of 300 ml/min.

Three main factors can cause injuries to skin and thereby leading to wounds. These factors include environmental, mechanical and chemical. The environmental factors include wind, temperature irregularities, humidity and sunlight. Mechanical injuries may result due to friction, shear force, pressure and epidermal stripping. The chemical injuries are caused by certain irritant chemical substances like corrosive acids, phenols, etc. [1].

Wounds are classified into three major categories, namely open, closed and burn. When the skin is torn, cut or punctured, it is categorized as open while closed wounds involve contusion caused by blunt force trauma. Burn wounds are due to exposure or contact with fire, heat, radiation, chemicals, sunlight, electricity, etc. The tissue injury or wound can follow the mechanism of normal repair, excessive healing or deficient healing depending on physiological, pathological, environmental and nutritional conditions. The normal repair or healing of wounds follows a predictable overlapping phases of inflammation, wound contraction, re-epithelization, tissue remodelling and angiogenesis with granulation tissue formation, in timely manner. The excessive wound healing process leads to the formation of scars, such as keloid. Sometimes, the wound healing process does not progress in the predictive and timely manner leading to impairment of the healing process, and these conditions lead to chronic wounds like venous ulcer.

2. Mechanism of wound healing

Wound healing takes place in four overlapping phases. These involve dynamic and interactive events with involvement of soluble mediators, blood cells, extracellular matrix and parenchymatous cells leading to the restoration of functional and anatomical integrity of skin. The phases of wound healing include inflammatory, proliferative, re-epithelization and remodelling [2, 3]. The cellular and biochemical aspects of these phases are discussed below.

2.1. The inflammatory phase

This phase initiates wound healing cascade and the duration is for about 1–5 days after injury. The phase plays a vital role of clearing the debris and preparation of wound for regeneration of new tissue. Immediately after wounding, within 5–10 minutes, local vasoconstriction takes place and simultaneously the platelets aggregate leading to fibrin clot formation via activation of the coagulation process and release inflammatory cytokines and growth factors. The fibrin clot acts as a matrix for migration of inflammatory cells and is directed to the wound site via chemotaxis from the growth factors such as platelet derived growth factor (PDGF), transforming growth factor-α (TGF-α) and adhesive glycoprotein fibronectin [4]. The brief period of vasoconstriction leads to ischemia of the surrounding tissue. This is followed by sustained vasodilation, in turn increased vascular permeability and migration of neutrophils to the wounded area. Enzymes, fluids and proteins are trapped in the extracellular space leading to inflammation.

This phase is also characterized by oxidative stress as the concentration of reactive oxygen species (ROS) increase. The reactive oxygen species have bacterio-static properties; however,

very high concentration of ROS and if the inflammatory conditions are sustained, these can lead to impairment of wound healing [5].

The migration of neutrophils and monocytes to the wound site is also accompanied by mast cells and chemotactic factors released within the 24 hours of injury. The monocyte specific chemotactic factors includes monocyte chemo attractant protein-1 and macrophage inflammatory protein-1. The other chemotactic factors generated during the coagulation stage include kellikerin, fibrinopeptide and fibrin degradation products. Tumour necrosis factor, histamine, cytokines such as interleukin, leukotrienes and proteases represent the inflammatory mediators that are released by mast cells. These serve to up-regulate the intercellular adhesion molecules both on leucocytes and endothelial cell surface through mediating inflammatory cell migration. This results in co-ordination of cell-cell and cell-matrix interactions and thus allowing neutrophils to perform their function of phagocytosis and invasion of microbes.

Proteases such as neutrophil elastase, neutrophil collagenase, known as matrix metalloproteinases (MMPs)-8, are released during the phagocytosis by leucocytes. The proteases initiate wound healing by removing damaged extracellular matrix components and these are replaced by new, intact extra cellular matrix components. The tumour necrosis factor and interleukin 1β, secreted by macrophages, stimulate endothelial cells of capillaries to express cell adhesion molecules and also induce production of cytokine and interleukin-8. These adhesion molecules enable the inflammatory cells to bind to vascular endothelial cells and traverse the capillary basement membrane to enter the surrounding tissues. The tumour necrosis factor also induces the macrophages to produce interleukin-1β and up-regulate MMP expression. Thus, both tumour necrosis factor-β and interleukin-1β directly influence deposition of collagen in wound through inducing collagen synthesis by fibroblasts. The cytokines downregulate expression of tissue inhibitors of MMPs. Macrophages release the angiogenic factor and a growth factor. The growth factor stimulates the fibroblasts production and collagen synthesis in the second phase of wound healing [6].

2.2. The proliferative phase

This phase occurs between days 3–12 after wounding. This is also termed as the granulation phase as it involves proliferation of fibroblast deposition of collagen and other extracellular matrix along with development of new blood vessels.

Cytokines derived from inflammatory cells and epithelial cells regulate the process of cellular migration and proliferation. Various growth factors include insulin like growth factor, basic fibroblast growth factor (bfGF), transforming growth factor (TGF)-β, PDGF and keratinocyte growth factor by fibroblast while TGF-β, TGF-β and interleukin (IL)-1β are synthesized by keratinocytes. Endothelial cells produce vascular endothelial growth factor, bfGF and PDGF. All these mediators are responsible for cell proliferation, synthesis of extracellular matrix proteins and capillary formation. If wound is not infected, the number of inflammatory cells decreases and the fibroblasts along with other cells migrate to the site of wound and proliferate. Fibroblasts get attached to the provisional fibrin matrix and start producing collagen [7–9].

The stages of biosynthesis of collagen include formation of pro-collagen chains, hydroxylation of proline and lysine further extracellular modifications take place leading to deposition and cross linking for formation of the extracellular matrix. Collagen gets its strength in the cross linking step.

2.3. Re-epithelization

This phase includes mitosis of epithelial cells at the wound margin and it begins 24 hours after the injury. The re-epithelization is stimulated by growth factors namely epidermal growth factor (EGF) and TGF-α secreted by the activated macrophages present in wounds. The epithelial cells now start covering the wound area and after completion of complete overlap of the wound area, enzymes are released to dissolve the attachment at the base of scab in turn its removal. Epithelial cell migration needs development of actin filaments within the cytoplasm of the migratory cells and disappearance of desmosomes, which link them to one another and to the basement membrane, respectively. If epidermal basement layer is intact, cells simply migrate over it, however in case of destroyed epidermal basement layer cells initially migrate over the provisional fibrin-fibronectin matrix. As these migrate across the matrix, the epithelial cells regenerate a new basement membrane [10].

2.4. Neoangiogenesis

Various high metabolic activities take place in the wound healing process; hence, there is great demand for oxygen and nutrients. Reduced pH due to the production of lactates and reduced oxygen levels initiate mediators leading to the formation of new blood vessels/capillaries. These are formed as a bud-like structure from pre-existing vessels adjacent to the wound. The new capillaries grow into red loops of blood vessels and give a granular appearance to the wound surface [11–13].

2.5. Remodelling phase

The final stage of wound healing involves formation of an immature collagen matrix into the scar tissue. The new collagen leads to an increase in the tensile strength of skin; however, the strength will be till 80–90% of the original tensile strength. After the formation of initial scar, there is a cessation of proliferation and neo-vascularization, and the remodelling phase begins in the wound. Matrix metalloproteinases secreted by fibroblasts degrade the matrix, and fibronectin gradually disappears and replaces the hyaluronic acid and other amino glycosides by proteoglycans. Proteoglycans are components of the extra cellular matrix, which play an important role in modulating the structure and functions of skin. Proteoglycans impart mechanical strength by their capacity to absorb water and fill the space between the collagen and elastin fibres and also influence collagen formation, cell proliferation, cell migration and cell adhesion during the wound healing process.

Fibroblasts also secrete lysyl oxidase that cross links components of extracellular matrix (ECM). As the scar matures, the angiogenesis decreases; thus, a decrease in the metabolic activin is

observed at the wound site and elimination of fibroblasts and macrophages takes place through apoptosis [14, 15].

Human body or animal body has its own capacity to repair the damage occurred to skin with its own pace, however any agent that promotes the process of regeneration can be termed to have wound healing potential. Clinically the main agents used for treatment of wounds include antibiotics to prevent infection and some bio-molecules like collagen. Another source of wound healing agents is plant sources. Many plants have been utilized for treatment of wounds in traditional and folklore medicines. The molecules or the plant as whole or extracts thereof have multiple mechanisms in the regeneration of the skin in injuries. Many such plants have been investigated for their potential in promoting the wound healing process. The knowledge of biochemical, cellular changes that occur during various phases of wound healing help in identifying targets for evaluation of wound healing activity by both *in vivo* and *in vitro* techniques.

3. *In vitro* evaluation of wound healing activity

Various targets are identified to develop a target specific drug, which is an agent that inhibits the molecular target, such as MMP inhibitors and protease inhibitors. These targets are central to the disease mechanism of interests. Cellular targets include the cells involved in the process of wound healing, such as keratinocytes, fibroblasts and immune cells.

3.1. Targets of cellular activity

Any agent, which decreases the bleeding time, that is the first stage of wound healing, has the potential to promote wound healing, as these have a positive effect on integrity of blood vessels or active participation of platelets in forming haemostatic plug. The effect of any agent or extract on angiogenesis is studied by Chick Chorioallantoic Membrane Assay (CAM) [16].

The proteases released are helpful as wound diagnostics. Proteases are also known as proteinases, which play a vital role in normal wound healing processes. One of the important families of proteases, matrix metalloproteinases (MMPs), plays an important role in wound healing. These preferentially breakdown proteins comprise of extracellular tissues and require zinc at the active centre of the ion. MMPs are produced by activated inflammatory cells (neutrophils and macrophages) and wound cells (epithelial cells, fibroblasts and vascular endothelial cells). Most of the MMPs are secreted in the extracellular matrix; some are also associated with cell membranes and are known as membrane type MMPs [17].

The important functions of proteases in the inflammatory phase include removal of the damaged extracellular matrix. In phase II of wound healing, that is proliferation stage, proteases degrade the capillary basement membrane to promote angiogenesis, and also aid in detachment. MMP-1 is responsible for migration of cells. In the last phase, that is the remodelling phase, proteases are responsible for contraction and remodelling of the scar extracellular matrix. The amounts of proteases are very critical as the excess amount of proteases lead to

degradation of the newly formed extracellular matrix and growth factors leading to delayed wound healing. MMP-9 has shown inverse correlation with the wound healing process and the most non-healing wounds are found to have high levels of MMP-9 [18]. The levels of inflammatory markers, high levels of proteases including MMPs, and diminished growth factor activity can be treated as markers of the wound healing process [19, 20].

3.2. Skin cells: targets for wound healing

Fibroblasts are the key players in the process of wound healing through contraction, synthesis and deposition of the extracellular matrix; hence, the fibroblast *in vitro* model is apt to correlate contractile events of wound healing. *In vitro* fibroblast bioassay involves isolation of human dermal fibroblasts from post auricular surgery, followed by culturing in laboratories. These fibroblast cultures are then incubated with the test substances, and the content of hydroxy-proline/100 mg of DNA is estimated [21].

Keratinocytes assay involves isolation of keratinocytes from human foreskin or residual skin samples removed during surgery, followed by culturing in laboratories. Wound is inflicted in the culture through gentle scrapping. The cells are then incubated with the test sample. The effect of test sample on proliferation and motility of keratinocytes is studied. The greater the proliferation and motility as compared to the untreated cells indicate the promotion of the wound healing process [22].

4. *In vivo* models for wound healing

The *in vivo* studies are generally carried out in animals like mice, rats, guinea pigs, through inflicting wounds of various types like excision, incision, burn, dead space, diabetic wounds, etc. The most popularly studied wound models include excision, incision and dead space. The general method of evaluation of any test substance for its wound healing potential includes evaluation of acute dermal toxicity of the base utilized for preparation of topical preparation and the test substance in its highest concentration that would be incorporated into the base. The acute dermal toxicity is evaluated in animals like albino rats using the protocols described in OECD Guidelines number 434. Once the substance and the base is proved to be safe, it is then evaluated using the following standard models.

The animals are divided into three main groups, namely control indicating the normal wound healing mechanism of body without any treatment, vehicle control is treated with the base only and the test group is treated with the test substance(s).

4.1. Excision wound model

The animals utilized for wound healing studies are depilated on their thoracic region and excision wounds are inflicted on the thoracic region using sharp gadgets covering an area of about 500 mm^2 and a depth of about 2 mm (**Figure 1**). The wounds are cleaned using cotton swab and are treated most of the time topically but it is also observed that sometimes studies

are carried out by oral treatment with the test substance. The day of infliction of wound is considered as zero and the area of the wound is traced on an OHP sheet. The percentage wound closure is determined by tracing the wound on alternative days and calculating the area of wound. The results of the test substances are compared with the untreated or vehicle treated wounds. The reduction in the number of days for closing the wounds as compared to the untreated wound indicates the promotion of the wound healing process [23]. The progress of healing of wound is also monitored by period of epithelization, histological studies and estimation of biochemical parameters like hydroxyproline, hexosamine, collagen content in granulation tissue on day 11 after wounding [24–26].

Figure 1. Excision wound inflicted in the thoracic region of the albino rat.

4.2. Incision wound model

The model simulates surgical wounds. These are inflicted by giving longitudinal paravertebral incisions through the skin and cutaneous muscles at a distance of about of about 1.5 cm from midline on either side of the vertebral column. After cleaning of the wound, the parted skin is stitched with interrupted sutures (**Figure 2**). The wounds are treated either locally or orally with the test substance and the stitches are opened on day 10. The tensile strength of the treated skin is measured as a parameter for the repairing of skin [27]. The tensile strength of the repaired skin can be measured by a tensiometer indicated in **Figure 3**, which was constructed in our laboratory. Higher tensile strength of the repaired skin as compared to the untreated skin is an indication of promotion of the wound healing process.

Figure 2. Sutured incision wound inflicted on the back of the albino rat.

Figure 3. Tensiometer for determination of tensile strength of repaired skin of the albino rat.

4.3. Dead space wound model

The model is useful to study physical and mechanical changes in granulation tissue. In this model, subcutaneous dead space wounds are inflicted on either side of axilla and groin on the ventral surface of animal by creating a pouch through a small nick in skin followed by insertion of a sterile cylindrical object made up of glass or polypropylene or sterile cotton pallet in the pouch. The wounds are then sutured and treated with the test substance either locally or orally for 10 days [28]. The objects inserted are taken out through surgical procedures and the physical changes in the granuloma tissues are studied along with the determination of biochemical markers, like hydroxyl proline, hexosamine, etc.

Histopathological studies on the granulation tissue also reveal the extent of healing. Sections of granulation tissue are formed during the healing process [29].

4.4. Burn wound model

Burn wounds are inflicted using heated gadgets like hot metal cylinder, metal plate or using hot molten wax [30]. Promotion of healing by the test substance is monitored in the same way as described in the excision wound model.

5. Phyto-constituents with wound healing activity

Plants have been used in many traditional systems of medicine for healing wounds. With the advent of elucidation of the mechanism of wound healing at cellular and molecular levels and based on the knowledge of various plants used in traditional systems of medicines for treatment of wounds, scientific investigations were triggered to get agents/drugs for healing wounds. There is another major reason for the research in wound healing agents, is non-availability of specific wound healing agents, except the use of antibiotics, anti-inflammatory and analgesic drugs in the allopathic system of medicines.

Plants are a good source of chemically diverse phyto-constituents and these are assimilated by human and animal bodies easily as compared to the synthetic molecules and hence render a pharmacophore for the development of drugs. Several papers have been published indicating the investigation of plant extracts for their potential as wound healing agents, however very few plants have been investigated in detail explaining the constituents responsible and the mechanism of action of wound healing.

Chemically phyto-constituents are classified into major categories like alkaloids, glycosides, terpenoid, quinines, flavonoids, polyphenols, sulphur-containing compounds, polyacety-lenes, polyketides and steroids. It is observed that the compounds having anti-inflammatory, antibacterial, astringent, antioxidant activities and immunomodulatory activities, indicated to promote the wound healing process.
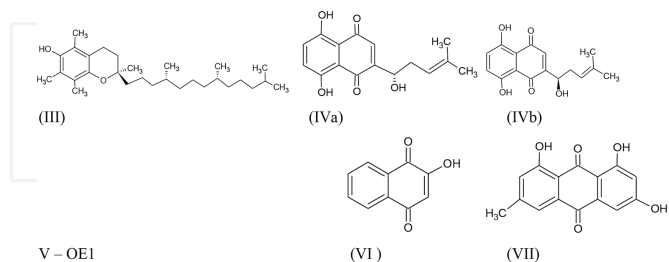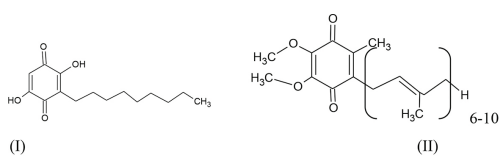
5.1. Quinones

Quinones are oxidized derivatives of phenols and catechols. Three types of quinones occur in nature namely Benzo (1,2/1,4), naphtha (1,4) and anthra (9,10). Benzoquinones occurring in nature include embelin (I) from berries of *Embelia ribes* and Co-enzyme Q10 (Co-Q10) (II) which is also known as ubiquinone is a biosynthesized quinone with 10 isoprene side chains in humans. Radhakrishnan et al. [31] in their study on the effect of Co-Q10 Radhakrishnan on cutaneous healing of skin incised mice revealed that the compound has antioxidant activity in which it is converted to reduced (CoQ10H) in the presence of some kinds of intracellular reducing agents and it has increased levels of collagen-like polymer in the granulation tissue of animals.
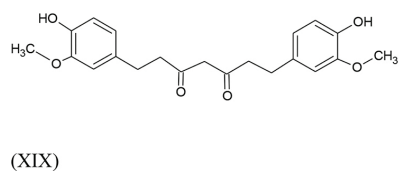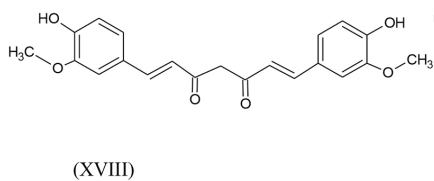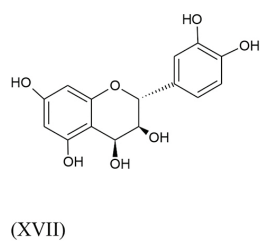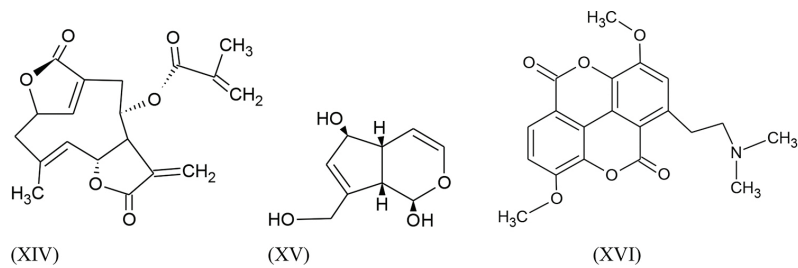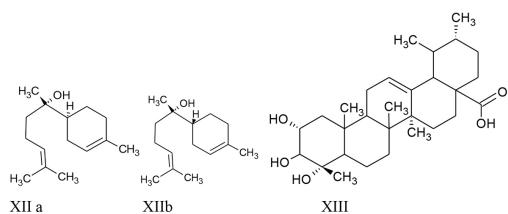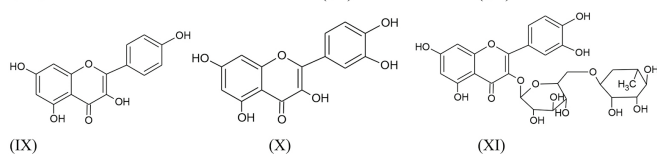
Embelin is a well-known anthelmintic agent and also reported to have antibacterial activity and wound healing activity [32]. Incision wound treated with embelin indicated a higher rate of wound contraction and tensile strength with increased cross linking of collagen fibres was observed in granulation tissues as compared to the standard skin ointment, Framycetin. Embelin has structural resemblance to vitamin E that is alpha tocopherol (III) which is a well-known antioxidant. Lin et al. [33] studied the effect of application of topical tocopherol cream on cutaneous wound healing in Streptozotocin-induced diabetic rats. It was revealed that the cream containing 0.29% w/w of tocopherol could promote healing of the excision wounds in diabetic rats.

Many naphthaquinone derivatives found in plants have been observed to possess wound healing activity. The popular one include alkannins and shikonins [34]. Alkannins and shikonins (IV a & b) are chiral-pairs of naturally occurring isohexenylnaphthazarins. They are found in the external layer of the roots of at least a hundred and fifty species that belong mainly to the genera *Alkanna, Lithospermum, Echium, Onosma* and *Arnebia* of the Boraginaceae family. The alkannin, shikonin and their derivatives possess potent antimicrobial and anti-inflammatory properties and are revealed to actively support the proliferative phase of wound healing. Papageorgiou et al. [35, 36] isolated alkannin, shikonin and derivatives to be active constituents from roots of Boraginaceae family and carried out clinical studies on alkannins and shikonins for treatment of non-healing venous wounds and reported the molecules could effectively heal the wounds. Dimer of naphthaquinone (V) [37] was isolated and the structure of the same was elucidated by Gawand et al. and reported it to be active wound healing agent through animal studies. Lawsone (VI) obtained from Leaves of *Lawsonia alba* and *L. inermis* was complexed with zinc and the wound healing activity of the complex [38] was evaluated using excision and incision wound models. The complex indicated good antimicrobial activity and displayed to promote the proliferative phase.

One of the anthraquinone derivatives Emodin (VII) [1,3,8– trihydroxy—6–methyl anthraquinone] obtained from rhizomes of, is reported to promote the repair of excision wounds in animals via a complex mechanism involving stimulation of tissue regeneration and regulating Smad-mediated TGF-beta(1) signalling pathway [39].

(I)

(II)

(III)

(IVa)

(IVb)

V – OE1

(VI)

(VII)

(IX)

(X)

(XI)

XII a

XIIb

XIII

(XIV)

(XV)

(XVI)

(XVII)

(XVIII)

(XIX)

5.2. Phenolics and polyphenolics

Phenolic compounds include simple phenolic compounds, phenyl propanoids, lignans, lignins, flavonoids and polyphenols include tannins. Tannins have mainly antibacterial, antioxidants and astringent activities. Phenyl propanoid glycosides namely verbascoside and teopolioside are structurally characterized to contain caffeic acid and 4,5-hydroxyl ethanol [40] were isolated from *Syringa vulgaris* and *Ajuga reptans* plant cell lines, respectively, were studied for wound healing activity by both *in vitro* and *in vivo* techniques. The compounds exhibited good anti-inflammatory, antioxidant and wound healing activities. Both the compounds were extremely effective inhibitors of chemokine and growth factor expression by cultured human keratinocytes treated with pro-inflammatory cytokines, TNF-α and interferon gamma.

Another group of phenolic compounds include flavonoids and these are one of the vast groups of phyto-constituents with large structural diversity. The chemical nuclei of flavonoids described as Benzopyran. Kaempferol (IX) and Quercetin (X) occurring in onion extract are reported [41] to increase type-1 collagen through increased expression of MMP-1, revealing their role in the anti-scar effect in skin. One of the popular flavonoidal glycoside of Quercetin is Rutin (Quercetin-3-o-rutinoside) (XI) commercialized to be administered orally, supported wound healing through enhanced production and accumulation of extracellular matrix. Rutin conjugated hydrogels in wound dressing was found to promote the wound healing process. Anthocyanins are reported to stimulate wound induced VEGF production in fibroblast and keratinocytes, also reduce the adhesion of inflammatory monocytes to endothelial cells [42].

Fruit pulp of *Musa sapientum* exhibited wound healing activity in animals, increased tensile strength, collagen formation which was revealed through the elevated levels of hydroxyproline, hexosamine and the wound contraction rate. Docking studies on Leucocyanidin (XVII) from the fruit indicated inhibition of MMPs, like collagenase, gelatinase, elastase and stromelysine [43].

The astringent property of tannins plays a vital role in wound contraction and increased rate of epithelization in phase three [40]. The tannin extract from *Terminalia arjuna* exhibited angiogenic activity through up-regulating VEGF-A expression in the inflammatory phase [44]. Experiments conducted with tannic acid cross-linked collagen scaffolds demonstrated a significant effect on the wound healing process. The other tannins like proanthocyanidin from grape seed displayed accelerated wound contraction and closure associated with the hyper-proliferative epithelial region [45]. Proanthocyanidins are high molecular weight oligomers consisting of 4–11 units of flavan-9-ol. These form complexes with proteins and precipitate them leading to enzyme inhibition involved in vascular tissue degradation [46]. Extracts of grape seed proanthocyanidins are reported to stimulate the expression of VEGF in cultured keratinocytes and thus promote the wound healing process [45, 47].

5.3. Terpenoids

Terpenoids form a broad class of phyto-constituent derived from acetate mevalonic acid pathway. The isoprene (C_5H_8) unit is a monomer for various terpenoids. Based on the number of isoprene units in a molecule decide the type of terpenoid, namely for two isoprene units it

is mono-terpene, three isoprene units it is sesquiterpene, four isoprene units it is di-terpene, for four isoprene units it tri-terpene and for eight isoprene units it tetraterepenes or also referred to carotenoids.

Mono-terpenes and sesquiterpenes generally form the major constituents of volatile oils. *Matricaria chamomilla* is popularly used as nutraceutical for its anti-inflammatory activity. The volatile oil of flowers is rich in (+) epi-α-bisabol and its (-)enantiomer (XII a & b). Wound healing studies on the bisabols revealed to shorten the healing period in cutaneous burns of guinea pigs exposed to UV light [48] and the probable mechanism for wound healing activity reported in another study involved increasing cell migration [49]. Asiaticoside (XIII) isolated from *Centella asiatica* was found to increase the rate of wound healing through collagen synthesis in turn increasing tensile strength [50]. An active sesquiterpene lactone deoxyelephantopin (XIV) was isolated from ethanolic extract of leaves of *Elephantopus scaber* by Singh et al. [51] and reported that the presence of alpha methylene gama lactone is an essential structural feature for wound healing activity. The compound exhibited wound healing activity at all the three phases. Carotenoids like retinoids are found to reverse the impaired healing of wound by acting on growth factors and collagen deposition.

Iridoids are classified under the category of mono- terpenoids and occur as glucosides. These are structurally bicyclic cis fused cyclopentane-pyrans and have diverse pharmacological activities like anti-inflammatory, antimicrobial, anti-viral, hepatoprotective, etc. One of the iriod glycosides Aucubin (XV) (0.1% solution) isolated from *Aucuba japonica* was reported to have an anti-inflammatory effect on oral mucosal wound healing, and to promote early re-epithelialization and collagen matrix formation [52].

5.4. Alkaloids

Alkaloids form one of the vast categories of phyto-constituents. These are basic secondary metabolites with presence of nitrogen either in the heterocyclic ring or outside the ring and exhibit marked physiological actions when administered to animals or human beings. These have varied pharmacological actions like anticancer, emetic, hypotensive, etc. One of the well investigated alkaloids is Tapsine (XVI) obtained from *Croton lechleri* (*Euphorbiaceae*) is found to be potent wound healing agent as earlier phases of wound healing presumably by increasing the migration of fibroblasts to the wounded area [49, 53]. Aconite alkaloids exhibited wound healing activity through stimulation of growth of colonies from fibroblasts precursors [54, 55].

5.5. Steroids

Steroids are non-polar secondary metabolites and are described to possess a cyclopentano-perhydrophenanthrene nucleus. These are biosynthesized through the acetate mevalonic acid pathway. Stigmastatone was isolated from bark of *Celastrus paniculatus* and was studied for enzyme target glycogen synthase kinase-3-β (GSK-3-β), an important regulatory enzyme whose inhibition promotes wound healing through β-catanin dependent Wnt signalling pathway. The molecule was docked with GSK-3-β, and it was observed that the molecule leads to the inhibition of the enzyme with an IC_{50} value of 1.21 Nm while nitrofurazone indicated

an IC_{50} value of 1.35 nM. Further evaluation of *in vivo* models of excision, incision and dead space wounds, the compound exhibited wound healing activity with increased tensile strength and collagenation of granulation tissue [56].

5.6. Polysaccharides

Aloe species are known for the presence of anthraquinone glycosides and the derivatives are popularly utilized for purgative actions. Aloe species also contain glucomannans which find their applications in the cosmetic industry in moisturising and anti-ageing products. *Aloe vera* is reported to heal second degree burn wounds and this property is attributed to the presence of mannose-6-phosphate. It is also reported that glucomannans interact with growth factor receptors of fibroblasts and stimulate its activity and proliferation in turn increase collagen synthesis when administered by both oral and topical route. Acetylated mannose is also termed as acemannan forms a major component of *Aloe vera* and this accelerates wound healing and also reported to have potential to stimulate release of fibrogenic cytokines [57–60].

A phyto-constituent from the class of arylheptanoids is curcumin (XVIII), a colouring matter obtained from the rhizomes of *Curcuma longa*, a very important molecule as it has array of pharmacological activities. The molecule is very well investigated and lot of research has been envisaged for formulations to improve bioavailability of the molecule. It is a good anti-inflammatory agent and has been used since ages to treat wounds. It is also proved to be good wound healing agent [61, 62].

The mechanism of enhancing the wound healing rate was reported [63] to be through increased cellular proliferation and collagen synthesis. It was observed that there is increase in DNA, total protein and type III collagen in wound tissues. The increased collagen synthesis was also responsible for increased tensile strength of repaired skin. The antioxidant property of collagen also played a vital role in promoting wound healing. The antioxidant property of the molecule was revealed through decreased levels of lipid peroxides and increased levels of superoxide dismutase, catalase and glutathione peroxidase.

Tetrahydrocurcumin (XIX), a metabolite of curcumin, also exhibits many pharmacological actions. The poor bioavailability of the molecule has been overcome by preparation of a water soluble derivative, glycosylated tetrahydrocurcumin. The product has an advantage of being colourless and also increased cutaneous absorption of the molecule thereby exhibiting good wound healing properties [64–66].

6. Conclusion and summary

Currently antibacterial and antibiotics are utilized for treatment of wound. Based on the knowledge of traditional medicines and folklore medicines, many plants have been screened for wound healing activity. Some plants are investigated in detail to get an active wound healing agent; however, very few have reached till clinical use. There is still scope to carry out more research in the area and get molecules with clinical applications. The following chart

indicates the summary of the mechanism of wound healing and various targets, which can be utilized in development of wound healing agent (**Figure 4**).
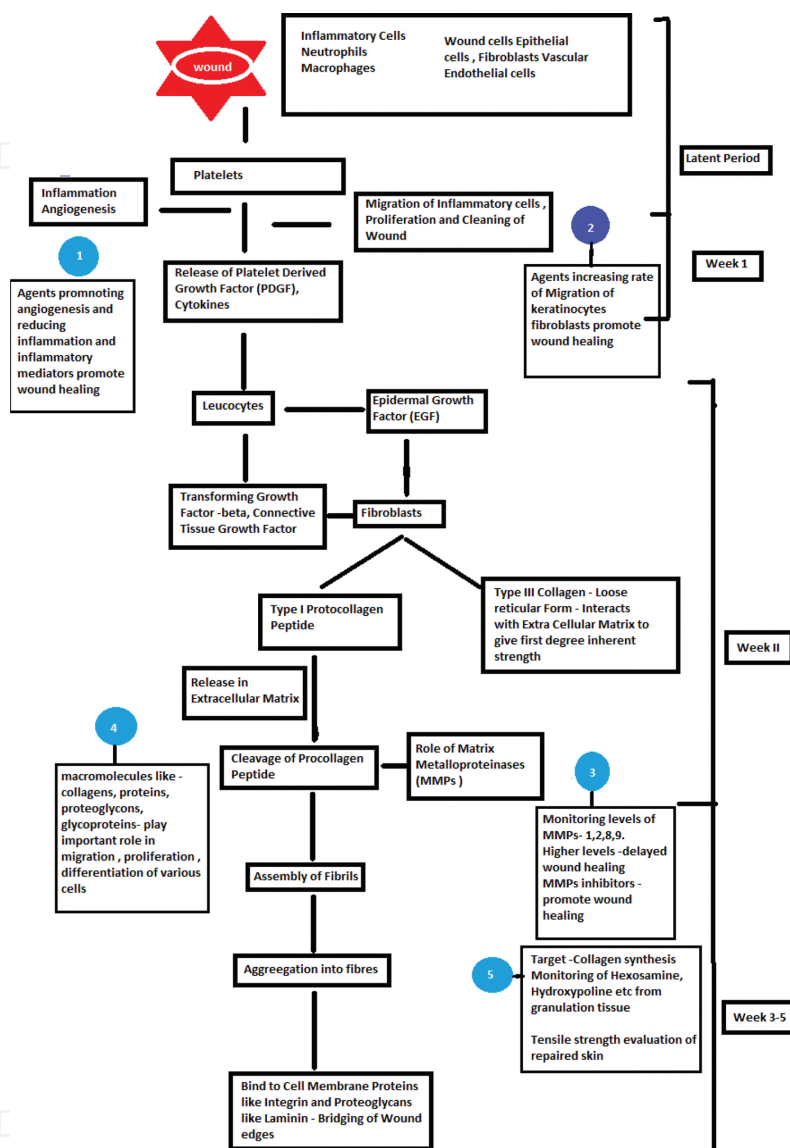
Figure 4. Summary of the mechanism of wound healing and various targets.

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