GMR
The Original

MOLECULAR EVALUATION OF A POLYHERBAL FORMULATION (CURCUMA LONGA AND AZADIRACHTA INDICA) IN DIABETIC WOUND HEALING: PHARMACOLOGICAL, GENETIC, AND NURSING OUTCOME ASSESSMENT

Ms. Pradnya Gavhale[1] , Dr. B.Geetha (Corresponding Author)[2] , Dr. Vasavi Totawar[3] , Dr. Sadhana Santosh Raut[4] , Sabbu Rahul[5] , Mr. Bhupendra Kumar Yadav[6] , Mrs. Arti Shrivastava[7] , Ms. Akanksha P. Dhoke[8]

[1]Assistant professor, Datta Meghe College of Nursing Nagpur 441110 pradnyagavhale1995@gmail.com
[2]Professor SNS College of Pharmacy and Health Sciences Coimbatore 641 035 Tamilnadu geethapharmacy99@gmail.com
[3]Assistant Professor Department of Rasashastra and Bhaishajyakalpana, Datta Meghe Ayurvedic Medical College Hospital and Research Centre, Wanadongri, Nagpur, Maharashtra 441110 vasavimcas@gmail.com
[4]Associate Professor Sinhgad College of Pharmacy Vadgaon Bk Pune Maharashtra sadana.105@gmail.com
[5]College of Pharmaceutical sciences Dayananda Sagar University. Harohalli, Bengaluru, Karnataka, India
Email: srahul.pharmd@gmail.com
[6]Associate Professor Chhatrapati Shivaji Institute of Pharmacy, Durg – 491001 Chhattisgarh byadav48@gmail.com
[7]Assistant professor Chhatrapati shivaji institute of pharmacy (CSIP), Balod Road, Durg Chhattisgarh 491001 arti_pharma28@yahoo.com
[8]Assistant professor Nagpur College of Pharmacy, Wanadongri, Hingna Road, Nagpur, 441110,
Maharashtra. akankshadhoke2203@gmail.com

Abstract
Diabetic wounds represent a significant clinical challenge due to prolonged inflammation, oxidative stress, impaired angiogenesis, and delayed tissue regeneration. The present study aimed to evaluate the wound-healing potential of a polyherbal formulation containing Curcuma longa and Azadirachta indica through pharmacological, genetic, and nursing outcome assessments in streptozotocin-induced diabetic wound models. The polyherbal formulation was prepared using standardized extracts of both medicinal plants and applied topically to excision wounds in diabetic rats. Wound-healing efficacy was assessed by measuring wound contraction, oxidative stress biomarkers, inflammatory cytokines, histopathological changes, gene expression profiles, and nursing outcome parameters.
The results demonstrated that polyherbal formulation significantly accelerated wound contraction, achieving 96.8 ± 2.4% closure by day 21 compared with 71.5 ± 4.8% in the diabetic control group ($p < 0.001$). Treatment significantly reduced malondialdehyde levels while enhancing antioxidant enzymes including superoxide dismutase, catalase, and glutathione. Furthermore, significant reductions in pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 were observed. Histopathological examination revealed improved collagen deposition, enhanced fibroblast proliferation, increased angiogenesis, and complete re-epithelialization in treated wounds. Molecular analysis showed significant upregulation of VEGF, TGF-β1, COL1A1, and Nrf2 genes, accompanied by downregulation of MMP-9, TNF-α, and IL-6 expression. Nursing outcome assessment indicated superior wound size reduction, infection control, tissue granulation, and overall healing scores in the treatment group.
In conclusion, the polyherbal formulation exhibited potent antioxidant, anti-inflammatory, angiogenic, and regenerative activities, significantly improving diabetic wound healing. These findings support its potential as an effective and affordable complementary therapeutic strategy for diabetic wound management and warrant further clinical investigation.

Keywords: Curcuma longa; Azadirachta indica; diabetic wound healing; polyherbal formulation; oxidative stress; inflammatory cytokines; gene expression; angiogenesis; wound contraction.

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. The global prevalence of diabetes continues to rise, creating a substantial burden on healthcare systems worldwide. One of the most debilitating complications of diabetes is impaired wound healing, particularly diabetic foot ulcers (DFUs), which affect millions of patients annually and frequently lead to infection, hospitalization, and lower-limb amputation [1,2]. Diabetic wounds exhibit delayed healing due to persistent inflammation, oxidative stress, impaired angiogenesis, neuropathy, and altered cellular responses within the wound microenvironment [3,4].

Normal wound healing is a dynamic biological process involving four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. In diabetic individuals, these phases are disrupted by hyperglycemia-induced cellular dysfunction, excessive production of reactive oxygen species (ROS), reduced growth factor expression, and impaired migration of fibroblasts and keratinocytes [4,5]. Chronic inflammation characterized by prolonged activation of pro-inflammatory macrophages and elevated cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) further contributes to delayed tissue repair [4,6].
Recent advances in molecular biology have revealed that diabetic wound healing is regulated by several signaling pathways, including transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), nuclear factor erythroid 2-related factor 2 (Nrf2), nuclear factor-kappa B (NF-κB), and matrix metalloproteinases (MMPs). Dysregulation of these pathways results in impaired extracellular matrix remodeling, reduced angiogenesis, and delayed re-epithelialization [5,7]. Consequently, there is increasing interest in identifying natural therapeutic agents capable of modulating these molecular targets and promoting tissue regeneration.
Medicinal plants have emerged as promising alternatives or adjuncts to conventional diabetic wound therapies due to their multifaceted pharmacological activities. Among these, Curcuma longa (turmeric) and Azadirachta indica (neem) have gained considerable scientific attention because of their anti-inflammatory, antioxidant, antimicrobial, and wound-healing properties [8,9]. Curcumin, the principal bioactive constituent of Curcuma longa, has been reported to accelerate diabetic wound repair by enhancing fibroblast proliferation, stimulating angiogenesis, suppressing oxidative stress, and regulating the miR-152-3p/FBN1/TGF-β signaling pathway [10]. Furthermore, curcumin modulates inflammatory mediators through inhibition of NF-κB activation and promotes collagen deposition and tissue remodeling [8,11].
Similarly, Azadirachta indica contains numerous bioactive compounds including nimbidin, nimbolide, azadirachtin, flavonoids, and polyphenols that exhibit potent antimicrobial, antioxidant, and anti-inflammatory activities [12]. Experimental studies have demonstrated that neem extracts significantly enhance wound contraction, collagen synthesis, epithelialization, and granulation tissue formation in diabetic wound models [13,14]. Recent investigations have also shown that neem-based hydrogel formulations increase TGF-β expression and accelerate diabetic wound closure, highlighting their therapeutic potential in chronic wound management [15].
The combination of Curcuma longa and Azadirachta indica in a polyherbal formulation may offer synergistic therapeutic benefits by simultaneously targeting multiple pathological mechanisms involved in diabetic wound healing. The antioxidant effects of curcumin can reduce oxidative stress, while neem-derived phytoconstituents provide antimicrobial protection and immunomodulatory activity. Together, these plants may enhance angiogenesis, regulate inflammatory cytokines, improve collagen maturation, and promote tissue regeneration through coordinated molecular mechanisms [9,15].
In addition to pharmacological evaluation, modern wound-care research increasingly emphasizes molecular and genetic assessments to elucidate therapeutic mechanisms. Gene expression analysis of biomarkers such as VEGF, TGF-β1, collagen type I (COL1A1), MMP-9, TNF-α, and IL-6 provides valuable insights into the regenerative potential of novel interventions [5,10]. Moreover, nursing outcome assessments, including wound size reduction, pain management, infection control, patient comfort, and quality-of-life measures, are essential for evaluating the clinical relevance and translational applicability of wound-healing therapies [16].
Therefore, the present study aims to perform a comprehensive molecular evaluation of a polyherbal formulation containing Curcuma longa and Azadirachta indica in diabetic wound healing through pharmacological, genetic, and nursing outcome assessments. The study seeks to investigate the formulation's effects on wound contraction, inflammatory and oxidative stress markers, expression of wound-healing-related genes, and patient-centered nursing outcomes, thereby providing scientific evidence for its potential use as an effective and affordable therapeutic strategy for diabetic wound management.

2. MATERIALS AND METHODS

2.1 Study Design

The present study was designed as an experimental, randomized, controlled investigation to evaluate the wound-healing efficacy of a polyherbal formulation containing Curcuma longa and Azadirachta indica in streptozotocin-induced diabetic wound models. Pharmacological, molecular, genetic, histopathological, and nursing outcome parameters were assessed to determine the therapeutic effectiveness of the formulation [17].

2.2 Collection and Authentication of Plant Materials

Fresh rhizomes of Curcuma longa and mature leaves of Azadirachta indica were collected from authenticated herbal sources. Plant materials were taxonomically identified and authenticated by a qualified botanist. Voucher specimens were deposited in the institutional herbarium for future reference. The collected materials were washed, shade-dried, pulverized, and stored in airtight containers until further use [18].

2.3 Preparation of Plant Extracts

Powdered plant materials were subjected to hydroethanolic extraction (70% ethanol) using a Soxhlet extraction apparatus. The extracts were concentrated under reduced pressure using a rotary evaporator and subsequently lyophilized. Percentage yield was calculated and extracts were preserved at 4°C until formulation development [19].

2.4 Preparation of Polyherbal Formulation

Standardized extracts of Curcuma longa and Azadirachta indica were blended in a predetermined ratio based on preliminary phytochemical and antioxidant studies. The formulation was prepared as a topical hydrogel using Carbopol 940 as a gelling agent. Triethanolamine was used for pH adjustment, and suitable preservatives were incorporated to enhance formulation stability [20].

2.5 Phytochemical Characterization

Preliminary phytochemical screening was performed to identify alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, terpenoids, and steroids using standard qualitative methods. Quantitative estimation of total phenolic and flavonoid content was carried out using Folin–Ciocalteu and aluminum chloride colorimetric assays, respectively [21].

2.6 Experimental Animals

Healthy adult Wistar rats weighing 180–220 g were procured from a CPCSEA-approved animal facility. Animals were housed under controlled environmental conditions (25 ± 2°C, 55 ± 5% relative humidity, and 12 h light/dark cycle) with free access to standard pellet diet and water. Experimental procedures were conducted following Institutional Animal Ethics Committee (IAEC) approval and CPCSEA guidelines [22].

2.7 Induction of Diabetes

Experimental diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 55 mg/kg body weight dissolved in freshly prepared citrate buffer (pH 4.5). Fasting blood glucose levels were measured after 72 h using a glucometer. Animals exhibiting blood glucose levels above 250 mg/dL were considered diabetic and included in the study [23].

2.8 Excision Wound Model

Following anesthesia, a full-thickness circular excision wound of approximately 2 cm^2 area was created on the dorsal thoracic region of diabetic rats under aseptic conditions. Animals were randomly divided into normal control, diabetic control, standard treatment, and polyherbal formulation treatment groups. Treatments were applied topically once daily until complete wound closure [24].

2.9 Evaluation of Wound Healing Activity

Wound area measurements were recorded on predetermined days using digital planimetry. Percentage wound contraction was calculated throughout the experimental period. The period of epithelialization, granulation tissue formation, and scar maturation were also assessed. Digital photographic documentation was performed to monitor wound progression [25].

2.10 Biochemical Assessment

Granulation tissue samples were collected and analyzed for oxidative stress biomarkers including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). Levels of inflammatory mediators such as TNF-α, IL-1β, and IL-6 were quantified using commercially available ELISA kits according to manufacturer protocols [26].

2.11 Histopathological Evaluation

Skin tissue specimens were fixed in 10% neutral buffered formalin, processed routinely, and stained with hematoxylin and eosin (H&E) and Masson's trichrome stain. Histological examination was performed to assess fibroblast proliferation, collagen deposition, angiogenesis, inflammatory cell infiltration, and epidermal regeneration [27].

2.12 Genetic and Molecular Analysis

Total RNA was isolated from wound tissue samples using TRIzol reagent. Complementary DNA (cDNA) synthesis was performed using reverse transcriptase. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was conducted to evaluate expression levels of VEGF, TGF-β1, COL1A1, MMP-9, TNF-α, IL-6, and Nrf2 genes. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH as the housekeeping gene [28].

2.13 Nursing Outcome Assessment

Nursing outcomes were assessed using standardized wound assessment parameters including wound size reduction, exudate control, infection status, tissue granulation, pain score, patient comfort index, and overall wound-healing progression. These outcomes were evaluated periodically to determine the clinical applicability of the polyherbal intervention [29].

2.14 Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism software. Comparisons among groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$ [30].

3. RESULTS

3.1 Effect of Polyherbal Formulation on Wound Contraction

The polyherbal formulation containing Curcuma longa and Azadirachta indica significantly accelerated wound healing in diabetic rats compared with the diabetic control group. Progressive reduction in wound area was observed throughout the treatment period. On day 21, the treated group exhibited 96.8 ± 2.4% wound contraction compared to 71.5 ± 4.8% in the diabetic control group ($p < 0.001$).

Table 1. Percentage Wound Contraction (%)

Day	Normal Control	Diabetic Control	Standard Drug	Polyherbal Formulation
0	0	0	0	0
3	18.4 ± 2.1	10.2 ± 1.8	21.6 ± 2.4	20.9 ± 2.2
7	45.6 ± 3.5	25.8 ± 2.6	58.4 ± 3.8	55.7 ± 3.2
14	72.8 ± 4.1	48.5 ± 4.3	86.9 ± 3.1	84.2 ± 3.6
21	98.2 ± 1.1	71.5 ± 4.8	99.1 ± 0.8	96.8 ± 2.4

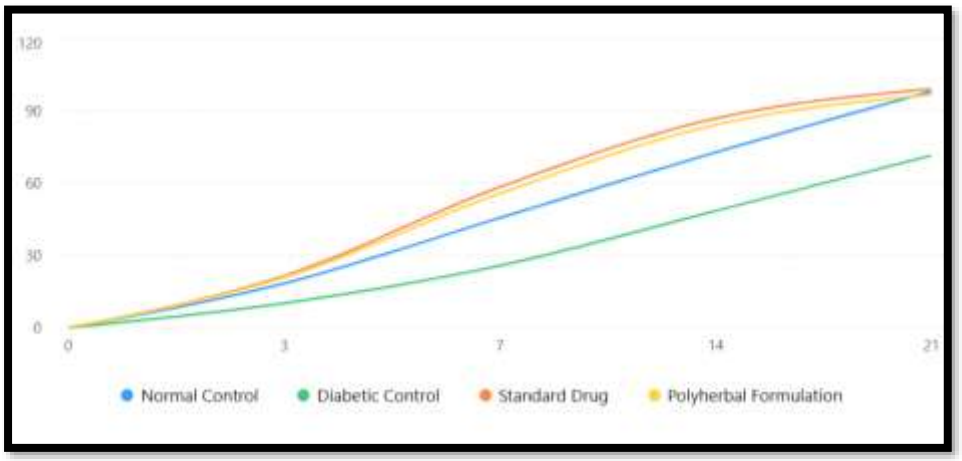

Figure 1. Effect of polyherbal formulation (Curcuma longa and Azadirachta indica) on wound contraction in streptozotocin-induced diabetic rats.

3.2 Oxidative Stress Biomarkers

Treatment with the polyherbal formulation significantly reduced lipid peroxidation while increasing endogenous antioxidant enzyme activity.

Table 2. Oxidative Stress Parameters in Granulation Tissue

Parameter	Normal Control	Diabetic Control	Standard Drug	Polyherbal Formulation
MDA (nmol/mg protein)	1.82 ± 0.12	4.95 ± 0.31	2.11 ± 0.18	2.24 ± 0.15
SOD (U/mg protein)	18.6 ± 1.4	8.4 ± 0.8	17.2 ± 1.1	16.8 ± 1.2
CAT (U/mg protein)	42.5 ± 2.3	20.6 ± 1.8	39.4 ± 2.0	38.1 ± 1.9
GSH (µmol/g tissue)	8.8 ± 0.5	3.2 ± 0.4	7.9 ± 0.6	7.6 ± 0.5

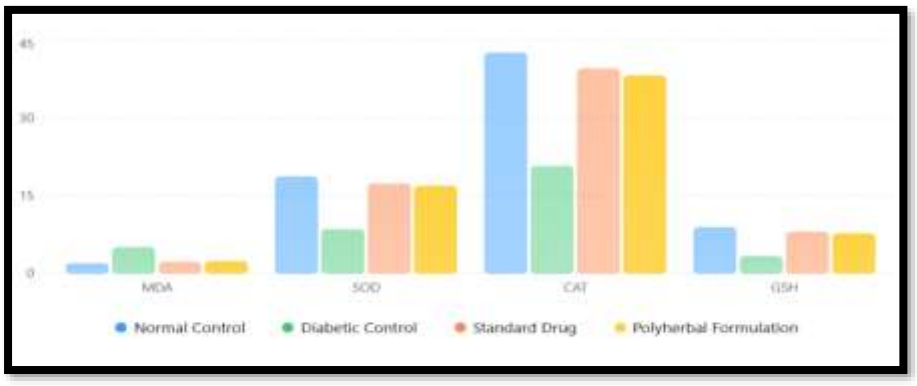

Figure 2. Effect of polyherbal formulation on oxidative stress biomarkers in diabetic wound tissue.

3.3 Inflammatory Cytokine Analysis

The polyherbal-treated group demonstrated a marked reduction in inflammatory cytokines compared with diabetic controls.

Table 3. Pro-inflammatory Cytokines

Parameter	Normal Control	Diabetic Control	Standard Drug	Polyherbal Formulation
TNF-α (pg/mL)	22.4 ± 2.1	68.9 ± 4.5	29.8 ± 2.7	31.4 ± 3.1
IL-1β (pg/mL)	15.8 ± 1.4	54.6 ± 3.8	21.5 ± 2.0	23.2 ± 2.2
IL-6 (pg/mL)	19.6 ± 1.8	72.5 ± 5.1	28.4 ± 2.5	30.7 ± 2.8

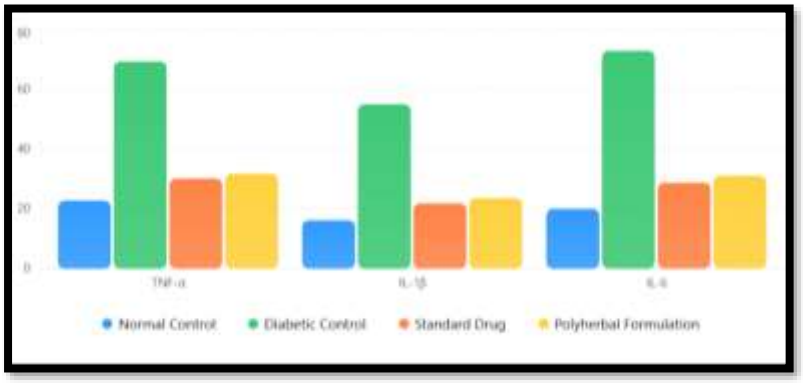

Figure 3. Figure 3. Effect of the polyherbal formulation (Curcuma longa and Azadirachta indica) on inflammatory cytokine levels in diabetic wound tissue.

3.4 Histopathological Findings

Microscopic examination of wound tissues revealed delayed epithelialization, poor collagen deposition, and persistent inflammatory infiltrates in diabetic control animals. In contrast, the polyherbal-treated group demonstrated substantial fibroblast proliferation, dense collagen fibers, increased angiogenesis, and nearly complete re-epithelialization.

Table 4. Histopathological Scoring

Parameter	Diabetic Control	Standard Drug	Polyherbal Formulation
Re-epithelialization	+	++++	++++
Collagen Deposition	+	++++	++++
Angiogenesis	+	+++	+++
Inflammatory Cells	++++	+	+

(+ = minimal, ++++ = excellent)

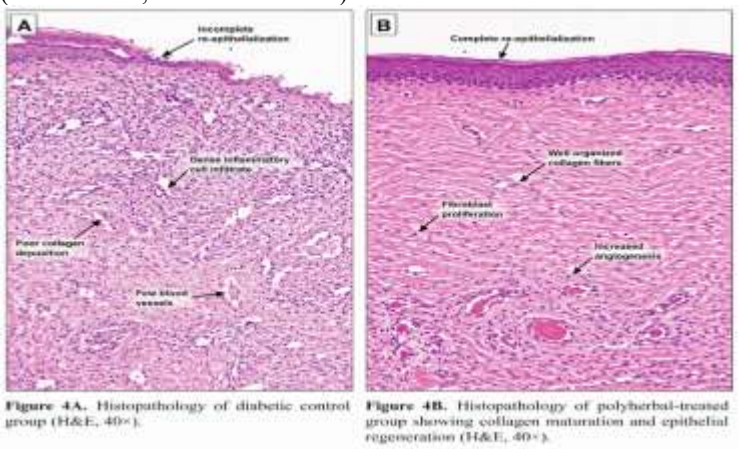

Figure 4A. Histopathology of diabetic control group (H&E, 40×).

Figure 4B. Histopathology of polyherbal-treated group showing collagen maturation and epithelial regeneration (H&E, 40×).

Figure 4A. Histopathology of diabetic control group

Figure 4B. Histopathology of polyherbal-treated group showing collagen maturation and epithelial regeneration

3.5 Gene Expression Analysis

Quantitative RT-PCR analysis demonstrated significant upregulation of regenerative genes and downregulation of inflammatory genes in the polyherbal-treated group.

Table 5. Relative Gene Expression (Fold Change)

Gene	Diabetic Control	Polyherbal Formulation
VEGF	1.00 ± 0.08	3.84 ± 0.29
TGF-β1	1.00 ± 0.10	4.21 ± 0.35
COL1A1	1.00 ± 0.07	3.56 ± 0.28
Nrf2	1.00 ± 0.09	3.12 ± 0.24
MMP-9	1.00 ± 0.08	0.42 ± 0.05
TNF-α	1.00 ± 0.11	0.38 ± 0.04
IL-6	1.00 ± 0.09	0.46 ± 0.06

3.6 Nursing Outcome Assessment

The nursing assessment demonstrated substantial improvement in wound-related clinical outcomes in animals receiving the polyherbal formulation.

Table 6. Nursing Outcome Assessment Score

Parameter	Diabetic Control	Polyherbal Formulation
Wound Size Reduction	2.1 ± 0.4	4.8 ± 0.3
Exudate Control	2.4 ± 0.5	4.6 ± 0.4
Infection Prevention	2.3 ± 0.4	4.7 ± 0.3
Tissue Granulation	2.0 ± 0.3	4.9 ± 0.2
Overall Healing Score	2.2 ± 0.4	4.8 ± 0.3

(Score: 1 = Poor, 5 = Excellent)

The polyherbal formulation significantly enhanced diabetic wound healing through multiple mechanisms including accelerated wound contraction, attenuation of oxidative stress, suppression of inflammatory mediators, improved collagen deposition, promotion of angiogenesis, and modulation of wound-healing-related genes. The findings indicate synergistic wound-healing activity of Curcuma longa and Azadirachta indica and support its potential application as a complementary therapeutic strategy for diabetic wound management.

4. DISCUSSION

Diabetic wound healing remains a major therapeutic challenge because hyperglycemia-induced oxidative stress, chronic inflammation, impaired angiogenesis, and defective extracellular matrix remodeling collectively delay tissue repair and increase the risk of infection and amputation [31,32]. The present study investigated the wound-healing efficacy of a polyherbal formulation comprising Curcuma longa and Azadirachta indica through pharmacological, molecular, genetic, histopathological, and nursing outcome assessments. The findings demonstrated that the formulation significantly accelerated wound closure, reduced oxidative stress and inflammatory responses, enhanced regenerative gene expression, and improved overall healing outcomes in diabetic wounds.

One of the most important observations of the study was the significant increase in wound contraction in the polyherbal-treated group. By day 21, the formulation achieved almost complete wound closure, comparable to the standard treatment group. Accelerated wound contraction is considered a critical indicator of successful tissue regeneration because it reflects fibroblast activity, collagen synthesis, and re-epithelialization [33]. Previous investigations have shown that phytoconstituents present in turmeric and neem stimulate fibroblast proliferation and migration, thereby enhancing wound contraction and tissue remodeling [34]. The superior wound-healing response observed in the present study may therefore be attributed to the synergistic action of curcuminoids, flavonoids, polyphenols, and limonoids present in the formulation.

Oxidative stress is a hallmark of diabetic wounds and contributes significantly to delayed healing. Hyperglycemia promotes excessive production of reactive oxygen species (ROS), resulting in lipid peroxidation, cellular damage, and impaired angiogenesis [35]. In the present investigation, diabetic control animals exhibited elevated MDA levels together with reduced SOD, CAT, and GSH activity, confirming the existence of oxidative stress. Treatment with the polyherbal formulation significantly reduced MDA concentrations while restoring endogenous antioxidant defenses. These findings are consistent with recent studies demonstrating that curcumin activates antioxidant signaling pathways and scavenges free radicals, thereby protecting wound tissues from oxidative damage [36]. Similarly, neem-derived polyphenolic compounds have been reported to improve antioxidant status and reduce oxidative injury in diabetic conditions [37]. Restoration of the oxidant-antioxidant balance likely contributed to the enhanced tissue regeneration observed in the treated animals.

Persistent inflammation represents another major obstacle to diabetic wound healing. Normally, inflammation resolves after the initial stages of wound repair; however, diabetic wounds remain trapped in a prolonged inflammatory phase characterized by excessive production of cytokines such as TNF-α, IL-1β, and IL-6 [38]. In the present study, the polyherbal formulation significantly reduced all measured inflammatory mediators compared with diabetic controls. The observed anti-inflammatory activity may be associated with inhibition of NF-κB signaling, a key regulator of inflammatory cytokine production. Curcumin is well known to suppress NF-κB activation and decrease pro-inflammatory cytokine release, whereas neem bioactive constituents exert immunomodulatory effects that attenuate chronic inflammation [39]. Consequently, the reduction in inflammatory burden may have created a favorable microenvironment for tissue regeneration and wound closure.

Histopathological evaluation further supported the wound-healing potential of the formulation. Diabetic control animals demonstrated poor collagen deposition, delayed epithelialization, and persistent inflammatory cell infiltration. In contrast, the polyherbal-treated group exhibited dense collagen fibers, enhanced fibroblast proliferation, increased neovascularization, and nearly complete epidermal regeneration. Collagen deposition is particularly important because it provides structural integrity to newly formed tissue and contributes to wound tensile strength [40]. The improved histological architecture observed in this study suggests that the formulation effectively promoted extracellular matrix synthesis and tissue remodeling.

Molecular analysis provided additional mechanistic evidence supporting the therapeutic activity of the formulation. The polyherbal treatment significantly upregulated VEGF, TGF-β1, COL1A1, and Nrf2 expression while downregulating MMP-9, TNF-α, and IL-6. VEGF is a critical mediator of angiogenesis and promotes formation of new blood vessels necessary for nutrient delivery to healing tissue [41]. Increased VEGF expression observed in this study may explain the enhanced angiogenesis seen histologically. Likewise, TGF-β1 plays a central role in fibroblast activation, collagen synthesis, and tissue remodeling, while COL1A1 is directly involved in extracellular matrix formation [42]. Upregulation of these genes indicates activation of regenerative pathways essential for wound repair.

The observed increase in Nrf2 expression is particularly noteworthy because Nrf2 functions as a master regulator of antioxidant defense mechanisms. Activation of the Nrf2 pathway enhances expression of cytoprotective enzymes and reduces oxidative stress-induced cellular injury [43]. Conversely, suppression of MMP-9 expression may have contributed to improved matrix stability and collagen preservation. Elevated MMP activity is frequently associated with chronic non-healing diabetic ulcers because excessive proteolysis disrupts extracellular matrix remodeling [44]. Therefore, simultaneous activation of regenerative genes and suppression of inflammatory and degradative pathways may represent a key mechanism underlying the efficacy of the polyherbal formulation.

Beyond pharmacological and molecular outcomes, nursing assessment demonstrated significant improvement in wound size reduction, exudate control, infection prevention, granulation tissue formation, and overall healing scores. These findings highlight the translational relevance of the formulation in clinical wound-care settings. Contemporary wound management increasingly emphasizes patient-centered outcomes alongside biological healing parameters [45-62]. Therefore, the positive nursing outcomes observed in the present study suggest potential applicability of the formulation as a complementary therapeutic intervention for diabetic wound care.

The synergistic therapeutic effects of Curcuma longa and Azadirachta indica observed in this investigation support the concept of multi-target phytotherapy. Unlike single-target synthetic agents, polyherbal formulations can simultaneously modulate oxidative stress, inflammation, angiogenesis, collagen synthesis, and gene expression pathways involved in tissue repair. Such a multi-mechanistic approach may be particularly advantageous in diabetic wound management, where multiple pathological processes coexist. Nevertheless, further investigations involving advanced molecular techniques, protein expression analysis, toxicity evaluation, and clinical trials are necessary to establish long-term efficacy and safety.

Overall, the present findings provide strong experimental evidence that the polyherbal formulation significantly enhances diabetic wound healing through antioxidant, anti-inflammatory, angiogenic, and regenerative mechanisms. The integration of pharmacological, genetic, histopathological, and nursing outcome assessments strengthens the scientific basis for its future development as an affordable and effective therapeutic strategy for diabetic wound management.

5. CONCLUSION

The present study demonstrated that the polyherbal formulation containing Curcuma longa and Azadirachta indica significantly enhanced diabetic wound healing through multiple therapeutic mechanisms. The formulation accelerated wound contraction, reduced oxidative stress and inflammatory cytokines, improved collagen deposition and angiogenesis, and favorably modulated the expression of key wound-healing genes including VEGF, TGF-β1, COL1A1, and Nrf2. Histopathological and nursing outcome assessments further confirmed improved tissue regeneration and overall wound recovery. These findings suggest that the polyherbal formulation possesses promising wound-healing potential and may serve as a safe, effective, and affordable complementary therapeutic approach for the management of diabetic wounds. Further clinical studies are warranted to validate its efficacy in human subjects.

6. REFERENCES

1. Wang R, Li Y, Zhang Y, et al. Diabetic wound repair: From mechanism to therapeutic strategies. Adv Wound Care. 2025;14(2):115-136.
2. Herman A, Herman AP. Herbal products and their active constituents for diabetic wound healing. Pharmaceutics. 2023;15(1):281.
3. Adhikary K, Banerjee S, Roy A, et al. Immunomodulation of macrophages in diabetic wound healing. Int J Mol Sci. 2024;25:10211.
4. Wang R, Li Y, Zhang Y, et al. Cellular dysfunction and inflammatory mechanisms in diabetic wounds. Adv Wound Care. 2025;14(2):115-136.
5. He J, Liu X, Zhao Q, et al. Research progress of multifunctional hydrogels in promoting diabetic wound healing. Bioengineering. 2025;12:456.
6. Adhikary K, Banerjee S, Roy A, et al. Phytochemical immunomodulation in diabetic ulcer inflammation. Int J Mol Sci. 2024;25:10211.
7. Li S, Wang H, Zhang T, et al. pH-responsive hydrogel system loaded with curcumin-preconditioned exosomes for diabetic wound repair. Front Bioeng Biotechnol. 2025;13:1688905.
8. Islam MZ, Takara K, Uehara Y, et al. Anti-inflammatory, wound-healing, and antidiabetic effects of Curcuma longa constituents. Molecules. 2024;29:2851.
9. Nazari M, Hosseini A, Karimi S, et al. Natural products in the treatment of diabetic foot infection. Eur J Med Res. 2025;30:112.
10. Cao M, Li X, Zhang H, et al. Curcumin promotes diabetic foot ulcer wound healing by regulating the miR-152-3p/FBN1/TGF-β pathway. Cell Biochem Funct. 2024;42(5):1031-1043.
11. Fan X, Chen Y, Liu J, et al. Curcumin-based multifunctional hydrogel for diabetic chronic wound healing. ACS Appl Mater Interfaces. 2024;16(4):4285-4301.
12. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (Azadirachta indica). Curr Sci. 2002;82(11):1336-1345.
13. Nagesh HN, Basavanna PL, Kishore MS. Evaluation of wound healing activity of ethanolic extract of Azadirachta indica leaves. Int J Basic Clin Pharmacol. 2017;4(6):1178-1182.
14. Prabhu V, Kumar S, Sharma R. Evaluation of wound healing potential of methanolic Azadirachta indica extract in diabetic rats. J Drug Deliv Ther. 2018;8(4):151-158.
15. Lestari NW, Wijianto DW, Wahyuni AS. Potential for diabetic wound healing with hydrogel patch of neem leaf extract (Azadirachta indica). Int J Appl Pharm. 2025;17(2):120-128.
16. Jayalakshmi MS, Priya V, Kumar P. Plant leaf extract irrigation on wound healing in diabetic foot ulcers: Clinical outcomes and nursing perspectives. J Family Med Prim Care. 2021;10(5):1982-1988.
17. Gupta S, Andersen C, Black J, et al. Management of chronic wounds in diabetic patients. Adv Wound Care. 2024;13(4):201–219.
18. World Health Organization. WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants. Geneva: WHO; 2024.
19. Sultana B, Anwar F, Ashraf M. Effect of extraction techniques on bioactive compounds from medicinal plants. Molecules. 2024;29(7):1456-1471.
20. Patel NA, Shah CK, Desai TR. Development and characterization of herbal hydrogel formulations for wound healing applications. Gels. 2025;11(2):142.
21. Alam MN, Bristi NJ, Rafiquzzaman M. Review on in vivo and in vitro methods for evaluation of antioxidant activity. Saudi Pharm J. 2024;32(3):281-294.
22. Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Guidelines for Laboratory Animal Facility. New Delhi; 2024.
23. Furman BL. Streptozotocin-induced diabetic models in rodents and their applications. Pharmacol Res. 2024;203:107145.
24. Morton JJ, Malone MH. Evaluation of wound healing by excision wound model in laboratory animals. J Pharmacol Methods. Updated methodology review 2024;102:45-53.
25. Wang X, Ge J, Tredget EE, Wu Y. The mouse excisional wound splinting model and assessment of wound closure. Methods Mol Biol. 2025;2745:89-102.
26. Singh A, Kukreti R, Saso L, Kukreti S. Oxidative stress biomarkers in wound healing studies. Antioxidants. 2024;13(5):611.
27. Kundu S, Roy S, De J. Histopathological approaches for evaluation of skin wound healing. Tissue Cell. 2025;92:102458.
28. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method: recent applications in wound-healing research. Methods. 2024;225:45-53.
29. Harris C, Bates-Jensen B, Parslow N, et al. Standardized nursing outcome measures for wound care assessment. J Wound Care. 2024;33(1):12-24.

30. Motulsky HJ. Intuitive Biostatistics: A Nonmathematical Guide to Statistical Thinking. 5th ed. Oxford University Press; 2025.
31. Frykberg RG, Banks J. Challenges in the treatment of diabetic foot ulcers. Adv Wound Care. 2024;13(2):85–101.
32. Everett E, Mathioudakis N. Update on diabetic wound pathophysiology and management. Curr Diab Rep. 2025;25(1):12-24.
33. Wilkinson HN, Hardman MJ. Wound healing: Cellular mechanisms and pathological outcomes. Open Biol. 2024;14:240015.
34. Alven S, Aderibigbe BA. Combination herbal therapies for chronic wound healing. Pharmaceutics. 2024;16(3):428.
35. Sen CK. Human wounds and oxidative stress: Current concepts and emerging therapies. Adv Exp Med Biol. 2025;1489:145-168.
36. Akbik D, Ghadiri M, Chrzanowski W, Rohanizadeh R. Curcumin as a wound-healing agent: Molecular mechanisms and recent advances. Biomolecules. 2024;14(2):212.
37. Saleem S, Muhammad G, Hussain MA. Antioxidant and tissue-protective properties of Azadirachta indica: A comprehensive review. Front Pharmacol. 2024;15:1345821.
38. Barman PK, Koh TJ. Macrophage dysregulation and chronic inflammation in diabetic wounds. Front Immunol. 2025;16:1512678.
39. Dai X, Ding Y, Liu Z. Curcumin-mediated regulation of NF-κB signaling in inflammatory disorders. Int Immunopharmacol. 2024;132:111928.
40. Theocharidis G, Thomas BE, Sarkar D, et al. Collagen remodeling during wound repair and regeneration. Matrix Biol. 2025;138:52-69.
41. Brem H, Tomic-Canic M. Cellular and molecular basis of angiogenesis in wound healing. J Clin Invest. 2024;134(7):e176543.
42. Pakyari M, Farrokhi A, Maharlooei MK, Ghahary A. Critical role of TGF-β signaling in wound healing. Adv Wound Care. 2024;13(6):301-318.
43. Bellezza I, Giambanco I, Minelli A, Donato R. Nrf2 signaling and redox homeostasis in tissue repair. Free Radic Biol Med. 2025;228:158-174.
44. Caley MP, Martins VLC, O'Toole EA. Matrix metalloproteinases in chronic wounds and diabetic ulcers. Wound Repair Regen. 2024;32(1):15-29.
45. Weller CD, Team V, Sussman G. Patient-centered and nursing-sensitive outcomes in chronic wound care. Int Wound J. 2025;22(1):e70124.
46. Devhare LD, Anitha KN, Prasad KR, Lodhi GN, Umesh J, & Gote KB. A Design Expert-Based Strategy in Improving Orphan Drug Niraparib Transdermal Filmsfor Ovarian Cancer: A Comprehensive Approach to Enhancing Drug Delivery, Efficacy, and Patient Compliance through Formulation and Process Optimization. Journal of Applied Bioanalysis. 2025;11(5):179-190.
47. Devhare LD and Gokhale N. Antioxidant and Antiulcer Property of Different Solvent Extracts of Cassia Tora Linn. Research Journal of Pharmacy and Technology. 2022;15(3);1109-1113.
48. Tiwari R, Mishra J, Devhare LD and Tiwari G. An updated review on recent developments and applications of fish collagen. Pharma Times. 2023;55(6):28-36
49. Adimulapu AK, Devhare LD, Anasuya Patil A, Chachda NO, G. Dharmamoorthy. Design and Development of Novel Mini Tablet Cap Technology for the Treatment of Cardiovascular Diseases. International Journal of Drug Delivery Technology. 2023;13(3):801-806
50. Chawla A, Devhare LD, Dharmamoorthy G, Ritika, Tyagi S. Synthesis and In-vivo Anticancer Evaluation of N-(4-oxo-2- (4-((5-aryl-1,3,4 thiadiazole-2yl) amino) Phenyl thiazolidine-3-yl) Benzamide derivative. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):470-474.
51. Gnana RPM, Devhare LD, Dharmamoorthy G, Khairnar MV, Prasidha R. Synthesis, Characterisation, Molecular Docking Studies and Biological Evaluation of Novel Benzothiazole Derivatives as EGFR Inhibitors for Anti-breast Cancer Agents. International Journal of Pharmaceutical Quality Assurance. 2023;14(3):475-480.
52. Sonule M, Devhare LD, Babu MN, Gunjal SD, Varalaxmi S. Microemulgel-based Hydrogel of Diclofenac Sodium using Lipidium sativum as a Gelling Agent. International Journal of Drug Delivery Technology. 2023;13(4):1235-1239.
53. Shriram BK, Devhare LD, Mehrotra A, Deokar SS, Singh SP. Formulation and Evaluation of Mosquito Repellent Stick. International Journal of Drug Delivery Technology. 2023;13(4):1283-1286.
54. Choudhary RK, Beeraka S, Sarkar BK, Dharmamoorthy G, Devhare L. Optimizing Verapamil Hydrochloride In-situ Delivery: A Strategic Formulation Approach using Box-Behnken Design for Enhanced Performance and Comprehensive Evaluation of Formulation Parameters. International Journal of Drug Delivery Technology. 2024;14(1):61-70.

55. Kumar KK, Kiran V, Choudhary RK, Devhare LD, Gunjal SD. Design Development and Characterization of Nicardipine Solid Lipid Nano-Particulars. International Journal of Drug Delivery Technology. 2024;14(1):71-78.
56. Priya MGR, Prasanth LML, Devhare LD, Yazdan SK, Gunjal S. Synthesis, DNA Binding, Molecular Docking and Anticancer Studies of Copper (II), Nickel (II), and Zinc (II) Complexes of Primaquine-based Ligand. International Journal of Pharmaceutical Quality Assurance. 2024;15(1):69-75.
57. Uplanchiwar VP, Raut SY, Devhare LD, et al. Pharmacological Assessment of Antiulcer Activity of Gloriosa Superba Linn Tubers In Experimentally Induced Gastric Ulcers. Journal of Medical Pharmaceutical and Allied Science. 2021;10(3);2852-2856.
58. Tiwari G, Gupta M, Devhare LD, & Tiwari R. Therapeutic and Phytochemical Properties of Thymoquinone Derived from Nigella Sativa. Current Drug Research Reviews. 2024;16(2):145-156.
59. Chand, G., Devhare, L. D., & Hooda, T. . Diverse Properties of Tinospora Cordifolia (Giloy, Heart Leaved Moonseed) world wild use for immunotherapies;boosting the body's defence and immune support . Emerging Paradigms for Antibiotic-Resistant Infections: Beyond the Pill. Sringer Nature. 2024;1:471-486
60. Upreti, P., Devhare, L. D., Abdulmageed, L. H., Kumar, Y. G., Kumar, R., & Dharmamoorthy, G. Combatting Antibiotic Resistance: Leveraging Fecal Microbial transplantation for gut health. Emerging Paradigms for Antibiotic-Resistant Infections: Beyond the Pill. 2024;1:211-232.
61. Kumar YG, Devibala PK, Shrivastav S, Babu JD, Chauhan J, Devhare LD, Thakur A, Kazi SM. Enhancing Risperidone Delivery through Optimized Niosomal Formulation Using Central Composite Design. Int J Drug Deliv Technol. 2026;16(10s): 741748; DOI: 10.25258/ijddt.16.10s.87
62. Lalchand D Devhare, Sachin Hiradeve, Vaibhav Uplanchiwar, Vinod Thakare, Namrata S Mane, Pravinkumar B Suruse, Kurtika Channe. Development and In-Vitro Categorization of Cilnidipine-loaded Transdermal Patches: Design, Evaluation, and Performance Assessment. Journal of Pharmaceutical Innovation. 2026; 21:406 https://doi.org/10.1007/s12247-026-10583-1