

A Detailed Assessment of Medicinal Plants in Wound Healing in Rats

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ABSTRACT:

A wound is a physical trauma that involves tearing, cutting, or puncturing the skin. A microorganism enters the site upon exposure to air, causing wound contamination and ultimately a spread of infection. Healing process is very complicated and involves various steps- inflammation, proliferation and remodeling. There are underlying factors that affect the process of wound healing like age, sex, infection, stress etc. Wound healers, a substance that accelerates the pace of healing, are in demand since the healing process is sluggish and delayed. Due to their adverse effects, several synthetic medications used to treat wounds are restricted and we chose medicinal plants over synthetic ones. Here in this review, we enlisted various medicinal plants as wound healers and this has gained much importance due to its various bioactive constituents present. This review article focuses on wound healing in different rat models by the use of various medicinal plants.

Keywords: Inflammation, Medicinal Plants, Rat Models, Wound Healing

INTRODUCTION

The complex organ known as skin covers the surface of the entire body. It protects harmful microorganisms and serves as a physical shield and barrier between the body and the external environment, stopping the loss of water and electrolytes. Due to inadequate healing and the possibility of microbial infections, which can occasionally be fatal, wound healing is a serious public health concern in developed countries. ^[1]

Physical injuries that cause the skin to break down or open up are called wounds. For the skin's impaired functional status and damaged anatomical stability to be restored, wounds must heal properly. A series of processes, including inflammation, cell migration, and proliferation, take place during the repair of damaged tissues.

1. As soon as an injury occurs, the inflammatory stage starts with vasoconstriction, which promotes homeostasis and releases inflammatory mediators.
2. Granulation tissue proliferation, mostly caused by fibroblasts and the angiogenesis process, ultimately defines the proliferative phase.

3. Reformulations and improvements in the collagen fiber's component parts that increase its tensile strength is what define the remodeling stage.^[2]

For thousands of years, people have used plant-based medicine to cure a variety of ailments. Medicinal plants are vital to healing and contain bioactive substances that are used to treat a variety of human disorders (Wadood et al. 2013; Putra et al. 2020; Nugroho et al. 2020). The bioactive component of medicinal plants has a bigger impact on how cells proliferate throughout the healing process of wounds (Lordani et al. 2018). Secondary metabolite-enriched medicinal plants may include endophytic microorganisms that contribute to the pathophysiology of certain diseases. In order to live and thrive in their natural habitat, these plants produce a wide range of physiologically active substances, some of which serve as defences against abiotic stressors such as temperature, water, mineral nutrient availability, and insect pests (Egamberdieva et al. 2017). Dermatological issues and cutaneous wound healing are the main uses for phyto-therapeutic substances. Essential oils found in medicinal plants can prevent a wide variety of harmful bacteria from growing (Akhtar et al. 2014). Because allopathic medicines are not only available to tribal communities, but their widespread use has long-term negative effects that are well-known (Kumarasamayraja et al. 2012). Researchers used the problem as a focus to find a different way to treat the wound infection—using the plant. Because plant compounds support the repair mechanisms in a very natural way, they may be able to cure wounds (Farah et al. 2018).^[3]

TYPES OF WOUNDS

Many factors can be used to categorise wounds. When it comes to wound healing and damage treatment, time is important. Therefore, depending on how long it takes for a wound to heal, it can be clinically classified as either acute or chronic.^[4]

1. **Acute wounds:** Acute wounds are defined as wounds that heal on their own and proceed according to a scheduled, systematic healing process, ultimately leading to both functional and anatomical restoration. Healing typically takes place within 30 days, or between 5 to 10 days.^[4]
2. **Chronic wounds:** Chronic wounds are ones that are unable to heal through the regular phases and cannot be effectively, rapidly, or cleanly repaired. Numerous factors that prolong one or more phases of homeostasis, inflammation, proliferation, or remodelling impair and disrupt the healing process. These elements consist of exudate, necrosis, tissue hypoxia, infection, and high concentrations of inflammatory cytokines.^[5]
3. **Complicated wounds:** Combining an infection with a tissue defect creates a unique wound known as a complex wound.^[4] A continuous threat to the wound is infection. On the other hand, the traumatic or post-infectious aetiology, or a large tissue resection (e.g. in cancer management), evolves as the cause of the problem.

The classification of wounds also considers the aetiology, level of contamination, morphological features, and communication with solid or hollow organs.^[4]

Based on the trigger component, aetiology divides wounds into:

- contusions,
- abrasions,
- avulsions,
- lacerations,
- cuts,
- stab wounds,

- crush wounds,
- shot wounds and
- burns.

Based on the degree of contamination, wounds are classified into three groups as follows:

1. aseptic wounds (bone and joint operations)
2. contaminated wounds (abdominal and lung operations) and
3. septic wounds (abscesses, bowel operations, etc).

In addition, wounds can be characterised as open, in which the underlying tissue is exposed but the skin layer has been injured or as closed, in which the underlying tissue has been traumatised but the skin has not been destroyed. [4,6,7,8]

PHYSIOLOGICAL MECHANISMS OF WOUND HEALING

The wound healing process can be divided into three phases: inflammatory, proliferative, and remodelling.

Inflammatory phase

The successive infiltration of neutrophils, macrophages, and lymphocytes is what defines the inflammatory response. Although these cells produce chemicals like proteases and reactive oxygen species (ROS), which can cause some damage, their primary role in the wound is the removal of microorganisms and cellular debris. Macrophages play a crucial part in the healing of wounds. When a wound initially appears, macrophages release cytokines that draw and activate leukocytes, which in turn sets off an inflammatory response. In addition to stimulating and eradicating apoptotic cells, such as neutrophils, macrophages also help to reduce inflammation. In order to enhance the environment and encourage keratinocytes, fibroblasts, and angiogenesis to support tissue regeneration, apoptotic cells undergo phenotypic changes. Macrophages facilitate the shift of the healing phase into the proliferative phase. T lymphocytes go into the wound after macrophages and inflammatory cells, peaking in the early remodelling or late proliferative phases.^[9]

T cells started to show up on the fifth day after the damage and continued to do so until the seventh day. Fibroblasts are impacted by the cytokines that lymphocytes produce, including fibroblast activating factor and IL-2. Additionally, T cells generate interferon- γ (IFN- γ), which prompts macrophages to release cytokines like TNF- α and IL-1. T cells are involved in the long-term repair of wounds.^[10] While some studies report that CD4+ cells (T helper cells) have a positive role in wound healing, others report that CD8+ cells have a positive role in wound healing. Still other studies link impaired wound healing to delayed T cell infiltration followed by a decrease in the concentration of T cells in the wound. (Cytotoxic-Suppressor T lymphocytes) are involved in preventing the healing of wounds. Research on mice with low levels of both T and B cells demonstrates a decrease in scar development. Gamma-delta T lymphocytes also control inflammation, pathogen defence, and tissue integrity, among other elements of wound healing. Because of their distinctive dendritic shape, these cells are also known as dendritic epidermal T-cells (DETC). In order to support keratinocyte proliferation and cell survival, dendritic epidermal T-cells are activated by stress, injury, or keratinocytes. They then create fibroblast growth factor 7 (FGF-7), keratinocyte growth factors, and insulin-like growth factor-1. Moreover, chemokines and cytokines that are involved in starting and controlling the inflammatory response during wound healing are induced by dendritic epidermal T-cells. Keeping DETC and keratinocytes in balance promotes healthy skin and wound healing. A DETC deficiency is associated with reduced keratinocyte proliferation in the wound and delayed wound healing. Moreover, chemokines and cytokines that are involved in starting and controlling

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Proliferative phase

During this phase, there is a significant increase in cell migration and proliferation as well as the production of granulation tissue, which is made up of fibroblasts, endothelial cells, macrophages, and extracellular matrix.^[11] To encourage the growth of fibroblasts, keratinocytes, and endothelial cells, injured cells release FGF, VEGF, EGF (epidermal growth factor), and TGF β 1 (transforming growth factor- β 1). In order to facilitate cell migration into the wound, fibroblasts also produce extracellular matrix components such as type III collagen, proteoglycans, and fibronectin.^[12,13] After an injury, vascularization in the wound starts right away. However, during the proliferative phase, it becomes more active to supply the oxygen and nutrients required for cell migration, proliferation, and the production of extracellular matrix components. The vascular system at the wound site is reorganised and endothelial cell proliferation is stimulated by secreted mediators including VEGF and angiopoietin. Re-epithelialization takes place during the proliferative phase to close the epithelial gap and reestablish the skin's defensive function.^[13, 14] Growth factors induce keratinocytes along the wound border, which lead to their proliferation and differentiation. This stimulus accelerates the migration of these cells across the extracellular matrix, causes keratinocyte adhesion molecules to be lost, and prevents physical interaction with desmosomes and hemidesmosomes.^[14,15]

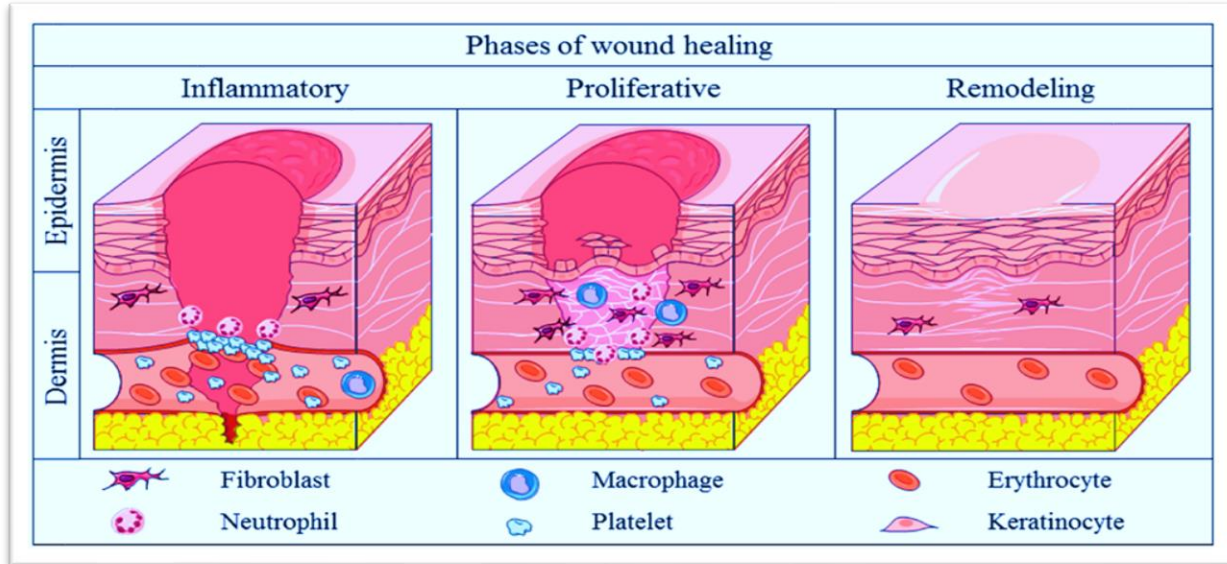
Final phase (Remodelling)

Wound healing proceeds into a remodelling phase following extracellular matrix formation and proliferation. Capillary regression takes place during this phase, allowing the wound's vascular density to recover to normal. In order to attain a normal tissue architecture, the extracellular matrix remodelling phase is the most crucial remodelling step. Additionally, wounds contract thanks to the presence of contractile fibroblasts, or myofibroblasts, within the wound. The function of stem cells in tissue regeneration and wound healing, with particular attention to adult stem cells including bone marrow (BM)-derived cells and epidermal stem cells (BMDCs). Hair follicles and the basal layer of the epidermis include epidermal stem cells, which elevate keratinocytes in preparation for their migration into the wound. Hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) are the two primary types of stem cells found in bone marrow. Different cell types, including adipocytes, osteoblasts, chondrocytes, fibroblasts, and keratinocytes, may be differentiated from BM-MSCs. Key players in neovascularization are endothelial progenitor cells (EPCs), which are generated from HSC. Both BM-MSC and EPC contribute to the healing of wounds. An essential part of the neovascularization process is wound-induced hypoxia, which acts as a catalyst for EPC mobilisation into the circulation.^[9]

Inflammatory phase: Acute inflammation and homeostasis of the wound area are caused by the local release of growth factors, cytokines, and leukocyte migration.

Proliferative phase: enhanced keratinocyte, fibroblast, endothelial cell, and leukocyte migration and proliferation within the wound. enhanced angiogenesis, re-epithelialization, and synthesis of extracellular matrix components.

Remodelling phase: remodelling of the extracellular matrix, wherein collagen I is substituted for collagen III. MMP activity has increased. Endothelial cells, fibroblasts, and myofibroblasts undergo transient apoptosis in response to damage. [16]



FACTORS AFFECTING WOUND HEALING

Local factors

Oxygen

Oxygen is essential for wound healing as well as for cell metabolism, which uses it to create ATP, that is how energy is produced in cells. Induced angiogenesis, increased keratinocyte differentiation, migration, and re-epithelialization, increased fibroblast cell proliferation and collagen synthesis, and wound contraction are all dependent on oxygen. [17,18] Furthermore, polymorphonuclear leukocytes' production of superoxide—a critical component of oxidative pathogen killing—depends heavily on oxygen. Hypoxia, the microenvironment in oxygen-deficient wounds, and higher metabolism can all lead to damaged blood vessels and high oxygen demand. Vascular abnormalities can be brought on by a number of systemic illnesses, such as diabetes and advanced age, which can lead to inadequate tissue oxygenation. Hypoxia can occur in a wound during the healing process due to inadequate perfusion. [19]

Poor oxygenation of wounds will prevent them from healing. While chronic and protracted hypoxia can impair wound healing, temporary hypoxia brought on by an accident can hasten the healing process. [17, 18]

Hypoxia in acute wounds can act as a signal to accelerate the healing process. Macrophages, keratinocytes, and fibroblast cells can all produce growth factors and cytokines in response to hypoxia. In response to hypoxia, several cytokines are released, such as promoter cells, which are crucial for angiogenesis, chemotaxis, cell migration, proliferation, and wound healing, as well as TGF- β , VEGF, TNF-, and endothelin-1. [18] Sufficient oxygen levels are necessary for the best possible wound healing. Because hypoxia can result in the release of growth factors and angiogenesis, which are necessary for the maintenance of the wound healing process, hypoxia promotes wound healing. [17]

Infection

When the skin is wounded, a localized bacterial infection may arise, delaying the healing process. [20] *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and other *Streptococcal* species account for the majority of bacteria that cause infection. The human body starts inflammatory processes in reaction to infection,

including leukocyte migration and cytokine release. On the other hand, leukocytes with phagocytic activity induce bacteria to release endotoxins, which leads to localized inflammation and necrosis because of elevated pro-inflammatory cytokines, elevated metalloproteinase activity, and reduced growth factor release.^[11, 21] The first biological process of healing is the inflammatory response. Chronic inflammation can delay wound retraction and tissue remodelling, interfere with re-epithelialization, and impede the healing process.^[9,11]

Systemic factors

Age: One of the main risk factors for poor wound healing is aging. Age-related changes in metabolism and the body can also result in a thinner layer of epidermis.^[22] The inflammatory response in the aged is altered in a number of ways, including a decrease in growth factor/cytokine production, a delay in leukocyte recruitment to the region, and a reduction in macrophage activity during phagocytosis.^[20, 22] Additionally, re-epithelialization, delayed angiogenesis, decreased fibroblast activity, and collagen remodelling can all be caused by aging.^[9]

Sex hormones: By lowering leukocyte infiltration and the production of pro-inflammatory cytokines, estrogen can have anti-inflammatory effects.^[23] Furthermore, keratinocyte and endothelial cell migration and proliferation can be influenced by estrogen molecules, which promotes angiogenesis and re-epithelialization during wound healing.^[24] Due to their persistent inflammatory effect on wounds, androgen hormones (testosterone and 5 α -dihydrotestosterone) might impede the healing process of wounds by increasing inflammatory cytokines and leukocyte migration..^[23,24]

Stress: Stress can impede the healing of wounds and a number of systemic illnesses by disrupting the balance of endocrine hormones. Because stress affects the hypothalamus and neurological system, it can cause an increase in the release of cortisol, glucocorticoids, adrenaline, and norepinephrine.^[9, 25] These compounds have the ability to reduce leukocyte immunological response and cytokine release, which can disrupt inflammatory processes and slow the healing of wounds.^[25]

Diabetes: Impaired wound healing is one of the multiple systemic diseases associated with diabetes mellitus. Chronic inflammation is caused by this condition, which alters leukocyte migration and activation and can increase the release of pro-inflammatory cytokines. Diabetes can also cause a hypoxic environment and reduced angiogenesis in the skin microvasculature. Furthermore, this illness may alter keratinocyte and fibroblast proliferation and differentiation, which would postpone extracellular matrix remodelling and re-epithelialization.^[22]

Obesity: Being obese is a chronic illness that impedes wound healing in a number of ways. Obese wounds frequently include pressure and venous ulcers linked to hematoma, edema, seroma development, and local infection.^[26,27] Obesity-related cellular and molecular factors linked to poor wound healing include reduced immunological response, increased release of pro-inflammatory cytokines, and decreased skin microperfusion.^[9,26]

Drugs: Numerous medications have the ability to impede the process of wound healing by disrupting the coagulation cascade, inflammatory pathways, or the proliferation of cells.^[9] Corticosteroids are commonly used as both an anti-inflammatory and an immune response modulator. However, their systemic anti-inflammatory effects may cause a reduction in growth factors and cytokines, which may affect wound healing mechanisms and fibroblast proliferation.^[28] In addition to being used systemically to treat pain and inflammation, non-steroidal anti-inflammatory medicines (NSAIDs) have been shown to have a deleterious effect on wound healing. This is because they inhibit fibroblast proliferation, cause wound

retraction, and slow down angiogenesis. NSAID compositions applied topically may accelerate wound healing and lessen localized pain. [30,29]

Alcohol: Drinking alcohol, whether acutely or chronically, can hinder the healing of wounds.^[9] Suppression of host immunity and increased vulnerability to infection are two of the processes involved. Research indicates that alcohol use before the onset of inflammation reduces neutrophil recruitment and activity as well as pro-inflammatory cytokines. Later in the healing process, however, alcohol consumption increases leukocyte and cytokine levels.^[27] Furthermore, alcohol use affects the proliferative phase by expressing VEGF receptors, which reduces angiogenesis in the wound area.^[9,27] Consequently, oxidative stress molecules and free radicals develop in the wound region, creating a hypoxic environment. Additionally, drinking alcohol affects collagen synthesis, remodelling processes, and extracellular matrix metalloproteinase concentrations.^[9]

Smoke: Smoking has been linked to a higher chance of developing a number of illnesses, including poor wound healing. Research indicates that the constituents included in tobacco cigarettes, namely nicotine, carbon monoxide, and hydrogen cyanide, have the potential to impact the processes involved in wound healing.^[31] One of the primary mechanisms linked to smoking is hypoxia, which can hinder the healing of wounds and reduce angiogenesis, blood flow, oxygenation, erythrocyte proliferation, and wound oxygenation.^[9] In addition to raising blood viscosity and platelet aggregation and adhesiveness, smoking raises the risk of thrombosis and embolism. In addition, substances included in cigarettes may potentially inhibit fibroblast migration, proliferation, and collagen remodelling.^[9,27] Smoking affects the immune system, increasing the risk of infection by decreasing the function of neutrophils, macrophages, and lymphocytes.^[9,31]

TREATMENT OF WOUNDS

Although the body naturally heals itself from tissue damage, the process is extremely sluggish and there is a significant risk of microbial infection. This increases the need for a drug that quickens the healing process.^[32] Various treatments have been applied both locally and systemically to promote wound healing. Antibiotics and antiseptics, desloughing agents (chemical debridement, such as hydrogen peroxide, eusol, and collagenase ointment), wound healing promoters, certain materials like tissue extracts, vitamins, and minerals, and a variety of plant products are among the various agents used to promote wound healing. Due to their adverse effects, several synthetic medications have restrictions. By using them, wound healers reduce the need for additional medications such as antibiotics and decrease the probability of their negative effects (Lazarus, et al., 1994).^[32] The use of medicinal plants for wound healing has gained popularity throughout time due to its decreased adverse effects and ability to control wounds.^[33]

Medicinal plants help treat wounds by quickening the healing process, preventing infection, and encouraging blood clotting. It can be said that plants and plant-derived chemicals enhance therapy and control the healing of wounds^[33]. Medicinal herbs have a variety of processes by which they influence wound healing, including changes in collagen deposition, bacterial count reduction, fibroblast and fibrocyte stimulation, and more. Tribals and folklore traditions in India employ a wide variety of botanicals, plant extracts, decoctions, and pastes to cure burns, wounds, and cuts.^[32]

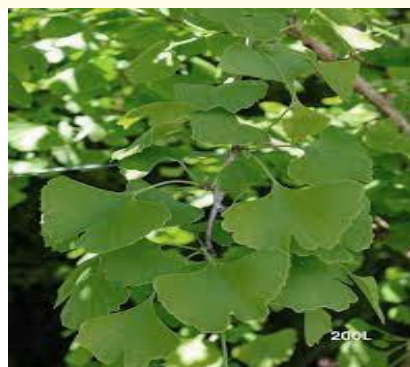
Commonly used medicinal plants as wound healers:

Aloe vera



The perennial herb *Aloe Vera*, sometimes referred to as Kumari, is a member of the Liliaceae family. This plant, sometimes known as the plant of immortality or the lily of the desert, is endemic to Africa. Aloe vera extract has several advantageous qualities that can improve mature granulation tissue, reduce inflammation, and hasten the healing of wounds [34]. Additionally, it lowers blood sugar, which is advantageous for diabetic wounds [35]. Gels with a 96% water content, essential oil, amino acids, minerals, vitamins, enzymes, and glycoproteins have long been known to exist. Additionally, because aloe vera extract has anti-inflammatory properties, it aids in the healing of wounds. Because tannic acid and a certain kind of polysaccharide are present in aloe vera extract [36] that help in healing wounds. By reducing the inflammatory phase and providing more mature granulation tissue, aloe vera extract has been shown to have positive benefits on wound healing. This ultimately encourages healing and may result in a sound, well-remodelled scar. [34] Because alkaloids and indoles are among the components that give aloe vera leaf gel its antioxidant qualities, it helps promote wound healing. [37] Spectrophotometric tests reveal the presence of phytosterols, indoles, and non-flavonoid polyphenols in aloe vera. Additionally, these substances exhibit antimicrobial qualities that can slow down the healing process in infected wounds.

Ginkgo biloba



It's a member of the Ginkgoaceae family and is also referred to as the Kew tree. It is extensively grown in China and Korea. Vegetative mechanisms and seeds are two types of propagation. It has been discovered that *Ginkgo biloba* significantly affects male rat models of excision wounds and dead space. A 50 mg/kg dose has been reported to decrease the epithelization duration in the excision wound model and to have considerably increased the breaking strength and hydroxyproline content of granulation issues in dead space wounds (Bairy et al., 2001). It is also stated that the reason for G.B.'s effectiveness is because of its high amino acid content, which enters the bloodstream quickly and, when combined with vitamins, delivers vital nutrients to the wound site to aid in healing. [32]

Cinnamon



Cinnamomum verum, is a member of the Lauraceae family. In conventional medical systems, cinnamon has long been employed. Cinnamon bark is utilized as a flavoring, condiment, and spice. Antioxidant, antiulcer, antibacterial, antidiabetic, hypoglycemia, hypolipidemic, and anti-inflammatory activity are some of its characteristics^[38], which can be helpful for diabetic and infected wounds, among other types of wounds. In addition to the previously listed qualities, cinnamon has a high concentration of polyphenols, which may improve an animal's ability to absorb glucose.^[39] In mouse adipocytes, it raises the amounts of the glucose transporters-1 (GLUT-1) mRNA.^[40] Research has demonstrated that the antioxidant qualities of cinnamon alcoholic and aqueous extracts speed up the healing of wounds^[41, 42]. However, the anti-inflammatory properties of cinnamon, such as quercetin, 2-hydroxycinnamaldehyde, and cinnamyl, can hasten the healing of wounds.^[43]

Centella asiatica



Centella asiatica is a little trailing herb that typically grows abundantly in moist environments and bears white to crimson blooms. It is commonly referred to as "Brahmi" and is spread through the use of seeds and vegetables. According to clinical trials, when *Centella asiatica* aqueous extracts are applied topically to open wound sites three times a day for a period of twenty-four days, the products (ointment, cream, and gels) exhibit positive results. In comparison to the control wound, the treated wound epithelized more quickly and contracted at a higher rate. When compared to the other two formulations, the gel formulation yields superior outcomes (Kumar et al., 1998). According to reports, asiaticosides and madicassoides are the active ingredients in *Centella asiatica* that give it its characteristic properties (Shetty et al., 2006).^[32]

Curcuma longa



Curcumin, is a constituent that is bright yellow, comes from *Curcuma longa* L. (turmeric) plants of the Zingiberaceae family. Scientists Vogel and Pelletier of Harvard College's laboratory discovered curcumin in the rhizomes of *Curcuma longa*, or turmeric, over 200 years ago^[44]. Turmeric has been used in

traditional herbal therapy to treat gastrointestinal and skin inflammation, as well as weight loss ^[45]. Three bioactive curcuminoids found in turmeric—curcumin, desmethoxycurcumin, and bisdemethoxycurcumin—have been demonstrated to have anti-inflammatory, anti-cancer, and anti-aging effects. ^[46] A prior study demonstrated that mice's wounds healed quickly after topical Curcumin treatment, forming well-formed granulation tissue primarily made of deposited collagen and renewing epithelium. Additionally, by regulating the quantities of several cytokines, curcumin administration dramatically decreased matrix metalloproteinase-9 and tumor necrosis factor alpha and accelerated the healing of wounds in mice ^[47]. Combining curcumin and ginger extract improves skin function and wound healing in hairless rats whose skin has been damaged by corticosteroids. This reduces the likelihood of non-healing wounds developing. ^[48]

Calendula officinalis



In ethnopharmacology, flower extracts from *Calendula officinalis*, sometimes known as pot marigold, have a long history. Lipophilic and aqueous *Calendula* alcoholic extracts are traditional remedies for mild skin inflammation and for accelerating the healing of small wounds ^[49]. The most notable pharmacological effects associated with extracts of *C. officinalis* are its anti-inflammatory, anti-edematous, antioxidant, antibacterial, antifungal, and immunostimulant qualities. The *C. officinalis*' plant is composed of terpenoids, flavonoids, phenolic acids, carotenoids, coumarins, quinones, volatile oils, amino acids, and lipids as constituents. ^[50] Additional pharmacological characteristics of *C. officinalis* include wound healing abilities, antimicrobial and antiviral properties, potent anti-gastric ulcer activity, antioxidant and anti-immunomodulatory activity, effective treatment for breast cancer, treatment of acne, treatment of bacterial infections in animals, and hepatoprotective and reno-protective activity ^[51]. The benefits of *calendula officinalis* on wounds from rat excision were investigated both topically and orally. The findings demonstrated that the extract-treated group had a 90.0% wound closure rate on the eighth day following the formation of the wound (as opposed to the control group's 51.1% wound closure), and that the extract-treated group's hydroxyproline and hexosamine contents were significantly higher than those of the untreated group ^[52]. Furthermore, as calendula ointment greatly expedites the healing of cesarean wounds, it can be utilized to speed up the recuperation process following a cesarean section. ^[53]

Eucalyptus



Plantations of eucalyptus reduce demand on tropical forests and associated biodiversity while producing high-quality woody biomass for a range of industrial uses ^[54]. Eucalyptus is considered a very successful replanting tree species because of its rapid growth and exceptional adaptation to a variety of situations ^[55]. Numerous species of Eucalyptus plants exist ^[56]. Worldwide, people have used the essential oil derived from Eucalyptus globulus leaves as an antiseptic and to relieve the symptoms of various ailments, including sore throats, coughs, and colds ^[57]. Rats were used to study wound healing, estimate collagen, and assess histology using an improved nano-emulsion of Eucalyptus essential oil (EEO) as opposed to pure EEO and ordinary gentamycin. Rats treated with the improved EEO nano-emulsion demonstrated significant wound healing activity. ^[58] According to additional research, in order to promote greater cell proliferation and the best outcomes for wound healing, eucalyptus alba leaves should be dried at a temperature of no more than 30 °C and extracted in ethanol. ^[59].

Trigonella foenum



Trigonella foenum-graecum, often known as fenugreek, is widely used in Ayurvedic medicines and is well recognized for its hypocholesterolaemic and antiulcer properties. *Trigonella foenum-graecum*, also known as fenugreek, is frequently used in culinary preparations and as a spice. It is well known that fenugreek has hypoglycemic properties. ^[60] Certain polysaccharides, including diosgenin, yamogenin, gitogenin, tigogenin, and neotigogens, are present in fenugreek seeds. Steroid effects produced by saponins have the ability to reduce inflammation in the body. Additional bioactive components of fenugreek include alkaloids, flavonoids, volatile oils, mucilage, and amino acids. 4-hydroxyisoleucine is fenugreek's other active component. According to reports, fenugreek reduces inflammation by releasing an anti-inflammatory chemical into the wound area. ^[61] Furthermore, fenugreek's antibacterial qualities might boost its anti-inflammatory effects. According to a study, the antibacterial qualities of flavonoids and triterpenoids may facilitate the healing of wounds ^[62]. It is well known that fenugreek contains antioxidants, which help hasten the healing of wounds ^[63]. Use of fenugreek seed topically predominantly improved the kinetics of wound contraction and epithelialization. ^[64].

Tulsi



This extract is made from the *Ocimum sanctum* plant, which is a member of the Labiatae family. It is frequently planted in gardens and is grown all over the world. *Ocimum sanctum* has historically been used to treat liver infections, stomach problems, and malarial fevers. The leaves of *Ocimum sanctum* are also used to treat earaches, ringworm, bronchitis, and other cutaneous conditions. The leaves are used to improve memory and as a nerve tonic. Along with the volatile oil, *Ocimum sanctum* leaves also contain alkaloids, glycosides, and saponins, in addition to an abundance of tannins such as gallic acid and chlorogenic acid. The primary active ingredient found in holy basil leaves is ursolic acid. According to Udupa et al. (2006), it has 70% eugenol, carvenol, and eugenol-methyl-ether.^[32]

Neem



Neem alcoholic extract helps with ringworm, scabies, and eczema. Seed oil and extracts from neem leaves have been shown to have antimicrobial properties. This prevents microbes from causing secondary infections to any wounds or lesions. Additionally, neem reduces inflammation just as well as cortisone acetate, according to clinical research, which speeds up the healing of wounds. Margosic acid, butyric acid, glycerides of fatty acids, and trace amounts of valeric acid are all present in neem oil. Neem alcoholic extract helps with ringworm, scabies, and eczema. Seed oil and neem leaf extracts have been shown to have antibacterial properties. This prevents bacteria from causing subsequent infections to any wounds or lesions. Additionally, neem reduces inflammation just as well as cortisone acetate, according to clinical investigations; this effect hastens the healing of wounds (Raina et al.2008).^[32]

St. John wort



Known commonly as St. John's Wort, *Hypericum perforatum* is a perennial blooming plant that has been used for a long time in traditional medicine. It belongs to the family Hypericaceae. Fresh tincture or tea are popular uses for the flowering tops. Rats' wounds are said to heal when *Hypericum perforatum*'s methanolic extract is applied. The extract was tested in rat excision and incision wound models in the form of ointment (5% w/w and 10% w/w o arial portion). When compared to the control, both ointment concentrations demonstrated a notable response in both types of wounds. The results are similar to those of conventional medications (Mukherjee et al., 2000, Harsh et al., 2000).^[32]

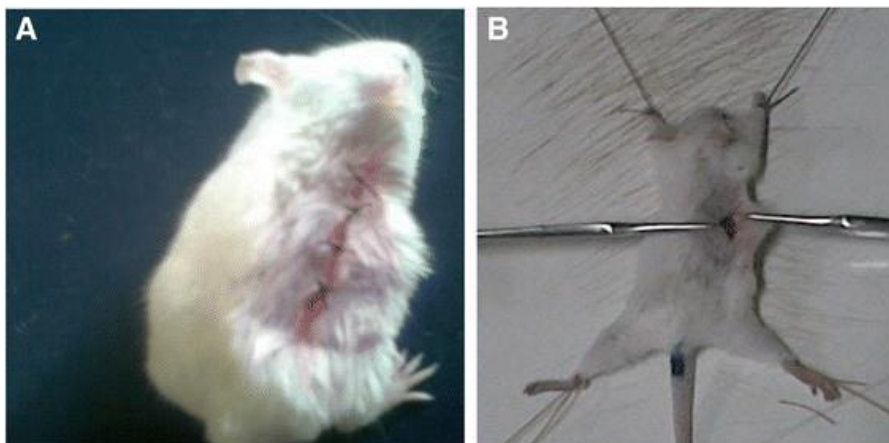
IN-VIVO MODELS FOR WOUND HEALING

In vivo models entail inflicting wounds on lab animals and tracking the healing process. Modifications to the wound environment that are physical, chemical, or biological can also be included.^[65,66] Experiments on cutaneous wounds are conducted on a variety of animals, including pigs, rabbits, and rodents (rats, mice).

Because they provide a realistic depiction of the wound environment, encompassing different cell types, environmental cues, and paracrine interactions, in vivo models continue to be the best predictive models for researching wound healing.^[67] The model selected should take into account factors like the lesion's precise reproducibility, the ability to obtain multiple biopsy samples, the possibility of multiple investigations, compatibility with animal facilities, ease of handling, and the amount of time needed to obtain meaningful results.^[68] A model that accurately captures some features of human physiology without requiring human subjects for testing is the ideal scenario.^[69] Because small animals heal more quickly than humans do, experiments with them often last for days rather than the weeks or months that human research do. Rats and mice are the most commonly used species.^[70]

The in-vivo models for wound healing in rats is categorised into three:

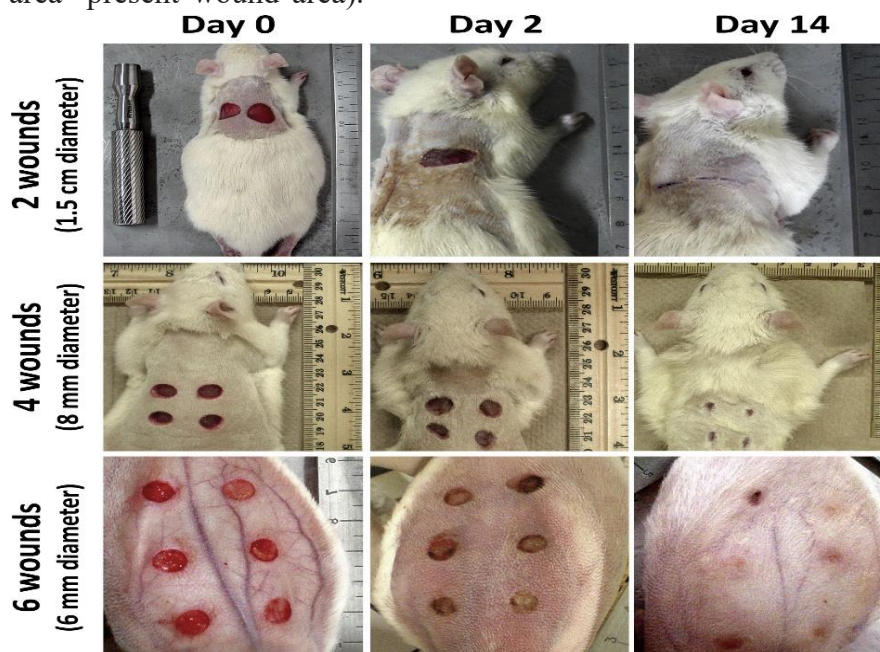
Incision Wound Model: After administering anaesthesia, two parallel, six-centimetre paravertebral incisions were made through the entire thickness of the skin, one centimetre laterally to the midline of the spinal column^[71]. Incisions were sealed using surgical sutures spaced 1 cm apart. On the seventh day following the wound, the sutures were taken out. On the tenth day following the injury, rats under anaesthesia had their wound breaking strength (WBS) tested. Three millimetres distant from the wound, a line was drawn on either side of the incision line. The line facing each other was securely gripped with two Allis forceps. One forceps was fixed, while the other was attached to a lightweight, graded polypropylene container that was suspended freely by a line that was run over to a pulley. The container was filled gradually and steadily with standard weights. Weight was gradually applied to the incision site, causing the margins of the wound to separate. The weight was halted and recorded as soon as the wound started to open.



Incision wound creation (a) and tensile strength measurement (b): Tensile strength was assessed using a continuous water flow approach on the tenth post-wound day, after the sutures were taken out on the seventh post-incision day.

Excision Wound Model: Rats were dosed with ketamine (30 mg/kg, *i.p*) to induce unconsciousness, and a standard ring was used to mark an area on the rat's back that measured roughly 500 mm². The marked skin was then gently sliced across its whole thickness. On 1 mm² graph paper, wounds were traced on the day they were inflicted, then every 4 days until the 14th day, and then on alternate days until healing was finished. The following formula was used to calculate the rate of wound contraction based on regular measurements of changes in the wound area. By comparing the test groups healed wound areas on different days with the control group's healed wound areas, one can determine the significance of the test groups' wound healing. Furthermore, noted was the epithelization phase, or the day the eschar fell and the scar area. [71]:

$\% \text{ wound contraction} = \frac{\text{Healed area} - \text{Total wound area}}{\text{Healed area} - \text{original wound area}} \times 100$



Dead Space Wound Model: Rats were treated with ketamine anaesthesia, and the dorsal lumbar region of their backs was incised by 1 cm. In the dead area of the rat's lumbar region, two 0.5 × 2.5 cm² polypropylene tubes were inserted into each side, and the incisions were sutured shut. The animals had been killed on the tenth day following the wounding, and the granulation tissue that had developed on and around the implanted tubes was systematically removed, weighed, and processed in order to estimate the levels of antioxidants, free radicals, and collagen tissue characteristics. [71].

CONCLUSION

Wounds are the physical injuries to skin that involves tearing, puncturing of the skin. In this review, we explained the wound healing process and the factors affecting that in detail which shows that healing is slow, delayed and complicated. So, to fasten the process of healing “wound healers” are essential. Considering the main disadvantages of synthetic substances, plants a natural gift with traditional knowledge of use make good raw materials for treating a wide range of illnesses and ailments. There are a number of plants/plant extracts in the form of different formulations which are used traditionally by tribal and old people in India for the treatment of cuts and wounds. This review is focused on various

medicinal plants and their pharmacological actions acting as wound healers in human and animals i.e. on rats. They have been used in different rat models to heal the wounds. Similar to the allopathic medical system, there are wound healers on hand; nevertheless, traditional knowledge, found in literature, offers numerous home remedies and customs for the same objectives. Plant-based natural compounds may be used to find some new therapeutics for wounds, according to preliminary scientific research.

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