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Review

Medicinal Plants in Diabetic Wound Healing: Mechanistic Insights from *In-Vitro* Studies

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Abstract: Diabetic wound healing remains a major clinical challenge due to the combined effects of persistent hyperglycaemia, oxidative stress, chronic inflammation, impaired cell migration, and increased susceptibility to infection. Conventional wound-care strategies often provide incomplete outcomes, as they primarily address isolated aspects of this complex pathology. In recent years, medicinal plants have gained increasing attention as potential supportive agents in diabetic wound management owing to their multi-targeted biological activities. The present review critically examines mechanistic evidence from *in-vitro* studies to elucidate the role of medicinal plants in diabetic wound healing. Emphasis is placed on key pathological mechanisms, including oxidative stress imbalance, prolonged inflammatory responses, microbial burden, and dysfunction of fibroblast and keratinocyte migration. *In-vitro* findings indicate that plant-derived phytoconstituents, particularly phenolics and flavonoids, exhibit antioxidant and anti-inflammatory properties, inhibit wound-associated pathogens, and enhance cellular migration in scratch wound models. These activities collectively contribute to the modulation of processes essential for effective wound repair under diabetic conditions. The review also highlights commonly employed *in-vitro* models used to evaluate herbal wound-healing agents and discusses their strengths and limitations. Although *in-vitro* evidence cannot fully replicate the complexity of *in-vivo* wound healing, it provides valuable mechanistic insights and a rational basis for further investigation. Overall, this review underscores the potential of medicinal plants as multi-functional agents in diabetic wound healing and supports their continued exploration through standardised experimental and formulation-based research approaches.

Keywords: medicinal plants; diabetes; wound healing; *in-vitro*

1. Introduction

Wound healing is often impaired in people with diabetes, making the management of diabetic ulcers a continuing clinical challenge. Persistent hyperglycaemia can disturb several processes involved in tissue repair, including inflammatory regulation, cellular proliferation, new blood vessel formation and extracellular matrix remodelling. Oxidative stress and an increased risk of infection may further delay wound closure. The combined effects of these changes can prevent wounds from progressing through the normal stages of repair [1,2].

Conventional wound management remains important for protecting the wound, controlling infection and maintaining an appropriate healing environment. However, the biological disturbances associated with diabetes have encouraged researchers to investigate additional approaches that may influence more than one aspect of tissue repair [2,3]. Medicinal plants are among the materials being studied for this purpose because their extracts and isolated constituents have shown different biological activities in experimental systems.

The findings reported for medicinal plants are not uniform. Some studies examine antioxidant or antimicrobial activity, whereas others investigate cellular viability, migration or wound closure in laboratory models. These outcomes provide different levels of evidence and should not be interpreted as equivalent. For example, activity in a free-radical scavenging assay does not, by itself, demonstrate that a plant extract improves wound repair under diabetic conditions. The plant part used, extraction procedure, concentration and experimental model can also influence the results. A closer examination of the existing literature is therefore needed to establish which biological effects have been demonstrated directly and which remain preliminary observations. This distinction is particularly important when interpreting proposed mechanisms of action and considering the relevance of laboratory findings to diabetic wounds.

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The present review examines *in-vitro* research on medicinal plants and their bioactive constituents in relation to diabetic wound healing. Particular attention is given to the mechanisms investigated experimentally, the cellular and microbiological models employed, and the limitations of the available evidence. By comparing findings across studies, the review aims to identify promising research directions while distinguishing experimental observations from mechanisms that still require confirmation [4,5].

2. Literature Search Strategy and Study Selection

Relevant literature was examined to understand how medicinal plants and plant-derived compounds may contribute to diabetic wound repair, particularly through mechanisms investigated in *in-vitro* experiments. The review focused on processes commonly disrupted in diabetic wounds, such as excessive oxidative stress, persistent inflammation, microbial infection, impaired cell migration, and reduced tissue regeneration.

The literature search used combinations of terms related to diabetic wounds and plant-based interventions, including diabetic wound healing, diabetic foot ulcer, medicinal plants, plant extracts, phytoconstituents, *in-vitro* wound healing, antioxidant activity, anti-inflammatory activity, and fibroblast migration. Boolean operators (AND and OR) were used to combine relevant terms, and the reference lists of selected papers were examined for additional studies. Databases searched, the search period, and the final search date should be reported according to the actual search performed.

Studies were considered relevant when they examined a medicinal plant, plant extract, or isolated phytoconstituent and reported outcomes associated with wound repair. These outcomes included cell viability, wound-gap closure, inflammatory responses, oxidative stress, antimicrobial effects, collagen production, and related cellular processes. Greater emphasis was placed on original *in-vitro* research when discussing possible mechanisms. Animal experiments, clinical studies, and review articles were used where appropriate to provide additional context and assess the potential relevance of laboratory findings to wound management.

Publications were excluded when they had no clear connection to diabetic wound repair, investigated interventions unrelated to plant-derived materials, duplicated other records, or provided insufficient methodological details for interpretation. Studies reporting general antioxidant, antimicrobial, or anti-inflammatory activity were not treated as direct evidence of diabetic wound-healing efficacy unless their findings were relevant to the wound-healing context.

Information was recorded, where available, on the plant species and part used, extraction procedure, principal phytoconstituents, experimental model, treatment concentration, measured outcomes, reported effects, proposed mechanisms, and study limitations. The findings were then grouped by their relevance to the biological processes involved in diabetic wound repair. Since the studies differed in their experimental models, extract composition, concentrations, and outcome measures, their results were interpreted individually and discussed narratively rather than assumed to be directly comparable.

3. Pathophysiological Mechanisms Involved in Diabetic Wound Healing

Oxidative stress and impaired redox balance: Oxidative stress is one of the central pathological factors responsible for delayed wound healing in diabetic conditions. Persistent hyperglycaemia leads to excessive production of reactive oxygen species (ROS) through multiple biochemical pathways, including glucose auto-oxidation, activation of the polyol pathway, and mitochondrial dysfunction. While controlled levels of ROS play a physiological role in normal wound healing by aiding cell signalling and host defence, their excessive accumulation in diabetes creates a hostile microenvironment that interferes with tissue repair [6]. In diabetic wounds, elevated ROS levels overwhelm the endogenous antioxidant defence system, resulting in an imbalance between oxidant generation and antioxidant capacity. This impaired redox balance causes oxidative damage to cellular lipids, proteins, and nucleic acids, ultimately affecting the viability and function of skin cells involved in wound repair. Fibroblasts and keratinocytes exposed to high oxidative stress show reduced proliferation and migration, leading to delayed re-epithelialisation and poor collagen deposition [6,7]. Oxidative stress also negatively influences growth factor signalling and angiogenesis, both of which are essential for wound closure. Excess ROS can inactivate nitric oxide and disrupt vascular endothelial function, thereby impairing blood vessel formation and reducing oxygen and nutrient supply to the wound site. In addition, oxidative damage prolongs the inflammatory phase of wound healing by activating redox-sensitive transcription factors, which further delays progression to the proliferative stage [8]. Evidence from *in-vitro* studies has consistently demonstrated that cells cultured under hyperglycaemic or oxidative stress inducing conditions exhibit compromised wound-healing responses. As a result, modulation of oxidative stress has emerged as a critical target in diabetic wound management. Strategies aimed at restoring redox balance, particularly through agents with antioxidant properties,

are considered essential for improving cellular responses associated with wound repair. Understanding the role of oxidative stress in diabetic wound pathology provides a mechanistic basis for exploring medicinal plants and phytoconstituents that possess free radical scavenging and antioxidant activities. Such compounds may help mitigate oxidative damage, protect skin cells, and create a more favourable environment for wound healing, as supported by a growing body of *in-vitro* [9].

Prolonged inflammation and cytokine imbalance: Inflammation is a necessary and tightly regulated phase of normal wound healing, responsible for clearing debris and preventing infection at the injury site. However, in diabetic conditions, this phase is often exaggerated and prolonged, contributing significantly to delayed wound repair. Persistent hyperglycaemia alters immune cell function and promotes continuous activation of inflammatory pathways, preventing timely transition from the inflammatory to the proliferative phase of healing. Diabetic wounds are characterised by elevated levels of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). While these mediators are essential in the early stages of wound response, their sustained expression disrupts cellular homeostasis and inhibits the activity of fibroblasts and keratinocytes. Prolonged inflammatory signalling also reduces the expression of growth factors necessary for cell proliferation, extracellular matrix formation, and angiogenesis [10]. Another important consequence of chronic inflammation in diabetic wounds is increased protease activity. Matrix metalloproteinases remain excessively active, leading to degradation of newly formed extracellular matrix components and growth factors. This imbalance between tissue synthesis and degradation further delays wound closure and weakens newly formed tissue. In addition, inflammatory mediators can induce oxidative stress, creating a feedback loop that exacerbates cellular damage and impairs healing [11]. *In-vitro* studies using hyperglycaemic or cytokine-rich culture conditions have demonstrated that prolonged exposure to inflammatory mediators significantly reduces cell migration and viability. These findings highlight inflammation as a critical mechanistic barrier to effective wound healing in diabetes. Therefore, therapeutic strategies that can modulate inflammatory responses without completely suppressing the necessary immune functions are of particular interest [12]. Medicinal plants and their bioactive constituents have been reported to exhibit anti-inflammatory properties through modulation of cytokine production and inhibition of pro-inflammatory signalling pathways. By attenuating excessive inflammation, such agents may help restore a balanced wound- healing environment and facilitate progression toward tissue regeneration, as suggested by various *in-vitro* experimental studies [13].

Impaired cell migration and proliferation: Efficient wound healing depends on the coordinated migration and proliferation of key skin cells, particularly fibroblasts and keratinocytes, which are responsible for re-epithelialisation and formation of new connective tissue. In diabetic conditions, these cellular processes are markedly impaired, contributing to delayed wound closure and poor tissue regeneration. Hyperglycaemia, oxidative stress, and chronic inflammation collectively alter the cellular behaviour required for effective wound repair [14]. Fibroblasts play a central role in synthesising extracellular matrix components such as collagen, which provide structural support to newly formed tissue. In a diabetic environment, fibroblasts exhibit reduced proliferative capacity and diminished migratory activity. High glucose levels and oxidative stress interfere with cytoskeletal organisation and intracellular signalling pathways, limiting the ability of these cells to migrate into the wound bed and produce adequate extracellular matrix. As a result, granulation tissue formation is often weak and disorganized [15].

Keratinocyte migration is equally critical for wound closure, as these cells are responsible for covering the wound surface during re-epithelialisation. In diabetic wounds, keratinocytes show delayed migration and reduced responsiveness to growth factors. Prolonged exposure to inflammatory cytokines further suppresses keratinocyte function, preventing timely resurfacing of the wound. *In-vitro* scratch assays conducted under diabetic-simulated conditions have consistently demonstrated slower wound gap closure, reflecting impaired cellular movement and proliferation [16]. Cellular dysfunction in diabetic wounds is also linked to altered growth factor expression and receptor signalling. Reduced availability of growth factors such as epidermal growth factor and transforming growth factor-β limits activation of pathways essential for cell cycle progression and migration. Additionally, increased matrix degradation and an unfavourable extracellular environment hinder cell attachment and movement [17]. These observations underscore impaired cell migration and proliferation as key mechanistic barriers to wound healing in diabetes. Consequently, agents capable of supporting fibroblast and keratinocyte viability and enhancing their migratory behaviour are of significant interest. *In-vitro* studies have shown that certain plant-derived compounds can positively influence these cellular processes, providing a mechanistic rationale for their investigation in diabetic wound-healing research [12].

Increased susceptibility to microbial infection: Diabetic wounds are highly susceptible to microbial infection, which represents a major barrier to effective wound healing. Several factors associated with diabetes, including impaired immune function, reduced blood supply, and elevated glucose levels in wound exudates, create

favourable conditions for microbial growth. Once established, infection further delays healing by intensifying inflammation, increasing tissue damage, and disrupting normal cellular activities involved in wound repair [18]. In diabetic conditions, the innate immune response is often compromised. Neutrophil chemotaxis, phagocytosis, and microbial killing are impaired, reducing the body's ability to clear invading pathogens. At the same time, poor vascularisation limits the delivery of immune cells and antimicrobial agents to the wound site. These factors allow bacteria to persist and, in many cases, form biofilms that are difficult to eradicate and highly resistant to conventional antibiotics [19]. Common wound-associated pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* are frequently isolated from diabetic wounds. Their presence prolongs the inflammatory phase and increases the production of proteolytic enzymes and reactive oxygen species, which further damage surrounding tissue. Infection also interferes with fibroblast and keratinocyte function, thereby inhibiting cell migration, collagen synthesis, and re-epithelialisation [20]. *In-vitro* antimicrobial studies have demonstrated that exposure of skin cells to bacterial toxins or inflammatory mediators released during infection significantly reduces cell viability and wound-healing responses. These findings highlight the importance of infection control as an integral component of diabetic wound management. However, reliance on conventional antibiotics alone is increasingly challenged by the emergence of antimicrobial resistance and potential cytotoxic effects on host cells [21]. Medicinal plants have been widely investigated for their antimicrobial properties, with many extracts and phytoconstituents showing inhibitory activity against wound-associated pathogens in *in-vitro* studies. In addition to direct antimicrobial effects, some plant-derived compounds may interfere with biofilm formation or reduce microbial virulence. Such multifaceted actions provide a mechanistic basis for considering herbal interventions as supportive agents in the management of infected diabetic wounds [22].

The major pathophysiological mechanisms involved in diabetic wound healing and their cellular consequences are summarised in Table 1.

Table 1. Major pathophysiological mechanisms in diabetic wound healing and their cellular consequences [18–22].

Pathological Mechanism	Key Molecular Mediators	Cellular Targets	Resulting Impairment	Overall Effect on Wound Healing
Oxidative Stress	ROS, O_2^-, OH^-	Fibroblasts, Keratinocytes, Endothelial cells	Reduced proliferation, impaired migration, NO inactivation	Delayed re-epithelialisation, poor angiogenesis
Chronic Inflammation	TNF-α, IL-1β, IL-6	Fibroblasts, Keratinocytes	Growth factor suppression, ECM degradation	Prolonged inflammatory phase
Impaired Cell Migration	Reduced EGF, TGF-β	Fibroblasts, Keratinocytes	Weak granulation tissue formation	Delayed wound closure
Microbial Infection	S. aureus, P. aeruginosa	Skin cells, immune cells	Biofilm formation, protease activation	Persistent non-healing wound

4. Mechanism-Based Role of Medicinal Plants in Diabetic Wound Healing

Antioxidant mechanisms of medicinal plants: Given the central role of oxidative stress in delaying wound repair under diabetic conditions, antioxidant intervention represents one of the most important therapeutic targets in diabetic wound management. Medicinal plants have been extensively investigated for their antioxidant potential, largely due to their rich content of phenolic compounds, flavonoids, tannins, and other redox-active phytoconstituents. These compounds are capable of modulating oxidative imbalance through multiple complementary mechanisms rather than acting via a single pathway [23]. At the cellular level, plant-derived antioxidants can directly scavenge reactive oxygen species, thereby reducing oxidative damage to membrane lipids, structural proteins, and nucleic acids. In *in-vitro* models, herbal extracts have been shown to lower intracellular oxidative stress, leading to improved viability and functional activity of fibroblasts and keratinocytes exposed to hyperglycaemic or oxidative conditions. Restoration of redox balance helps preserve cytoskeletal integrity and cellular signalling pathways essential for migration and proliferation during wound repair [24]. Beyond direct free radical scavenging, several phytochemicals influence endogenous antioxidant defence systems. *In-vitro* studies have demonstrated that certain plant extracts can enhance the activity or expression of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione-related enzymes. By supporting these intrinsic defence mechanisms, medicinal plants contribute to sustained protection against oxidative injury in the wound microenvironment, which is particularly relevant in chronic diabetic wounds where continuous oxidative stress is present [25]. Antioxidant activity of medicinal plants is commonly evaluated using standard *in-vitro* assays such as DPPH, ABTS, and ferric reducing antioxidant power (FRAP) tests. Although these assays do not fully replicate *in-vivo* conditions, they provide valuable comparative data on the redox-modulating capacity of herbal extracts and isolated phytoconstituents. Compounds such as curcumin, quercetin, and other polyphenols consistently demonstrate strong antioxidant profiles in these assays, supporting their relevance in diabetic wound-healing

research. Importantly, reduction of oxidative stress has indirect benefits on other wound-healing mechanisms. Lower oxidative burden is associated with attenuation of prolonged inflammation, improved growth factor responsiveness, and enhanced cell migration. Thus, the antioxidant effects of medicinal plants form a mechanistic foundation upon which other biological activities, such as anti-inflammatory and pro-regenerative actions, can operate. This multifaceted role explains why antioxidant-rich herbal preparations are frequently explored as supportive agents in the management of diabetic wounds based on *in-vitro* evidence [26].

Anti-inflammatory effects of medicinal plants in the diabetic wound environment: Chronic and unresolved inflammation is a defining feature of diabetic wounds and represents a major obstacle to effective tissue repair. As discussed earlier, prolonged elevation of pro-inflammatory cytokines and mediators prevents the normal transition from the inflammatory to the proliferative phase of wound healing. In this context, medicinal plants have attracted considerable attention for their ability to modulate inflammatory responses in a controlled and balanced manner, as evidenced by numerous *in-vitro* studies [27]. Many plant-derived phytoconstituents exert anti-inflammatory effects by regulating key signalling pathways involved in cytokine production and inflammatory cell activation. *In-vitro* experiments using macrophages, fibroblasts, or keratinocytes exposed to inflammatory stimuli have shown that certain herbal extracts can reduce the expression of pro-inflammatory mediators such as TNF-α, IL-1β, and IL-6. This downregulation helps to limit excessive inflammatory signalling, thereby creating a more favourable environment for cell proliferation and migration [28]. Unlike conventional anti-inflammatory drugs, which often suppress inflammation broadly and may interfere with normal healing processes, medicinal plants tend to exhibit a modulatory effect rather than complete inhibition. This distinction is particularly important in wound healing, where an initial inflammatory response is necessary for debris clearance and host defence. By attenuating excessive or prolonged inflammation without abolishing essential immune functions, plant-based agents may support a more physiologically appropriate healing response under diabetic conditions [29]. *In-vitro* studies have also demonstrated that some phytochemicals can inhibit the activation of transcription factors associated with chronic inflammation and oxidative stress. This dual action is especially relevant in diabetic wounds, where inflammation and oxidative stress are closely interconnected and often reinforce one another. Reduction in inflammatory mediator levels has been associated with improved fibroblast viability, enhanced keratinocyte migration, and preservation of extracellular matrix components in cell-based wound models. Overall, the anti-inflammatory properties of medicinal plants provide a mechanistic explanation for their potential benefits in diabetic wound healing. By restoring balance to the inflammatory phase, these agents may facilitate progression to subsequent stages of wound repair. The growing body of *in-vitro* evidence supporting these effects highlights the relevance of herbal interventions as multi-targeted modulators within the complex diabetic wound environment [30].

Antimicrobial action of medicinal plants against diabetic wound pathogens: Microbial infection is a frequent and serious complication in diabetic wounds, often leading to prolonged inflammation, tissue destruction, and failure of wound closure. The compromised immune response associated with diabetes, combined with reduced vascular perfusion and elevated glucose levels, creates an environment that favours microbial colonisation and persistence. Consequently, effective antimicrobial control is a critical component of successful diabetic wound management [31]. Medicinal plants have been widely explored for their antimicrobial properties, with *in-vitro* studies demonstrating inhibitory activity against a range of wound-associated pathogens. Plant extracts rich in phenolics, flavonoids, terpenoids, and essential oils have shown effectiveness against common bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. These antimicrobial effects are often attributed to multiple mechanisms, including disruption of microbial cell membranes, interference with enzyme activity, and inhibition of nucleic acid synthesis [32]. An important advantage of plant-derived antimicrobials is their broad-spectrum and multi-targeted mode of action. Unlike conventional antibiotics that often act on a single molecular target, phytochemicals may affect several microbial processes simultaneously. This characteristic reduces the likelihood of rapid resistance development and may be particularly beneficial in the context of chronic diabetic wounds, where long-term antimicrobial exposure is common. *In-vitro* antimicrobial assays such as agar diffusion and minimum inhibitory concentration tests provide valuable preliminary evidence for these effects [33]. In addition to direct antimicrobial activity, some medicinal plants have been reported to interfere with biofilm formation, a major challenge in diabetic wound infections. Biofilms protect microbial communities from host defences and antibiotic treatment, thereby prolonging infection and inflammation. *In-vitro* studies suggest that certain herbal extracts can reduce biofilm formation or weaken established biofilms, indirectly supporting wound healing by limiting persistent microbial stress on the tissue [34]. Control of microbial burden has downstream benefits for wound repair, as reduced infection is associated with lower inflammatory mediator levels and improved cell viability. By contributing to both antimicrobial defence and modulation of the wound environment, medicinal plants may support multiple aspects of the healing process. The *in-vitro* evidence highlighting these

antimicrobial mechanisms strengthens the rationale for incorporating herbal agents into strategies aimed at managing diabetic wounds [35].

Enhancement of cell migration and proliferation by medicinal plants: Restoration of effective cell migration and proliferation is a critical requirement for successful wound closure, particularly in diabetic conditions where these processes are markedly compromised. As discussed earlier, fibroblasts and keratinocytes exposed to hyperglycaemia, oxidative stress, and chronic inflammation exhibit reduced motility and impaired proliferative capacity. In this context, medicinal plants have been increasingly investigated for their ability to support cellular behaviours directly involved in re-epithelialisation and tissue regeneration [36]. *In-vitro* studies using fibroblast and keratinocyte cell lines have shown that several herbal extracts and phytoconstituents can enhance cell viability and promote migration across artificial wound gaps created in scratch assays. These effects are often concentration-dependent and occur without inducing cytotoxicity, indicating a favourable safety profile for topical application. Improved cell migration observed in such models reflects enhanced cytoskeletal organisation, cell matrix interactions, and activation of signalling pathways associated with wound closure [37]. Medicinal plants may influence cell proliferation and migration through modulation of growth factor signalling and extracellular matrix dynamics. Certain phytochemicals have been reported to stimulate pathways involved in cell cycle progression and survival, thereby supporting the expansion of fibroblast and keratinocyte populations at the wound site. At the same time, preservation of extracellular matrix components creates a supportive scaffold that facilitates cell attachment and directional movement, both of which are essential for effective re-epithelialisation [38]. Importantly, enhancement of cell migration by medicinal plants is closely linked to their antioxidant and anti-inflammatory properties. Reduction of oxidative stress and excessive inflammatory signalling creates a more permissive cellular environment, allowing skin cells to respond more effectively to endogenous repair cues. *In-vitro* scratch assays performed under diabetic-simulated conditions consistently demonstrate faster wound gap closure in the presence of selected herbal extracts compared with untreated controls, supporting this mechanistic interplay [39]. Overall, the ability of medicinal plants to promote cell migration and proliferation represents a key mechanism through which they may contribute to improved wound healing in diabetic settings. Although *in-vitro* models cannot fully capture the complexity of *in-vivo* wound repair, the observed enhancement of cellular responses provides meaningful mechanistic evidence. These findings reinforce the relevance of medicinal plants as multi-functional agents capable of addressing core cellular deficits associated with diabetic wound healing [38,39].

The major biological activities of medicinal plants and their mechanisms of action in diabetic wound healing are presented in Table 2.

Table 2. Mechanistic actions of medicinal plants in diabetic wound healing (based on *in-vitro* evidence) [23–39].

Biological Activity	Target Mechanism	Cellular Effect	Experimental Model Used	Therapeutic Relevance
Antioxidant	ROS scavenging, ↑ SOD, CAT	Improved fibroblast viability	DPPH, ABTS, FRAP, Cell ROS assay	Restores redox balance
Anti-inflammatory	↓ TNF-α, IL-1β, IL-6	Reduced inflammatory signalling	Cytokine assay, macrophage models	Enables transition to proliferative phase
Antimicrobial	Membrane disruption, biofilm inhibition	Reduced microbial burden	MIC, agar diffusion	Infection control
Pro-migratory	Growth factor modulation	Enhanced scratch closure	Scratch wound assay	Faster wound gap closure

5. *In-Vitro* Evidence Supporting Herbal Interventions

Common *in-vitro* models used in diabetic wound research: *In-vitro* experimental models play a central role in investigating the wound-healing potential of medicinal plants, particularly in the context of diabetic conditions where ethical and practical limitations may restrict early *in-vivo* evaluation. These models offer controlled environments in which specific biological processes relevant to wound repair can be examined systematically, allowing researchers to establish mechanistic links between herbal interventions and cellular responses [40]. Antioxidant assays are among the most widely used *in-vitro* tools in diabetic wound research. Methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP) assays are commonly employed to assess the free radical–scavenging and redox-modulating capacity of plant extracts and isolated phytoconstituents. Although these chemical assays do not involve living cells, they provide important preliminary insights into the ability of herbal agents to counter oxidative stress, a key pathological factor in diabetic wound healing [41]. Cell-based assays offer more biologically relevant information by directly evaluating the effects of herbal preparations on skin-related cells. Cytotoxicity assays, such as MTT or similar viability tests, are routinely used to assess the safety of plant extracts toward fibroblasts and keratinocytes. These assays ensure that observed biological effects are not a consequence of cellular damage and help identify concentration ranges suitable for further wound-healing evaluation [42]. The *in-vitro*

scratch wound assay is one of the most commonly used models to study cell migration and wound closure. In this method, a linear "scratch" is created in a confluent monolayer of fibroblasts or keratinocytes, and the ability of cells to migrate and close the wound gap in the presence of herbal extracts is monitored over time. Under diabetic-simulated conditions, such as high-glucose media or oxidative stress induction, this assay provides valuable information on how plant-derived compounds influence impaired cell migration associated with diabetes [43]. Antimicrobial assays also form an important component of *in-vitro* diabetic wound research. Agar diffusion methods and minimum inhibitory concentration studies are frequently employed to evaluate the activity of herbal extracts against common wound-associated pathogens. These models help determine whether medicinal plants can contribute to infection control, which is critical for preventing prolonged inflammation and tissue damage in diabetic wounds [44]. Together, these *in-vitro* models provide complementary insights into the antioxidant, antimicrobial, cytocompatible, and pro-migratory effects of medicinal plants. While each model has inherent limitations, their combined use allows a more comprehensive and mechanistic evaluation of herbal interventions in diabetic wound healing research [40–44].

Strengths and limitations of *in-vitro* models: *In-vitro* models offer several important advantages for studying the wound-healing potential of medicinal plants, particularly in the context of diabetic conditions. One of the primary strengths of these models is the high level of experimental control they provide. Variables such as glucose concentration, oxidative stress levels, exposure time, and extract concentration can be precisely regulated, allowing researchers to isolate specific mechanisms and directly assess cellular responses to herbal interventions [45]. Another significant advantage is reproducibility. *In-vitro* assays can be standardised and repeated with minimal biological variability compared with *in-vivo* models. This reproducibility is especially valuable when comparing different plant extracts, formulations, or concentrations. Additionally, *in-vitro* studies are ethically favourable and cost-effective, making them accessible within academic and student research settings while still generating scientifically meaningful data [46]. *In-vitro* models are also well suited for early-stage screening. They enable rapid identification of potentially effective and non-toxic herbal candidates before more complex studies are considered. Assays such as antioxidant tests, cytotoxicity evaluations, and scratch wound models provide mechanistic insights into how medicinal plants may influence key processes involved in diabetic wound healing, including oxidative stress modulation, cell viability, and migration. Despite these strengths, *in-vitro* models have inherent limitations that must be acknowledged. They cannot fully replicate the complex physiological environment of a living organism, where multiple cell types, immune responses, vascular factors, and systemic metabolic influences interact dynamically. For example, *in-vitro* scratch assays do not account for angiogenesis, immune cell infiltration, or extracellular matrix remodelling that occur during *in-vivo* wound healing. Similarly, antimicrobial assays performed under laboratory conditions may not accurately reflect microbial behaviour within chronic wound biofilms. Furthermore, results obtained from *in-vitro* studies should be interpreted cautiously, as positive findings do not always translate directly to *in-vivo* or clinical outcomes. Differences in bioavailability, metabolism, and tissue penetration can influence the effectiveness of herbal agents beyond the *in-vitro* setting. Recognising these limitations is essential for maintaining scientific integrity and avoiding overinterpretation of results. Overall, *in-vitro* models represent a valuable and necessary foundation for diabetic wound-healing research involving medicinal plants. When used thoughtfully and interpreted within their limitations, these models provide critical mechanistic evidence that can guide formulation development and inform future preclinical investigations. Together, these models provide a structured framework for preliminary mechanistic evaluation of herbal wound-healing agents under diabetic-simulated conditions [45,46].

6. Formulation Considerations for Herbal Wound-Healing Agents

Importance of topical delivery systems: Topical delivery systems play a crucial role in the effective application of herbal agents for wound healing, particularly in the management of diabetic wounds. Unlike systemic administration, topical formulations allow direct delivery of bioactive compounds to the wound site, thereby maximizing local therapeutic effects while minimizing systemic exposure and potential side effects. This targeted approach is especially important in diabetic wounds, where impaired circulation may limit the effectiveness of systemically administered agents [47]. An ideal topical wound-healing formulation should maintain a moist environment, protect the wound from external contaminants, and facilitate the controlled release of active constituents. Moist wound conditions are known to promote cell migration, enhance epithelialization, and reduce tissue dehydration. Herbal formulations designed for topical use can support these requirements while simultaneously delivering phytochemicals with antioxidant, anti-inflammatory, and antimicrobial properties [47,48]. Various topical delivery systems, including gels, hydrogels, creams, ointments, and advanced wound dressings, have been explored for incorporating herbal extracts. Among these, gels and hydrogels are particularly attractive

for diabetic wound applications due to their cooling effect, ease of application, and ability to provide sustained contact with the wound surface. Their high-water content also supports oxygen diffusion and helps maintain a favourable microenvironment for wound repair [49]. From a formulation perspective, the choice of excipients and polymeric matrices can significantly influence the stability and biological activity of herbal constituents. Proper formulation helps protect sensitive phytochemicals from degradation and ensures consistent release at the wound site. In addition, topical systems can be optimised to enhance skin compatibility and reduce irritation, which is important for chronic wounds requiring prolonged treatment [50]. Overall, the design of an appropriate topical delivery system is a critical step in translating the biological potential of medicinal plants into practical wound-healing applications. Consideration of formulation aspects alongside mechanistic *in-vitro* evidence strengthens the relevance of herbal interventions for diabetic wound management and supports their further development [47,51].

Influence of formulation on biological activity: The biological effectiveness of herbal wound-healing agents is not determined solely by the intrinsic activity of the plant extracts or phytoconstituents, but is also strongly influenced by the formulation in which they are delivered. Proper formulation can enhance the stability, availability, and interaction of bioactive compounds with the wound environment, thereby significantly affecting their observed *in-vitro* and potential therapeutic outcomes [52]. Formulation parameters such as the type of polymer, viscosity, pH, and excipient composition play a crucial role in modulating the release and diffusion of herbal constituents. In topical systems like gels and hydrogels, the polymer matrix can control the rate at which active compounds are released, influencing the duration of their biological effects. Sustained and controlled release is particularly advantageous in diabetic wounds, where prolonged exposure to antioxidant and anti-inflammatory agents may support continuous modulation of the impaired healing process [53]. *In-vitro* studies have demonstrated that the same herbal extract may exhibit different levels of biological activity depending on its formulation. For instance, incorporation into a suitable gel base can improve the apparent antioxidant or antimicrobial efficacy by enhancing solubility and contact with the test medium or cells. Similarly, well-formulated systems can reduce local cytotoxicity by preventing high local concentrations of phytochemicals, thereby improving cell compatibility in cytotoxicity and scratch wound assays [54]. Formulation also influences the interaction between herbal agents and skin cells. Factors such as pH and osmolarity affect cell viability and migration, while the presence of humectants and penetration enhancers can facilitate better cellular uptake of active compounds in *in-vitro* models. These considerations are essential when interpreting experimental data, as differences in formulation may account for variations in biological responses observed across studies [55]. The evidence for plant-derived compounds in diabetic wound healing varies according to the experimental model and the outcome investigated. Studies using high-glucose cell culture conditions can provide mechanistic information about cellular responses associated with diabetes, whereas conventional antioxidant and antimicrobial assays address narrower biological activities. These findings should therefore be interpreted separately. Table 3 summarises selected phytochemicals and the experimental evidence available for their potential role in wound repair, with particular attention to the model used and the outcomes reported.

Table 3. Key phytochemicals investigated in diabetic wound healing (*in-vitro* evidence) [56,57].

Phytochemical	Source Plant	Potential Mechanism	*In-Vitro* Model	Reported Outcomes
Curcumin	Curcuma longa L.	Antioxidant and anti-inflammatory effects; modulation of cellular responses associated with wound repair	High-glucose-treated fibroblast cells; assays of cell viability, apoptosis and migration	Improved fibroblast proliferation and migration and reduced apoptosis under the tested experimental conditions

Overall, formulation acts as a critical bridge between the mechanistic potential of medicinal plants and their practical application in wound healing. Recognition of the formulation-dependent nature of biological activity underscores the importance of integrating pharmaceutical design principles with mechanistic *in-vitro* evidence. Such an integrated approach enhances the relevance and translational value of herbal wound-healing research, particularly in the context of diabetic wound management.

7. Limitations of Current Evidence

Despite the growing body of *in-vitro* research supporting the potential role of medicinal plants in diabetic wound healing, several limitations within the existing literature must be carefully considered. One of the primary challenges is the lack of standardisation across studies. Variations in plant species, plant parts used, extraction methods, solvent systems, and phytochemical composition make direct comparison between studies difficult. Such heterogeneity can influence biological outcomes and limits the ability to draw generalised conclusions regarding efficacy [58]. Another important limitation relates to the diversity of *in-vitro* models employed. While antioxidant

assays, antimicrobial tests, cytotoxicity evaluations, and scratch wound assays are widely used, differences in experimental conditions such as glucose concentration, exposure duration, and cell type can significantly affect results. In many cases, diabetic conditions are simulated using high-glucose media or oxidative stress inducers, which may not fully replicate the complex and dynamic microenvironment of diabetic wounds in vivo [59]. Interpretation of *in-vitro* findings also requires caution, as positive results do not necessarily translate into comparable effects in living systems. *In-vitro* models lack key physiological components such as immune cell interactions, vascular responses, and systemic metabolic influences, all of which play critical roles in wound healing. Consequently, the magnitude and consistency of biological effects observed in cell-based assays may differ when evaluated under more complex biological conditions [60]. Additionally, many studies focus on isolated biological activities without integrating multiple mechanisms relevant to diabetic wound pathology. For example, antioxidant or antimicrobial effects may be reported independently, without assessing their combined influence on cell migration or tissue regeneration. This fragmented approach can obscure the broader therapeutic relevance of herbal agents, which often exert multi-targeted actions [61]. Finally, reporting quality and experimental transparency vary across studies. Incomplete methodological details, limited statistical analysis, and selective reporting of outcomes can reduce reproducibility and confidence in the findings. Recognising these limitations is essential for balanced interpretation of the available evidence and for guiding future research efforts toward more robust and standardised *in-vitro* investigations [62].

8. Critical Appraisal of the Available Evidence

The potential role of medicinal plants in diabetic wound healing should be evaluated in relation to the experimental evidence supporting each proposed mechanism. Antioxidant and antimicrobial assays provide useful preliminary information about the biological properties of plant-derived compounds, but these findings alone do not establish wound-healing efficacy. Cell-based experiments can offer further insight into cellular viability, migration, proliferation, and inflammatory responses, particularly when high-glucose conditions are used to simulate aspects of the diabetic environment. However, the relevance of these findings depends on the experimental model, treatment concentration, duration of exposure, and outcomes measured. Results obtained with purified compounds may also differ from those produced by crude extracts because their chemical composition and concentrations of active constituents vary. Consequently, evidence from biochemical assays, cell cultures, animal models, and clinical studies should be interpreted separately. A critical comparison of these evidence types is necessary to identify promising candidates without overstating their therapeutic potential.

9. Future Perspectives

Future research on medicinal plants for diabetic wound healing should aim to address the limitations identified in current *in-vitro* studies and move toward a more integrated and standardised approach. One of the key priorities is the development of uniform experimental protocols for *in-vitro* evaluation. Standardisation of extraction methods, concentration ranges, glucose-simulated conditions, and assay parameters would improve reproducibility and allow meaningful comparison across studies. Greater emphasis should also be placed on multi-mechanistic evaluation rather than isolated biological activities. Since diabetic wound healing is influenced by interconnected processes such as oxidative stress, inflammation, infection, and impaired cell migration, future *in-vitro* studies should be designed to assess these mechanisms in combination. Such an approach would better reflect the complex nature of diabetic wound pathology and provide stronger mechanistic justification for herbal interventions. Integration of formulation science with biological evaluation represents another important direction for future research. Optimised topical delivery systems, such as hydrogels or advanced wound dressings, can significantly influence the stability, release, and cellular interaction of herbal constituents. Evaluating herbal agents within suitable formulations rather than as crude extracts alone may yield more clinically relevant insights and enhance translational potential. Advances in cell culture techniques also offer opportunities to improve the predictive value of *in-vitro* models. Co-culture systems involving multiple skin-related cell types, three-dimensional skin models, and models incorporating microbial components may provide a more realistic representation of the wound environment. Although more complex, such systems could bridge the gap between conventional *in-vitro* assays and *in-vivo* studies. Ultimately, well-designed *in-vitro* research should serve as a foundation for subsequent preclinical and clinical investigations. By generating robust mechanistic evidence and identifying promising herbal candidates and formulations, future studies can facilitate rational progression toward *in-vivo* validation and, eventually, safe and effective therapeutic options for diabetic wound management.

10. Conclusions

In-vitro studies provide a useful starting point for investigating medicinal plants and their constituents in relation to diabetic wound healing. The reported experiments address different aspects of wound repair, including antioxidant capacity, antimicrobial activity, cellular viability and migration. These findings can help identify materials for further investigation, but they do not provide the same level of evidence, and results from general laboratory assays should not be interpreted as proof of efficacy in diabetic wounds.

Future research should prioritise well-characterised plant extracts and isolated compounds, reproducible experimental methods, appropriate diabetic wound-related models and the direct measurement of proposed molecular mechanisms. Findings should also be validated through complementary experimental approaches before their therapeutic relevance is inferred. A careful distinction between demonstrated effects and proposed mechanisms will help establish a more reliable evidence base for the development of plant-derived interventions for diabetic wound management.

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