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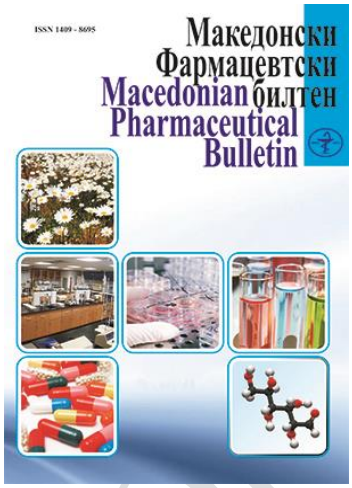

Title: Medical plants with potential wound-healing properties

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DOI:
Received date: March 2026
Accepted date: May 2026
UDC:
Type of paper: Review paper
Mac. Pharm. Bull. 72 Vol. (2) 2026

Please cite this article as:

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*Corresponding author e-mail: iskradavkova@ff.ukim.edu.mk

doi: *Review paper*

Medical plants with potential wound-healing properties

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Abstract

Chronic wound management relies on biological activities such as antimicrobial action to prevent infection, antioxidant capacity to counteract oxidative stress, and anti-inflammatory effects to support tissue repair and regeneration. Medicinal plants have been used from traditional healing practices to modern research as valuable sources of such activities. This review integrates historical use with contemporary scientific evidence to highlight medicinal plants with potential applications in wound treatment, with particular emphasis on their biological activities and reported therapeutic effects. Special attention is given to five selected medicinal plants: *Calendula officinalis* L., *Hypericum perforatum* L., *Achillea millefolium* L., *Plantago major* L., and *Verbena officinalis* L. Available experimental and clinical evidence demonstrates that these plants exhibit pronounced antioxidant, anti-inflammatory, and antimicrobial properties across various models. In this context, growing evidence suggests that multicomponent herbal mixtures may offer enhanced therapeutic benefits by simultaneously targeting multiple pathological mechanisms involved in chronic wounds. Therefore, the therapeutic potential of combined herbal formulations and possible synergistic interactions warrants further focused investigation to support the development of effective, evidence-based strategies for chronic wound management.

Key words: antioxidant, anti-inflammatory, antimicrobial activity, healing plants

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Introduction

Wounds are defined as discontinuities in the integrity of the skin and underlying tissues caused by physical, chemical, thermal or biological trauma. They can be acute or chronic. Acute wounds heal within a reasonable period of time (4-6 weeks), while chronic wounds require a significantly longer period to reduce in size and achieve healing. Chronic wounds are characterized by a prolonged inflammatory phase, reduced ability to regenerate, and an increased risk of infection. These wounds require long-term therapy and, if not treated appropriately, can lead to complications such as limb loss or systemic infections (Martinengo et al., 2019).

Chronic wounds represent a significant global health problem, affecting millions of people worldwide. They are potentially much more threatening than they are realistically presented. For example, the five-year mortality rate for patients with chronic wounds is significantly higher than for patients with colorectal, breast, and prostate cancer (Mahmoudi and Gould, 2020), as medicine has developed greater focus, experience, and knowledge in diagnosing these diseases. However, when it comes to chronic wounds, the complexity of their causes, environmental influences, and contributing factors makes diagnosis and treatment more challenging (Mahmoudi and Gould, 2020; Martinengo et al., 2019; Sen, 2019).

The treatment of chronic wounds is a complex and challenging process that demands a multidisciplinary approach to ensure effective healing. It is essential that the selected therapy does not worsen existing health conditions due to the fact that chronic wounds are often associated with multiple comorbidities. Therefore, any therapeutic strategy must prioritize safety, avoiding toxicity or harmful side effects, since negative outcomes can significantly affect the wound and compromise the patient's overall health, including pre-existing conditions (Martinengo et al., 2019). Effective treatment of chronic wounds requires several essential biological actions: antimicrobial activity to prevent infection, antioxidant properties to reduce oxidative stress that can delay healing, and anti-inflammatory effects to reduce inflammation and promote tissue regeneration. As a result of the increasing resistance of existing antibiotics, there is a need for new antibacterial agents that will have both antioxidant and anti-inflammatory effects. The fact that it is much easier to discover an existing and at the same time new agent, guides

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us toward research in herbal medicine and natural, biochemically active components (Zulkefli et al., 2023).

Given the complexity of chronic wound management and the need for multifunctional therapeutic action, herbal treatments have emerged as a promising alternative or complement to conventional methods. One of the key advantages of herbal treatments lies in their broad-spectrum activity, derived from the multicomponent nature of plants. In contrast to synthetic drugs, which typically rely on a single active ingredient, herbal preparations contain a diverse array of bioactive compounds that act synergistically to enhance therapeutic effects (Kaya et al., 2022). Moreover, while synthetic drugs often carry a risk of severe side effects, herbal treatments tend to be safer and less aggressive toward the body. This allows for a more holistic approach that not only targets the wound locally but also supports the patient's overall well-being (Maver et al., 2015).

Therefore, the aim of this review is not to identify a single most potent medicinal plant, but rather to justify and systematize the selection of complementary plant species whose combined activities address the multifactorial pathophysiology of chronic wounds, thereby supporting more effective and rational wound management.

Plants with Potential for Chronic Wound Treatment

Methodology

This review article is based on a comprehensive analysis and synthesis of relevant scientific literature addressing the use of medicinal plants in the treatment of chronic wounds. The methodological approach was designed to integrate historical knowledge with contemporary pharmacological, phytochemical and biological evidence, providing a structured overview of the bioactive potential of selected medicinal plants.

A systematic literature search was conducted using the following scientific databases: PubMed, Scopus, ScienceDirect, SpringerLink, Web of Science and Google Scholar.

The search strategy employed combinations of the following keywords and phrases: *Calendula officinalis* L., *Hypericum perforatum* L., *Achillea millefolium* L., *Plantago major* L., *Verbena officinalis* L., bioactive compounds, antioxidant activity, antimicrobial activity, anti-inflammatory activity, wound healing, and chronic wounds.

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Peer-reviewed scientific articles published within the last 10 years were primarily considered. However, older publications of recognised scientific or historical importance were also included when relevant, particularly for the ethnopharmacological context and traditional use. Only studies published in peer-reviewed journals with clearly described methodologies were included in the analysis.

In addition to primary research articles, EMA/HMPC assessment reports were used as authoritative sources for chemical composition and traditional use of selected medicinal plants, where available.

The review included *in vitro*, *in vivo* and clinical studies reporting quantified outcomes related to antioxidant, anti-inflammatory, antimicrobial and wound-healing activities. Studies lacking verified data on chemical composition, biological activity or scientific validation were excluded.

The selected studies were systematically organised and grouped according to their primary focus, including antioxidant, antimicrobial, anti-inflammatory and regenerative activities relevant to chronic wound pathophysiology. A comparative analysis of the reported results was performed to assess consistency, relevance and complementarity among different plant species.

All data were critically evaluated and synthesised to provide an integrated overview of the bioactive potential of the selected medicinal plants. This approach enabled the identification of complementary mechanisms of action and supported the rationale for investigating multicomponent herbal combinations rather than isolated single-plant preparations in the management of chronic wounds.

Background for plant selection

Based on the available literature, several medicinal plants have attracted considerable scientific interest and show promising potential in the treatment of chronic wounds, including *Calendula officinalis* L., *Hypericum perforatum* L., *Achillea millefolium* L., *Plantago major* L., and *Verbena officinalis* L.

The selection of medicinal plants included in this review research was based on documented historical and traditional use in the treatment of wounds, burns, and inflammatory skin conditions, as well as on their long-standing application in European and ethnopharmacological medical systems (Table 1 and 2).

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Table 1.

In order to better understand the biological basis underlying their reported therapeutic effects and to provide a mechanistic context for their wound-healing activities, it is essential to consider the phytochemical profiles of these plants and the major classes of bioactive compounds they contain (Table 3).

Table 2

Table 3

Wound healing potential of Calendula officinalis L.

Calendula officinalis L., commonly known as marigold, is a well-known medicinal plant that has been valued since ancient times for its therapeutic properties. Historical records and traditional practices from various cultures document its use in the treatment of numerous skin ailments. Today, *C. officinalis* L. continues to be widely incorporated into modern dermatological and cosmetic formulations, particularly those intended for sensitive, irritated, or damaged skin.

Numerous studies have highlighted the antioxidant, anti-inflammatory, and antibacterial properties of *C. officinalis* L., making it a potential natural remedy for chronic wound treatment. Available literature indicates that various extracts of this plant, obtained using solvents such as ethanol, methanol, or ethyl alcohol, demonstrate significant antioxidant activity through free-radical scavenging mechanisms and effectively reduce oxidative stress (Khairnar et al., 2013; Preethi et al., 2006). As described by Arora et al. (2013), this activity may be primarily due to the inhibition of lipoxygenase by isorhamnetin-*O*-glucosides. Moreover, *Calendula officinalis* extract has demonstrated inhibitory effects on monoamine oxidases (MAOs), enzymes implicated in oxidative stress and the pathogenesis of neurodegenerative disorders. This activity is primarily attributed to its flavonoid constituents, including rutin, apigenin, kaempferol, vitexin, and luteolin (Shahane et al., 2023). Additionally, phenolic acids such as caffeic acid, vanillic acid, chlorogenic acid, and p-coumaric acid, also present in *C. officinalis* flower extracts, exhibit notable free radical scavenging activity through their hydrogen-

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donating capacity, further supporting their potential in the treatment of oxidative stress-related conditions (Butnariu and Coradini, 2012).

Several studies support the potent anti-inflammatory properties of Mary's gold. The plant exhibits anti-inflammatory activity by modulating cytokine levels (Khairnar et al., 2013) and inhibiting the enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (Garrido-Suárez et al., 2022). Based on these findings, Mary's gold is expected to regulate excessive inflammation, promote healing, and reduce inflammation, pain, and swelling at the affected site.

C. officinalis extract has also demonstrated significant antibacterial activity against a range of pathogens (Khairnar et al., 2013; Patil et al., 2022; Balciunaitiene et al., 2022). This efficacy has been confirmed against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans* and *Aspergillus niger* (Ashwlayan et al., 2018).

Numerous *in vivo* studies provide evidence supporting the wound-healing activity of *Calendula officinalis* L. The plant has demonstrated significant wound-healing activity through mechanisms such as the adhesion and augmentation of fibroblasts in the affected area (Shahane et al., 2023), enhancement of connective tissue synthesis, increased collagen cross-linking, and the inhibition of collagen-degrading enzymes (Preethi and Kuttan, 2009). Furthermore, an aqueous extract of Mary's gold has been shown to promote wound healing in the oral mucosa by stimulating neovascularization and accelerating hyaluronic acid deposition (Roveroni-Favaretto et al., 2009). The EMA/HMPC assessment report identifies at least six clinical studies evaluating the topical application of Mary's gold in wound and skin lesion management, indicating a consistent clinical signal of wound-healing activity**.** Topical application of *C. officinalis* ethanolic extract on patients with venous ulcers resulted in a notable reduction of ulcer surface area (Duran et al., 2005). In a randomized clinical trial involving 111 women, the topical application of *Calendula officinalis* and *Aloe vera* ointment significantly reduced redness, edema, ecchymosis, discharge and improved wound approximation following episiotomy (Eghdampour et al., 2013). Similarly, a randomized controlled open trial on 156 patients with partial- to full-thickness burns demonstrated that daily topical application of *Calendula* ointment significantly reduced fibrin and hyperemia, accompanied by increased collagen deposition and enhanced angiogenesis in the wound

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bed (Lievre et al., 1992). Animal studies further support these findings. Topical application of Mary's gold gel enhanced fibroblast proliferation and blood vessel formation at incision sites, leading to complete re-epithelialization and significantly greater tensile strength of the healed wounds (Shafeie et al., 2015). Moreover, topical use of *C. officinalis* L. has proven effective in treating diaper dermatitis in infants (Panahi et al., 2012). The application of *C. extract* gel also showed positive effects in reducing lesion size in patients with homogeneous leukoplakia (Singh and Bagewadi, 2017). Additionally, *Calendula officinalis* L. has been associated with faster secondary intention healing in patients with acute hand and finger wounds (Giostri et al., 2021).

Wound healing potential of Hypericum perforatum L.

Hypericum perforatum L., also known as St. John's Wort, is a well-known medicinal plant used for centuries in traditional medicine. It is most commonly associated with mood support, but it also has a wide range of other beneficial effects. Studies have shown that it possesses antioxidant, anti-inflammatory, antibacterial, and wound-healing properties.

Its antioxidant activity is attributed primarily to its flavonoid content, which contributes to free radical scavenging (Zou et al., 2004). Application of *H. perforatum* oil cream has been shown to reduce levels of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), suggesting its role in modulating oxidative stress *in vivo* (Han et al., 2017).

As previously mentioned, this plant exhibits significant anti-inflammatory activity. It increases the number of fibroblasts, enhances the volume of collagen bundles (fibers), and enlarges both the average diameter and volume of blood vessels. These effects collectively support fibroblast proliferation, collagen synthesis, and revascularization (Jarić et al., 2018; Öztürk et al., 2007). St. John's Wort extract also inhibits the production of pro-inflammatory mediators such as prostaglandin E2 (PGE2) and tumor necrosis factor-α (TNF-α). The anti-inflammatory activity is largely due to flavonoids like quercetin and amentoflavone, together with the bioactive compounds pseudohypericin and hypericin (Hammer et al., 2007). In addition, an ethanol extract of *H. perforatum* aerial parts has been shown to shorten the inflammatory phase of wound healing and enhance resistance to infection (Süntar et al., 2010). Similarly, a methanol extract of *H.*

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perforatum leaves demonstrates notable antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* (Jarić et al., 2018). Other studies confirm its broad-spectrum antimicrobial effects against both Gram-positive and Gram-negative bacteria, including *Enterococcus faecalis* and *Pseudomonas aeruginosa* (Cecchini et al., 2007). Moreover, aqueous suspension, chloroform, and methanol extracts of *H. perforatum* have also demonstrated potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Oil- based creams and ointments containing St. John's Wort extract have shown notable antibacterial activity in various *in vivo* studies (Han et al., 2017; Samadi et al., 2010). Furthermore, numerous *in vivo* studies support the wound-healing activity of this plant. Topical application of 5% and 10% *Hypericum perforatum* gel improved tissue regeneration in diabetic skin injuries in rats by promoting fibroblast proliferation, collagen bundle synthesis, and revascularization (Yadollah-Damavandi et al., 2015). It also enhances collagen deposition, reduces inflammation, inhibits excessive fibroblast migration, and promotes epithelialization by increasing the number and activity of polygonal fibroblasts and collagen fibers. It modulates immune response and inhibits inflammatory mediators such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS), thereby accelerating the wound healing process (Farasati Far et al., 2024).

Other *in vivo* studies further confirm *Hypericum perforatum's* ability to enhance fibroblast migration and collagen deposition in both excision and incision wound models (Süntar et al., 2010), inhibit lipid peroxidation, improve local blood circulation, strengthen collagen fibers, and prevent cellular damage by stimulating DNA synthesis (Samadi et al., 2010). Additionally, *H. perforatum* L. has been shown to reduce inflammatory infiltration, promote epithelial regeneration, and minimize (Prisăcaru et al., 2013), as well as accelerate epithelialization in oral wound models (Altan et al., 2018).

According to the European Medicines Agency (EMA/HMPC) assessment report, at least four to five clinical studies in humans have evaluated the topical application of *Hypericum perforatum* preparations in the treatment of wounds and inflammatory skin conditions. These studies primarily addressed surgical and post-operative wounds, superficial burns and inflammatory skin lesions, demonstrating accelerated epithelialization, reduced inflammation and improved local wound healing following topical application of *Hypericum perforatum* preparations.

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Wound healing potential of Achillea millefolium L.

The genus *Achillea* comprises numerous species, each with a history of traditional medicinal use across different regions. Among these, *A. millefolium* L. or yarrow is the most extensively studied and widely utilized species, supported by a substantial body of data highlighting its therapeutic applications. While various *Achillea* species have demonstrated beneficial properties, *A. millefolium* remains the primary focus due to its broad range of documented uses.

All parts of *Achillea millefolium* L., including the leaves, flowers, seeds, stems, and aerial parts, have demonstrated notable antioxidant activity (Candan et al., 2003; Fraisse et al., 2011; Georgieva et al., 2015; Keser et al., 2013; Vitalini et al., 2011). *A. millefolium* antioxidant potential is primarily attributed to its high content of flavonoids and phenolic compounds, including rutin, luteolin-7-glucoside, chlorogenic acid, as well as caffeoyl derivatives (Fraisse et al., 2011; Vitalini et al., 2011).

In addition to its antioxidant properties, yarrow is well-documented in the literature for its significant anti-inflammatory properties. Methanol and aqueous extracts from this plant's aerial parts demonstrated *in vitro* anti-inflammatory effects, through the inhibition of inflammation-related proteases such as neutrophil elastase (HNE) and matrix metalloproteinases (MMP-2 and MMP-9) (Benedek and Kopp, 2007). Moreover, aqueous extract and 70% ethanolic extract of the leaves also suppress the expression of inducible nitric oxide synthase (iNOS), an enzyme responsible for nitric oxide production triggered by lipopolysaccharide (LPS), while simultaneously increasing the production of TNF-α and IL-10 (David et al., 2010; Lopes et al., 2003). In addition, extracts from dried flower heads of *Achillea millefolium* L. have been shown to reduce inflammation in mouse paw edema models, indicating *in vivo* anti-inflammatory activity (Goldberg et al., 1969).

In addition to its well-documented anti-inflammatory and antioxidant properties, *A. millefolium* L. has also demonstrated notable antimicrobial activity. Several studies have reported that extracts from the plant's aerial parts exhibit inhibitory effects against a wide range of microorganisms, including *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enteritidis, Aspergillus niger and Candida albicans* (Candan et al., 2003; Stojanovic et al., 2005).

According to the European Medicines Agency (EMA/HMPC) assessment report, several clinical studies in humans have evaluated the topical application of *Achillea*

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millefolium preparations in the treatment of wounds and inflammatory skin conditions. These studies mainly addressed minor wounds, inflammatory skin lesions and postoperative skin conditions, demonstrating reduced inflammation, improved wound contraction and enhanced local healing following topical application of *Achillea millefolium* preparations. In Hajhashemi's study, an ointment containing both Hypericum perforatum and Achillea millefolium extracts significantly reduced pain, redness, edema, and ecchymosis in primiparous women following episiotomy (Hajhashemi et al., 2017).

Wound healing potential of Plantago major L.

Plantago major L. has been used in the treatment of chronic wounds since ancient times. In central Italy, fresh flowers of *Plantago major* L. are traditionally applied to promote the reabsorption of pus, while in the coastal region of northwestern Italy (Liguria), the leaves of *P. major* and *P. lanceolata* are used as antiseptics on wounds and ulcers, as well as to treat bee stings (Adom et al., 2017).

The antioxidant components in *Plantago major* extract contribute significantly to wound healing, primarily by protecting cells from damage caused by inflammatory mediators involved in tissue injury. Flavonoids such as plantaginin, baicalein, and hispidulin are key contributors to this antioxidant activity (Adom et al., 2017). The genus *Plantago* has demonstrated the capacity to reduce redox imbalance and prevent oxidative cellular damage. Moreover, *Plantago major* extract has been shown to increase nitric oxide (NO) and tumor necrosis factor-alpha (TNF-α) production by macrophages in the absence of lipopolysaccharide (LPS) and interferon-gamma (IFN-γ), while inhibiting cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and 12-lipoxygenase (12-LOX) enzymes (Ringbom et al., 1998). The antioxidant activity was also confirmed *in vitro* through enhanced free radical scavenging, primarily attributed to the presence of phenolic and flavonoid compounds (Kobeasy et al., 2011).

Plantago major extract also exhibits anti-inflammatory activity, mainly attributed to iridoid glycosides such as aucubin and flavonoids, including baicalein and hispidulin. Hispidulin acts as a 5-lipoxygenase inhibitor (Adom et al., 2017). Various extracts from *P. major* leaves have demonstrated anti-inflammatory effects by inhibiting pro-inflammatory cytokines such as interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), and TNF-α (Hussan et al., 2015).

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Beyond its antioxidant and anti-inflammatory benefits, *Plantago major* L. shows effective antibacterial properties that contribute to its therapeutic potential. Regarding antibacterial activity, *Plantago major* L. has shown effectiveness against *Staphylococcus aureus* (Ghezavat et al., 2014). While the leaf extract exhibited weak antibacterial activity *in vitro*, stronger effects were observed *in vivo*, with treatments eliminating infections more effectively than some antibiotics (Jarić et al., 2018). This antibacterial efficacy has been confirmed by several studies using different solvent extracts (Velasco-Lezama et al., 2006; Metiner et al., 2012).

In vivo studies support the wound-healing activity of *Plantago major* L., documenting epidermal regeneration at wound sites in Swiss mice treated with hydroalcoholic extracts of *Plantago major* and *Siparuna guianensis* (Thome et al., 2012). *In vitro* studies have further demonstrated that *P. major* leaf extracts stimulate proliferation and regeneration of the H400 oral epithelial cell (OEC) line in scratch wound assays (Zubair et al., 2012). *Ex vivo* studies using a porcine wound healing model also confirmed the wound healing stimulation of *Plantago major* leaf extracts, corroborating traditional medicinal uses (Zubair et al., 2015).

Although clinical evidence for *Plantago major* L. in wound healing is limited compared to other medicinal plants, several clinical studies in humans have investigated its topical application in the treatment of wounds and skin lesions. In a randomized triple-blind clinical trial involving patients with stage I pressure ulcers, topical application of a *Plantago major* formulation resulted in a significantly higher rate of lesion resolution compared with the control treatment (Akbari et al., 2021). In addition, a small open-label clinical trial evaluated the safety and wound-healing potential of a 10% *Plantago major* ointment in patients with second-degree burn wounds, reporting favourable clinical outcomes and good tolerability, although differences in healing time were not statistically significant (Karimi et al., 2022). Furthermore, in a randomized clinical trial involving patients with diabetic foot ulcers, topical application of a Plantavera gel containing *Plantago major* and *Aloe vera* resulted in a significant reduction in ulcer surface area compared with placebo treatment (Rafiei et al., 2018).

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Wound healing potential of Verbena officinalis L.

Unlike the previously discussed medicinal plants, *Verbena officinalis* L. has been comparatively less investigated, and the available literature provides fewer data regarding its pharmacological properties. Across existing studies, various solvents have been used for the extraction of *Verbena officinalis* compounds; however, verbascoside is consistently reported as the main bioactive component responsible for the plant's biological activity. Several studies have confirmed the antioxidant potential of this plant (Billia et al., 2008; Rechecho et al., 2011; Speroni et al., 2007), along with its antibacterial (Ahmed et al., 2017) and anti-inflammatory properties (Calvo, 2006; Deepak and Handa, 2000). Moreover, an *in vivo* study demonstrated that the aerial parts of *Verbena officinalis* L. exhibit significant wound-healing activity (Speroni et al., 2007), highlighting the plant's potential for further research in wound management studies.

Currently, no clinical trials in humans have directly assessed the wound-healing efficacy of *Verbena officinalis* preparations.

Pathophysiology of chronic wounds: bridging historical use and contemporary scientific evidence

There is an integrated synthesis of historical/traditional evidence and contemporary literature data, linking key pathophysiological mechanisms of chronic wounds with the reported biological activities of the selected medicinal plants (Table 4). Historical records provide the initial background for the use of these species in wound management, while modern pharmacological and preclinical studies further elucidate their antioxidant, anti-inflammatory, antimicrobial and regenerative effects. By combining these two levels of evidence, the table highlights both the continuity of traditional use and the scientific relevance of the selected plants in the context of chronic wound healing. Importantly, the data demonstrate that each plant predominantly targets specific, yet complementary, aspects of wound pathophysiology, supporting the concept that effective management of chronic wounds requires a multicomponent approach rather than reliance on single-plant preparations.

Table 4

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Discussion

Given the complex and multifactorial nature of chronic wound pathophysiology, numerous medicinal plants have been traditionally and experimentally investigated for their wound-healing potential (Maver et al., 2015; Pazyar et al., 2014). However, the majority of these plants primarily target isolated aspects of the healing process, most commonly inflammation control or tissue regeneration, rather than addressing the full spectrum of pathological mechanisms involved in chronic wounds. To provide a broader comparative perspective, a qualitative overview of frequently used medicinal plants in wound and burn treatment was constructed based on available literature data (Fig. 1).

Fig. 1

Rather than ranking medicinal plants according to efficacy (Fig. 1), the analysis emphasizes the diversity and complementarity of biological activities reported for commonly used wound-healing plants. The figure is intended to illustrate complementary activity profiles among different plant species. The presented scores reflect the relative consistency and frequency of evidence reported in ethnopharmacological, preclinical, and clinical literature and do not constitute quantitative measures of efficacy. This comparative overview supports the concept that effective chronic wound management requires the simultaneous modulation of multiple interconnected biological pathways, rather than reliance on a single "superior" plant. Although several medicinal plants, such as *Aloe vera, Centella asiatica, Curcuma longa* and *Symphytum officinale*, have been traditionally employed in wound healing, their reported biological activities are often concentrated within one or two dominant mechanisms, most frequently anti-inflammatory or regenerative effects (Hammam et al., 2008; Panchatcharam et al., 2006; Somboonwong et al., 2012; Staiger, 2012). In contrast, the plant species selected in the present review: *Calendula officinalis*, *Hypericum perforatum*, *Achillea millefolium*, *Plantago major* and *Verbena officinalis* are distinguished by their long-standing traditional use specifically in wound management, combined with documented antioxidant, anti-inflammatory, antimicrobial and regenerative activities (Ahmed et al., 2017; Deepak et al., 2000; Farasati et al., 2024; Fraisse et al, 2011; Giostri et al., 2021;Goldberg et al., 1969; Metiner

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et al., 2012; Rehecho et al., 2011; Ringbom et al., 1998; Stojanovic et al., 2005; Zubair et al., 2015).

In order to provide a phytochemical basis for the reported biological activities and to better understand the mechanisms underlying wound-healing effects, the major classes of bioactive constituents present in the selected medicinal plants were systematically reviewed and summarised in Table 1 (EMA/HMPC, 2016, 2017, 2019; Keshavarzi et al., 2022; EMA/HMPC, 2022; Samuelsen, 2000; Speroni et al., 2007). As summarised in Table 2, all selected plants exhibit a consistent historical record of topical application for wounds, burns, ulcers and inflammatory skin conditions. These traditional indications closely correspond to key pathophysiological features of chronic wounds, including persistent inflammation, impaired tissue regeneration, oxidative stress and increased susceptibility to infection. Building upon this historical foundation, Table 2 integrates traditional knowledge with contemporary pharmacological and experimental evidence, demonstrating that each plant predominantly targets specific, yet complementary, aspects of chronic wound pathophysiology (EMA/HMPC, 2016, 2017, 2019, 2022; Keshavarzi et al., 2022; Samuelsen, 2000; Speroni et al., 2007).

Taken together, the evidence clearly indicates that no single medicinal plant is capable of fully addressing the complex biological processes underlying chronic wound development and persistence. While individual species exhibit pronounced activity within particular domains, such as inflammation modulation, antioxidant defence, antimicrobial protection or tissue regeneration, the effective healing of chronic wounds necessitates the coordinated regulation of multiple biological pathways. The complementary and partially overlapping mechanisms reported for the selected plants provide a strong scientific rationale for investigating multicomponent herbal mixtures.

Such a multicomponent approach may enhance therapeutic efficacy through synergistic interactions, improve coverage of critical wound-healing mechanisms, and better reflect the holistic principles underlying traditional medical practices, while remaining consistent with contemporary pharmacological understanding. Furthermore, although EMA/HMPC assessment reports primarily address *Plantago lanceolata*, this review emphasises *Plantago major* to provide a more comprehensive overview of its traditional use, phytochemical profile and biological activities relevant to wound healing, as supported by extensive ethnopharmacological and experimental literature (Akbari et

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al., 2021; Hussan et al., 2015; Ghezavat et al., 2014; Karimi et al., 2022; Keshavarzi et al., 2022; Metiner et al., 2012; Najafian et al., 2019; Samuelsen, 2000; Thomé et al., 2012; Velasco-Lezama et al., 2006; Zubair et al., 2015).

Conclusion

Calendula officinalis L., *Hypericum perforatum* L., *Achillea millefolium* L., *Plantago major* L., and *Verbena officinalis* L. contain a wide range of bioactive compounds responsible for their pronounced antioxidant, anti-inflammatory, and antimicrobial activities, which contribute to their effects on different stages of the wound healing process. From all of the above-mentioned data, it can be concluded that their potential role in the treatment of chronic wounds should not be overlooked. Rather, future research should prioritise the systematic investigation of multicomponent herbal mixtures, including possible synergistic interactions, supported by well-designed preclinical and clinical studies, in order to more effectively address the multifactorial nature of chronic wounds and optimise therapeutic outcomes.

References

Adom, M.B., Taher, M., Mutalabisin, M.F., Amri, M.S., Kudos, M.B.A., Sulaiman, M.W.A.W., Sengupta, P. and Susanti, D., 2017. Chemical constituents and medical benefits of Plantago major. Biomedicine & Pharmacotherapy 96, 348-360.

Ahmed, D., Qasim, K.A., Ashraf, C.M. and Maab, H., 2017. Verbena officinalis: A herb with promising broad-spectrum antimicrobial potential. Cogent Chemistry 3(1), 1363342. https://doi.org/10.1080/23312009.2017.1363342

Ahmed, D., Qasim, K.A., Ashraf, C.M. and Maab, H., 2017. Verbena officinalis a herb with promising broad spectrum antimicrobial potential. Cogent Chemistry 3(1), 1363342. https://doi.org/10.1080/23312009.2017.1363342

Akbari, Z., Haghani, S., Abedi, H.A., Alavi Majd, H. and Yaghmaei, F. 2021. Effect of Plantago major on healing of stage I pressure ulcers: A randomized triple-blind clinical trial. International Journal of Nursing Practice 27(1), e12887. https://doi.org/10.1111/ijn.12887

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Altan, A., Aras, M.H., Damlar, İ., Gökçe, H., Özcan, O. and Alpaslan, C., 2018. The effect of Hypericum perforatum on wound healing of oral mucosa in diabetic rats. European Oral Research 52(3), 143–149.

Arora, D., Rani, A. and Sharma, A., 2013. A review on phytochemistry and ethnopharmacological aspects of genus Calendula. Pharmacognosy Reviews 7(14), 179. https://doi.org/10.4103/0973-7847.120520

Ashwlayan, V.D., Kumar, A., Verma, M., Garg, V.K. and Gupta, S.K., 2018. Therapeutic potential of Calendula officinalis. Pharmacology & Pharmacy International Journal 6(2), 149–155. https://doi.org/10.15406/ppij.2018.06.00171

Balciunaitiene, A., Puzeryte, V., Radenkovs, V., Krasnova, I., Memvanga, P.B., Viskelis, P., Streimikyte, P. and Viskelis, J., 2022. Sustainable–green synthesis of silver nanoparticles using aqueous Hyssopus officinalis and Calendula officinalis extracts and their antioxidant and antibacterial activities. Molecules 27(22), 7700. https://doi.org/10.3390/molecules27227700

Benedek, B. and Kopp, B., 2007a. Achillea millefolium L. revisited: recent findings confirm the traditional use. Wiener Medizinische Wochenschrift 157, 312–331.

Bilia, A.R., Giomi, M., Innocenti, M., Gallori, S. and Vincieri, F.F., 2008. HPLC–DAD–ESI–MS analysis of the constituents of aqueous preparations of verbena and lemon verbena and evaluation of the antioxidant activity. Journal of Pharmaceutical and Biomedical Analysis 46(3), 463-470.

Butnariu, M. and Coradini, C.Z., 2012. Evaluation of biologically active compounds from Calendula officinalis flowers using spectrophotometry. Chemistry Central Journal 6(1), 35.

Calvo, M.I., 2006. Anti-inflammatory and analgesic activity of the topical preparation of Verbena officinalis L. Journal of Ethnopharmacology 107(3), 380–382. https://doi.org/10.1016/j.jep.2006.03.037

Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sökmen, A. and Akpulat, H.A., 2003. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp. millefolium Afan.(Asteraceae). Journal of Ethnopharmacology 87(2-3), 215-220.

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Cecchini, C., Cresci, A., Coman, M.M., Ricciutelli, M., Sagratini, G., Vittori, S., Lucarini, D. and Maggi, F., 2007. Antimicrobial activity of seven Hypericum entities from central Italy. Planta medica 73(06), 564-566.

David, R.B., Zbigniew, A.C. and Sasha, M.D., 2010. Aqueous extract of Achillea millefolium L. (Asteraceae) inflorescences suppresses lipopolysaccharide-induced inflammatory responses in RAW 264.7 murine macrophages. Journal of Medicinal Plants Research 4.

Deepak, M. and Handa, S.S., 2000. Antiinflammatory activity and chemical composition of extracts of Verbena officinalis. Phytotherapy Research 14(6), 463–465. https://doi.org/10.1002/1099-1573(200009)14:6

Duran, V., Matic, M., Jovanovc, M., Mimica, N., Gajinov, Z., Poljacki, M. and Boza, P., 2005. Results of the clinical examination of an ointment with marigold (Calendula officinalis) extract in the treatment of venous leg ulcers. International Journal of Tissue Reactions 27(3), 101–106.

Eghdampour, F., Jahdie, F., Kheyrkhah, M., Taghizadeh, M., Naghizadeh, S. and Hagani, H., 2013. The impact of Aloe vera and Calendula on perineal healing after episiotomy in primiparous women: a randomised clinical trial. Journal of Caring Sciences 2(4), 279–286.

European Medicines Agency (EMA), Committee on Herbal Medicinal Products (HMPC), 2017. Assessment report on Calendula officinalis L., flos. EMA/HMPC/603409/2017.

European Medicines Agency (EMA), Committee on Herbal Medicinal Products (HMPC), 2016. Assessment report on Hypericum perforatum L., herba. EMA/HMPC/244315/2016.

European Medicines Agency (EMA), Committee on Herbal Medicinal Products (HMPC), 2019. Assessment report on Achillea millefolium L., herba. EMA/HMPC/376415/2019.

European Medicines Agency (EMA), Committee on Herbal Medicinal Products (HMPC), 2022. Assessment report on Plantago lanceolata L., folium. EMA/HMPC/887981/2022.

Farasati Far, B., Gouranmohit, G., Naimi Jamal, M.R., Neysani, E., El Nashar, H.A.S., El Shazly, M. and Khoshnevisan, K., 2024. The potential role of Hypericum

UNEDITED PROOF

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perforatum in wound healing: a literature review on the phytochemicals, pharmacological approaches, and mechanistic perspectives. Phytotherapy Research 38(7), 3271–3295. https://doi.org/10.1002/ptr.8204

Fraisse, D., Felgines, C., Texier, O. and Lamison, J.L., 2011. Caffeoyl derivatives: major antioxidant compounds of some wild herbs of the Asteraceae family. Food and Nutrition Sciences 2, 181–192.

Garrido-Suárez, B.B., Garrido, G., Menéndez, A.B., Merino, N., Valdés, O., Paz, N., Romero, A., Delgado L., Fernández, M., Piñeros, O. and Delgado-Hernández, R., 2022. Topical Calendula officinalis L. inhibits inflammatory pain through antioxidant, anti-inflammatory and peripheral opioid mechanisms. Journal of Integrative Medicine 21(18), 100002. https://doi.org/10.1016/j.joim.2022.10.002

Georgieva, L., Gadjalova, A., Mihaylova, D. and Pavlov, A., 2015. Achillea millefolium L. phytochemical profile and in vitro antioxidant activity. International Food Research Journal 22, 1347–1352.

Ghezavat, K., Mohamadi Sani, A. and Yavarmanesh, M., 2014. Assessment of the Plantago major extract for antimicrobial activities. BioTechnology: An Indian Journal 10(1), 5–10.

Giostri, G.S., Novak, E.M., Buzzi, M. and Guarita-Souza, L.C., 2021. Treatment of acute wounds in hand with Calendula officinalis L.: a randomized trial. Tissue Barriers 10(1), 1994822. https://doi.org/10.1080/21688370.2021.1994822

Goldberg, A.S., Mueller, E.C., Eigen, E. and DeSalva, S.J., 1969. Isolation of the anti-inflammatory principles from Achillea millefolium (Compositae). Journal of Pharmaceutical Sciences 58(8), 938–941.

Hajhashemi, M., Ghannadi, A., Hajiloo, M., Hashemi, M. and Rajaei, M., 2017. The effect of Achillea millefolium and Hypericum perforatum ointments on episiotomy wound healing in primiparous women. The Journal of Maternal-Fetal & Neonatal Medicine 31(1), .63–69. https://doi.org/10.1080/14767058.2016.1275549

Hamman, J.H., 2008. Composition and applications of Aloe vera leaf gel. Molecules 13(8), 1599–1616. https://doi.org/10.3390/molecules13081599

Hammer, K.D., Hillwig, M.L., Solco, A.K., Dixon, P.M., Delate, K., Murphy, P.A., Wurtele, E.S. and Birt, D.F., 2007. Inhibition of prostaglandin E_2 production by anti-inflammatory Hypericum perforatum extracts and constituents in RAW264.7

UNEDITED PROOF

mouse macrophage cells. Journal of Agricultural and Food Chemistry 55(18), 7323–7331.

Han, M.C., Durmuş, A.S., Sağlıyan, A., Günay, C., Özkaraca, M., Kandemir, F.M., Çomaklı, S. and Öztopalan, D.F., 2017. Effects of Nigella sativa and Hypericum perforatum on wound healing. Turkish Journal of Veterinary & Animal Sciences 41(1), Article 15. https://doi.org/10.3906/vet-1603-26

Hussan, F, Mansor, A.S, Hassan, S.N, Kamaruddin, T.N.E, Budin, S.B. and Othman, F, 2015. Anti-inflammatory property of Plantago major leaf extract reduces the inflammatory reaction in experimental acetaminophen-induced liver injury. Evidence-Based Complementary and Alternative Medicine 2015, 347861. https://doi.org/10.1155/2015/347861.

Jarić, S., Kostić, O., Mataruga, Z., Pavlović, D., Pavlović, M., Mitrović, M. and Pavlović, P., 2018. Traditional wound-healing plants used in the Balkan region (Southeast Europe). Journal of Ethnopharmacology. https://doi.org/10.1016/j.jep.2017.09.018

Karimi, A., Rahmani, N., Hashemi, S.A. and Ghorbani, A. 2022. Evaluation of the safety and efficacy of topical Plantago major ointment in patients with second-degree burn wounds: An open-label clinical trial. Burns 48(6), 1402–1409. https://doi.org/10.1016/j.burns.2022.03.006

Kaya, S., Doğan, Ş. and Ateş, M., 2022. Effect of combination of Hypericum perforatum, Calendula officinalis and Aloe vera plant extracts on incisional wound healing. Mehmet Akif Ersoy University Journal of Health Sciences Institute 10(3), 161–168.

Keser, S., Celik, S., Turkoglu, S., Yilmaz, O. and Turkoglu, I., 2013. Antioxidant activity, total phenolic and flavonoid content of water and ethanol extracts from Achillea millefolium L. Turkish Journal of Pharmaceutical Sciences 10, 385–392.

Keshavarzi, Z., Rahimi, M., Keshavarzi, F., Heidari, R. and Ghorbani, A., 2022. Topical application of Plantago major extract accelerates wound healing in patients with second-degree burn injuries. Burns 48(6), 1371–1379. https://doi.org/10.1016/j.burns.2022.02.006

Khairnar, M.S., Pawar, B., Marawar, P.P. and Mani, A., 2013. Evaluation of Calendula officinalis as an anti-plaque and anti-gingivitis agent. Journal of Indian Society of Periodontology 17(6), 741–747. https://doi.org/10.4103/0972-124X.124491

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Kobeasy, M.I., Abdel-Fatah, O.M., Abd El-Salam, S.M. and Mohamed, Z.A., 2011. Chemical constituents and antioxidant activity of Plantago major L. Natural Product Research 25(7), 683–693. https://doi.org/10.1080/14786419.2010.529550

Kubica, P., Szopa, A., Ekiert, H. and Tomczyk, M., 2021. Secondary metabolites of the genus Verbena (Verbenaceae): Distribution and biological activity. Planta Medica 87(8–9), 653–670. https://doi.org/10.1055/a-1232-5758

Lievre, M., Marichy, J., Baux, S., Foyatier, J.L., Perrot, J. and Boissel, J.P., 1992. Controlled study of three ointments for the local management of 2nd and 3rd degree burns. Clinical Trials Metaanalysis 28(1), 9–12.

Lopes, F.C.M., Benzatti, F.P., Moreira, R.R.D., Santos, L.E. and Carlos, I.Z., 2003. Evaluation of the immunological activity of Achillea millefolium L. Revista Brasileira de Farmacognosia 13, 11–13.

Mahmoudi, M. and Gould, L.J., 2020. Opportunities and challenges of the management of chronic wounds: a multidisciplinary viewpoint. Chronic Wound Care Management and Research 7, 27–36.

Martinengo, L., Olsson, M., Bajpai, R., Soljak, M., Upton, Z., Schmidtchen, A., Car, J. and Järbrink, K., 2019. Prevalence of chronic wounds in the general population: systematic review and meta-analysis of observational studies. Annals of Epidemiology 29, 8–15.

Maver, T., Maver, U., Stana Kleinschek, K., Smrke, D.M. and Kreft, S., 2015. A review of herbal medicines in wound healing. International Journal of Dermatology 54(7), 740–751. https://doi.org/10.1111/ijd.12766

Metiner, K., Yildirim, E., Cakmak, O., and Gulmez, M., 2012. Antibacterial effects of ethanol and acetone extract of Plantago major L. on gram-positive and gram-negative bacteria. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 18(3), 503–505.

MKobeasy, M.I., Abdel-Fatah, O.M., El-Sayed, S.A.A. and Abd El-Baky, A.A., 2011. Biochemical studies on Plantago major L. and Cyamopsis tetragonoloba L. International Journal of Biodiversity and Conservation 3(3), 83–91.

Öztürk, N., Korkmaz, S. and Öztürk, Y., 2007. Wound-healing activity of St. John's Wort (Hypericum perforatum L.) on chicken embryonic fibroblasts. Journal of Ethnopharmacology 111(1), 33-39.

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Panahi, Y., Sharif, M.R., Sharif, A., Beiraghdar, F., Zahiri, Z., Amirchoopani, G., Marzony, E.T. and Sahebkar, A., 2012. A randomized comparative trial on the therapeutic efficacy of topical Aloe vera and Calendula officinalis on diaper dermatitis in children. Scientific World Journal 2012, 810234. https://doi.org/10.1100/2012/810234

Panchatcharam, M., Miriyala, S., Gayathri, V.S. and Suguna, L., 2006. Curcumin improves wound healing by modulating collagen and decreasing reactive oxygen species. Molecular and Cellular Biochemistry 290(1–2), 87–96. https://doi.org/10.1007/s11010-006-9170-2

Patil, K., Sanjay, C., Doggalli, N., Devi, K.R. and Harshitha, N., 2022. A review of Calendula officinalis magic in science. Journal of Clinical and Diagnostic Research 16(2), ZE23–ZE27. https://doi.org/10.7860/JCDR/2022/16024

Pazyar, N., Yaghoobi, R., Rafiee, E., Mehrabian, A. and Feily, A., 2014. Skin wound healing and phytomedicine: a review. Skin Pharmacology and Physiology 27(6), 303–310. https://doi.org/10.1159/000357477

Preethi, K.C. and Kuttan, R., 2009. Wound healing activity of flower extract of Calendula officinalis. Journal of Basic & Clinical Physiology & Pharmacology 20(1), 73–79.

Preethi, K.C., Kuttan, G. and Kuttan, R., 2006. Antioxidant potential of an extract of Calendula officinalis flowers in vitro and in vivo. Pharmaceutical Biology 44(9), 691–697.

Prisăcaru, A.I., Andriţoiu, C.V., Andriescu, C., Hăvârneanu, E.C., Popa, M., Motoc, A.G. and Sava, A., 2013. Evaluation of the wound-healing effect of a novel Hypericum perforatum ointment in skin injury. Romanian Journal of Morphology and Embryology 54(4), 1053–1059.

Najafian, Y., Khorasani, Z.M., Najafi, M.N., Hamedi, S.S., Mahjour, M. and Feyzabadi, Z., 2019. Efficacy of Aloe vera/Plantago major gel in diabetic foot ulcer: a randomized double-blind clinical trial. Current Drug Discovery Technologies 16(2), 223–231. https://doi.org/10.2174/1570163815666180115093007

Rehecho, S., Hidalgo, O., de Cirano, M.G.I., Navarro, I., Astiasarán, I., Ansorena, D., Cavero, R.Y. and Calvo, M.I., 2011. Chemical composition, mineral content and antioxidant activity of Verbena officinalis L. LWT-Food Science and Technology 44(4), 875-882.

UNEDITED PROOF

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Ringbom, T., Segerback, D., Noreen, Y., Bohlin, L. and De Las Heras, B., 1998. Ursolic acid from Plantago major, a selective inhibitor of cyclooxygenase-2 catalyzed prostaglandin biosynthesis. Journal of Natural Products 61(10), 1212–1215. https://doi.org/10.1021/np980122t

Roveroni-Favaretto, L.H., Lodi, K.B. and Almeida, J.D., 2009. Topical Calendula officinalis L. successfully treated exfoliative cheilitis: a case report. Cases Journal 2, 9077. https://doi.org/10.1186/1757-1626-2-9077

Samadi, S., Khadivzadeh, T., Emami, A., Moosavi, N.S., Tafaghodi, M. and Behnam, H.R., 2010. The effect of Hypericum perforatum on the wound healing and scar of cesarean. Journal of Alternative and Complementary Medicine 16(1), 113–117. https://doi.org/10.1089/acm.2009.0317

Samuelsen, A.B., 2000. The traditional uses, chemical constituents and biological activities of Plantago major L.: A review. Journal of Ethnopharmacology 71(1–2), 1–21. https://doi.org/10.1016/S0378-8741(00)00212-9

Sen, C.K., 2019. Human wounds and its burden: an updated compendium of estimates. Advances in Wound Care 8(2), 39–48.

Shafeie, N., Tabatabai Naini, A. and Kargar Jahromi, H., 2015. Comparison of different concentrations of Calendula officinalis gel on cutaneous wound healing. Biomedical & Pharmacology Journal 8(2), 979–992. https://doi.org/10.13005/bpj/850

Shahane, K., Kshirsagar, M., Tambe, S., Jain, D., Rout, S., Ferreira, M.K.M., Mali, S., Amin, P., Srivastav, P.P., Cruz, J. and Lima, R.R., 2023. An updated review on the multifaceted therapeutic potential of Calendula officinalis L. Pharmaceuticals 16(4), 611. https://doi.org/10.3390/ph16040611.

Singh, M. and Bagewadi, A., 2017. Comparison of effectiveness of Calendula officinalis extract gel with lycopene gel for treatment of tobacco-induced homogeneous leukoplakia: a randomized clinical trial. International Journal of Pharmaceutical Investigation 7(2), 88–92. https://doi.org/10.4103/jphi.JPHI_17_17

Somboonwong, J., Kankaisre, M., Tantisira, B. and Tantisira, M.H., 2012. Wound healing activities of different extracts of Centella asiatica in incision and burn wound models: an experimental animal study. BMC Complementary and Alternative Medicine 12, 103. https://doi.org/10.1186/1472-6882-12-103

UNEDITED PROOF

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Speroni, E., Cervellati, R., Costa, S., Guerra, M., Utan, A., Govoni, P., Berger, A., Müller, A. and Stuppner, H., 2007. Effects of differential extraction of Verbena officinalis on rat models of inflammation, cicatrization and gastric damage. Planta Medica 73(3), 227–235. https://doi.org/10.1055/s-2007-967116

Speroni, E., Cervellati, R., Costa, S., Guerra, M.C., Utan, A. and Govoni, P., 2007. Analgesic and anti-inflammatory activity of Verbena officinalis extracts. Journal of Ethnopharmacology 114(2), 224–229. https://doi.org/10.1016/j.jep.2007.08.006

Staiger, C., 2012. Comfrey: a clinical overview. Phytotherapy Research 26(10), 1441–1448. https://doi.org/10.1002/ptr.4612

Stojanovic, G., Radulović, N., Hashimoto, T. and Palić, R., 2005. In vitro antimicrobial activity of extracts of four Achillea species: the composition of Achillea clavennae L. (Asteraceae) extract. Journal of Ethnopharmacology,101, 185–190.

Süntar, I.P., Akkol, E.K., Yılmazer, D., Baykal, T., Kırmızıbekmez, H., Alper, M. and Yeşilada, E., 2010. Investigations on the in vivo wound healing potential of Hypericum perforatum L. Journal of ethnopharmacology 127(2), 468-477.

Thomé, R.G., Scalon, M.C., Lana, R.M.Q., Sousa, J.P.B., Faria, F.S.E., and Casadei, D.L., 2012. Evaluation of healing wound and genotoxicity potentials from hydroalcoholic extracts of Plantago major and Siparuna guianensis. Experimental Biology and Medicine (Maywood) 237(12), 1379–1386.

Velasco-Lezama, R., Tapia-Aguilar, R., Gallardo-Casas, C.A., Diaz-Zagoya, J.C. and Juárez-Oropeza, M.A., 2006. Effect of Plantago major on cell proliferation in vitro. Journal of Ethnopharmacology, 103(1), 36–42. https://doi.org/10.1016/j.jep.2005.05.050

Vitalini, S., Beretta, G., Iriti, M., Orsenigo, S., Basilico, N., Dall'Acqua, S., Iorizzi, M. and Fico, G., 2011. Phenolic compounds from Achillea millefolium L. and their bioactivity. Acta Biochimica Polonica, 58, pp.203–209.

Yadollah-Damavandi, S., Chavoshi-Nejad, M., Jangholi, E., Nekouyian, N., Hosseini, S., Seifaee, A., Rafiee, S., Karimi, H., Ashkani-Esfahani, S., Parsa, Y. and Mohsenikia, M., 2015. Topical Hypericum perforatum improves tissue regeneration in full-thickness excisional wounds in diabetic rat model. Evidence-Based Complementary and Alternative Medicine 2015(1), 245328.

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Zou, Y., Lu, Y. and Wei, D., 2004. Antioxidant activity of a flavonoid-rich extract of Hypericum perforatum L. in vitro. Journal of Agricultural and Food Chemistry 52(16), 5032–5039. https://doi.org/0.1021/jf049571r

Zubair, M., Khan, M.S., Ahmad, M., Shakil, S., Mohammad, F. and Alam, M.S., 2015. Promotion of wound healing by Plantago major L. leaf extracts – ex vivo experiments confirm experiences from traditional medicine. Natural Product Research 29(7), 622–624.

Zubair, M., Khan, M.S., Ahmad, M., Shakil, S., Mohammad, F., and Alam, M.S., 2012. Effects of Plantago major L. leaf extracts on oral epithelial cells in a scratch assay. Journal of Ethnopharmacology 141(3), 825–830.

Zulkefli, N., Che Zahari, C. N. M., Sayuti, N. H., Kamarudin, A. A., Saad, N., Hamezah, H. S., Bunawan, H., Baharum, S. N., Mediani, A., Ahmed, Q. U., Ismail, A. F. H. and Sarian, M. N., 2023. Flavonoids as potential wound-healing molecules: emphasis on pathways perspective. International Journal of Molecular Sciences 24(5), 4607. https://doi.org/10.3390/ijms24054607

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doi: *Review paper*

Резиме

Лековити растенија со потенцијал за третман и заздравување на рани

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Клучни зборови: антиоксидативно, антиинфламаторно, антимикробно дејство, лековити растенија

Третманот на хроничните рани се темели на биолошки активности како што се: антимикробното дејство за превенција на инфекции, антиоксидативното дејство за намалување на оксидативниот стрес и антиинфламаторното дејство за обновување и регенерација на ткивото. Лековитите растенија, од традиционалната медицина до современите научни истражувања, претставуваат значајни извори на вакви биолошки активности. Овој ревијален труд ја опфаќа историската употреба и современите научни сознанија со цел да ги истакне лековитите растенија со потенцијал за примена во третманот на рани, со посебен акцент на нивните биолошки активности и докажани терапевтски ефекти. Посебно внимание е посветено на пет избрани лековити растенија: *Calendula officinalis* L., *Hypericum perforatum* L., *Achillea millefolium* L., *Plantago major* L. и *Verbena officinalis* L. Достапните експериментални и клинички докази укажуваат дека овие растенија поседуваат изразени антиоксидативни, антиинфламаторни и антимикробни својства во различни експериментални и клинички модели. Во овој контекст, сè поголем број истражувања укажуваат дека мултикомпонентните хербални мешавини можат да обезбедат подобрени терапевтски ефекти преку истовремено делување врз повеќе патолошки механизми вклучени во хроничните рани. Терапевтскиот потенцијал на комбинираните хербални формулации и можните

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синергистички интеракции укажуваат на потребата од понатамошни детални истражувања со цел развој на ефикасни и научно засновани стратегии за третман на хронични рани.

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Table 1. Historical and traditional background for the selection of medicinal plants with wound healing potential

Medical plant	Documented traditional use related to skin, wounds and burns	Historically reported activities relevant to chronic wound healing	Source of historical evidence
Calendula officinalis L. (flos)	Long-standing use in European traditional medicine for the treatment of wounds with poor healing tendency, leg ulcers (ulcus cruris), minor wounds, burns and inflammatory skin conditions.	Anti-inflammatory, wound-healing and regenerative activities; promotion of epithelialisation and tissue repair.	EMA/HMPC Assessment Report - *Calendula officinalis*
Hypericum perforatum L. (herba)	Traditionally topic application for wounds, burns, abrasions and sunburns, particularly in the form of oil-based preparations used to support skin recovery.	Anti-inflammatory, antioxidant, antimicrobial and regenerative effects; stimulation of granulation and epithelialisation.	EMA/HMPC Assessment Report - *Hypericum perforatum*
Achillea millefolium L. (herba)	Historically used in European and Asian traditional medicine for wound healing, ulcers and skin injuries, including applications for damaged and inflamed skin.	Anti-inflammatory, astringent, haemostatic and wound-healing properties; supportive role in ulcerated and inflamed wounds.	EMA/HMPC Assessment Report - *Achillea millefolium*
Plantago lanceolata L. (folium)	Traditionally used for wounds, ulcers and inflammatory skin conditions, often applied topically as a soothing and haemostatic remedy.	Anti-inflammatory, haemostatic, soothing and regenerative activities; support of wound repair processes.	EMA/HMPC Assessment Report - *Plantago lanceolata*
Plantago major L. (folium)	Widely used in traditional medicine across different cultures for wound healing and burns, with topical application of fresh or processed leaves to damaged skin.	Wound-healing, anti-inflammatory, antioxidant and antimicrobial activities; facilitation of tissue regeneration.	Literature data (Samuelsen, 2000; Keshavarzi et al., 2022)
Verbena officinalis L. (herba)	Traditionally used in European folk medicine for skin burns, abrasions and inflammatory skin conditions.	Anti-inflammatory, antioxidant and cicatrising activities; traditional support of wound closure and tissue recovery.	Literature data (Speroni et al., 2007)

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Table 2. Historical use of selected medicinal plants and key pathophysiological mechanisms of chronic wounds

Chronic wound pathology	**Historical/traditional therapeutic target**	**Medicinal plants traditionally addressing this mechanism**
Persistent inflammation	Reduction of redness, swelling and skin irritation	*Calendula officinalis, Hypericum perforatum, Achillea millefolium, Plantago lanceolata, Plantago major, Verbena officinalis*
Oxidative stress	Protection of damaged tissue and promotion of recovery	*Hypericum perforatum, Verbena officinalis, Plantago major, Calendula officinalis*
Impaired granulation and epithelialisation	Promotion of tissue regeneration and wound closure	*Calendula officinalis, Hypericum perforatum, Achillea millefolium*
Risk of infection	Traditional cleansing and protective wound applications	*Hypericum perforatum, Plantago species, Achillea millefolium*
Poor haemostasis and exudation	Control of bleeding and wound secretions	*Achillea millefolium, Plantago lanceolata*

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Table 3. Chemical composition of selected medicinal plants with wound healing potential

Medicinal plant	**Main chemical constituents**	**Source**
Calendula officinalis L. (flos)	Triterpene saponins (2–10%, oleanolic acid derivatives); triterpene alcohols (α- and β-amyrin, lupeol, taraxasterol; faradiol and arnidiol esters); carotenoids (up to 4.7%, mainly lutein and zeaxanthin); flavonoids (0.3–0.8%, isorhamnetin and quercetin glycosides); coumarins (scopoletin, umbelliferone, esculetin); essential oil (0.2–0.3%, mainly sesquiterpenes); water-soluble polysaccharides (up to 15%).	EMA/HMPC Assessment Report - *Calendula officinalis*
Hypericum perforatum L. (herba)	Phloroglucinol derivatives (0.2–4%, hyperforin and adhyperforin); naphthodianthrones (0.03–0.4%, hypericin and pseudohypericin); flavonoids (2–4%, quercetin glycosides: hyperoside, rutin, isoquercitrin, quercitrin; biflavones such as biapigenin and amentoflavone); procyanidins/tannins (6–15%); essential oil (0.07–0.25%); phenolic acids (e.g. chlorogenic acid).	EMA/HMPC Assessment Report - *Hypericum perforatum*
Achillea millefolium L. (herba)	Tannins (3–4%); essential oil (0.3–1.4%, containing linalool, borneol, camphor, β-caryophyllene, 1,8-cineole); sesquiterpene lactones and proazulenes (guaianolides and germacranolides); flavonoids (apigenin, luteolin, isorhamnetin, rutin); phenolic acids (caffeic and salicylic acid); coumarins; phytosterols (β-sitosterol).	EMA/HMPC Assessment Report - *Achillea millefolium*
Plantago lanceolata L. (folium)	Iridoid glycosides (approximately 2–3%, mainly aucubin and catalpol); mucilage polysaccharides (rich in arabinose and galactose); flavonoids (apigenin and luteolin derivatives); phenylethanoid glycosides (acteoside/verbascoside, plantamajoside); phenolic acids and tannins.	EMA/HMPC Assessment Report - *Plantago lanceolata*
Plantago major L. (folium/herba)	Polysaccharides (mucilage); iridoid glycosides (notably aucubin); phenolic acids and caffeic acid derivatives; flavonoids (including plantaginin); lipids and minor terpenoid constituents.	Literature data (Samuelsen, 2000, Kobeasy et al., 2011.)
Verbena officinalis L. (herba/aerial parts)	Phenylpropanoid and phenylethanoid glycosides (predominantly verbascoside/acteoside); iridoid glycosides (e.g. verbenalin, hastatoside); flavonoids (luteolin and apigenin derivatives); phenolic acids; minor amounts of terpenoids and coumarins.	Literature data (Speroni et al. 2007, Kubica et al., 2021, Ahmed et al., 2017)

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Table 4. Integrated historical andlLiterature-based evidence on selected medicinal plants in chronic wound healing

Pathophysiological aspect of chronic wounds	***Calendula officinalis* L.**	***Hypericum perforatum* L.**	***Achillea millefolium* L.**	***Plantago major* L.**	***Verbena officinalis* L.**
Persistent inflammation	Modulation of cytokine levels; inhibition of COX-1 and COX-2	Inhibition of PGE_2, TNF-α, IL-6, COX-2 and iNOS; shortening of inflammatory phase	Inhibition of HNE, MMP-2 and MMP-9; suppression of iNOS expression	Inhibition of COX-1, COX-2, 12-LOX and pro-inflammatory cytokines (IL-1α, IL-1β, TNF-α)	Reported anti-inflammatory activity, likely mediated by verbascoside
Oxidative stress	Strong free-radical scavenging via flavonoids and phenolic acids; inhibition of lipoxygenase	Flavonoid-mediated antioxidant activity; reduction of lipid peroxidation and modulation of oxidative stress markers	High antioxidant capacity attributed to flavonoids and phenolic acids	Reduction of redox imbalance; flavonoid-mediated antioxidant protection	Confirmed antioxidant activity; verbascoside as the main bioactive antioxidant
Extracellular matrix degradation	Inhibition of collagen-degrading enzymes; increased collagen cross-linking	Enhanced collagen fibre strength and organisation	Inhibition of MMP-2 and MMP-9, protecting collagen integrity	Indirect protection through inflammation control	Limited data available
Impaired fibroblast proliferation and tissue regeneration	Increased fibroblast adhesion and proliferation; enhanced connective tissue synthesis	Increased fibroblast number and activity; enhanced collagen bundle formation	Supportive role in tissue protection rather than direct stimulation	Stimulation of epithelial and fibroblast proliferation in vitro and *ex vivo*	*In vivo* evidence of wound-healing activity
Impaired angiogenesis	Promotion of neovascularisation and angiogenesis	Increased blood vessel diameter and volume;	Limited evidence	Indirect support via improved tissue regeneration	Limited data available

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Pathophysiological aspect of chronic wounds	***Calendula officinalis* L.**	***Hypericum perforatum* L.**	***Achillea millefolium* L.**	***Plantago major* L.**	***Verbena officinalis* L.**
		improved local circulation			
Risk of infection	Broad-spectrum antibacterial and antifungal activity	Broad-spectrum antimicrobial activity, including MRSA	Antibacterial and antifungal activity against wound-related pathogens	Antibacterial activity, particularly effective *in vivo*	Reported antibacterial activity
Delayed epithelialisation and wound closure	Accelerated epithelialisation; clinical evidence in ulcers, burns and surgical wounds	Accelerated epithelialisation and wound closure in multiple in vivo models	Supportive role via inflammation and protease control	Enhanced epithelial regeneration in *in vitro*, *in vivo* and *ex vivo* models	Demonstrated wound-healing activity *in vivo*

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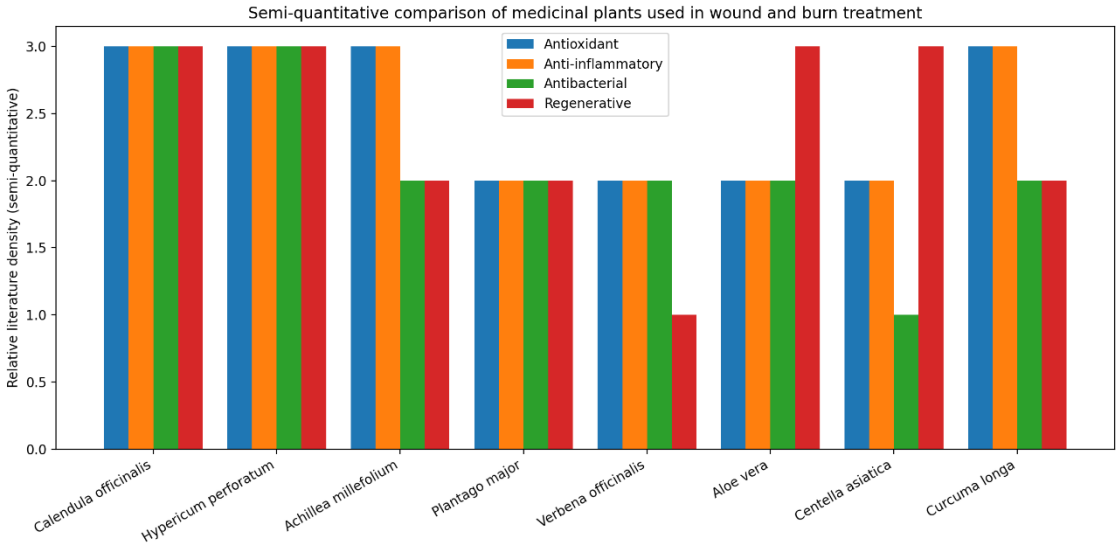

Fig. 1. Semi-quantitative, literature-based comparison of medicinal plants commonly used in the treatment of skin wounds and burns, classified according to reported antioxidant, anti-inflammatory, antibacterial and regenerative activities.

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