Research Article

Wound Healing and Tissue Regeneration Effects of *Vitex pinnata* Leaf Extract Gel in Streptozotocin-Induced Diabetic Rats: An *In Vivo* and *In Silico* Study

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Abstract | Diabetes mellitus (DM) is often associated with impaired wound healing, leading to chronic ulcers with limited therapeutic efficacy. This study investigated the wound healing activity of *Vitex pinnata* leaf extract gel in streptozotocin (STZ)-induced diabetic rats through phytochemical, in vivo, and in silico evaluations. LC-HRMS profiling identified phenolic compounds, including gallic acid, isoferulic acid, vanillin, and apocynin, while ADME–Lipinski screening highlighted Isoferulic acid and Apocynin as drug-like candidates. Molecular docking revealed strong binding affinities to EGFR1 (–7.2 kcal/mol), FGFR1 (–7.7 kcal/mol), and MMP-1 (–7.5 kcal/mol), suggesting possible involvement in angiogenic and tissue-regeneration pathways. In vivo, Wistar rats (n=30) were allocated into six groups: normal, diabetic placebo, positive control (Bioplacenton®), and three gel formulations (1%, 2%, 4%). Wound diameter measurements showed significant contraction in all treatment groups compared with diabetic placebo ($p < 0.001$). The 4% gel achieved near-complete closure by day 12 (1.0 ± 0.1 mm) and full closure by day 15, showing a healing pattern similar to Bioplacenton®. Serum analysis confirmed a significant reduction of TNF-α ($p < 0.001$) and marked increases in FGF-7 and VEGF ($p < 0.001$), particularly in the 4% group, approaching normal values. Histological evaluation supported these findings, showing epidermal thickening, reduced fibrosis, and dense collagen fibers in treated groups, with optimal restoration in the 4% formulation. These results demonstrate that *V. pinnata* gel accelerates diabetic wound healing via anti-inflammatory, proliferative, and angiogenic mechanisms in a dose-dependent manner. The 4% gel showed the highest efficacy, statistically comparable to standard therapy. Further toxicity and clinical studies are warranted to validate its translational potential.

Keywords | *Vitex pinnata*, Wound, *In silico, In vivo*, Streptozotocin, Gel

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from impaired insulin secretion, insulin action, or both. One of the most severe complications of diabetes is impaired wound healing, which often leads to chronic ulcers and infections, significantly increasing morbidity and mortality among patients (Rodríguez-Rodríguez *et al.*, 2022). Diabetic ulcers are associated with prolonged inflammation, excessive oxidative stress, impaired angiogenesis, and microbial invasion, all of which contribute to delayed tissue

regeneration and poor clinical outcomes (Patel *et al*., 2024). Current treatment options, including topical antimicrobials and growth factor therapies, are often limited by high cost, side effects, and variable efficacy, thereby driving the need for alternative therapeutic strategies.

Medicinal plants have long been explored as sources of bioactive compounds for wound management due to their diverse pharmacological actions, affordability, and availability. Among these, *V. pinnata*, a plant widely distributed in tropical regions, has been traditionally used in ethnomedicine for treating infections, inflammation, and skin-related ailments (Kamal *et al*., 2022; Uthirapathy, 2021). Phytochemical studies have revealed that *V. pinnata* contains phenols, flavonoids, alkaloids, saponins, terpenoids, and sterols, all of which have been associated with antioxidant, anti-inflammatory, antimicrobial, and tissue regenerative properties (Sonia *et al*., 2022; Thenmozhi and Subasini, 2016). These multi-target activities suggest its potential as a natural agent for accelerating wound closure and promoting tissue repair in diabetic conditions.

Recent advances in analytical techniques such as liquid chromatography–high resolution mass spectrometry (LC-HRMS) have facilitated comprehensive profiling of plant metabolites, enabling the identification of hundreds of compounds that may contribute synergistically to therapeutic effects. In addition, in silico approaches provide valuable insights into molecular mechanisms by predicting interactions between bioactive compounds and biological targets relevant to wound healing, such as growth factors, inflammatory mediators, and extracellular matrix remodeling enzymes. Combining in vivo and *in silico* approaches thus provides a robust framework for evaluating both the pharmacological efficacy and mechanistic pathways of potential phytotherapeutics.

Despite numerous studies exploring herbal therapies for wound healing, no previous research has developed or evaluated a topical gel formulation of *V. pinnata* specifically for diabetic wounds. This study fills that gap by combining LC-HRMS-based metabolite profiling, molecular docking of wound-healing-related proteins, and in vivo evaluation in a streptozotocin-induced diabetic rat model. The integrated design provides a comprehensive understanding of both the chemical composition and biological efficacy of *V. pinnata*. While this study does not directly quantify molecular synergy, the observed outcomes across antioxidant, anti-inflammatory, and angiogenic pathways collectively support the plant's multi-target therapeutic potential for diabetic wound management.

Given the increasing prevalence of diabetic ulcers and the limitations of conventional therapies, this study was designed to investigate the wound healing and tissue regeneration effects of *V. pinnata* leaf extract gel in streptozotocin-induced diabetic rats. Phytochemical screening and LC-HRMS metabolite profiling were performed to identify the active constituents, while in vivo experiments assessed wound contraction, histological changes, and tissue regeneration. Furthermore, in silico analysis was conducted to predict the molecular interactions underlying the observed biological effects. The outcomes of this study are expected to provide scientific evidence supporting the development of *V. pinnata* as a phytopharmaceutical candidate for diabetic wound management.

MATERIALS AND METHODS

Materials

The materials and tools used in this study included *V. pinnata* leaves (300 g) extracted with 70% ethanol (3000 mL; Merck). The formulation of gels consisted of Carbopol-980 (2%), propanol (5%), glycerin (5%), triethanolamine (q.s.), methyl paraben and propyl paraben (q.s.), and distilled water up to 10 g for each preparation containing 1%, 2%, and 4% extract, respectively. Sterilized instruments such as tweezers, knives, and scissors were used in the wound healing experiments. Histopathological analysis was performed using hematoxylin and eosin (H&E) staining solutions. For cytokine serum analysis, an ELISA microplate reader was employed with specific antibodies for VEGF, TNF-α, and FGF-7 (Solarbio). Additional materials included 0.1 M citrate buffer (pH 4.5), ketamine (50 mg/kg BW), and 0.9% w/v NaCl solution.

Sample collection and phytochemical screening of *Vitex pinnata leaves* extract

Fresh leaves of *V. pinnata* were obtained from a garden in Bagan Serdang Village, Deli Serdang District, at an altitude of 100–150 meters above sea level. The gathered material was air-dried, pulverized, and extracted using the maceration procedure in accordance with previously documented protocols (Nugraha *et al*., 2024). In summary, 300 g of powdered leaves were submerged in 3,000 mL of 70% ethanol at room temperature (~27 °C) for 24 hours, preserving a solvent-to-sample ratio of 10:1. The extract was further filtered and concentrated at lower pressure utilizing a rotary evaporator. Qualitative phytochemical analyses were conducted to identify the presence of essential secondary metabolite categories, including alkaloids, flavonoids, glycosides, tannins, saponins, and terpenoids/steroids (Bhardwaj et al., 2024). Additionally, Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS) was utilized for the comprehensive identification and characterisation of bioactive substances following published techniques (Oosthuizen *et al*., 2018).

Pharmacokinetic screening of bioactive compounds in *Vitex pinnata* leaf extract

The pharmacokinetic properties of the compounds present in *V. pinnata* leaf extract were evaluated in silico using ADME (Absorption, Distribution, Metabolism, and Excretion) analysis via the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP; https://old.tcmsp-e.com/tcmsp.php, accessed 25 September 2025). Compounds demonstrating oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 were selected as potential bioactive candidates (Li *et al*., 2022).

Molecular docking

Molecular docking was employed to investigate the binding interactions between selected bioactive compounds of *V. pinnata* and key protein targets associated with wound healing and tissue regeneration, including Epidermal Growth Factor Receptor 1 (EGFR1), Fibroblast Growth Factor Receptor 1 (FGFR1), and Matrix Metalloproteinase 1 (MMP-1). These proteins were selected due to their well-established roles in keratinocyte proliferation, fibroblast migration, angiogenesis, and extracellular matrix remodeling critical processes underlying wound closure and tissue regeneration. Protein crystal structures were retrieved from the RCSB Protein Data Bank Protein Data Bank (https://www.rcsb.org/, accessed 25 September 2025), prepared using Biovia Discovery Studio 2021, and validated by re-docking their native co-crystallized ligands; RMSD values < 2.0 Å confirmed docking reliability. Docking simulations were then performed using AutoDockTools 4.2 (http://autodock.scripps.edu/, accessed September 2025), and binding energies and interaction modes were analyzed with Discovery Studio Visualizer 2021 (Botelho *et al*., 2020).

Gel formulation preparation

The gel formulations of *V. pinnata* leaf extract were prepared in three variations (F1, F2, and F3) with different extract concentrations, as shown in Table 1. The preparation began by dispersing Carbopol-980 (2% w/w) in a portion of distilled water under continuous stirring until completely swollen. Glycerin (5% w/w) and propanol (5% w/w) were then added as humectants and co-solvents, followed by the addition of methyl paraben and propyl paraben (q.s.) as preservatives. The *V. pinnata* extract was incorporated at concentrations of 1%, 2%, and 4% for F1, F2, and F3, respectively, and mixed until homogenous. Neutralization was achieved by dropwise addition of triethanolamine (q.s.) to adjust the pH and form the gel matrix. Finally, the volume was adjusted with distilled water to a total weight of 10 g for each formulation, and the gels were homogenized to obtain a uniform consistency.

Table 1: Composition of gel formulations (% w/w).

Component	F1	F2	F3
V. pinnata leaf extract (active)	1%	2%	4%
Carbopol-980	2%	2%	2%
Propanol	5%	5%	5%
Glycerin	5%	5%	5%
Triethanolamine	q.s.	q.s.	q.s.
Methyl paraben & propyl paraben	q.s.	q.s.	q.s.
Distilled water (to final weight)	10 g	10 g	10 g

Animal preparation

The experimental animals employed in this study were Wistar rats weighing 150–180 g, obtained from the Faculty of Pharmacy, Universitas Sumatera Utara, Indonesia. All animal handling followed the ethics guidelines approved by the Animal Research Ethics Committee, Institut Kesehatan Deli Husada Deli Tua (approval number: 0311/KE/PPM/2025).

Wound healing experiment procedure

The minimum sample size per group was calculated using the Federer formula, yielding 30 rats that were randomly allocated into six groups via a computer-generated randomization technique to mitigate selection bias. The experimental design included: (1) normal control rats, (2) STZ-induced negative control rats without intervention, (3) positive control rats administered Bioplacenton® cream (0.1 g/day), a clinically validated topical formulation containing neomycin sulfate and placenta extract with regenerative and antimicrobial properties, (4) STZ-induced rats treated with Formula 1 gel, (5) Formula 2 gel, and (6) Formula 3 gel. Diabetes was established via intraperitoneal injection of streptozotocin (STZ, 50 mg/kg body weight) dissolved in 0.1 M citrate buffer (pH 4.5) (Sahlan *et al*., 2020). Blood glucose levels were observed throughout a 21-day period, with readings over 180 mg/dL classified as diabetic (Igbashio et al., 2024). Following the diagnosis of diabetes, rats were sedated with ketamine (50 mg/kg body weight), the dorsal area was shaved, and a standardized full-thickness excisional wound (1 cm in diameter) was made using a sterile biopsy punch. Wounds were created in the dorsal thoracic region to reduce movement variability, involving the excision of the epidermis and partial dermis while preserving the underlying muscle (Greenwood et al., 2022). Treatments (0.1 g/day) were administered topically following wound decontamination with a 0.9% NaCl solution. The development of wound healing was evaluated every day for 21 days by measuring wound diameter using a digital caliper with a precision of ±0.01 mm. Upon completion of the experiment, histological assessment was performed via hematoxylin–eosin (H & E) staining, and blood concentrations of VEGF, TNF-α, and FGF-7 were quantified via ELISA with a microplate reader (Gondaliya

et al., 2022).

Statistical analysis

All data were initially assessed for normality utilizing the Shapiro-Wilk test. One-way ANOVA was employed for group comparisons, succeeded by Tukey's HSD post-hoc test ($p < 0.05$). All statistical analyses were performed using GraphPad Prism 9. The histogram data were generated utilizing GraphPad Prism Software 9.0 (Kim, 2017).

RESULT AND DISCUSSION

Results of phytochemical screening

The preliminary phytochemical screening of *V. pinnata* leaf extract confirmed the presence of major classes of secondary metabolites (Table 2). The extract tested positive for tannins, flavonoids, alkaloids, saponins, and terpenoids/steroids. These bioactive groups are widely recognized for their pharmacological activities relevant to wound healing, such as antioxidant, anti-inflammatory, antimicrobial, and tissue-regenerative properties (Joseph *et al.*, 2018).

Table 2: Results of phytochemical screening of *V. pinnata* extract.

No	Secondary metabolite	Reagent	Result
1	Tannins	$FeCl_3$ 1–5%	+
2	Flavonoids	Mg + HCl / NaOH + HCl	+
3	Alkaloids	Mayer / Dragendorff / Boucarhdat	+ /+/+
4	Saponins	Distilled water (foam test)	+
5	Terpenoids/ Steroids	Liebermann–Burchard/Steroid test	+ / +

Metabolite profiling by LC-HRMS revealed a diverse range of phenolic compounds in *V. pinnata* extract (Figure 1; Table 3). A total of 38 phenolic derivatives were identified from 604 total compound, comprising simple phenols, phenolic acids, aldehydes, coumarins, and polyphenolic structures. Prominent compounds included gallic acid, chlorogenic acid, isoferulic acid, vanillin, tyrosol, salicylic acid, coniferylaldehyde, and apocynin. These metabolites belong to multiple sub-classes such as benzoic acid derivatives, cinnamic acid derivatives, phenylpropanoids, and flavonoid-related phenolics.

The results demonstrate that *V. pinnata* contains a broad spectrum of phenolic metabolites with established roles in wound healing. Compounds such as gallic acid, chlorogenic acid, and isoferulic acid are well-documented antioxidants that can neutralize free radicals, thereby reducing oxidative stress in diabetic wounds. Vanillin and tyrosol contribute antimicrobial and anti-inflammatory activities, which help prevent infection and modulate prolonged inflammatory phases common in chronic wounds (Kafali *et al.*, 2024; Yadav *et al.*, 2020). Similarly, cinnamic acid derivatives support fibroblast proliferation, angiogenesis, and extracellular matrix remodeling (De Aquino *et al.*, 2021). The presence of multiple classes of phenolics suggests a synergistic mechanism in accelerating wound repair. These compounds may act on different stages of the healing process, from reducing oxidative damage and inflammation to enhancing granulation tissue formation and collagen deposition. Collectively, the phytochemical diversity of *V. pinnata* underpins its potential as a multi-target herbal candidate for diabetic wound management, aligning with earlier ethnopharmacological evidence of its traditional use in skin-related ailments.

Antibacterial potential of *Vitex pinnata* leaf extract against *Staphylococcus aureus*

The antibacterial assay demonstrated that the inhibition zone of *V. pinnata* leaf extract against *Staphylococcus aureus* increased in a concentration-dependent manner, with the largest effect observed at 20 mg/mL (11.27 ± 0.32 mm) and the smallest at 0.625 mg/mL (5.73 ± 0.06 mm) (Table 4).

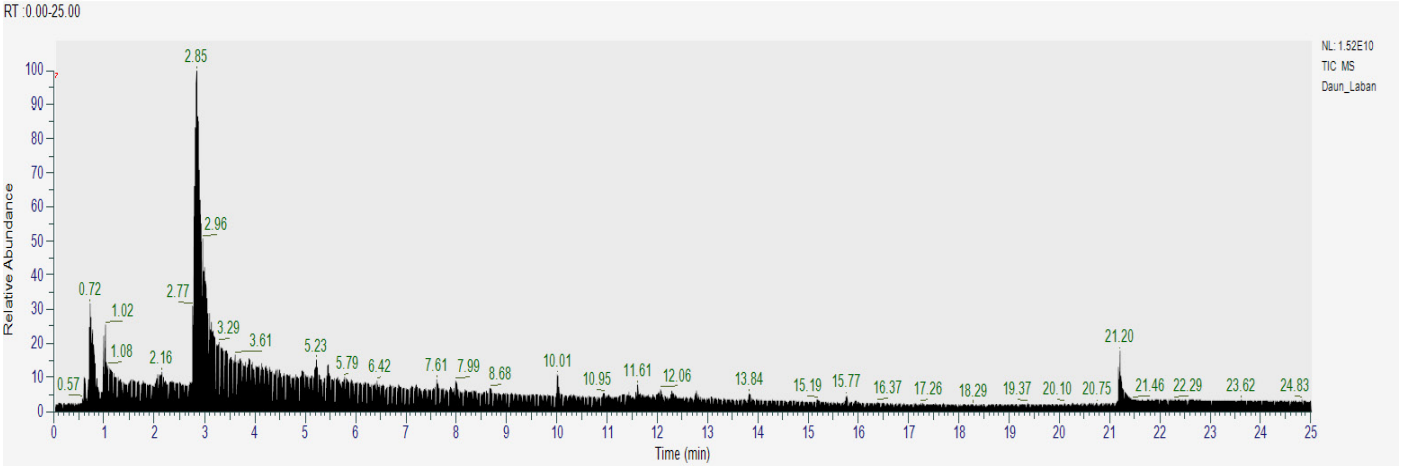

Figure 1: Total ion chromatogram (TIC) of *V. pinnata* leaf extract.

Table 3: Phenolic compounds identified in *V. pinnata* extract.

No	Compound	Subclass/ Description
1	Isoferulic acid	phenol
2	Apocynin	1,2-dihydroxybenzene
3	Phloroglucinol	1,3,5-trihydroxybenzene
4	Tyrosol (2-(4-hydroxyphenyl) ethanol)	phenylethylphenol
5	Vanillin (4-hydroxy-3-methoxybenzaldehyde)	aromatic phenolic aldehid
6	4-Hydroxybenzoic acid	phenolic acid
7	2,4-Dihydroxybenzoic acid	phenolic acid
8	3-Hydroxybenzoic acid	phenolic acid
9	Salicylic acid (2-hydroxybenzoic acid)	phenolic acid
10	6-Formylsalicylic acid	formylated salicylic acid
11	Gallic acid	trihydroxybenzoic acid
12	3,4-Dihydroxyphenylacetic acid	phenolic acid (dopamine derivative)
13	3,4-Dihydroxyphenylpropionic acid	dihydroxy phenylpropionic acid
14	3-(3,4-Dihydroxyphenyl)pyruvate	phenolic keto acid
15	3,4-Dihydroxymandelaldehyde	phenolic aldehyde
16	3,4-Dihydroxyphenylethyleneglycol	phenolic glycol
17	4-Hydroxyphenylacetic acid	phenolic acid
18	4-Hydroxybenzaldehyde	phenolic aldehyde
19	4-Hydroxybenzyl alcohol	phenolic benzyl alcohol
20	2-Anisic acid (p-anisic / 4-methoxybenzoic acid)	anisic acid (methoxybenzoate)
21	4-Anisic acid	anisic acid (isomer)
22	3-Methoxy-4-hydroxyphenylethyleneglycol	vanillyl-glycol
23	3-Methoxytyramine	O-methoxy phenylethylamine (vanillamine)
24	5-Hydroxyindole-3-acetic acid	indole phenolic (serotonin metabolite)
25	8-Hydroxyquinoline	heteroaryl phenol (quinolinol)
26	8-Hydroxyquinoline-5-sulfonic acid	quinolinol deriv

Table 4: Antibacterial activity of *V. pinnata* leaf extract against *Staphylococcus aureus.*

Concentration %	Mean ± SD (mm)	Significance vs. placebo (–)
0.625	5.73 ± 0.06	ns
1.25	7.13 ± 0.15	*
2.5	8.57 ± 0.15	**
5	9.43 ± 0.15	**
10	10.47 ± 0.15	***
20	11.27 ± 0.32	***
Positive control (+)	26.10 ± 1.57	***
Placebo control (–)	6.00 ± 0.00	–

Note: ns = not significant ($p > 0.05$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (ANOVA followed by Tukey's test).

Although the extract showed lower activity compared to the positive control (26.10 ± 1.57 mm), it was significantly higher than the negative control (6.17 ± 0.15 mm). The progressive increase in inhibition zone diameter is clearly illustrated in Figure 2, which highlights the concentration response relationship of the extract.

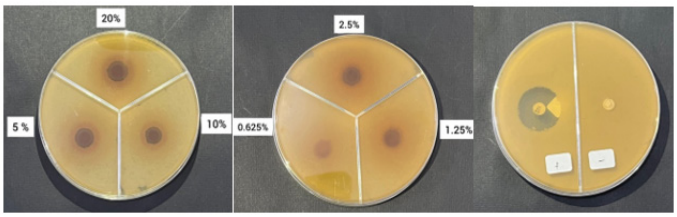

Figure 2: Antibacterial activity of *V. pinnata* leaf extract against *Staphylococcus aureus.*

The antibacterial assay was conducted to determine the minimum inhibitory concentration (MIC) of *V. pinnata* leaf extract against *Staphylococcus aureus* as a preliminary step in formulation design. The extract exhibited concentration-dependent inhibition, with the MIC determined at 1.25% (w/v), which served as the basis for selecting gel concentrations of 1%, 2%, and 4% for *in vivo* testing. As shown in Table 4, the antibacterial activity of *V. pinnata* extract was considerably lower than that of the positive control (Bioplacenton®), indicating

that its direct antimicrobial potency is limited. Therefore, the observed wound-healing activity is unlikely due to antibacterial effects but rather to the extract's antioxidant, anti-inflammatory, and angiogenic mechanisms. This limitation highlights the need for further optimization of concentration or potential combination with stronger antimicrobial agents in future formulations.

Additionally, the antibacterial activity of *V. pinnata* leaf extract against *S. aureus* can be mechanistically linked to its phytoconstituents, particularly flavonoids, tannins, alkaloids, saponins, and terpenoids. These metabolites are known to disrupt bacterial cell wall integrity, alter membrane permeability, and inhibit key metabolic enzymes, thereby exerting a synergistic antimicrobial effect (Lobiuc *et al.*, 2025). The concentration-dependent response observed in this study reinforces the pharmacological relevance of these compounds, supporting the potential role of *V. pinnata* as a complementary antibacterial agent in wound healing applications.

Analysis of selected ligands based on ADME parameters and Lipinski's rule of five

Pharmacokinetic evaluation using ADME parameters and Lipinski's Rule of Five confirmed that Isoferulic acid, and Apocynin are drug-like molecules with favorable physicochemical properties. All compounds demonstrated acceptable oral bioavailability (OB ≥ 30%), drug-likeness (DL ≥ 0.18), and TPSA < 140 Å², supporting their potential to cross biological membranes and exert systemic effects.

Table 5: Selected ligands and ADME–Lipinski profile.

No	Compound	OB (%)	DL	TPSA (Å²)	Lipinski RO5
1	Isoferulic acid	45	0.21	67	Pass
2	Apocynin	45	0.24	46	Pass

Binding affinity analysis

Molecular docking simulations revealed favorable interactions of all three ligands with wound-healing-related proteins EGFR1, FGFR1, and MMP-1. Result showed in Table 6 and Figure 3.

Table 6: Binding energies (kcal/mol) of selected ligands docked to wound-healing-related proteins.

No	Compound	EGFR1	FGFR1	MMP-1
1	Isoferulic acid	–4.7	–5.2	–6.4
2	Apocynin	–5.1	–6.1	–6.5

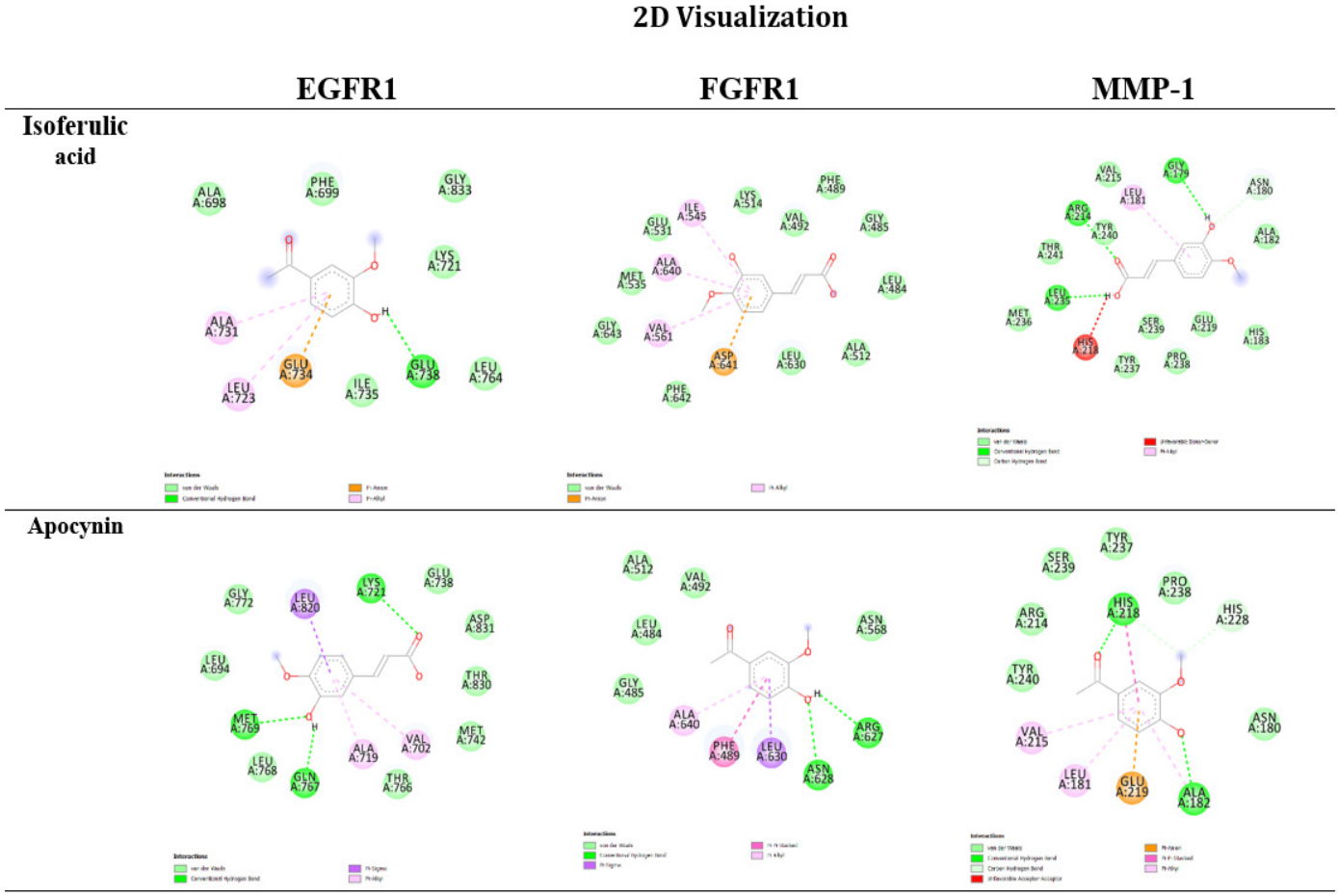

Figure 3: Two-dimensional visualization of binding interactions of the selected ligandsIsoferulic acid, and Apocynin with wound-healing-related protein targets EGFR1, FGFR1, and MMP-1.

Table 7: Evaluation of gel formulations.

Parameter	F1	F2	F3
Color	Light brown	Medium brown	Dark brown, homogeneous
Odor	Herbal characteristic	Herbal characteristic	Herbal characteristic
Homogeneity	Homogeneous, with particles	Moderately homogeneous, slight sediment	Homogeneous, no sediment
pH (± SD)	5.1 ± 0.1	5.4 ± 0.1	5.6 ± 0.1
Viscosity (cPs)	8,500 ± 120	9,200 ± 110	10,400 ± 135
Spreadability (cm)	6.2 ± 0.1	5.9 ± 0.2	5.5 ± 0.2
Centrifugation	Phase separation	Slight separation	No separation

The molecular docking results presented in Table 6 were reinterpreted following correction of the target selection rationale. The selected targets EGFR1, FGFR1, and MMP-1 are known to play critical roles in cell proliferation, angiogenesis, and extracellular matrix remodeling during wound repair (Zhao *et al*., 2020; Mir *et al*., 2023). The binding affinities of isoferulic acid and apocynin indicate potential modulatory interactions with these proteins, suggesting supportive roles in tissue regeneration. However, as these findings are based solely on *in silico* predictions, they should be regarded as preliminary indicators rather than confirmatory evidence of biological activity, pending experimental pathway validation.

Apocynin showed moderate affinity (–5.1 to –6.5 kcal/mol), consistent with its known role as an NADPH oxidase inhibitor that reduces ROS accumulation and limits oxidative damage in diabetic wounds (Cano Sanchez *et al*., 2018). Isoferulic acid, although with relatively weaker binding energies (–4.7 to –6.4 kcal/mol), has been reported to exhibit anti-inflammatory properties by suppressing NF-κB signaling and promoting collagen stabilization (Neopane *et al*., 2023). Collectively, these findings support the hypothesis that *V. pinnata*-derived ligands act on multiple molecular targets relevant to diabetic wound healing, including regulation of oxidative stress, inflammation, and extracellular matrix remodeling.

Evaluation of gel formulations

Organoleptic, physicochemical, and stability parameters of the three gel formulations (F1–F3) were systematically evaluated (Table 7). All gels exhibited a brown coloration characteristic of *V. pinnata* extract and a natural herbal odor. Homogeneity assessment revealed that F1 contained visible particles, F2 presented slight sedimentation, while F3 was fully homogeneous without sediment. The pH values (5.1–5.6) were within the optimal topical range (4.5–6.5), confirming suitability for skin application. A placebo gel containing the same base composition (Carbopol, glycerin, and 5% propanol) but without extract served as the control for safety evaluation. All treated animals were observed daily, and no visible irritation, erythema, or edema was detected throughout the experimental period.

Viscosity values increased progressively with the concentration of the gelling agent, with F3 demonstrating the highest viscosity (10,400 ± 135 cPs), followed by F2 (9,200 ± 110 cPs) and F1 (8,500 ± 120 cPs). This increase was inversely related to spreadability, where higher viscosity corresponded to reduced spreading capacity. However, all formulations maintained spreadability within the acceptable range for topical preparations (5–7 cm). Centrifugation tests further supported formulation stability, showing complete phase separation in F1, slight separation in F2, and no separation in F3. These findings are consistent with formulation theory, which indicates that increasing the proportion of thickening agents such as Carbopol enhances viscosity, reduces spreadability, and improves gel stability (Estanqueiro *et al*., 2016). Additionally, humectants (glycerin and propylene glycol) contributed to maintaining gel hydration and penetration properties, while methyl paraben served as a preservative.

Wound healing activity

The progression of wound closure in normal, diabetic, and treatment groups was monitored over 15 days. The quantitative changes in wound diameter are summarized in Table 8, while the visual observation of wound healing progression is presented in Figure 4.

The wound diameter measurements demonstrated distinct healing patterns among groups. The normal control group achieved complete closure by day 12, while the diabetic placebo group showed only minimal reduction, with a wound diameter of 6.5 ± 0.7 mm persisting until day 15, confirming impaired healing under hyperglycemic conditions. The positive control (Bioplacenton®) displayed strong efficacy, achieving complete healing by day 15. The *V. pinnata* gel formulations exhibited dose-dependent wound healing effects. The 1% gel required up to day 15 for full closure, whereas the 2% and 4% gels accelerated wound contraction, showing marked reductions from day 6 onward and achieving complete healing by day 15. Notably, the 4% gel demonstrated comparable activity to the positive control, with wound diameter reduced to 1.0 ± 0.1 mm by day 12 prior to full closure. These findings highlight the concentration-dependent wound healing activity of *V.*

pinnata gel, which may be attributed to its rich content of flavonoids, phenols, tannins, and terpenoids. These phytochemicals are known to exert antioxidant and anti-inflammatory effects, as well as to stimulate angiogenesis and collagen deposition (Zulkefli *et al*., 2023). This aligns with the pathophysiological concept that diabetic wounds are characterized by prolonged oxidative stress and inflammation; thus, multi-target phytochemicals can accelerate the transition from the inflammatory phase to the proliferative and remodeling phases of wound healing (Pham *et al*., 2020).

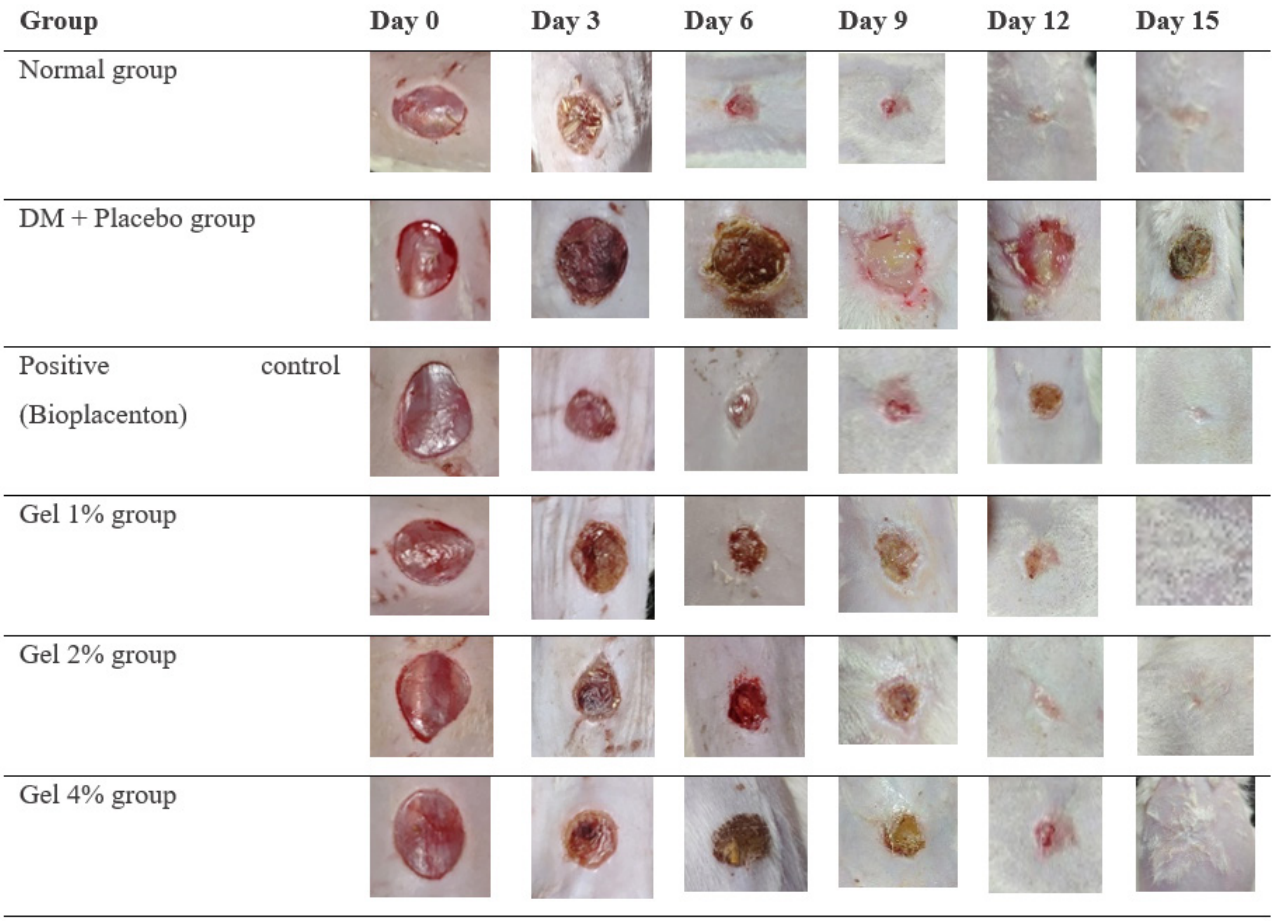

Figure 4: Observation of wound healing in STZ-induced diabetic rats.

Table 8: Wound diameter measurement during the healing process.

Day	Normal		DM + Placebo		Positive (Bioplacenton)		Gel 1%		Gel 2%		Gel 4%	
	Diameter	% Closure	Diameter	% Closure	Diameter	% Closure	Diameter	% Closure	Diameter	% Closure	Diameter	% Closure
0	10.0±0.0 (ns)	0.0	10.0±0.0	0.0	10.0 ± 0.0 (ns)	0.0	10.0 ± 0.0 (ns)	0.0	10.0 ± 0.0 (ns)	0.0	10.0 ± 0.0 (ns)	0.0
3	7.5±0.8 (***)	25.0	9.0±0.9	10.0	7.0 ± 0.7 (***)	30.0	8.0 ± 0.8 (**)	20.0	8.2 ± 0.8 (**)	18.0	8.5 ± 0.9 (*)	15.0
6	4.0±0.4 (***)	60.0	8.5±0.9	15.0	4.0 ± 0.4 (***)	60.0	6.0 ± 0.6 (***)	40.0	5.5 ± 0.6 (***)	45.0	5.8 ± 0.6 (***)	42.0
9	2.0±0.2 (***)	80.0	8.0±0.8	20.0	2.5 ± 0.3 (***)	75.0	4.0 ± 0.4 (***)	60.0	3.5 ± 0.4 (***)	65.0	3.2 ± 0.3 (***)	68.0
12	0.0±0.0 (***)	100.0	7.5±0.8	25.0	1.0 ± 0.1 (***)	90.0	2.0 ± 0.2 (***)	80.0	1.5 ± 0.2 (***)	85.0	1.0 ± 0.1 (***)	90.0
15	0.0±0.0 (***)	100.0	6.5±0.7	35.0	0.0 ± 0.0 (***)	100.0	0.0 ± 0.0 (***)	100.0	0.0 ± 0.0 (***)	100.0	0.0 ± 0.0 (***)	100.0

Note: Values are expressed as mean ± SD. Statistical significance compared with DM + placebo: ns = not significant ($p > 0.05$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (one-way ANOVA with Tukey's post-hoc test).

Biochemical serum analysis

Biochemical serum analysis (Figure 5) demonstrated a significant elevation of TNF-α levels in the DM + placebo group compared to the normal control, indicating chronic inflammation triggered by hyperglycemia. This finding aligns with the established concept that persistent hyperglycemia induces excessive ROS generation and NF-κB activation, resulting in the upregulation of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6.

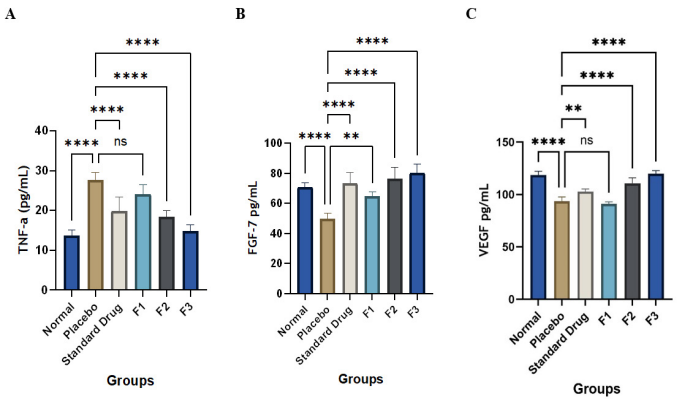

Figure 5: Serum levels of inflammatory marker (TNF-α), epithelial proliferation marker (FGF-7), and angiogenic marker (VEGF) in diabetic ulcer model rats following treatment with *V. pinnata* leaf extract gel compared with normal, placebo, and standard drug groups. Data are expressed as mean ± SD, with statistical significance indicated in the graph (**p < 0.01; ***p < 0.001; ****p < 0.0001; ns = non-significant).

Treatment with the standard drug showed significant improvement relative to placebo, whereas the *V. pinnata* gel formulations (F1, F2, F3) markedly reduced TNF-α levels, with F3 producing the most pronounced effect, approaching normal values. This reduction may be attributed to the antioxidant and anti-inflammatory properties of flavonoids, phenols, and terpenoids present in *V. pinnata*, which are known to inhibit NF-κB activation and modulate the expression of pro-inflammatory enzymes (Yahfoufi *et al*., 2018).

In contrast, FGF-7 and VEGF levels were significantly decreased in the placebo group, reflecting impaired epithelial proliferation and angiogenesis typically observed in diabetic ulcers. This is consistent with earlier reports indicating that diabetes suppresses critical growth factors such as FGF-7, essential for keratinocyte proliferation, and VEGF, which regulates angiogenesis via VEGFR2 signaling in endothelial cells (Abhinand *et al*., 2016). Notably, treatment with *V. pinnata* gel significantly restored both biomarkers, with F3 showing the highest levels, nearly comparable to normal controls. These effects may be explained by polyphenols enhancing nitric oxide (NO) bioavailability, improving endothelial function, and stimulating VEGF secretion, thereby facilitating angiogenesis and tissue vascularization (Treggiari *et al*., 2018). Collectively, these findings highlight that *V. pinnata* extract accelerates diabetic wound healing through a multi-target mechanism: suppressing inflammation (via TNF-α reduction), promoting tissue proliferation (via FGF-7 elevation), and enhancing angiogenesis (via VEGF upregulation). Such outcomes are consistent with the modern paradigm of wound healing, which emphasizes therapies that simultaneously address inflammation, proliferation, and remodeling phases.

The significant changes observed in serum VEGF, FGF-7, and TNF-α levels following topical administration of *V. pinnata* gel are most likely secondary effects of enhanced local tissue repair rather than direct systemic modulation. The extract may act through local anti-inflammatory and angiogenic mechanisms, reducing oxidative stress and promoting fibroblast and endothelial cell activity at the wound site, which in turn can lead to measurable systemic biomarker shifts. However, since local tissue concentrations of these cytokines were not assessed, the present data should be interpreted as an indirect reflection of wound-healing progression rather than evidence of systemic pharmacodynamic effects. This limitation will be addressed in future studies through local tissue ELISA or immunohistochemistry analyses.

Histological analysis

Histological evaluation of rat skin revealed distinct differences between treatment groups (Figure 6 and Table 9). In the normal control, the epidermal structure appeared intact with well-organized layers, dense collagen bundles, and the absence of inflammatory infiltration, representing healthy tissue architecture. In contrast, the DM + placebo group showed marked epidermal thinning, extensive fibrous tissue formation, and sparse, irregular collagen fibers, consistent with the impaired wound healing typically observed in diabetic ulcers (Jais, 2023). Administration of Bioplacenton® as a positive control resulted in partial improvement, characterized by increased epidermal thickness and reduced fibrous tissue, although collagen density remained relatively low. Treatment with *V. pinnata* gel at 1% demonstrated initial tissue repair with reduced fibrosis and the presence of thin collagen fibers, though the healing process was not yet optimal. The 2% gel formulation produced more pronounced effects, with thicker epidermis, reduced inflammation, and denser, more organized collagen fibers, indicating effective remodeling (Spielman *et al*., 2023). The 4% gel showed the most significant histological recovery, with epidermal structure approaching normal, minimal fibrous tissue, and dense, well-aligned collagen bundles.

Table 9: Histological findings of rat skin in different treatment groups.

Group	Description
Normal	The skin tissue showed a normal structure with intact epidermal thickness, well-organized layers, and no signs of inflammation. Collagen fibers appeared dense, aligned, and compact, with no evidence of fibrous tissue. This condition represents healthy skin without impaired regeneration.
DM + Placebo	Epidermal thinning was observed, indicating impaired regeneration. Extensive fibrous tissue formation was evident due to chronic inflammation. Collagen fibers appeared sparse, irregular, and less dense. This corresponds to the pathophysiology of diabetic wounds, which are difficult to heal.
Positive (Bioplacenton)	Epidermal thickness began to improve compared to the placebo group, although not yet comparable to normal. Fibrous tissue decreased, but collagen fibers remained sparse and loosely arranged. Bioplacenton facilitated tissue regeneration, although the effect was still limited.
Gel 1%	The epidermis showed improvement compared to placebo and the positive control, with reduced fibrous tissue. Collagen began to form, although still thin and loosely arranged. This suggests potential tissue repair at a low dose, but the outcome was not yet optimal.
Gel 2%	The epidermis exhibited increased thickness and more closely resembled normal conditions. Fibrous tissue was further reduced, indicating decreased inflammation. Collagen appeared denser and more organized, reflecting more effective tissue proliferation and remodeling.
Gel 4%	The epidermis nearly resembled normal structure with intact layers. Fibrous tissue was minimal, and inflammation was markedly reduced. Collagen fibers were dense, aligned, and comparable to normal tissue. This dose provided the most optimal effect on skin tissue restoration.

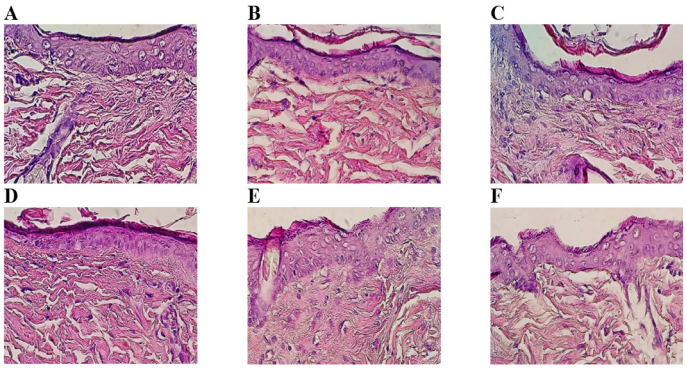

Figure 6: Histological appearance of the epidermal and dermal layers for each treatment group, stained with Hematoxylin–Eosin at 400× magnification. Panels A–F correspond respectively to the normal, diabetic control, positive control (Bioplacenton®), and *V. pinnata* gel formulations at 1%, 2%, and 4% concentrations.

These findings confirm that the wound healing activity of *V. pinnata* gel is dose-dependent, with the 4% formulation providing the most optimal restoration of skin architecture. This outcome is consistent with theoretical frameworks suggesting that successful healing of chronic wounds depends on the proper balance of inflammation, proliferation, and tissue remodeling phases (Raziyeva *et al*, 2021; Yang *et al*., 2021).

Limitation and future directions

This study establishes a correlation between the topical application of *V. pinnata* gel and improvements in wound-healing parameters and systemic biomarkers (TNF-α, VEGF, and FGF-7); however, it does not provide direct mechanistic evidence that these molecular pathways mediate the therapeutic effect. The observed cytokine modulation may represent secondary responses to accelerated tissue repair rather than primary targets of the treatment. Future studies should therefore employ pathway-specific inhibition of TNF-α or VEGF blockade, local tissue quantification through ELISA or immunohistochemistry, and gene expression profiling to verify whether *V. pinnata* acts through direct cytokine regulation or other intermediary mechanisms.

CONCLUSION

This study confirm the combination of phytochemical profiling, in silico prediction, and in vivo validation to explore the wound-healing potential of *Vitex pinnata* leaf extract in diabetic rats. LC-HRMS identified phenolic compounds such as isoferulic acid and apocynin, which showed strong interactions with wound-healing targets (EGFR1, FGFR1, MMP-1). Topical gel formulations (1%, 2%, and 4%) enhanced wound contraction, improved tissue regeneration, and modulated inflammatory and angiogenic responses. These results indicate that *V. pinnata* gel promotes wound repair through multi-target biological activities. However, further mechanistic, tissue-level, and safety studies are needed to confirm causality and support future clinical application.

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NOVELTY STATEMENT

This study is the first to formulate and evaluate a Vitex pinnata leaf extract gel for diabetic wound healing using an integrated phytochemical, in silico, and in vivo approach. LC-HRMS profiling, ADME screening, and molecular docking to EGFR1, FGFR1, and MMP-1 were combined with biological validation in streptozotocin-induced diabetic rats. The gel showed dose-dependent wound healing, angiogenic, and anti-inflammatory effects, with the 4% formulation demonstrating efficacy comparable to standard therapy.

AUTHOR'S CONTRIBUTION

Delisma Marsauli Simorangkir conducted the experiments and drafted the manuscript. Rika Puspita Sari and Hindri Syahputri assisted with extract preparation, formulation, and data analysis. Sony Eka Nugraha contributed to data interpretation and in silico analysis

Generative AI and AI-assisted technology statement

Generative AI and AI-assisted technologies were used only to improve language clarity, grammar, and readability of the manuscript. The authors take full responsibility for the scientific content, data interpretation, and conclusions presented in this work.

Conflict of interest

The authors have declared no conflict of interest.

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