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Review Article

Signal transduction in wound healing: The effects of plant-derived biologically active substances

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Abstract

Wounds are among the most common skin diseases. They are formed after tissue injury and result in severe damage to the epidermis. The application of plants for wound healing is not only a cheap and accessible way of treatment, but also provides a reliable natural resource of medicinal substances with fewer side effects. Biologically active substances such as alkaloids, essential oils, flavonoids, tannins and phenolic compounds demonstrate a wide spectrum of action: wound healing, anti-inflammatory, antibacterial and antioxidant effects.

This review article examines the chemical composition of three plants - *Calendula officinalis*, *Marrubium vulgare*, *Vitis vinifera* and the signal transduction in skin wound healing. Studies have shown that they have a strong antioxidant effect, hemostatic activity, powerful vasoconstrictor effect, pro-angiogenic and cell-protective effects.

Because of the mentioned benefits of these plants, more scientific research and well-designed clinical trials are needed to establish their wider use in wound treating medicaments.

Keywords

Calendula officinalis, *Marrubium vulgare*, signal transduction, *Vitis vinifera*, wound healing

Anatomical features of the skin

The skin consists of three main layers: the outermost layer, the epidermis, followed by the dermis and the hypodermis. It is considered to be the largest organ of the human body, which covers and protects the body from external harmful agents. The skin reduces electrolyte loss, regulates body temperature, and acts as an immune defense against microorganisms (Marques et al. 2023).

The epidermis consists of a flat 0.1 mm thick epithelium without blood vessels. The main cells are keratinocytes, which are almost 95% (Gantwerker and Hom 2012). Keratinization - the process that leads to the differentiation of keratinocytes, starts from the inner basal layer of the epidermis, where these cells actively divide. The epidermis is subdivided into four sublayers: *stratum germinativum*, *stratum spinosum*, *stratum granulosum* and *stratum corneum*. An additional sublayer called the *stratum lucidum* can be found between the *stratum corneum* and *stratum granulosum* of the epidermis of the palms and soles. In addition to keratinocytes, melanocytes, Merkel cells, Langerhans cells are also found in the epidermal layer, but in a smaller amount (Mahmoud et al. 2022).

The dermis gives the skin its durability, strength and flexibility. It is divided into two layers. The first layer, the most superficial – the papillary dermis, is made up of connective tissue, elastic fibers, collagen, vessels and nerves. The second dermal layer is known as the reticular dermis and includes fibroblasts, mast cells, nerve endings, lymphatics and muscles, and also contains thick bundles of collagen and blood vessels. According to different stages of development and health status, other types of cells may be present in the dermal layer. These cells can be extravasated leukocytes common during inflammation or infection, histiocytes or reticulin cells (Mahmoud et al. 2022).

The hypodermis is highly vascularized and made up of adipocytes. It is located between the dermis and the underlying muscle and plays a crucial role in thermoregulation, protection from mechanical injury and energy reserve. The thickness of this layer has anatomical and individual specificity, reflecting the eating habits of each individual (Khavkin and Ellis 2011).

The physiology of wounds

Wounds are among the most common diseases that affect the skin. They are formed after tissue injury, usually by external agents causing severe damage to the epidermis and underlying connective tissue (Lordani et al. 2018). Disruption of the normal function and structure of the skin leads to several types of wounds – burns, contusions, ulcers and cuts (Yazarlu et al. 2021; Mssillou et al. 2022). Skin lesions can also occur as secondary complications of other diseases such as cancer and diabetes. Wounds are classified as acute or chronic depending on the factors leading to the injury, signaling inhibitors, type of skin wound and individual skin condition (Lordani et al. 2018). Acute wounds normalize in a few days depending on the severity of the injury, rejecting the hypothetical growth of microorganisms (Mssillou et al. 2022). Proliferation of bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* leads to treatment delays, complications and increased healing time (Yazarlu et al. 2021). Chronic non-healing or difficult-to-heal wounds are more challenging clinical conditions. These are mostly associated with circulatory problems and diabetes, where the normal healing process is often arrested by excessive neutrophil infiltration, inflammation, increased production of reactive oxygen species (ROS) and even necrosis (Baali et al. 2022).

Wound healing

Functional restoration and mechanical integrity of the skin consists of four sequential stages: hemostasis, inflammation, proliferation, and wound remodelling.

Hemostasis

Hemostasis begins immediately after injury and lasts from 1 to 3 hours. The repairing process begins with vasoconstriction, followed by the release of platelet granules with subsequent aggregation into a platelet plug that disrupts blood flow. This becomes possible after the release of several growth factors, cytokines and low molecular weight substances from the serum of damaged blood vessels and degranulating platelets (Mssillou et al. 2022). The blood clot includes cross-linked fibrin and extracellular matrix (ECM) proteins such as fibronectin, vitronectin and thrombospondin (Velnar et al. 2009). Inflammatory cells are attracted to the area by chemokines and cytokines released by platelets. This induces an invasion of neutrophils, macrophages and lymphocytes that will lead to the next stage of the healing process (Werner and Grose 2003; Marques et al. 2023).

Inflammation

The inflammatory stage begins with the influx of neutrophils, macrophages, and lymphocytes to the site of injury. This stage can last from 24 to 48 hours or even a week. During this stage, the focus is on controlling bleeding, preventing bacterial growth, and removing any cellular debris from the wound area. Neutrophils at the wound site begin the process of phagocytosis. This process is continued by macrophages, which result from the differentiation of monocytes present in the blood. Consequently, by-products of neutrophil apoptosis and cell fragments are phagocytosed by macrophages (Hart 2002). Besides eliminating bacteria and cellular debris from the wound site, macrophages also release several growth factors and other molecules that stimulate fibroplasia and angiogenesis (Tsourdi et al. 2013). These factors are cytokines, such as interleukin IL-6, IL-1β, IL-10, matrix metalloproteinases (MMPs), and growth factors such as vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), basic fibroblast growth factor (b-FGF), epidermal growth factor (EGF), and transforming growth factor (TGF-β) (Rodrigues et al. 2019). At the end of this stage, lymphocytes appear at the wound site to help upregulate collagenase, which is crucial for collagen remodeling in subsequent phases (Marques et al. 2023) (Fig. 1).

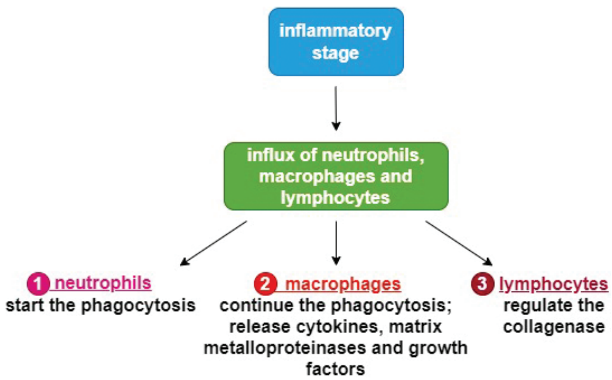

Figure 1. Inflammatory stage of early healing.

Proliferation

Once the wound is free of damaged cells and debris, the proliferative phase begins, it can take up to 20 days in acute wounds. It includes different subphases such as re-epithelialization, angiogenesis and granulation tissue formation. During re-epithelialization, mesenchymal stem cells (MSCs) differentiate into keratinocytes, which then migrate and proliferate to cover the wound and form a new epidermal layer. The next phase is angiogenesis, which is promoted by growth factors secreted during the hemostasis phase such as VEGF, platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) (Sorg et al. 2017; Marques et al. 2023).

Remodelling

Remodelling is the final phase of the wound healing process and involves cytokine production and wound contracture. It can last from 21 days to a year after the wound was inflicted. Thus, in response to cytokines and growth factors present at the wound site, fibroblasts produce MMPs that act by destroying the temporary extracellular matrix (ECM) or by synthesizing new ones composed of different types of collagen, proteoglycans, hyaluronic acid, glycosaminoglycans, fibrin and fibronectin (desJardins-Park et al. 2018). This process strengthens the newly formed tissue. Myofibroblast differentiation also improves wound closure and scar formation. (Yazarlu et al. 2021). As the wound heals, fibroblasts and macrophages undergo apoptosis, which aims to reduce their presence at the wound site. (Marques et al. 2023) (Table 1).

Table 1. Growth factors in wound healing and their functions (Chhabra et al. 2017).

Growth factor	Origin	Function
epidermal growth factor - **EGF**	platelets	Stimulates the proliferation of epithelial cells, fibroblasts and vascular endothelial cells
platelet-derived growth factor - **PDGF**	fibroblasts	Attracts fibroblasts, smooth muscle cells, monocytes and neutrophils to the wound
	vascular endothelial cells	
vascular endothelial growth factor - **VEGF**	macrophages	Stimulates angiogenesis
	keratinocytes	
	macrophages	
fibroblast growth factor - **FGF**	nervous tissue	A mitogen for tissues of mesenchymal and neural origin
	fibroblasts	
	endothelial cells	
transforming growth factor - **TGF-α**	macrophages	Inhibits the proliferation of a lot of cell types in vitro, including keratinocytes, endothelial cells and macrophages.
	lymphocytes	
	fibroblasts	
	keratinocytes	
	platelets	

Plants with an effect on wound healing

The application of plants in wound healing (in the form of decoctions, tinctures, syrups, oils, ointments and infusions) is not only a cheap and accessible way of treatment, but also provides a reliable natural resource of medicinal substances with fewer side effects compared to chemical agents (Kumar et al. 2007). Studies of biologically active substances from plants show that they have a lot of advantages in terms of rapid healing. These biologically active substances include different chemical families such as alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins and phenolic compounds, which demonstrate a wide range of actions: wound healing, anti-inflammatory, antibacterial and antioxidant (Jarić et al. 2017). Three of the most famous and widespread plants used for treating skin wounds are *Calendula officinalis*, *Marrubium vulgare* and *Vitis vinifera.*

Calendula officinalis (Asteraceae)

Chemical composition

Calendula officinalis (*C. Officinalis*) or pot marigold is a common garden plant which belongs to the Asteraceae family. The marigold is native to southern Europe, grows up to 60 cm tall and produces large yellow or orange flowers.

Flower extracts of the plant have a long history in ethnopharmacology. Traditional remedies used to heal minor wounds and treat minor skin inflammations include lipophilic and aqueous alcoholic extracts. The chemical composition of *C. Officinalis* consists of terpenoids, flavonoids, phenolic acids, carotenoids, coumarins, quinones, volatile oils, amino acids and lipids (Cruceriu et al. 2018).

Effects of *Calendula officinalis* on skin wounds

The pharmacological effects of *Calendula officinalis* are anti-inflammatory, antioxidant, antibacterial and immunostimulating (Table 2). *C. Officinalis* is mainly used in the treatment of acne, gastric ulcer, wound healing and bacterial infections (Albahri et al. 2023).

Table 2. Chemical composition of *Calendula officinalis* (Jan et al. 2017).

Active components	Constituents	Effect
Terpenoids	Lupeol, Ψ-taraxasterol Erythrodiol, Calenduloside Calendulaglycoside A Calendulaglycoside B	- Anti-Inflammatory activity
		- Cytotoxicity against, melanoma leukemia and colon cancer (Calenduloside)
Flavonoids	Quercetin, Isorhamnetin Isoquercitrin, rutin, calendoflavoside Isorhamnetin-3-O-β-D glycoside, narcissin	- Anti-proliferative, anti-inflammatory and anti-allergy activity
		- Vasorelaxation activity (Isorhamnetin-3-O-β-D glycoside)
Coumarins	Esculetin,scopoletin,umbelliferone	-Antioxidant and antimicrobial activity
Carotenoids	a-carotene, ß-carotene. lutein, zeaxanthin, neoxanthin, lycopene	-Antioxidant activity

Protein deficiency during wound healing can reduce the development of new capillaries, fibroblast proliferation, collagen synthesis, wound remodelling, and sometimes suppress the immune system. When collagen is broken down, hydroxyproline and its peptides are released. It is used as a biochemical marker to assess collagen content in tissues during the wound healing process. Increased hydroxyproline levels in granulation tissue is an indicator of increased collagen turnover, indicating better collagen proliferation. In the wound healing process, collagen synthesized by fibroblasts is the main component of the extracellular matrix, and the presence of collagen at the wound site is important for the final wound repair (Kumar Srivastava et al. 2016).

The effect of three different calendula flower extracts (n-hexane, ethanol and water) on the inflammatory phase of wound healing was investigated in human keratinocytes and dermal fibroblasts. N-hexane and ethanol extracts of calendula flowers affect the inflammatory phase by activating the transcription factor NF-κB and increasing the amount of IL-8, both at the transcriptional and protein levels, in human keratinocytes. The ethanol extract inhibited collagenase activity in vitro and increased the amount of collagen in the supernatant of human dermal fibroblasts (Nicolaus et al. 2017).

A study on rats' excision (4 cm^2) divided into two groups – untreated control and treated with 100 mg/kg/bw marigold extract was done. At the end of the tenth day, the lyophilized granuloma tissue was examined to assess the content of hydroxyproline and hexosamine. The content of hydroxyproline and hexosamine in granuloma tissue indicates the rate of tissue regeneration. The extract-treated group showed a significant increase in hydroxyproline and hexosamine content during the first days, proving that wound regeneration was significantly faster (Preethi and Kuttan 2009). The results of the same study showed that on the eighth day after wound formation, the extract-treated group had a 90% wound closure rate as opposed to 51.1% wound closure in the control group (Preethi and Kuttan 2009).

This promising plant needs to be investigated thoroughly and can be exploited for extraction of active ingredients that can be used in the synthesis of drugs used for wound healing (Jan et al. 2017).

Marrubium vulgare (Lamiaceae)

Chemical composition

Marrubium vulgare L. (*M. vulgare*) – white horehound, native to the area between the Mediterranean and Central Asia, is a widespread species that is now found on all continents. *M. vulgare* is a herbaceous plant with a tough, branching taproot or numerous fibrous lateral roots and erect stems - 20 to 100 cm tall. The essential oil content is between 0.03% and 0.06% with monoterpenes such as camphene, p-cymol, fenchene, limonene, α-pinene, sabinene and α-terpinolene (Zawiślak 2009). *M. vulgare* accumulates labdane-type diterpenes with marubiin being predominant (0.12–1%), followed by its precursor pre-marubiin (0.13%), 12(S)-hydroxymarubiin, 11-oxomarubiin, 3-deoxo-15(S)-methoxyvelutin, marubenol and others. It is also an abundant source of various phenolic compounds – phenolic acids, phenylpropanoid (cinnamic) acids and flavonoids. *M. vulgare* also contains tannins (up to 7%), with the estimated amount of condensed tannins being 16.55 mg catechin/100 g dry material (Aćimović et al. 2020). More detailed chemical analysis identifies gallic, gentisic, p-hydroxybenyoic, protocatechuic, and syringic acids. In the cinnamic acids subgroup of phenolic compounds, trans-cinnamic, ferulic, o-coumarinic, p-coumarinic and sinapic acids are determined. Hydroxycinnamic acid derivatives, phenylethanoid glycosides, such are acteoside (used as qualitative marker), alyssonoside, arenanoside, ballotetroside, marruboside, and others represent another subgroup of phenolic compounds present in *M. vulgare* extracts (Dewick 2009; Aćimović et al. 2020).

Effect of *Marrubium vulgare* on skin wounds

In vitro antioxidant properties of methanolic extract of *M. vulgare* determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay showed strong activity with a half maximal inhibitory concentration (IC50) value of 8.24–12.42 μg/mL (Yousefi et al. 2016). The antioxidant activity tested by the same method showed that the essential oil of *M. vulgare* showed an IC50 value of 153.84 μg/mL, which was about two times higher than a synthetic antioxidant - butyl hydroxytoluene (Abadi and Hassani 2013). A photochemiluminescence assay evaluating the antioxidant activity of the compound in the presence of superoxide radical and reactive oxygen species determined the strong antioxidant effect of methanol and acetone extract of *M. vulgare* (261.41 and 272.90 μmol TE/g, respectively).

Methanolic extract of *M. vulgare* is rich in polyphenolic compounds (flavonoids and several phenylethanoid glycosides) and marubiin (6.62%). A study showed that the extract has antioxidant and wound-healing properties by activating cell migration and proliferation (Amri et al. 2017) (Fig. 2). A study of hemostatic activity by the plasma recalcification time method confirmed a dose-dependent anticoagulant effect of the aqueous extract of *M. Vulgare* (Kadri et al. 2011). The investigated parameters, the content of condensed tannins and hemostatic activity also show the powerful vasoconstrictor property of the plant in the first stage of wound healing (Aćimović et al. 2020).

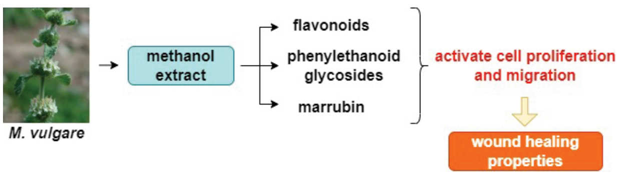

Figure 2. Content and effects of *M. vulgare* methanolic extract.

Vitis vinifera (Vitaceae)

Chemical composition

Vitis vinifera, the common grape, is found primarily in the Mediterranean region, central Europe and southwest Asia. The grapes can be eaten fresh or dried, and the leaves are used in the cuisine of many cultures. Fresh grapes can also be processed into juice through the fermentation process to make wine or vinegar. Grape skin is a valuable source of unsaturated fatty acids, fiber, polyphenols, proanthocyanidins (catechin/epicatechin), minerals and resveratrol (3,5,4'-trihydroxy-trans-stilbene). The fruits of *V. vinifera* are considered a major source of phenolic antioxidant compounds, including quercetin. The seeds also contain polyphenolic compounds, such as flavanols, resveratrol and proanthocyanidins (Ma and Zhang 2017).

Effects of *Vitis vinifera* on skin wounds

Tissue regeneration and revascularization in wounds are regulated by resveratrol-induced expression of VEGF and inhibited by the expression of proinflammatory factors such as TNF-α. In ulcers associated with microvasculopathy, such as diabetic foot ulcers, VEGF has a wide range of healing-related actions, ranging from capillary growth to increased cell migration, collagen deposition and epithelialization (Brem et al. 2009). Proinflammatory markers such as interleukin (IL)-1β, IL-6, and TNF-α prolong the inflammatory phase and delay wound healing. An increase in these markers leads to an upregulation of matrix metalloproteinases (MMPs), which are particularly elevated in chronic wounds. MMPs degrade the local extracellular matrix (ECM) and delay cell migration in wounds (Pignet et al. 2021). In patients treated with resveratrol, pro-inflammatory markers such as IL-1β, IL-6, TNF-α, MMP-2, -3, -9 and C-reactive protein (CRP) were reduced (Khodarahmian et al. 2021; Pignet et al. 2021).

Topical application of *V. vinifera* seed extract to excision wounds in animals showed a significant ($p < 0.001$) reduction in wound area compared to untreated wounds (Al-Warhi et al. 2022). Wound closure is a signal for re-epithelialization, angiogenesis, granulation, keratinocyte differentiation and fibroblast proliferation (Tang et al. 2007).

Gene expression analysis of samples at day 7 showed that the action of inflammatory markers such as TNF-α and IL-1β was decreased in wounds treated with *V. vinifera* seed extract compared to untreated wounds. Treatment with *V. vinifera* seed extract for 14 days showed a significant decrease in TNF-α and IL-1β mRNA expression compared to the untreated group ($p < 0.001$) (Al-Warhi et al. 2022).

VEGF plays an important role in the regeneration of new blood vessels, stimulates wound healing through various processes consisting of collagen deposition, angiogenesis and epithelialization and binds to the two VEGF receptors (VEGF-1 and VEGF-2) that are located on vascular endothelial cells (Ferrara 2004). Analysis of the relative expression of VEGF as well as type I collagen in wound samples at day 7 post-injury showed significantly increased levels in wounds treated with *V. vinifera* seed extract compared to untreated wounds. Furthermore, treatment with *V. vinifera* seed extract for 14 days revealed a more significant increase in the relative protein expression of VEGF and type I collagen genes compared to untreated wounds ($p < 0.001$) (Al-Warhi et al. 2022).

Resveratrol, which is contained in grapes, is a polyphenol, which, in addition to its well-known antioxidant potential, also has anti-inflammatory, proangiogenic and cell-protective effects. Resveratrol has been shown to have beneficial effects on human skin and has been used to accelerate wound healing and prevent the development of chronic wounds without systemic side effects (Pignet et al. 2021) (Fig. 3).

Several in vitro and in vivo studies demonstrate that resveratrol acts as a sirtuin 1-regulator (SIRT1). SIRT1 is a nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase located in the cell nucleus. Seven sirtuin isoforms have been identified - SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6 and SIRT7. SIRT1 can be activated by calorie restriction (CR) or pharmacologically by CR-mimetics such as dietary or topical resveratrol. The rate of deacetylation of SIRT1 in the presence of resveratrol is dependent on the fluorophore. No direct effects of resveratrol have been observed. This indicates that the in vivo effects mediated by resveratrol may not be based on direct activation of SIRT1, but on a different molecular mechanism (Kaeberlein et al. 2005). A study that tests whether the attached fluorophore mimics a hydrophobic pocket on native protein substrates that provokes higher affinity for SIRT1 was conducted. A native peptide substrate was used and direct detection and quantification methods such as high performance liquid chromatography (HPLC) were performed. Their results show that resveratrol does not activate SIRT1 in these natural substrates and therefore provide evidence that resveratrol can activate SIRT1 indirectly (Pacholec et al. 2010).

In vivo and in vitro experiments show that resveratrol can be a competitive inhibitor of cAMP-degrading phosphodiesterase (PDE). In the presence of low dose

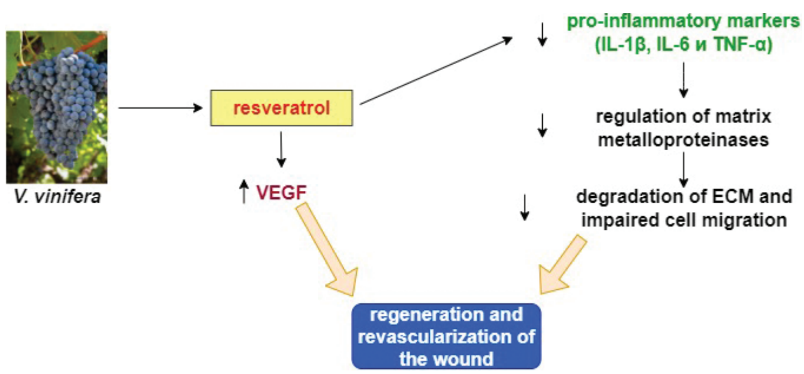

Figure 3. Effects of *V. Vinifera* on wound healing.

(≤50 μM) resveratrol, intracellular cAMP levels in C2C12 myotube cells were significantly increased. Elevation of cAMP levels further leads to activation of the Ca(2+)/calmodulin-dependent protein kinase-β-AMP-activated protein kinase (CamKKβ-AMPK) pathway. AMPK leads to an increase in NAD+ levels, followed by SIRT1 activation and deacetylation of SIRT1 target proteins. To confirm that resveratrol increases cAMP levels in vivo, an experiment was done by administering resveratrol to mice and measuring cAMP levels in skeletal muscle and white adipose tissue. An increase in cAMP levels was found in both tissues (Park et al. 2012).

Once SIRT1 is activated, it deacetylates several transcription factors that contribute to cellular regulation, such as forkhead box O3 (FOXO3), nuclear factor (NF-κB), and p53. SIRT1-regulated pathways affect cell survival, metabolism, stress resistance, endothelial function and circadian rhythm (Villalba and Alcaín 2012). In wound healing, SIRT1 has anti-inflammatory and antioxidant properties, induces collagen synthesis, and induces autophagy as well as angiogenesis (Huang et al. 2019; Pignet et al. 2021). More investigations need to be done in order to evaluate the precise mechanisms underlying healing properties.

Conclusion

A number of studies on *C.officinalis*, *M.vulgare*, *V.Vinifera* and their active ingredients prove their beneficial effects on signal transduction of wound healing. The application of plants for wound healing is not only a cheap and accessible way of treatment, but also provides a reliable natural resource of medicinal substances with fewer side effects. Biologically active substances such as alkaloids, essential oils, flavonoids, tannins, saponins and phenolic compounds demonstrate a wide spectrum of action: wound healing, anti-inflammatory, antibacterial and antioxidant effects. Because of the mentioned benefits of these plants, more scientific research and well-designed clinical trials are needed to establish their wider use in wound-treating medicaments.

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