SYSTEMATIC REVIEW

A Review of Wound Healing Mechanisms of Natural Products in Keratinocyte Cells [version 2; peer review: 1 approved with reservations, 2 not approved]

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Abstract

Background: Wound-healing in the skin is one mechanism that maintains homeostasis. Ineffective skin wound healing is a significant health problem that ultimately causes morbidity and mortality. The process of wound healing using traditional medicine has a good effect with various mechanisms of action. This review aims to provide insights related to the wound-healing mechanisms of several plants in HaCat cells.

Methods: The literature study method was used from primary and secondary libraries. The library search was conducted using online-based library search instruments from 1988 to 2021, such as NCBI-PubMed, Google Scholar, and Elsevier.

Results: The wound-healing mechanism includes processes that restore skin integrity through four stages: hemostasis, inflammation, multiplication, and remodeling. Many plants have been studied to have activity in wound healing by various mechanisms.

Conclusions: Several compounds in plants have shown the activity in wound healing in keratinocyte cells by various mechanisms.

Keywords

Wound-healing, Keratinocyte, Natural Products

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Introduction

Skin is the human body’s largest organ, about 15% of the body weight consisting of the epidermis, dermis, and subcutaneous (Figure 1). The epidermis is the outermost layer of the skin and maintains a vital barrier against external trauma. The main cellular content of the epidermis is keratinocytes (about 95% of the epidermis), and fibroblasts are the main cellular components of the dermis. The epidermis, mainly composed of keratinocytes, is classified into stratum corneum, granular layer, spinous layer, and basal layer, based on the stages of keratinocyte differentiation. Keratinocytes have an essential role in inflammation. The skin’s primary function is to protect the body from exogenous factors by forming a protective barrier that covers the body; therefore, any injury or damage to the skin must be repaired immediately to provide continuous protection to our body systems. The skin protects against environmental factors such as harmful UV rays and pathogens and prevents water loss.

Wound healing is one of the functions of skin. Wound healing maintains homeostasis. The physiological healing system involves four stages, which include homeostasis (blood clotting), inflammation, proliferation (new tissue growth), and maturation (remodeling). A wound-healing response is initiated when the epidermis is injured. Keratinocytes, the main cellular component of the epidermis, are responsible for restoring the epidermis after injury through epithelialization. Epithelialization is an essential component of wound healing and is a critical success parameter. Epithelialization is defined as the process of covering a denuded epithelial surface. The cellular and molecular processes involved in initiating, maintaining, and completing epithelialization are critical to successful wound closure. Without re-epithelialization, the wound cannot be considered healed. The successful step of re-epithelialization is considered an essential indicator of wound closure to prevent further infection and chronic wound development. Re-epithelialization is triggered to restore the damaged epidermis in response to skin damage. The most important cell types responsible for re-epithelialization are keratinocytes, which proliferate, differentiate, and migrate to heal open wounds.

The study of folklore medicine is recognized as one way to explore medicine’s potential in the future. Researchers have identified 122 compounds used in primary medicine derived from “ethnomedical” plant sources, and 80% of these compounds are used or closely related to traditional ethnomedical uses. Because the skin healing process is quite complex, there are limits to treating skin wounds entirely with a single compound. Thus, developing wound healing agents with natural ingredients can be an option for skin wound treatment. Using natural products as a wound healing agent has several advantages, such as low cost and high safety compared to other synthetic products. A large number of plants have been used, by tribes and folklore, in many countries to treat wounds and burns. The wound healing process is promoted efficiently using traditional medicine, mainly sourced from plants. These drugs have been shown to affect one or more stages of the healing process. In this context, traditional medicine provides a comprehensive source

![Figure 1. The structure of skin.](image-url)
for discovering native bioactive compounds and developing new pharmaceutical applications. Especially for the treatment of plant origin, both topical and systemic herbal medicines have been widely used in wound healing. Several properties, including anti-inflammatory, antioxidant, and antimicrobial activity, are required to be effective wound-healing agents. Thus, any herb with these properties should be investigated and used to develop effective wound-healing agents. Various health-supporting constituents in this plant have attracted scientists to examine it to know its potential wound-healing properties.

The epidermis is mainly composed of keratinocytes. Keratinocytes are known to have an essential role in inflammation. Keratinocytes are also a significant source of inflammatory mediators, including a group of the tumor necrosis factor (TNF)-α and interleukin (IL) families. Overproduction of pro-inflammatory mediators can lead to abnormal inflammatory responses. Therefore, in vitro studies of human skin’s epidermis and dermis have been commonly performed using HaCaT cells. HaCaT cells are immortalized human keratinocytes used to study dermatological conditions such as contact dermatitis, psoriasis, or skin cancer due to their high availability and ease of cell culture. HaCaT is the primary epithelial cell line from adult human skin to show normal differentiation and provides a promising tool for studying the regulation of keratinization in human cells. The HaCaT cell line has an altered phenotype in vitro (clonogenic on plastics and agar) but remains nontumorigenic. Despite the altered and unrestricted growth potential, HaCaT cells, similar to normal keratinocytes, reform the structured and differentiated epidermal tissue in an orderly manner when transplanted into model mice. Therefore, this review article aims to determine the wound-healing mechanism of several plant extracts on HaCaT cells.

Methods

Protocol and registration
The results are reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Scoping reviews purpose to; establish evidence available, elucidate key ideas, establish how research is done and determine knowledge gaps for a particular topic.

Eligibility criteria
Articles were included based on the following criteria:

1. Problem: Papers that match the research topic, namely the molecular mechanism of wound-healing of several compounds and extracts in HaCaT cells
2. Intervention: The studies using an in vitro approach
3. Outcome: Plants with wound-healing activity in HaCat cells
4. Design study: Literature non-experimental dan systematic review
5. Year published: 1988 - 2021
6. Language: English

Articles were excluded based on the following criteria:

1. Problem: Papers that were not matched with the topic, namely the molecular mechanism of wound-healing of several compounds and extracts in HaCaT cells
2. Intervention: The studies using an in vitro and in vivo approach
3. Outcome: Plants with no activity as wound healing agents in HaCat cell lines
4. Design study: Experimental
5. Year published: -
6. Language: non-English
Information sources
Articles relevant to the study were searched and retrieved electronically from PubMed (https://pubmed.ncbi.nlm.nih.gov/advanced/) and Google Scholar (https://scholar.google.com/) using advanced search builders. The search from the databases was lastly collected on 10th December 2021.

Search strategy
An advanced search in three databases, NCBI-PubMed, Google Scholar, and Elsevier, was searched to identify peer-reviewed articles on wound-healing and wound-healing mechanisms using compounds and extracts against HaCaT cells. Specifically, the search queries consisted of relevant medical subject titles (MeSH) and relevant keywords. The Search terms included:

Wound-healing AND HaCaT Cells AND Compounds AND Extracts

Selection of sources of evidence
The articles obtained were further verified using the search strategy and filters outlined in the search strategy section. Assessment of the resulting articles was done independently by all the reviewers. Disagreements between them were resolved through consensus. First, articles from the initial search were obtained. Duplicate references were removed through manual deduplication. The titles and abstracts of the retrieved articles were screened for relevance to the study topic. Full-text reports were examined for compliance with eligibility criteria.

Data chatting process
Data from the evidence sources was assessed independently and then discussed by the team to reach a consensus. The information abstracted was as shown in the table.

Data items
The selection of the review articles based on the molecular mechanism of action as the primary outcome domain was guided by the following items:

1. Title of study
2. Year of publication – 1988 - 2021
3. Study objectives – wound-healing mechanism in HaCaT cells
4. Study design – review
5. Results – summary of findings on molecular mechanisms
6. Discussion – detailed explanation of the results and limitations of the review
7. Conflict of interest – Authors declare no conflict of interest

Synthesis of results
Results from the selected articles were tabulated in the summary of findings. The methodologies and molecular mechanisms of action (interventions) were summarized.

Results
Wound-healing
Wound healing is a complex biological mechanism involving cellular interactions between cells, including smooth muscle cells, fibroblasts, endothelial cells, myofibroblasts, keratinocytes, and immune cells. Wound-healing processes restore skin integrity through four stages: The coagulation phase and forming of a platelet scab to cover the wound opening to prevent further blood loss or entry of pathogens. The inflammatory phase is the flow of inflammatory cells to the wound site for protection against pathogens and activates skin cells. During this phase, neutrophils and macrophages are activated by releasing pro-inflammatory cytokines such as Interleukin-1β, 6, 8, Tumor necrosis factor (TNF), and growth factors such as Platelet-derived growth factor (PDGF), Transforming growth factor (TGF-α, β, 1) and Fibroblast growth factor (FGF) (Figure 2). During the proliferation phase, skin cells multiply rapidly to replace lost cells. Restoring the basal keratinocyte layer in the basement membrane between the epidermis and the dermis begins to
proliferate through various signaling molecules during the proliferative stage. When a certain level of repair is reached, the cytoplasmic shape of the keratinocytes is altered to move to the upper layers of the epidermis, differentiate, and transform through different cell layers to reach the final maturation stage. Thus, the proliferation and migration of keratinocytes suture the wound site during wound healing.\textsuperscript{11} Remodeling Phase, In this phase, fibroblast and a vascular density decrease, old collagen fibers from the initial scar are replaced with matrix, and new collagen fibers are synthesized to form new tissue.\textsuperscript{12,20,21}

\textbf{Inflammation}

Tissue injury causes disruption of blood vessels and extravasation of blood. It will require hemostasis and provides a temporary extracellular matrix for cell migration by blood coagulation. Coagulation pathways activate complement pathways. In addition, the injured or activated parenchymal cells produce several vasoactive mediators and chemotactic factors. These substances carry inflammatory leukocytes to the site of injury. Neutrophil infiltration clears the wound site of foreign particles and bacteria and is then extruded with eschar or phagocytized by macrophages. Macrophages bind to specific extracellular matrix proteins by their integrin receptors, stimulating the phagocytosis of microorganisms and extracellular matrix fragments by macrophages. The extracellular matrix also stimulates monocytes to undergo metamorphosis into inflammatory macrophages and express colony-stimulating factor 1, a cytokine required for the survival of monocytes and macrophages; tumor necrosis factor-\(\alpha\), a potent inflammatory cytokine; and platelet-derived growth factor, a potent chemoattractant and mitogen for fibroblasts. Other important cytokines expressed by monocytes and macrophages are transforming growth factor \(a\), interleukin-1, transforming growth factor \(b\), and insulin-like growth factor I. Monocyte and macrophage-derived growth factors are required to initiate and propagate new tissue formation.\textsuperscript{22}

\textbf{Epithelialization}

Wound re-epithelialization begins within hours of injury. Epidermal skin cells, such as hair follicles, rapidly remove clotted blood and damaged stroma from the wound site. At the same time, the cells undergo phenotypic changes characterized by intracellular tonofilament retraction, dissolution of cellular desmosomes, which provide physical connections between cells, and formation of peripheral cytoplasmic actin filaments, which allow cell movement. Furthermore, the epidermal and dermal cells no longer adhere to each other due to the severance of the connection between the epidermis and the basement membrane, which allows lateral movement of the epidermal cells. Integrin receptors’ expression on cells interacts with various extracellular matrix proteins (e.g., fibronectin and vitronectin) with type I collagen in the wound stroma and thus form a fibrin clot. Plasminogen activator activates collagenase (matrix metalloproteinase 1) and facilitates the degradation of collagen and extracellular matrix proteins. One to two days after injury, epidermal cells at the wound margins begin to proliferate behind actively migrating cells. The absence of neighboring cells at the wound edges may signal migration and proliferation of epidermal cells. As re-epithelialization occurs, basement membrane proteins reappear in a highly ordered sequence from the wound edge inward, like a zipper.
Epidermal cells return to their normal phenotype, firmly adherent to the regenerated basement membrane and the underlying dermis.22

**Formation of granulation tissue**

New stroma, often called granulation tissue, begins to invade the wound site approximately four days after injury. Many new capillaries give new stroma with their granularity. Macrophages, fibroblasts, and blood vessels move into the wound site at the same time. Macrophages provide a source of growth factors needed to stimulate fibroplasia and angiogenesis, fibroblasts produce new extracellular matrix needed to support growth into cells, and blood vessels carry oxygen and nutrients needed to maintain cell metabolism. Primarily platelet-derived growth factor and transforming growth factor b1, together with extracellular matrix molecules, stimulate tissue fibroblasts around the wound to proliferate, express integrin receptors, and migrate into the wound site. The structural molecules of the newly formed extracellular matrix, called the transient matrix, contribute to the formation of granulation tissue by providing a scaffold or channel for cell migration. These molecules include fibrin, fibronectin, and hyaluronic acid. Movement of cells into the blood clot from fibrin crosslinks or into the extracellular matrix requires an active proteolytic system that can open pathways for cell migration. In addition to serum-derived plasmin, various fibroblast-derived enzymes include plasminogen activator, collagenase, gelatinase A, and stromelysin. After migrating into the wound, fibroblasts initiate extracellular matrix synthesis. The collagen matrix gradually replaces the temporary extracellular matrix. Once an abundant collagen matrix has been deposited in the wound, fibroblasts stop producing collagen, and the fibroblast-rich granulation tissue is replaced by relatively acellular scar tissue. Cells in the wound undergo signal-induced apoptosis.22

**Neovascularization**

Angiogenesis is a complex process that depends on the extracellular matrix in the wound bed and the migration and mitogenic stimulation of endothelial cells. Induction of angiogenesis is associated with acidic or basic fibroblast growth factors. Furthermore, many other molecules were also found to have angiogenic activity, including vascular endothelial growth factor, transforming growth factor b, angiogenins, angiotropins, angiopoietin 1, and thrombospondins. Low oxygen tension and increased lactic acid can also stimulate angiogenesis. The mentioned molecules induce angiogenesis by stimulating the production of essential fibroblast growth factors and vascular endothelial growth factors by macrophages and endothelial cells. Activated wound epidermal cells secrete large amounts of vascular endothelial cell growth factor. Essential fibroblast growth factor regulates the site for angiogenesis during the first three days of wound healing. In contrast, the vascular endothelial cell growth factor is critical for angiogenesis during granulation tissue formation at days 4 to 7. Cell disruption and the production of vascular endothelial cell growth factor by epidermal cells is stimulated by hypoxia. Proteolytic enzymes released into connective tissue degrade extracellular matrix proteins. These protein fragments carry peripheral blood monocytes to the site of injury, where macrophages are activated and release angiogenic factors. Certain macrophage angiogenesis factors, such as essential fibroblast growth factors, stimulate endothelial cells to release plasminogen activators and procollagenase. The plasminogen activator converts plasminogen to plasmin and procollagenase to activate collagenase, and together these two proteases digest the basement membrane. Fragmentation of the basement membrane allows endothelial cells stimulated by angiogenesis factors to migrate and form new blood vessels at the injury site. Once the wound is filled with new granulation tissue, angiogenesis stops, and many new blood vessels are destroyed due to apoptosis. This programmed cell death is regulated by various matrix molecules, such as thrombospondins 1 and 2, and antiangiogenic factors, such as angiostatin, endostatin, and angiopoietin 2.22

**Wound contraction and extracellular matrix reorganization**

Wound contraction involves complex interactions of cells, extracellular matrix, and cytokines. During the second week of healing, fibroblasts assume a myofibroblast phenotype characterized by extensive collections of actin-containing microfilaments placed along the cytoplasmic surface of the cell plasma membrane. The appearance of myofibroblasts corresponds to the initiation of connective tissue compaction and wound contraction. Contraction requires stimulation by altering growth factor b1 or b2 and platelet-derived growth factor, attachment of fibroblasts to the collagen matrix via integrin receptors, and crosslinking between collagens. Collagen remodeling during the transition from granulation tissue to scar tissue depends on the continuous synthesis and low levels of collagen catabolism. The degradation of collagen in wounds is controlled by several proteolytic enzymes called matrix metalloproteinases, secreted by macrophages, epidermal and endothelial cells, and fibroblasts. In the first three weeks, fibrillar collagen accumulates relatively rapidly and has been reshaped by wound contraction. After that, the rate of collagen accumulation is much slower, and collagen remodeling with the formation of larger collagen bundles and an increase in the number of intermolecular crosslinks increases. However, a wound never reaches its normal strength (the tension at which the skin breaks) like uninjured skin. The scar is only 70 percent stronger at maximum strength than normal skin.22

Plants are the potential to provide wound-healing activities. Many studies reported the activity of plants for wound-healing and its mechanism. Thereby, this study review several plants that exhibit wound-healing activity below.
Aristolochia bracteolata

Aristolochia bracteolata contains aristocram, aporphines, protobiberines, flavonoids, alkaloids, tannins, sterols, steroids, and several other compounds used for skin treatments, as well as utilized for its anti-inflammatory properties (Figure 3).23 A. bracteolata extract selectively inhibited cell proliferation at higher concentrations (>100 μg/mL) and lower concentrations (<25 μg/mL). This extract showed linear and dose-dependent cell proliferation. The wound healing study showed that wound closure was 50.38% ± 1.39 and 69.81% ± 1.89, respectively, at a 25 μg/mL concentration after 24 and 48 h. The extract was tested for anti-inflammatory activity by determining the inhibitory activity on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in 264.7 RAW cells. The results found that A. bracteolata had a strong inhibitory effect on the production of NO and tumor necrosis factor-α/TNF-α. A. bracteolata extract inhibited the expression of the inducible nitric oxide synthase (iNOS) gene by lipopolysaccharide (LPS). A. bracteolata showed decreased pro-inflammatory cytokine mRNA expression concentration-dependent, indicating a mechanism for iNOS inhibition, gene expression analyzed by Real-Time PCR.24

Boerhavia diffusa

The methanol extract (EM) of the leaves of Boerhavia diffusa significantly increased the viability and migration of human keratinocytes (HaCaT) cells. GC-MS analysis revealed the presence of caffeic acid, ferulic acid, and D-pinitol as the primary bioactive metabolites (Figure 4). The content of secondary metabolites in the extract of punarvana, such as...
phenolics and flavonoids, reduces lipid peroxidation, increases collagen fibrils’ survival by increasing collagen fibers’ strength, prevents cell damage, and accelerates DNA synthesis. Due to their antioxidant and antimicrobial properties, phenolics, flavonoids, and terpenoids enhance wound healing. Antioxidants enhance the healing process by reducing the damage caused by free oxygen radicals. D-pinitol, which is an insulinomimetic. Apply topical insulin promotes diabetic wound-healing by regulating wound inflammatory cells and improving cellular function. Bioactive insulin activates the IR/IRS/P3K/AKT pathway in skin wound-healing, leading to tissue regeneration, growth, proliferation, and migration of keratinocytes and fibroblasts. A previous study demonstrated that insulin applied topically enhances diabetic wound healing by regulating wound inflammatory cells and improving cellular function. In addition, Chen et al. (2012) reported that excision wound closure time was reduced from 7 to 5 days in insulin-treated animals. This finding was associated with increased inflammatory response, re-epithelialization, and collagen remodeling in the wound group of animals treated with the insulin solution. Azevedo et al. (2016) also investigated the effect of insulin cream (0.5 U/100 g) applied daily for 26 days on second-degree burns in control rats and diabetic rats; results showed that insulin cream increased inflammatory cell infiltration and collagen deposition in diabetic rats, whereas non-diabetic rats showed no such effect. In addition, caffeic and ferulic acids can promote wound healing mainly due to their potent antioxidant and anti-inflammatory properties.

*Achyrocline satureioides* (Lam.) extracts are medicinal plants from Brazil, Uruguay, Argentina, and Paraguay. This plant contains quercetin, luteolin, and 3-O-methylquercetin (Figure 5). The results showed a significant increase in the viability of HaCaT cells on ASE-loaded nanoemulsions (NEASE) (up to 5 μg/mL of flavonoids). Preliminary tests showed that NEASE was able to increase cell migration at low flavonoid concentrations. ASE did not induce HaCaT cytotoxicity and tended to increase keratinocyte cell viability compared to controls after 24 h of treatment for all concentrations tested (0.625-10 μg/mL). Luteolin decreases in protein expressions of inflammatory factors, including matrix metalloproteinase-9 (MMP-9), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1β and down-regulation of nuclear factor-κB (NF-κB).

*Calophyllum inophyllum* Linn.

Anti-inflammatory and wound healing activities of calophyllolide (CP) have been reported isolated from *Calophyllum inophyllum* Linn (Figure 6). CP was tested on model rats by performing surgical wounds and treated with phosphate buffer saline (PBS), povidone-iodine (PI), and CP to evaluate the wound healing rates. In addition, the anti-inflammatory activity was indicated with the MPO test. The results showed that CP did not affect the viability of HaCaT cells in the indicated concentrations. CP extract at 10-1000 ng/ml had a non-significant difference in cell viability. CP reduced fibrosis formation and effectively promoted wound healing in a mouse model without causing weight loss (p-value = 0.6524). CP decreased the fibrosis formation and accelerated the wound closure in the epidermis and dermis, which ultimately closed 14 days after treatment. The molecular mechanisms underlying wound repair reduce MPO activity and

![Figure 5. Wound healing mechanism of Achyrocline satureioides (Lam.).](image-url)
increase M2 macrophages. CP prevents a prolonged inflammatory process by downregulating pro-inflammatory cytokines—IL-1β, IL-6, and TNF-α—but upregulating anti-inflammatory cytokines, IL-10.32

**Ulmus parvifolia**

The bark of *Ulmus parvifolia* contains phenolic compounds and steroid glucosides, used to treat edema. This plant has been isolated, containing catechin-7-O-β-D-apiofuranoside (Figure 7).33 The results showed that HaCaT cells grown in the presence of *U. parvifolia* root bark extract showed a faster and dose-dependent growth rate than untreated cells. Collagen protein remodeling during wound healing may be affected by proteolytic activity in the extracellular matrix by matrix metalloproteinases (MMPs). MMPs play an essential role in all stages of wound healing during normal tissue remodeling and morphogenesis by modifying the wound matrix. Understanding the role of MMPs during infection and chronic tissue repair could pave the way for identifying potential targets for chronic wounds. In addition, MMPs also regulate cell-cell and cell-matrix signaling by releasing cytokines and growth factors sequestered in the extracellular matrix (ECM). TGF-β is a family of growth factors that play an essential role in wound healing by regulating the inflammatory response, keratinocyte proliferation and migration, angiogenesis, collagen synthesis, and ECM remodeling.6

**Aloe vera**

*Aloe vera* extract contains mannose-6-phosphate, increasing wound contraction and collagen synthesis. Isolated polysaccharides from *Aloe vera* also induce matrix metalloproteinase (MMP)-3 and metalloproteinase inhibitor-2 gene expression during wound repair (Figure 8).34 The gel was tested non-cytotoxic against nauplii and compatible with human blood and skin cells. *Aloe vera* promotes the attachment and proliferation of HaCaT and HFF1 cells. It also significantly accelerated wound closure through reepithelialization and wound.35 *Aloe vera* gel exhibited significant wound healing properties, as indicated by the statistically significant increase in the percentage of wound closure and migration rate for the two highest concentrations used.36
**Hibiscus syriacus**

*Hibiscus syriacus* (HS) contains flavonoids, including dihydroquercetin, herbacetin, and kaempferol (Figure 9). HS ethanol extract accelerated wound-healing activity in epithelial formation and fibronectin production. Fibronectin expression analysis with immunostaining revealed that fibronectin value after being treated with HS was 19.4 ± 6.7%. It was significant compared to the control group. During tissue repair, fibronectin is converted from a soluble inactive form to biologically active extracellular matrix (ECM) fibrils via a cell-dependent process. ECM fibronectin promotes many cellular processes essential for tissue repair and regulates the assembly of other proteins into the matrix. Reduced ECM fibronectin levels were indicated by unhealed wounds.

In addition, HS enhances the expression of genes involved in skin hydration and homeostasis. HS contains compounds which stimulate the expression of biomarkers relevant to skin regeneration and hydration, thereby counteracting the molecular pathways that cause skin damage and aging. Fibroblasts and keratinocytes are the keys to the wound-healing process in the skin. Treatment of HaCaT cells with 0.002% HS for 24 h significantly improved the wound healing response. 0.002 and 0.01% of HS increased by 50% and 20% of wound healing rate, respectively. Aquaporin 3 (Aqp3) is an integral membrane pore protein expressed more in the basal than in the upper layers of the epidermis. Specifically, the Aqp3 and filaggrin genes were increased by 20 and 58%, respectively. Aqp3 selectively conducts water molecules in and out of cells and prevents the passage of ions and other solutes. Filaggrin is a filament-associated protein that binds to keratin fibers and is responsible for the integrity and waterproofing capacity of the top layer of skin.

**Sideroxylon obtusifolium** (Roem. & Schult.)

N-Methyl-(2S,4R)-trans-4-Hydroxy-L-Proline (NMP) from the leaves of *Sideroxylon obtusifolium* (Brazilian medicinal species) has acted as an anti-inflammatory and wound-healing management (Figure 10). A previous study showed that the methanol fraction of *Sideroxylon obtusifolium* (MFSO) (50 μg/ml) stimulated HaCaT cells by increasing proliferation.
and migration rates in keratinocytes during wound-healing. MFSO demonstrated stimulation of human keratinocytes (HaCaT) cells and enhanced wound healing through modulation of inflammation in burns.40,41

**Alternanthera sessilis**

*Alternanthera sessilis* contains 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (8.92%), hexadecanoic acid (7.21%), palmitate (5.65%), and L-glutamic acid (5.04%) (Figure 11). The highest concentration of extract treatment (50 μg/ml) showed a migration rate of 99%. The extract showed a strong positive result of 65%, with a difference of 14% in the migration rate between the two. The extract may act on broad signaling receptors to promote proliferation and migration in HaCat. Higher concentrations are required for epithelial barrier stimulation, while lower doses are sufficient to trigger connective tissue cellular compounds.5

**Figure 10.** Wound healing mechanism of *Sideroxylon obtusifolium* (Roem. & Schult.).

**Figure 11.** Wound healing mechanism of *Alternanthera sessilis*. 
Wedelia trilobata L.

Grandiflorenic acid from Wedelia trilobata leaves was assessed for its possible activity on HaCaT keratinocyte proliferation and its effects on in vitro scratch tests, collagen content, TGF-β levels, and nitric oxide, TNF- and IL-1β determinations using Raw 264.7 cells. Grandiflorenic acid (2.5 μg/ml) resulted in a 106% percentage of HaCaT keratinocyte viability, induced a migration rate of 100% in the initial in vitro assay, and the collagen content increased to 171.2 μg/ml compared to the control (61.1 μg/mL) with human fibroblasts. Grandiflorenic acid has potential wound-healing activity due to fibroblast stimulation and inhibition of prolongation of the inflammatory phase of wound healing, as evidenced by a decrease in inflammatory cytokine levels from Raw 264.7 macrophage cells. Grandiflorenic acid and proteoglycans increased collagen production (Figure 12).15

Aegle marmelos L.

The active compounds isolated from the Maja flower (Aegle marmelos L.) are cineol, eugenol, cuminaldehyde, aegelin, 1-hydroxy-5,7-dimethoxy-2-naphthalene-carboxaldehyde (HDNC), and Luvangetin, which had been purified >98% (Figure 13). Treatment with Maja flowers for 24 h drastically increased cell motility and expression of keratinocytes in specific cell lines. It enhances protein expression in loricrin, filaggrin, and involucrin (a keratinocyte differentiation marker). Keratinocyte motility is enhanced by the ERK and Akt signaling pathways.42

Eriobotryae folium

The leaves of Eriobotrya japonica contain amygdalin (laetrile and vitamin B1) which has antioxidant activity with an IC50 value of 56.59 μg/ml (Figure 14). The ethanolic extract of Eriobotryae folium (EF) increases intracellular and extracellular PGE2 levels in HaCaT cells and inhibits 15-PGDH (ED50: 168.4 μg/ml) with relatively low cytotoxicity (IC50: 250.0 μg/ml). In the other study, EF extract suppressed LPS-induced nitric oxide and PGE2 production by inhibiting inducible nitric oxide synthase and COX-2 expression in lipopolysaccharides that stimulated RAW264 cells decreased MRP4 and PGT expression.44

Glycyrrhiza glabra

Glycyrrhiza glabra (GG) has a positive proliferative effect on keratinocytes. The larger the dose, the higher the rate of proliferation. GG inhibits abnormal cell proliferation and is anticarcinogenic. Although GG has been shown to increase the rate of cell proliferation and migration of keratinocytes and promote wound healing, the underlying mechanism is unclear. However, GG helps activate proliferation and cytoskeletal rearrangement proteins and promotes wound-healing. The antioxidant effect of some GG constituents, such as glycyrrhizin and glabridin, may also help enhance the wound healing ability of keratinocytes (Figure 15). G. glabra aqueous extract ointment could significantly (p ≤ 0.05) decrease

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**Figure 12. Wound healing mechanism of Wedelia trilobata L.**
the level of the wound site, total cell, macrophage, lymphocyte, and neutrophil, and enhance the level of wound contracture, fibrocyte, hexuronic acid, and hydroxyproline as compared with the basal ointment and control groups.46

**Calabrian honey**
BL1 (multifloral) and BL5 (orange) honey showed the best healing properties among the five kinds of honey tested. Pinocembrin, revealed in honey samples BL1 and BL5, is a flavanol with known biological activities, including...
wound-healing. At high concentrations or after prolonged contact, polyphenols can reduce the production of pro-inflammatory cytokines and interact with metabolism and cell proliferation, thereby healing wounds. Pinocembrin in vitro modulates the production of inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-10, by suppressing NF-κB and MAPK activation. Pinocembrin and its 7-linolenoyl derivative were found to be innovative wound-healing agents. Immunofluorescence and functional assays showed that GPR120 mediated the activity. Pinocembrin produced wound-healing of HaCaT cells after 6 and 24 h by about +30% compared to untreated. In contrast, the 7-linolenoyl derivative increased HaCaT wound closure by about +40% compared to untreated controls. Activation of GPR120 can impair by increasing levels of TGF-β, which triggers the synthesis of components of the extracellular matrix, thereby contributing to wound-healing induced by keratinocytes. Complex signaling pathways involved in the upregulation of MMPs and the turnover of extracellular matrix components stimulated by attendants can lead to tissue damage or repair processes. In particular, MMP-9 plays an essential role in cell migration and reepithelialization (Figure 16).

**Glycyrrhiza glabra**

Previous studies showed that Thyme oleoresin at 25 μg/ml and 50 μg/ml significantly promoted HaCaT cell migration, leading to wound closure. The upper part of the plant is reported to have significant components such as p-cymene.
α-terpinene, and thymol (Figure 17). The possible mechanisms in wound healing are its ability to maintain wound moisture, increase oxygenation by increasing blood supply, increase epithelial cell migration, rapid maturation of collagen and reduce inflammation, increase collagen synthesis, increase the synthesis of hyaluronic acid and dermatan sulfate in wound tissue.49

*Trapa japonica*

*Trapa japonica* contains fiber and polyphenols, such as ellagic acid, eugeniin, and gallic acid, which have antioxidant and anti-inflammatory activities (Figure 18). The results showed that the extract of *T. japonica* decreased the TNF-α, thus significantly decreasing MMP-1 and MMP-9 mRNA expression.50

*Gracilaria lemaneiformis*

*Gracilaria lemaneiformis* contains sulfated galactan, anti-inflammatory, and antioxidant (Figure 19). The purified *Gracilaria lemaneiformis* (GLP-2) fraction promoted cell proliferation and migration of HaCat cells through activation of PI3K/aPKC signaling during wound-healing of human keratinocytes. GLP-2 significantly increased wound-healing activity when compared to control cells. The results showed that GLP treatment could increase lamellipodium formation in migrating HaCaT cells. GLP-2 promotes cell migration with cell polarity and directional migration to accelerate wound healing of keratinocytes using early migration and transwell assays. GLP-2 significantly increased wound healing.
activity compared to control cells. Horikoshi et al. reported that upstream (upstream) regulation of the PI3K/aPKC signaling pathway promotes cell polarization in HaCaT cells. Increased Akt phosphorylation is considered an index of activation of the PI3K signaling pathway after injured cells. GLP-2 activates the PI3K/aPKC signaling pathway and promotes cell polarization in HaCaT cells. GLP-2 positively regulates Cdc-42, Rac-1, Par-3, and aPKC in HaCaT cells. Cdc42 induces filopodial extension at the cell periphery, which aids in directional cell migration. Par-3 and aPKC are considered proteins that regulate cell polarity involved in the regulation of cell polarization. Increased Akt phosphorylation was considered an index of activation of the PI3K signaling pathway after injured cells. The present study observed a significant increase in Akt phosphorylation in GLP-2-treated cells compared to control cells at 12 h during the wound-healing process.

Nerium indicum

Nerium indicum (NI) contains oleandrin, flavonoids, and tannins (Figure 20). These plants may vary on keratinocyte activity at the wound site at specific doses. Studies have shown that the test materials used, either alone or in combination, positively affect keratinocyte proliferation and migration, an essential factor required for proper wound closure and wound-healing.

Urtica dioica

Urtica dioica (UD) extract contains saponins, flavonoids, carbohydrates, ketoses, resins, and coumarins (Figure 21). The UD extract increased the proliferation rates of HEK-293 and HaCaT cells by 39% and 30% after 24 h, respectively, compared to control cells. The viability of extract-treated HEK-293 cells was increased compared to untreated (control) cells after 24 h of incubation (Figure 21). Maximum cell viability (i.e., 139 ± 2%) was achieved by the addition of 150 μg/ml of the extract. This value was statistically significant for p < 0.001. In the case of HaCaT cells (keratinocytes from human skin), cell viability was increased by 30% upon incubation with 100 μg/mL extract for 24 h. The extract increased the cell population in the G2/M phase by almost 10%. Moreover, the extract caused a twofold increase in the rate of cell migration of both cell lines compared to the control cells. In addition, the extract was found to have moderate anti-inflammatory and antioxidant properties that enhance the overall wound-healing potential.

Curcuma amarissima

Curcuma amarissima (CA) contains curcumenol, curdione and curzerenone (Figure 22). The cell viability test showed that the CA extract increased the viability of HaCaT cells. MTT cell viability test was performed to monitor changes in the
In addition, the extract was found to have moderate anti-inflammatory and antioxidant properties that enhance the overall wound-healing potential.

Figure 21. Wound healing mechanism of *Urtica dioica* L.

Figure 22. Wound healing mechanism of *Curcuma amarissima*. 

Cell monolayer wound-healing in HaCat.
viability of HaCat cells in media containing CA extract (CA extract in DMSO as solvent from stock solution) in the presence of 10% fetal bovine serum, FBS. The results showed that the CA extract at concentrations lower than 40 g/mL had no significant effect on cells cultured in FBS-rich media viability. However, extracts at 80 and 160 μg/ml caused a significant reduction in cell viability to about 30% and 10%, respectively. However, DMSO at all concentrations used did not cause changes in the viability of HaCaT cells. A similar experiment was also conducted in which treatment with CA extract was carried out in FBS-free media. The results showed that the viability of HaCaT cells in a serum-free medium was more sensitive to CA treatment. In particular, cell viability was significantly reduced when cells were treated with CA at 20 μg/mL, and cell viability was maximally decreased to about 20% in cells treated with CA at 40 μg/ml or more. This increase in cell viability was related to the CA extract’s pharmacological activity in inducing cell proliferation. CA extract rapidly induces ERK1/2 and Akt activation. Consistently, CA extract accelerated cell migration, rapidly healing the injured human keratinocyte monolayer. In particular, MEK inhibitors (U0126) or PI3K inhibitors (LY294002) blocked CA-induced enhancement of cell monolayer wound-healing. In addition, CA extract induces the expression of Mcl-1, an antiapoptotic protein, supporting that the CA extract enhances the survival of human keratinocytes.  

Clausena excavata
The methanol extract of Clausena excavate contains coumarins, flavonoids, and glycosides with various biological properties.54–56 C. excavata exhibits diverse therapeutic activities, which include anticancer, insecticidal, antifungal, antiplasmodial antiplatelet, antiinociceptive, immunomodulatory, antimycobacterial, and anti-HIV-1 activity.57–59 These compounds regulate inflammation through inhibition of the MAPK/NF-κB pathway. In addition, it was shown that methanol extract treatment increased TGF-β1 expression, the cytokine increased wound contraction, extracellular matrix deposition, and collagen formation in wound healing (Figure 23).54

Angelica gigas
Angelica gigas contains Coumarin, decursin, and decursinol angelate (Figure 24), which improves wound-healing with HaCaT human keratinocytes. ERK1/2 phosphorylation is essential for cell survival, proliferation, and inhibition of apoptosis. The expression of genes encoding ECM remodeling proteins, inflammatory cytokines, and growth factors is an essential step in human wound healing. The simultaneous expression of these genes can accelerate this process.60

Salvia haenkei
Salvia haenkei contains rosmarinic acid (Figure 25). Hydroalcoholic extract of S. haenkei effectively increases the wound closure rate in cultured keratinocytes with the almost total invasion of the scrapes after 48 h of treatment. Gene expression analysis showed that S. haenkei regulates the nuclear factor-κB (NF-κB) transcription factor signaling pathway. The results showed that the S. haenkei extract does not cause a statistically significant increase in the rate of fibroblast
Figure 24. Wound healing mechanism of *Angelica gigas*.

Figure 25. Wound healing mechanism of *Salvia haenkei*. 
migration. Specifically, this study analyzed the mRNA levels of several genes involved in the early inflammatory phase of skin repairs, such as the transcriptionally active subunit of the transcription factor NF-κB (RelA), the inflammatory cytokine interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), inducible nitric oxide synthase (iNOS or NOS2), and the inducible prostaglandin synthesis enzyme cyclooxygenase-2 (COX-2). Tn with S. haenkei increases the IL-6 in fibroblasts and keratinocytes (83.6 and 19.7-fold induction, respectively), whereas TNFα levels only have a mild increasing trend.16

*Crassocephalum crepidioides*  
*Crassocephalum crepidioides* (Benth.) S. Moore contains β-cubebene, α-farnesene, and α-caryophyllene, which exhibit antioxidant and anti-inflammatory activities (Figure 26). *C. crepidioides* (CC) extract exhibited anti-inflammatory in vitro assays on the macrophage cell line RAW 246.7. In addition, reduced inflammatory cell density in granulation tissue in 7-days wounds, combined with decreased TNF-α and NF-B1 mRNA expression. NF-B1 and TNF-α are essential markers for the degree of inflammation. High levels of TNF-α have been reported to inhibit wound re-epithelialization, myofibroblast formation, and smooth muscle actin (SMA-α). The results showed that CC could improve the wound-healing process through its anti-inflammatory activity. TGF-β1 mRNA was also found to be elevated in granulation tissue. TGF-β1 is involved in many essential effects on the wound-healing process. The activities of TGF-β1 include the induction of fibroblast proliferation, motivating the differentiation of fibroblasts into myofibroblasts, and increasing the synthesis, deposition, and maturation of collagen. The increase in the TGF-β1 gene may explain the increase in fibroblasts and the wound-healing effect.61

*Withania somnifera*  
*Withania somnifera* contains withaferin A, which has anti-inflammatory, antiangiogenic, antimetastatic, and anticancer activities (Figure 27). The results showed that ashwagandha extract (AE) significantly inhibited mRNA expression of inflammatory cytokines, including interleukin IL-8, IL-6, TNF-α, IL-1β, IL-12, and promoted mRNA expression of the anti-inflammatory cytokine TGF-β1 in HaCaT cells. Cytokine expression levels were investigated in ASH-WEX-treated HaCaT cells by RT-qPCR. The results showed that when cells were treated with ASH-WEX at concentrations <5 mg/ml, which was non-toxic in HaCaT cells, the mRNA expression levels of the inflammatory cytokines IL-8, IL-6, and IL-12 were significantly decreased. TNF- and IL-1β mRNA levels were also decreased. In contrast, treatment with ASH-WEX...
at concentrations < 5 mg/ml significantly increased the mRNA expression level of the anti-inflammatory cytokine TGF-β1. These results indicate that ASH-WEX has an anti-inflammatory effect on keratinocytes. In addition, AE inhibited lipopolysaccharide-induced phosphorylation of p38 and c-Jun N-terminal kinase, as well as NF-κB p65. The results showed that EA was not toxic to HaCaT cells up to a dose of 10 mg/mL. AE inhibits the MAPK/NF-κB pathway. The NF-κB and MAPK signaling pathways are strongly associated with the expression of inflammatory cytokines in HaCaT cells.

**Anemarrhena asphodeloides**

Mangiferin has been isolated from the plant *Anemarrhena asphodeloides* (Figure 28). *A. asphodeloides* (AA) extract promoted inhibiting Th2-type cytokines, pro-inflammatory cytokines, and filaggrin restoration in HaCaT cells. TNF-α/IFN-γ significantly increased mRNA expression of IL-4 in HaCaT keratinocytes. However, pretreatment with AA significantly suppressed the mRNA expression of IL-4. AA pretreatment of TNF-α/IFN-γ-stimulated HaCaT keratinocytes reduced IL-13 mRNA expression. However, pretreatment with AA significantly suppressed the mRNA expression of IL-6 in a dose-dependent manner. These results suggest that AA has a protective effect on skin keratinocytes by inhibiting the transcription of inflammatory cytokine levels associated with skin barrier dysfunction. TNF-α/IFN-γ co-stimulation decreased filagrin protein expression and mRNA levels, but pretreatment with AA significantly increased filagrin protein levels, although mRNA levels increased slightly. The results showed that AA had a filagrin-recovery effect on TNF-α/IFN-γ-stimulated HaCaT keratinocytes. Treatment with AA increased Keratinocyte HaCaT migration and inhibited the expression of iNOS protein levels. It is possible to assume that AA facilitates wound-healing in the skin barrier by inhibiting overexpression.

**Sasa veitchii**

*Sasa veitchii* is a traditional plant that contains lignin, polysaccharides, and chlorophyll (Figure 29). It has many pharmacological activities such as antioxidant, anti-inflammatory, antibacterial and anticancer. HaCaT cells treated with *S. veitchii* extract for 72 h showed significantly higher AQP3 expression and mitogen-activated p38 phosphorylated

![Wound healing mechanism of Anemarrhena asphodeloides.](Figure 28)
protein kinase (MAPK) than control cells. *S. veitchii* extract increases AQP3 expression and provides wound-healing and healing effects. The increase in AQP3 expression elicited by the Kumazasa extract may be due to increased transcription via activation of p38 MAPK signaling. It was also found that *S. veitchii* extract had a proliferative effect on HaCaT cells.\(^6^3\)

**Periplaneta americana**

*Periplaneta americana* contains polyalcohols, amino acids, pyrimidines, uracils, and proteoglycans (Figure 30). *P. americana* extract showed effects in wound-healing that depend on the Janus-activated kinase/signal transducer pathway and transcriptional activator 3 (JAK/STAT3) and Smad3 activity. Pretreatment with STAT3 inhibitors blocked cell proliferation and migration. This extract promotes the proliferation and migration of immortalized human keratinocyte HaCaT cells. The results showed increased keratinocyte proliferation and migration after treatment (0.3125 mg/mL) for 48 h. After treatment, JAK/STAT3 signaling expression and Smad3 activation, NF-κB/p65, and β-catenin were significantly upregulated in HaCaT cells and wound tissue. However, NF-κB and Wnt signaling appear to be minimally activated regardless of the limited expression of NF-κB/P65 and β-catenin upregulation or cell nuclear translocation.\(^6^4\)

**Figure 29. Wound healing mechanism of Sasa veitchii.**

**Figure 30. Wound healing mechanism of Periplaneta americana.**
Angelica tenuissima

Angelica tenuissima root contains decursin and Z-ligustilide (Figure 31). The root extract of A. tenuissima accelerates wound filling under basal conditions in the keratinocytes that make up the epidermal layer. It inhibits the mRNA expression of MMP-1 and elastase. It also increases the collagen content as indicated by the production and secretion of type I procollagen with or without UVB exposure. This extract could be beneficial in suppressing UVB-mediated wrinkling of skin formation and photoaging by increasing PIP levels and decreasing MMP-1 and elastase activity. A. tenuissima can play a role in attenuating the inflammatory response caused by UVB irradiation through upregulation of photo-protective hemeoxygease-1 and suppressing pro-inflammatory cyclooxygenase-2 expression.65

Astragali radix

Astragaloside VI (AS-VI) and cycloastragenol-6-O-beta-D-glucoside (CMG) (Figure 32) from Astragali radix enhance skin cell proliferation and migration via activation of the EGFR/ERK signaling pathway, resulting in enhanced wound-healing in vitro. AS-VI actively promotes the proliferation of human keratinocytes (HaCaT) by activating the ERK1/2 pathway rather than the JNK and p38 pathways. This plant shows that astragaloside can activate cellular processes involved in wound-healing. It is mediated, at least in part, by EGFR/ERK1/2, which could be beneficial in wound closure.66

Mimosa tenuiflora (Willd)

Mimosa tenuiflora bark contains high amounts of saponins and polyphenols such as arabinogalactan (Figure 33). Mimosa tenuiflora (Willd) aqueous extract at concentrations of 10 and 100 μg/ml indicated a loss of cell viability and proliferation of dermal fibroblasts. Isolated, ethanol-precipitated compounds (EPC) (10 μg/ml) have shown strong potential to increase viability by stimulating mitochondrial activity and dermal fibroblast proliferation. Stimulation of human keratinocytes was only found at a concentration of 100 μg/ml. EPC did not influence the expression of specific proliferation and

Figure 31. Wound healing mechanism of Angelica tenuissima.

Figure 32. Wound healing mechanism of Astragali radix.
Figure 33. Wound healing mechanism of *Mimosa tenuiflora* (Willd).

Figure 34. Wound healing mechanism of *Fitzroya cupressoides*.
differentiation-related genes. Fibroblasts in the connective tissue are the main targets of the arabinogalactan polymer compound from *Mimosa tenuiflora*. Intense fibroblast stimulation can initiate wound closure and production of extracellular and filling materials within the wound.

**Fitzroya cupressoides**

*Fitzroya cupressoides*, commonly called allerce, contains fatty acids, mono and sesquiterpenes, diterpenes, lignans, and phytosterols. Diterpenes and lignans were the most active compounds, with the biomolecules matairesinol, podophyllotoxin, and ferruginol (Figure 34). Allerce extract has a significant effect on wound healing. The results showed that the extract stimulated cell division in human skin epidermal cells in the context of wound healing. These results also indicated that allerce extract accelerated the healing process after 24 and 48 h treatment. This effect was promoted by stimulating HaCaT cell division in wound healing.

**Plantago australis**

The hydroethanolic extract of *Plantago australis* contains verbascoside (Figure 35). *P. australis* extract and verbascoside decreased cell viability at 1000 μg/mL and 100 μg/mL, respectively. The results showed approximately 81.06% wound closure (*P. australis* extract 25 μg/mL) and 58.7% and 57.77% (Verbascoside 5 and 10 μg/mL). *P. australis* extract showed a significant reduction in TNF-α level. These compounds have wound-healing activity, increase cell migration, and reverse the effects of oxidation in lipopolysaccharide-activated N9 cells. This effect may also be associated with decreased TNF-α, IL-6, IL-12p70, INF-γ, and MCP-1.

![Figure 35. Wound healing mechanism of *Plantago australis*.](image)

**Table 1. Plants with wound healing activity and its compounds.**

<table>
<thead>
<tr>
<th>No</th>
<th>Plants</th>
<th>Solvent</th>
<th>Compounds</th>
<th>References</th>
</tr>
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<tbody>
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<td>1</td>
<td><em>Aristolochia bracteolata</em></td>
<td>methanol</td>
<td>Aristoctam, Aporphines, Protobiberberines</td>
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<td><em>Boerhavia diffusa</em></td>
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<td>caffeic acid, ferulic acid, and D-pinitol</td>
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<td><em>Achyrocline satureioides</em> (Lam.)</td>
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<td>luteolin and 3-O-methylquercetin</td>
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<td><em>Calophyllum inophyllum</em> Linn</td>
<td>ethanol</td>
<td>calophyllolide</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td><em>Ulmus parvifolia</em></td>
<td>ethanol</td>
<td>catechin-7-O-β-D-apiofuranoside</td>
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<td><em>Aloe vera</em></td>
<td>aqueous</td>
<td>mannose-6-phosphate</td>
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<td>7</td>
<td><em>Hibiscus syriacus</em></td>
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<td>dihydroquercetin, herbacetin, and kaempferol</td>
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<td>8</td>
<td><em>Sideroxylon obtusifolium</em></td>
<td>ethanol fraction</td>
<td>N-Methyl-(2S,4R)-trans-4-Hydroxy-L-Proline</td>
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<td>9</td>
<td><em>Alternanthera sessilis</em></td>
<td>ethanol</td>
<td>2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, hexadecanoic acid, palmitate, and L-glutamic acid</td>
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</table>
### Table 1. Continued

<table>
<thead>
<tr>
<th>No</th>
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<th>Solvent</th>
<th>Compounds</th>
<th>References</th>
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</thead>
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<td>Grandiflorenic acid</td>
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<td>cineol, eugenol, cuminaldehyde, aegelin, 1-hydroxy-5, 7-dimethoxy-2 naphthalene-carboxaldehyde (HDNC), and Luvangetin</td>
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<tr>
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<td>ethanolic</td>
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<tr>
<td>13</td>
<td><em>Glycyrrhiza glabra</em></td>
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<td>glycyrrhizin and glabridin</td>
<td>45,46</td>
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<td><em>Calabrian honey</em></td>
<td>methanol</td>
<td>Pinocembrin</td>
<td>48</td>
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<td><em>Glycyrrhiza glabra</em></td>
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<td>48</td>
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<td><em>Glycyrrhiza glabra</em></td>
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<td>Pinocembrin</td>
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<td>curcumenol, curdione and curzerenone</td>
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<td>decursin, and decursin angelate</td>
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<td>Astragaloside VI (AS-VI) and cycloastragenol-6-O-beta-D-glucoside (CMG)</td>
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<td>31</td>
<td><em>Mimosa tenuiflora</em></td>
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<td>32</td>
<td><em>Fitzroya cupressoides</em></td>
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<td>matairesin, podophyllotoxin, and ferruginol</td>
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<tr>
<td>33</td>
<td><em>Plantago australis</em></td>
<td>hydroethanolic</td>
<td>verbascoside</td>
<td>61</td>
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</table>

### Conclusion
Several compounds in plants have been studied to have activity in wound healing by various mechanisms (Table 1). The wound-healing mechanism includes processes that restore skin integrity through four stages: hemostasis, inflammation, multiplication, and remodeling. Antioxidant and anti-inflammatory activities play an essential role in wound-healing mechanisms. Many compounds in plants have been studied to have activity in wound healing by various mechanisms.

### Acknowledgements
The authors would like to thank Thammasat University for the grant Thammasat Postdoctoral Fellowship contract number: TUPD/18 2021.


55. Nafrike L, Khairani VY: The effectiveness methanol extract clausena excovota on number of fibroblast and density of collagen fibers after tooth extraction. 2019


Open Peer Review

Current Peer Review Status: ☒ ☒ ☐

Version 1

Reviewer Report 01 August 2022

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Sisir Nandi
Department of Pharmaceutical Chemistry, Global Institute of Pharmaceutical Education and Research, Kashipur, Uttarakhand, India

Authors have carried out a critical review on the Wound Healing Mechanisms utilizing Natural Products. This is very significant and the article can be accepted after major revision.

1. The sentence "In general, the wound-healing process is divided into 4 phases: the coagulation and hemostasis phase, the inflammatory phase, the proliferative phase, and the remodeling phase." should be revised. See for example:

"It also involves phagocytosis, chemotaxis, neocollagenesis, collagen degradation, and collagen remodeling. The epithelization process initiates production of new glycosaminoglycans and proteoglycans are a few more important processes of healing of a wound."

Roy et al., Current Drug Discovery Technologies, 2020, 17, 534-541, DOI: 10.2174/1570163817666200123122532

2. Natural sourced chitosan is a very good composition for the formulation of wound healing. See, for example:

"Chitosan is a marrying sponge natural linear polysachharide that has been greatly used in the formulations like nanoparticles, hydrogels, implants, films, fibers, etc for the wound healing."


3. The introduction section should contain Alopathy generic medicine name commonly used
for the wound healing.

4. Authors should add a Table before results. Table should summarise the Sl. number, plant name, extracts, active component isolated if any and citation.

5. The authors have not been sincere in organizing and checking the manuscript. There are a lot of mistakes, English grammar as well as sentence structure.

6. Some natural formulations should be given in the results section.

7. Conclusion section should be re framed.

8. Include sketches, flow diagrams, table for applications describing mechanism of wound healing action that would be more informative to the readers

References

Are the rationale for, and objectives of, the Systematic Review clearly stated?
Yes

Are sufficient details of the methods and analysis provided to allow replication by others?
Yes

Is the statistical analysis and its interpretation appropriate?
Yes

Are the conclusions drawn adequately supported by the results presented in the review?
Yes

*Competing Interests*: No competing interests were disclosed.

*Reviewer Expertise*: Drug Design and development research

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Response 29 Sep 2022
David Leavesley, Agency for Science, Technology and Research, Singapore, Singapore
The authors should not be required to cite their reviewers work. I am confident alternative literature is available to substantiate the authors statements.

**Competing Interests:** None.

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**Reviewer Report 19 July 2022**

https://doi.org/10.5256/f1000research.133716.r141489

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**David Leavesley**

1 Skin Research Institute of Singapore, Agency for Science, Technology and Research, Singapore, Singapore
2 The University of Newcastle, Newcastle, NSW, Australia

This document claims to be a systematic review of human keratinocyte wound healing in response to exogenously applied products derived from natural materials; specifically, products manufactured from plants. Traditional and Complementary Medicine (T&CM) practices based on natural products provide essential health care for more than half of the world population (WHO). However, 17.5% (1 in 6) of WHO Member States include T&CM in their national essential medicines list. This review discusses including T&CM in the clinical management of cutaneous wound healing.

**Introduction**

I recommend that the authors expand their summary “Skin protects against environmental factors such as harmful UV rays and pathogens and prevents water loss”, to include less widely-recognised functions of skin. For example, skin is integral to our immune, nervous and endocrine systems, providing sensory functions (touch, vibration, temperature, electromagnetic energy, pain), thermoregulation, excretion (sweat), storage (water, blood, fats, glycans, xenobiotics), recognition and detection of pathogens, chemicals, toxins, and synthesizes many critical enzymes, antimicrobials, and biological response modifiers (e.g. hormones, cytokines, adipokines, chemokines, neuropeptides). (See Exp Dermatol. 2002; 11(2): 159–187)

It is not clear to me why “wound-healing” might be considered a “barrier” [Introduction]. Perhaps it is because wound-healing restores skin barrier functions?

I am also at a loss to comprehend why “epithelialization is referred to as faulty epidermis breakdown” [Introduction]. Epithelialisation is the process that demarcates initial responses to injury, that collectively attempt to reestablish barrier function and cutaneous homeostasis, from subsequent physiological events, that collectively attempt to restore skin structure and secondary functions.
Not all “traditional medicine” (T&CM), “is based on plant sources”. [Introduction] This statement is misleading.

It is not made clear that referred to “Secondary metabolites” are molecular species derived from processing compounds present in T&CM. [Introduction]

I fail to comprehend how “Growth rates” represent “a source of many biochemicals” (sic)? A rate describes something the changes over time. Is the author referring to biochemical products that change over time as a cause, or an effect, of wound healing processes?

HaCaT cells are epithelial. HaCaT cells are not capable of modelling mesenchymal cells, the cell type that constitute the human dermis. The statement is inaccurate. Importantly, HaCaT cells have been characterised as a premalignant phenotype (Am J Pathol. 2001; 159(4): 1567–1579)2. It is not clear how cells having this genotype / phenotype recapitulate normal human skin?

I disagree that “high availability and ease of cell culture” are robust, relevant criteria, to select HaCaT as suitable to model human skin in vitro.

Eligibility Criteria

I am confused. How can inclusion criteria specify “2. The studies using an in vitro approach”; yet exclusion criteria specify “3. In vitro studies”.

Search Strategy

Search strategy is claimed to be “advanced search in three databases, PubMed and Google Scholar...” using MeSH terms and Boolean operators: “Wound-healing AND HaCaT Cells AND Compounds AND Extracts”.

FYI, “Please identify the third database?

Searches of the database using the Boolean operator “AND” will severely limit results to strings that only include all three terms. Strings that did not include the words “Cells”, or “Compounds”, or “Extracts” would not be found. As Compounds” is not a recognised MeSH Term (www.nlm.nih.gov/mesh/meshhome), not data should be expected to be returned from the search, as described in this manuscript. Please revise using the accurate search strategy. In my opinion, this strategy (i.e. using Boolean operators), introduces bias into the search results. For example, works that instead used “tissue repair”, “skin repair”, “skin healing”, “traditional Chinese medicine”, “indigenous medicine”, “wound care”, “herbal medicine”, “wound treatment”, “supplement”, “botanical”, “mixture”, “formulation”, “preparation”, would not be returned.

What was the rationale for inclusion criteria: “3. studies were published from 8th October 2021 up to 31st March 2022”, but subsequently limiting selected publications to “2. Year of publication – 2009-2021”?

What effect did selecting articles using “3. Study objectives – wound-healing mechanism of several compounds and extracts in HaCaT cells”, have on studies reporting results acquire from analysis of single ‘compounds and extracts’?
Synthesis of Results

What is the origin of “different HaCaT cells”?

Results

The opening paragraph reporting Results, is a repeat of discussion previously reported in the Introduction.

Figure 1 reports the gross anatomy of human skin. It identifies the “Subcutaneous layer” (sic); however, no mention of the subcutaneous layer is present in the text.

Wound-healing

The data cited in support of “Wound-healing processes restore skin integrity through four stages: hemostasis, inflammation, multiplication, and remodeling” (sic) are inappropriate. Please replace with original references, for example Singer & Clark. N Engl J Med. 1999. 341(10):738-46.

The descriptor “multiplication” phase is ambiguous. The four phases of mammalian cutaneous wound-healing are usually described with adjectives. Thus, more commonly these are: hemostasis, inflammation, proliferation, and remodeling.

After identifying the four phases of wound-healing, the nomenclature is immediately changed to “Coagulation phase”, “Inflammatory phase”, “Proliferation phase” and “Remodeling phase”.

What are “IL-1?, IL-6, IL-8, and TNF, and growth factors such as PDGF, TGF-?, TGF-?, IGF-1, and FGF”? These abbreviations have not previously been described.

The statement “Restoring the basal keratinocyte layer in the basement membrane between the epidermis and the dermis begins to proliferate through various signaling molecules during the proliferative stage” (sic) does not make sense. How does the “basement membrane... begin to proliferate”?

What is “a certain level of repair”?

How does “the cytoplasmic shape of the keratinocytes... alter(ed) to move to the upper layers of the epidermis”? This statement is misleading; the shape of keratinocytes does not facilitate cell motility. Cell motility, also called cell migration, is a dynamic process involving intracellular second messenger molecules, cytoskeleton (actin microfilaments, tubulin microtubules, keratin intermediate filaments, and a large population of accessory molecules that include adhesive glycoproteins, crosslinking proteins, signalling molecules, ATPases, GTPases.

There is no evidence that keratinocytes “transform” as they migrate apically during squamous differentiation. The term “transform” has a specific meaning in human biology; it is unrelated to differentiation.

The metaphorical concept that “the proliferation and migration of keratinocytes suture the wound site during wound-healing”, is not widely supported. While this concept might be appropriate to
describe cutaneous wound-healing in rodents and mammals, where healing occurs primarily by contraction, it does not describe cutaneous wound-healing as it occurs in humans. In humans, cutaneous wound-healing is mediated primarily by reepithelialisation; only a minor contribution is from contraction. There is nothing akin to sutures in cutaneous wound-healing in humans.

Figure 2 is inaccurate and misleading. 1. Pathogens are located in the dermis, beneath an intact basement membrane. 2. Mast cells are illustrated, but not described in the text.

I recommend that Figures 3–35 be combined and presented as a single figure, or possibly as a table.

“Plants are the potential...l” is grammatically incorrect. It should read ‘Plants have the potential...’

It would be helpful for the reader to be informed what is the basis for selecting the plant species, subjects of subsequent discussion.

The statement “wound closure was 50,38%±1,39 and 69,81%±1,89, respectively” is meaningless without also reporting closure measures from untreated, control wounds evaluated in the same “wound-healing study” (sic). The wound-healing study is not identified. Please identify “The wound-healing study” (sic)!

The rationale for assaying “anti-inflammatory activity by determining the inhibitory activity on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in 264.7 RAW cells” (sic) escapes me. 264.7 RAW cells are a murine macrophage cell lines created by transformation with Abelson leukemia virus. LPS, also known as endotoxin, is a component of cell wall from Gram-negative bacteria. 264.7 RAW cells are very sensitive to LPS, and respond when recognised by the receptor TLR4, by generating ROS and NOS, part of macrophage’ antimicrobial pathways. It is not clear whether “The wound-healing study” evaluated wounds infected with Gram-negative bacteria, or uninfected wounds.

How do the “results found... (a strong inhibitory effect)? This is poor grammar.

How does “A. bracteolate showed decreased pro-inflammatory cytokine mRNA expression concentration-dependent, indicating a mechanism for iNOS inhibition, gene expression analyzed by Real-Time PCR” (sic)? The absence of methodology assures this statement cannot be tested and verified by others.

The discussion of collagen gene expression and synthesis under “Aristolochia bracteolate” has no relevance. No evidence is reported, nor is any evidence cited.

What is the evidence “The methanol extract (EM) of the leaves of Boerhavia diffusa significantly increased the viability and migration of human keratinocytes (HaCaT) cells”?

What is the evidence “…caffeic acid, ferulic acid, and D-pinitol (are) the primary bioactive metabolites”?

What is the evidence “…secondary metabolites in the extract of punarvana such as phenolics and flavonoids reduces lipid peroxidation, increases collagen fibrils’ survival by increasing collagen fibers’ strength, prevents cell damage, and accelerates DNA synthesis” (sic)?
What is the evidence “Phenolics, flavonoids, and terpenoids enhance wound-healing due to their antioxidant and antimicrobial properties”?

What is the evidence “Apply topical insulin promotes diabetic wound-healing by regulating wound inflammatory cells and improving cellular function” (sic)?

What is the evidence “The results showed a significant increase in the viability of HaCaT cells on ASE-loaded nanoemulsions” (sic)?

What is the evidence “ASE did not induce HaCaT cytotoxicity and tended to increase keratinocyte cell viability”? (What is ASE?)

What is the evidence “Anti-inflammatory and wound-healing activities have been reported of calophyllolide (CP) isolated from Calophyllum inophyllum Linn”? 

What is the evidence “results showed that CP did not affect the viability of HaCaT cells in the concentration range” (sic)?

What is the evidence “CP reduced fibrosis formation and effectively promoted wound closure in a mouse model without causing weight loss”?

What is the evidence “results showed that HaCaT cells grown in the presence of U. parvifolia root bark extract showed a faster and dose-dependent growth rate than untreated cells” (sic)?

What is the evidence “...polysaccharides from Aloe vera also induce matrix metallopeptidase (MMP)-3 and metallopeptidase inhibitor-2 gene expression during wound repair”?

What is the evidence “The gel was tested to be non-cytotoxic against nauplii and compatible with human blood and skin cells” (sic)?

What is the evidence “Aloe vera promotes the attachment and proliferation of HaCaT and HFF1 cells”?

What is the evidence “HS ethanol extract accelerated wound-healing activity in epithelial formation and fibronectin production”?

What is the evidence “HS enhances the expression of genes involved in skin hydration and homeostasis”? What are the “genes involved in skin hydration and homeostasis”?

What is the evidence “NMP from the leaves of Sideroxylon obtusifolium (Brazilian medicinal species) has activity as anti-inflammatory and wound-healing management”?

What is the evidence “The extract showed a strong positive result of 65%, with a difference of 14% in the migration rate between the two” (sic)?

What is the evidence “Grandiflorenic acid (2,5 μg/mL) resulted in a 106% percentage of HaCaT keratinocyte viability, induced a migration rate of 100% in the initial in vitro assay” (sic)?
What is the evidence “Treatment with Maja flowers for 24 hours drastically increases cell motility and expression of keratinocytes in specific cell lines” (sic)?

What is the evidence “ethanolic extract of Eriobotryae folium (EF) increases intracellular and extracellular PGE2 levels in HaCaT cells and inhibits 15-PGDH...”?

What is the evidence “GG inhibits abnormal cell proliferation and is anticarcinogenic”?

What are the “Previous studies (that) showed that Thyme oleoresin at 25 μg/mL and 50 μg/mL significantly promoted HaCaT cell migration, leading to wound closure”?

What is the evidence “The purified Gracilaria lemaneiformis (GLP-2) fraction promoted cell proliferation and migration of HaCat cells through activation of PI3K/aPKC signaling during wound-healing of human keratinocytes”?

What is the evidence “UD extract increased the proliferation rates of HEK-293 and HaCaT cells by 39% and 30%”?

Where are the “results (that) showed that the cell viability test showed that the CA extract increased the viability of HaCaT cells”?

What is the evidence “methanol extract of Clausena excavate contains coumarins, flavonoids, and glycosides with various biological properties”? What are these “various biological properties”?

What is the evidence Angelica gigas “extract improves wound-healing with HaCaT human keratinocytes”?

What is the evidence “reduced inflammatory cell density in granulation tissue in 7-day-old wounds, combined with decreased TNF-α and NF-B1 mRNA expression” (sic)?

Where are the “results (that) showed that ashwagandha extract (AE) significantly inhibited mRNA expression of inflammatory cytokines”?

What is the evidence “A. asphodeloides (AA) extract promoted inhibiting Th2-type cytokines, pro-inflammatory cytokines, and filaggrin restoration in HaCaT cells” (sic)?

The sentence “It is possible to assume that AA facilitates wound-healing in the skin barrier through the inhibition of overexpression” (sic) is incomplete.

What is the evidence “S. veitchii extract increases AQP3 expression and provides wound-healing and healing effects” (sic)?

What is the evidence “root extract of A. tenuissima accelerates wound filling under basal conditions in the keratinocytes” (sic)?

What is the evidence “This plant shows that astragaloside can activate cellular processes involved in wound-healing”?
What is the evidence “Mimosa tenuiflora (Willd) aqueous extract... indicated a loss of cell viability and proliferation of dermal fibroblasts“ (sic)?

What is the evidence “Allerce extract has a significant effect on wound-healing“?

References

Are the rationale for, and objectives of, the Systematic Review clearly stated?
No

Are sufficient details of the methods and analysis provided to allow replication by others?
No

Is the statistical analysis and its interpretation appropriate?
Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cutaneous wound healing and tissue repair; human epithelial cell physiology; extracellular interface.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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The abstract is not informative. The background should cover the rationale of using plant material in facilitating wound healing. The results should cover the common pathways of different plants in promoting wound healing.

○ Introduction: the anatomy of the skin and the process of wound healing should be presented here, not in the results.

○ Methods: PRISMA flow chart on article identification. The rationales for limiting years of publication to 2009-2021 were not presented. Other details like article management were not disclosed. This makes the search not replicable.

○ Results: There is no effort in the synthesis of search results.

○ There is no discussion, no schematic diagram to sum up the mechanisms of plant-derived substances involved in facilitating wound healing.

Are the rationale for, and objectives of, the Systematic Review clearly stated?
No

Are sufficient details of the methods and analysis provided to allow replication by others?
No

Is the statistical analysis and its interpretation appropriate?
Not applicable

Are the conclusions drawn adequately supported by the results presented in the review?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Natural products, bone and joint metabolism

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.
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