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Original Article

Efficacy of *Gynura procumbens* (Lour.) Merr. extract in enhancing diabetic wound healing through modulation of IL-1β, PDGFβ, and TGF-β in a rat model

[Eficacia del extracto de *Gynura procumbens* para mejorar la cicatrización de heridas diabéticas mediante la modulación de IL-1β, PDGFβ y TGF-β en un modelo de rata]

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Abstract

Context: Diabetic wound is characterized by prolonged inflammation and impaired tissue regeneration.

Aims: To investigate the efficacy of *Gynura procumbens* (Lour.) Merr. in modulating inflammatory and regenerative cytokines, including IL-1β, PDGFβ, and TGF-β in diabetic wound healing.

Methods: Gas chromatography-mass spectrometry (GC-MS) profiling identified key phytoconstituents of the extract. This was followed by antioxidant assays of 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), diphenyl picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP). A total of 36 male Wistar rats were induced with alloxan (160 mg/kg BW) to simulate diabetic wound and assigned to six groups, receiving topical treatments of moist exposed burn ointment (MEBO) or *G. procumbens* at a dose of 100, 200, or 300 mg/kg BW for 14 days. Histological analysis and immunohistochemistry were conducted to assess epithelial thickness, fibroblast count, and cytokine expression.

Results: The extract had a potent antioxidant capacity of IC_{50} < 100 µg/mL in both ABTS and DPPH assays, while GC-MS showed n-hexadecanoic acid (26.36%) as the dominant compound. Histology showed increased epithelial thickness and fibroblast density, specifically at a 300 mg/kg BW dose of the extract. At 200 mg/kg BW, epithelial thickness increased by 96%, IL-1β expression decreased by 45% ($p<0.01$), while PDGFβ and TGF-β expression levels respectively increased by 60% and 70%. Immunohistochemistry found significant downregulation of IL-1β as well as upregulation of PDGFβ and TGF-β expression ($p < 0.001$) in treated groups, comparable to MEBO.

Conclusions: *G. procumbens* extract enhanced diabetic wound healing by modulating oxidative stress and cytokine balance. The extract efficacy, supported by molecular and histological evidence, showed potential as a natural therapeutic agent for chronic wounds.

Keywords: *Gynura procumbens*; diabetic wounds; IL-1β; PDGF β; TGF-β.

Resumen

Contexto: La herida diabética se caracteriza por una inflamación prolongada y una regeneración tisular deficiente.

Objetivos: Investigar la eficacia de *Gynura procumbens* (Lour.) Merr. en la modulación de las citocinas inflamatorias y regenerativas, incluyendo IL-1β, PDGFβ y TGF-β, en la cicatrización de heridas diabéticas.

Métodos: El perfil de cromatografía de gases-espectrometría de masas (GC-MS) identificó los fitoconstituyentes clave del extracto. A continuación, se realizaron ensayos antioxidantes de 2,2-azino-bis(ácido 3-etilbenzotiazolin-6-sulfónico) (ABTS), difenil picrilhidrazilo (DPPH) y poder antioxidante reductor férrico (FRAP). Un total de 36 ratas Wistar macho fueron inducidas con aloxano (160 mg/kg de peso corporal) para simular una herida diabética y se asignaron a seis grupos. Recibieron tratamientos tópicos de ungüento húmedo para quemaduras expuestas (MEBO) o G. procumbens en dosis de 100, 200 o 300 mg/kg de peso corporal durante 14 días. Se realizaron análisis histológicos e inmunohistoquímicos para evaluar el grosor epitelial, el recuento de fibroblastos y la expresión de citocinas.

Resultados: El extracto presentó una potente capacidad antioxidante de CI_{50} < 100 µg/mL en los ensayos ABTS y DPPH, mientras que la cromatografía de gases-espectrometría de masas (GC-MS) mostró que el ácido n-hexadecanoico (26,36%) era el compuesto dominante. La histología mostró un aumento del grosor epitelial y de la densidad de fibroblastos, específicamente con una dosis de 300 mg/kg de peso corporal del extracto. A 200 mg/kg de peso corporal, el grosor epitelial aumentó un 96%, la expresión de IL-1β disminuyó un 45% ($p<0,01$), mientras que los niveles de expresión de PDGFβ y TGF-β aumentaron un 60% y un 70%, respectivamente. La inmunohistoquímica reveló una significativa disminución de la expresión de IL-1β, así como un aumento de la de PDGFβ y TGF-β ($p<0,001$) en los grupos tratados, comparable a MEBO.

Conclusiones: El extracto de *G. procumbens* mejoró la cicatrización de heridas diabéticas al modular el estrés oxidativo y el equilibrio de citocinas. La eficacia del extracto, respaldada por evidencia molecular e histológica, mostró potencial como agente terapéutico natural para heridas crónicas.

Palabras Clave: *Gynura procumbens*; heridas diabéticas; IL-1β; PDGFβ; TGF-β.

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INTRODUCTION

Diabetic wound is often characterized by increased inflammation and reduced angiogenesis. This is classified as a unique type of chronic wound, different from vascular and pressure ulcers (Jordan Holl et al., 2021). Diabetes has a complicated pathophysiology characterized by vascular, neuropathic, immunological, and metabolic factors, which contribute to the reported delayed healing of the condition (Mieczkowski et al., 2022). Hyperglycemia is a fundamental factor that prolongs the inflammatory and proliferative phases in diabetic wounds by compromising fibroblast functionality (Liu et al., 2022; Mieczkowski et al., 2022). Fibroblasts in people with diabetes are thought to sustain mitochondrial damage, leading to disruption of tissue composition and cellular function due to a high rate of programmed cell death or apoptosis (Liu et al., 2022). Fibroblasts, with contractile capabilities, are the principal cells responsible for wound tissue formation and healing, specifically in the synthesis and deposition of the extracellular matrix (ECM) (Darby et al., 2014; Liu et al., 2022). Moreover, hyperglycemia in diabetes is associated with the calcification of blood vessels, leading to diminished blood flow and compromised function of tiny arteries, which decrease oxygen delivery to tissues (Darby et al., 2014).

Fibroblasts are integral to the repair process in diabetic wounds by synthesizing structural proteins crucial for tissue regeneration (Darby et al., 2014). Interleukin-1β (IL-1β), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) all facilitate fibroblast proliferation throughout the standard healing process (Kwon et al., 2023). Additionally, granulation tissue formed during the inflammatory phase secretes transforming growth factor-β (TGF-β), which promotes fibroblast migration in the wound area (Kwon et al., 2023; Xu et al., 2013). The migration significantly influences healing and fibroblast proliferation in the affected area (Xu et al., 2013). The protracted healing of wounds in diabetic people is ascribed to a confluence of internal factors and external forces. The internal factors relate to biological processes, while external force variables include repetitive damage or mechanical stress (Xu et al., 2013). Chronic hyperglycemia impedes wound healing by generating advanced glycation end products (AGEs) and inducing the formation of senescent fibroblasts in the early phase of damage (Geng et al., 2021; Xu et al., 2013). This leads to the generation of inflammatory chemicals, namely TNF-α and IL-1, which subsequently extend the inflammatory phase and impede collagen synthesis (Geng et al., 2021).

Gynura procumbens (Lour.) Merr. (family *Asteraceae*) is a common ethnomedicinal plant in Southeast Asia, traditionally used for the management of inflammation, diabetes, and hypertension. Despite the popularity, the molecular mechanisms underlying the therapeutic potential, specifically in diabetic wound models, remain inadequately investigated (Tan et al., 2016). The plant is rich in bioactive components, including n-hexadecanoic acid, flavonoids, and phenolic acids, many of which have established antioxidant and anti-inflammatory properties essential for wound healing (Tahsin et al., 2022). The conventional applications of *G. procumbens* in diabetes management and skin injuries show that the phytoconstituents may significantly influence healing mechanisms, corresponding with the emphasis of this study on cytokines (IL-1β, PDGFβ, and TGF-β) dysregulated in diabetic wound. This study connects ethnopharmacology and contemporary biomedical research by scientifically confirming the traditional application. Although *G. procumbens* has medicinal potential, obstacles including inadequate solubility, instability, and low bioavailability of the phytochemicals prevent the clinical application. Nanoencapsulation with chitosan-sodium tripolyphosphate (TPP) attempts to improve delivery efficacy, prolong release, and facilitate tissue penetration, particularly crucial in chronic wound with compromised perfusion (Imam et al., 2021). The nano-formulation enhances the capacity for consistent, precise, and scalable treatments.

G. procumbens extract was nanoencapsulated to improve treatment delivery using a chitosan–sodium TPP system, which formed nanoparticles with a mean size of ~150 nm and a zeta potential of –30 mV, providing colloidal stability and enhancing skin penetration. Chitosan-encapsulated *G. procumbens* extract was hypothesized to promote diabetic wound healing by reducing IL-1β-driven inflammation as well as enhancing PDGFβ- and TGF-β-mediated tissue repair. The hypothesis was tested through phytochemical profiling, antioxidant assays, and *in vivo* wound-healing studies in an alloxan-induced diabetic rat model, which evaluated cytokine expression and histological outcomes.

MATERIAL AND METHODS

Chemical and reagents

Reagents used in immunohistochemistry included Invitrogen's IL-1β polyclonal antibody (Cat. PA5-119221; ThermoFisher Scientific), Monoclonal Mouse anti-Human PDGF β Antibody (clone 42G12, Cat. Number LS-B3667; LSbio company), and IHCeasy

TGF beta 1 Ready-To-Use IHC kit (Cat. KHC013; Proteintech Group). Furthermore, a phosphate-buffered saline (PBS) solution was used, which consisted of 50% glycerol and 1% bovine albumin (BSA) buffer (Cat. BS-0812R). Moist Exposed Burn Ointment (MEBO®) applied in this study was manufactured by Julphar Gulf Pharmaceutical Industries (UAE).

Vegetal material

The leaves of the *G. procumbens* plant were collected from the indigenous vegetation of the Southern Mountains karst area, specifically Bungur and Tulakan Village in Pacitan Regency, East Java, Indonesia, at coordinates of -8°10'22.8" latitude and 111°16'37.2" longitude, with an elevation range of 150–450 m above sea level across the 596,616 hectares constituting the area. To conduct the type of analysis, the samples were sent to the Herbarium Laboratory in USU and given voucher number 1321/MEDA/2023.

Preparation of the *G. procumbens* leaves

A total of 5 kg of *G. procumbens* leaves was dissolved in 5 L of 70% ethanol to produce a solution. Subsequently, maceration was carried out, and the liquid was evaporated to obtain 150 g of concentrated extract with a yield of approximately 3% w/w. *G. procumbens* extract was converted into nanochitosan extract by mixing chitosan with a glacial acetic acid solution in a 1:1 ratio. The mixture was filtered using Whatman paper after stirring for 24 hours. Additionally, sodium (Na)-TPP was dissolved in distilled water to form a solution after two hours of stirring. The extract was dissolved in the Na-TPP solution using a magnetic stirrer, then mixed with Connect Life chitosan for the nanoencapsulation process. The chitosan solution was stirred for 15 minutes at 28°C, followed by the addition of all the *G. procumbens* extract. The encapsulation efficiency was calculated using the indirect method and found to be 72.5%, based on the unencapsulated extract measured with a UV-Vis spectrophotometer. The chitosan nanoencapsulation of *G. procumbens* extract had a spherical morphology with dimensions between 140 and 185 nm. Particle measurement showed a mean size of about 150 nm, with a zeta potential of -30 mV, providing significant stability (Situmorang et al., 2025).

Examination of *G. procumbens* leaves using gas chromatography-mass spectrometry (GC-MS)

A GC-MS analysis was conducted using a mass spectrophotometer and combined gas chromatography equipment (Agilent; 19091-433HP). An HP-5 MS fused silica column with 5% phenyl methyl siloxane served as the technological component in this study, and the range of film thicknesses was 30–250 µm. Helium was used as a carrier gas by the 5675C MSD Inert, which comprised a Three-Axis Detector. The gas had a flow rate of 1.0 mL/min and was adjusted to fit the column, while the elution process required 47.5 minutes in total. The breakdown of each component was shown as a percentage, and the conclusion was reached by comparing the total area with the mean peak area. The MS Solutions software was provided by third parties contracted to manage the system and collect data.

Antioxidant activity assays

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method for antioxidant activity test

A stock solution of ABTS radical cation was prepared by dissolving 7 mg of ABTS salt and 3.5 mg of potassium persulfate in 5 mL of deionized water. This solution was incubated in the dark at room temperature for 12 to 16 hours to facilitate ABTS formation. Following the incubation, 1 mL of the ABTS stock solution was diluted to 10 mL with deionized water, generating the working solution. Subsequently, a UV-Vis spectrophotometer was used to measure the solution absorbance at the wavelength corresponding to the maximum absorbance between 400 and 800 nm. A stock solution of the antioxidant sample was prepared at a concentration of 1000 µg/mL in deionized water. Seven serial dilutions were carried out from the stock to obtain final concentrations of 12.5, 18.75, 25, 37.5, 50, 75, and 100 µg/mL. Each concentration of the sample was pipetted into brown vials and mixed with an equal volume of the ABTS working solution. The combination was left in the dark at room temperature for 30 minutes and incubated, then the absorbance of each sample was measured at a given wavelength, with a decrease in absorbance suggesting the presence of antioxidant activity (Sadeer et al., 2020; Wolosiak et al., 2022). During the ABTS assay, each concentration was tested in triplicate (n = 3) to ensure statistical reliability and reproducibility. The mean and standard deviation were calculated from these replicates.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of *G. procumbens* extract was evaluated using the DPPH radical scavenging assay. Approximately 2.0 mL of DPPH stock solution prepared at 0.1 mM in methanol was mixed with various extract concentrations (12.5, 25, 50, 100, 200, 300 µg/mL) and incubated in the dark for 30 minutes at room temperature. Subsequently, the absorbance was measured at 517 nm with a UV-Vis spectrophotometer, and ascorbic acid was used as the positive control. The IC_{50} value (the concentration required to inhibit 50% of the DPPH radicals) was calculated from the

dose-response curve using a four-parameter logistic (4PL) model (Woo et al., 2025). During the DPPH antioxidant assay, each concentration was tested in triplicate (n = 3) to ensure statistical reliability and reproducibility. The mean and standard deviation were calculated from these replicates.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was used to determine the reducing power of *G. procumbens* extract. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ at a ratio of 10:1:1. A volume of 100 µL of extract at various concentrations (12.5–300 µg/mL) was added to 3.0 mL of FRAP reagent and incubated at 37°C for 30 minutes. The absorbance was measured at 593 nm using a UV-Vis spectrophotometer (Minarti et al., 2024). The results were expressed in µmol Fe^{2+} equivalent per gram of extract using a standard curve of $FeSO_4 \cdot 7H_2O$. During the FRAP antioxidant assay, each concentration was tested in triplicate (n = 3) to ensure statistical reliability and reproducibility. The mean and standard deviation were calculated from these replicates.

Animal handling

This study used a total of 36 male *Rattus norvegicus* Wistar strain weighing 180 to 200 g when sexual maturity was attained, specifically between 11 and 14 weeks of age. Six male rats were kept in each cage and were ready to be tested after two weeks of acclimatization. The controlled conditions of the laboratory included a constant temperature of 25.3°C and a relative humidity of 35% to 60%. During the entire day, the male rats were fed a diet of corn and pellets in an environment with constant lighting, while being provided unfettered access to water. Acceptance of this study was granted by the Faculty of Mathematics and Natural Science, Medan Medical Research Ethics Committee at Universitas Sumatera Utara (reference number 0912/KEPH-FMIPA/2024).

Study design

The rats were fasted overnight and exposed to alloxan induction, except for the usual treatment group (control group). An intraperitoneal dosage of 160 mg/kg BW of alloxan was administered on day 0. Following three days of induction, blood glucose levels were assessed (Fajarwati et al., 2023). Animals under testing were diagnosed with diabetes when the fasting blood glucose levels were equal to or more than 200 mg/dL. The hair on the back of the rats was shaved completely, and a wound with a diameter of 5 mm was made using a punch biopsy tool. Wound formation started with anesthesia using ketamine 0.2-0.3 mL (150 mg/kg BW) intramuscularly (Mostafavinia et al., 2016). This study consisted of six groups, including group C, which served as a negative control to simulate normal conditions. There were two additional groups, namely C- comprising rats induced by alloxan only, and C+ comprising alloxan-induced rats administered with MEBO ointment.

MEBO was obtained from a certified pharmacy in Indonesia and applied topically once daily for 14 days. The product was stored at 25°C and protected from light, as recommended by the manufacturer. The total quantity used per animal was adjusted to cover the wound area uniformly without excess. MEBO was previously used in several studies as a reference topical agent for healing, including in diabetic wound models, due to the properties in modulating inflammation, promoting angiogenesis, and enhancing re-epithelialization (Gong et al., 2023; Zheng et al., 2020). D1 was the rat induced with alloxan + *G. procumbens* leaves at a dose of 100 mg/kg BW, while D2 was given alloxan + *G. procumbens* (GB) leaves at a dose of 200 mg/kg BW. D3 was treated with alloxan + *G. procumbens* leaves at a dosage of 300 mg/kg BW. This herbal remedy is often applied externally to treat sores in people with diabetes. Application of MEBO and *G. procumbens* ointment was carried out for 14 days, while a ketamine injection was administered for dissection. Euthanasia was performed by intramuscular injection of ketamine 150 mg/kg body weight (equivalent to approximately 0.2 mL for a 200 g rat), under deep anesthesia, in accordance with the institutional ethical guidelines (Mostafavinia et al., 2016). Subsequently, the diabetic wound skin was excised using surgical scissors for immunohistochemistry investigation.

Immunohistochemistry

The tissue was affixed to a specialized glass slide using Menzel Superfrost Plus adhesive and allowed to air dry overnight at 37°C for immunohistochemical examination. The objective lens was coated with poly-L-lysine, and the tissue was rehydrated in a decreasing series of alcohol solutions after being initially deparaffinized in xylene for two cycles of 15 minutes each. The tissue was rehydrated by submerging it in 96% ethanol for 2.5 minutes and 70% ethanol for an additional 2.5 minutes, which was repeated twice. The tissue was hydrated for 15 minutes, followed by a 3-hour and 5-minute wash with a TBS buffer. Subsequently, dehydration was performed through a succession of ethanol. The slides intended for staining with IL-1β, PDGF β, and TGF-β were segregated and labeled to avoid any confusion, while optimized dilutions were used to produce antibody solutions.

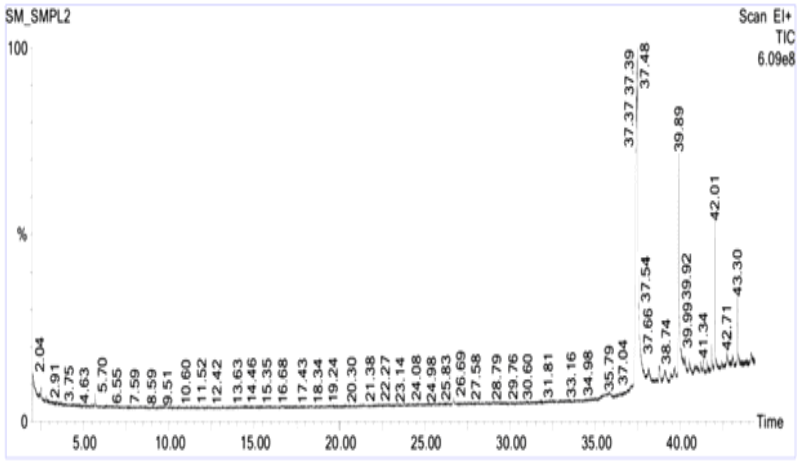


Figure 1. GC-MS chromatogram of the ethanolic leaf extract of *G. procumbens*, showing the separation of major phytochemical components across different retention times.

The procedure of antigen retrieval at a high temperature of 105°C was performed in a pressurized steam container called a decloaking chamber for 10-20 minutes, based on the requirements and characteristics of the antibody and tissue (Simanullang et al., 2022). The purpose of this phase was to enhance the interaction between the antibody and the specific protein (antigen) in the tissue, strengthening the antigen-antibody binding reaction.

Rehydration was performed to achieve antigen retrieval, and the sample was subjected to microwave heating in a citrate buffer for 5 minutes. Subsequently, the sample was rinsed three times for 5 minutes each using phosphate-buffered saline Tween 20 (PBST). A 3% solution of hydrogen peroxide (H_2O_2) was used to block the activity of endogenous peroxidase for 30 minutes, where the sample was rinsed three times with PBST for 5 minutes each time. To avoid non-specific binding, the tissue was treated with 1% fetal bovine serum (FBS) for 30 minutes, followed by three washes for 5 minutes each with PBST. Primary antibodies, including polyclonal antibodies against IL-1β, PDGF, and TGF-β, were applied to the tissue, which was incubated overnight at 40°C. Immunohistochemical staining was performed using primary antibodies specific for IL-1β (dilution 1:200), PDGFβ (dilution 1:150), and TGF-β (dilution 1:200). Positive controls consisted of tissue samples known to express the respective cytokines, obtained from healthy wound healing models. Negative controls were prepared by omitting the primary antibody during the staining procedure to confirm the specificity of immunoreactivity. A polymer-based secondary antibody system conjugated with horseradish peroxidase (HRP) was used for signal amplification. The chromogenic reaction was visualized using 3,3′-diaminobenzidine (DAB) as the substrate, generating a brown-colored precipitate at the site of antigen expression. Sections were counterstained with hematoxylin, dehydrated, and mounted for microscopic evaluation. After incubation, the slides were washed three times using PBST for 5 minutes each, rinsed with distilled water, dried off using a series of alcohol solutions of increasing concentration, and treated with xylol to remove any remaining impurities. The tissue on the object glass was affixed using a permount and covered with a cover glass (Situmorang et al., 2023).

Statistical analysis of data

Data were expressed as mean ± standard deviation, where parametric data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range post hoc test. The non-parametric counterparts were analyzed using the Kruskal-Wallis test, with the Mann-Whitney U test conducted for pairwise comparisons. Statistical significance was considered at $p<0.05$, and all analyses were performed using IBM SPSS Statistics version 24 (IBM Corp., Armonk, NY, USA).

RESULTS

Phytochemical composition

The chemical components of *G. procumbens* leaf extract were examined through GC-MS, with the results presented in Fig. 1 (chromatogram) and Table 1 (compound identification and relative abundance). A total of 40 chemical compounds were identified, each with unique retention durations and relative abundances (% area). The predominant chemical was n-hexadecanoic acid (palmitic acid), showing a relative area of 26.357% and a retention time (RT) of 37.462 minutes (Fig. 2). Octadecanoic acid (stearic acid) was the second most prevalent at 6.700%, followed by bis(2-ethylhexyl) phthalate at 1.512%.

Table 1. Chemical constituents identified in *G. procumbens* ethanolic leaf extract by GC-MS analysis**.**

Peak	RT	Compound name	Match	R.Match	Area	Area (%)	CAS Number
1	2.038	Methylhydrazine	727	844	19,289,394.0	4.975	60-34-4
2	2.424	Acetic acid, [(phenyl-methoxy)imino]-, trimethylsilyl ester	547	604	3,709,891.5	0.957	55494-08-1
3	2.539	o-Xylene (1,2-dimethyl-benzene)	882	929	10,633,417.0	2.742	95-47-6
4	2.834	p-Xylene (1,4-dimethyl-benzene)	746	909	5,309,052.0	1.369	106-42-3
5	2.944	Nitrosomethane	459	836	2,705,743.2	0.698	865-40-7
6	3.149	L-Alanyl-L-alanine	597	773	7,651,477.0	1.973	59247-16-4
7	3.364	1-Formyl-3-ethyl-6-β-D-ribofuranosyl-pyrazolo[4,5-b]imidazole	428	452	2,083,413.6	0.537	
8	3.454	3-Methyl-1-adamantanemethylamine	608	692	3,480,154.2	0.898	13392-28-4
9	3.569	2-Amino-4,6-dihydro-4,4,6,6-tetramethyl-thieno[2,3-c]furan-3-carbonitrile	414	502	1,608,339.6	0.415	447412-24-0
10	3.674	(3aR,6aS,7a,10a,10b)-3a,4,6a,7,9,10,10a,10b-octahydro-3a,10a-dihydroxy-5-(hydroxymethyl)-7-(1-hydroxy-1-methylethyl)-2,10-dimethyl-benz[e]azulene-3,8-dione	446	483	2,946,543.2	0.760	77590-91-1
11	3.814	Tetra-O-acetyl-D-xylonic nitrile	501	559	1,166,433.5	0.301	152434-78-1
12	3.899	6-Aza-2-(4-hydroxy-3-methoxy-phenyl)-2-propyl-heptadecanenitrile	464	604	1,018,335.8	0.263	
13	3.934	Lactic acid (2-hydroxy-propanoic acid)	425	575	2,199,873.8	0.39375	50-21-5
14	4.054	Glycolic acid (2-hydroxy-acetic acid)	431	759	2,656,753.0	0.685	152434-78-1
15	4.189	2,5-Difluoro-3,4,5-trihydroxy-N-methyl-benzene-1-ethanamine	534	615	3,932,554.2	1.014	152434-78-1
16	4.419	2,4-Dimethyl-benzo[h]quinoline	443	710	1,160,258.1	0.299	605-67-4
17	4.464	2-(5-Acetyl-3-cyano-6-methyl-pyridin-2-yl-sulfanyl)-N-(3-trifluoro-methyl-phenyl)acetamide	472	548	1,381,726.0	0.356	
18	4.579	N-Carbamoyl-acetamide (ureido-acetic acid)	570	933	1,103,846.9	0.285	585-05-7
19	4.634	Guanidinosuccinimide	469	655	1,725,517.4	0.445	
20	4.795	N-Methyl-1-butanamine	547	874	1,727,854.9	0.446	110-68-9
21	5.065	3-Methyl-1-adamantanemethylamine	535	724	1,109,483.5	0.286	13392-28-4
22	5.700	Eucalyptol (1,8-cineole)	778	802	2,001,851.5	0.516	470-82-6
23	5.770	2-(Aziridin-1-yl)ethylamine	566	841	1,061,471.4	0.274	4025-37-0
24	37.102	3-(Octadecyloxy)-1,2-propanediol diacetate	468	489	1,026,244.5	0.265	21994-81-0
25	37.462	n-Hexadecanoic acid (palmitic acid)	915	917	102,195,576.0	26.357	57-10-3
26	37.838	Guanidinosuccinimide	449	634	1,160,369.1	0.299	
27	37.943	Cystine (2,2'-dithiobis-[L-alanine])	413	489	1,089,016.0	0.281	56-89-3
28	38.098	Eicosanoic acid (arachidic acid)	506	565	2,111,417.5	0.545	506-30-9

Table 1. Chemical constituents identified in *G. procumbens* ethanolic leaf extract by GC-MS analysis (continued...)

Peak	RT	Compound name	Match	R.Match	Area	Area (%)	CAS Number
29	38.153	Actinobolin	473	599	1,876,194.2	0.484	24397-89-5
30	38.738	2-Hydroxy-1-(hydroxymethyl)-ethyl eicosanoate	511	579	2,739,422.0	0.707	55334-78-6
31	39.043	2-Methyl-hexadecan-1-ol	515	562	1,149,484.4	0.296	2490-48-4
32	39.663	Z-(13,14-epoxy)tetradec-11-en-1-yl acetate	543	601	1,461,495.0	0.377	56554-64-4
33	39.888	Octadecanoic acid (stearic acid)	888	893	25,976,434.0	6.700	57-11-4
34	40.268	Hexadecamethyl-heptasiloxane	679	804	1,521,843.9	0.392	541-01-5
35	40.504	1,1-Bis(dodecyloxy)hexadecane	578	610	1,162,560.6	0.300	56554-64-4
36	41.339	5-Tetradecyl-dihydro-2(3H)-furanone	619	656	1,126,752.4	0.291	502-26-1
37	41.819	Supraene	739	820		0.613	7683-64-9
38	42.009	Bis(2-ethylhexyl) hexanedioate (bis-2-ethylhexyl adipate)	886	915	9,008,273.0	2.323	103-23-1
39	42.709	Hexadecamethyl-heptasiloxane	664	772	1,713,485.9	0.442	541-01-5
40	43.305	Bis(2-ethylhexyl) phthalate	847	883	5,862,574.0	1.512	117-81-7

RT: Retention Time; Match: Library match score; R. Match: Reverse match score; Area (%): Relative abundance of each compound expressed as a percentage of total chromatogram area. The identification of compounds was based on a comparison with the NIST library database. N-Hexadecanoic acid was the most dominant compound detected in the extract.

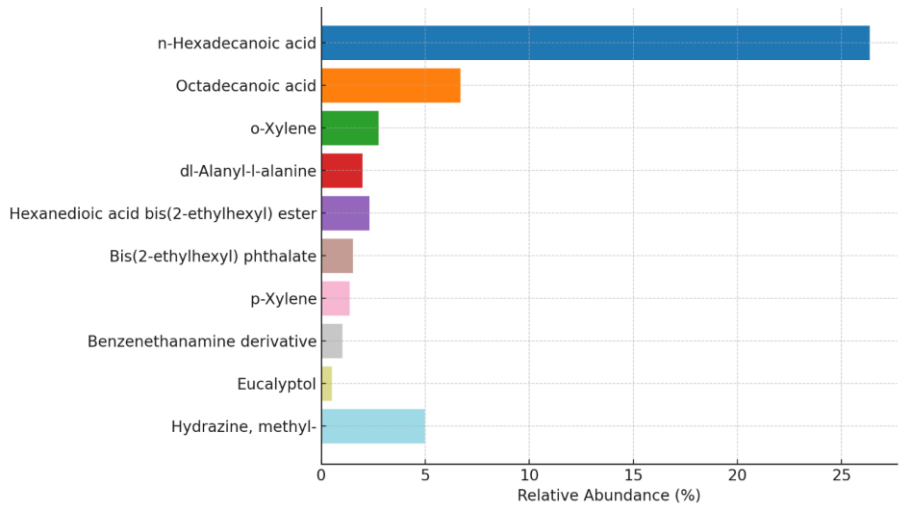


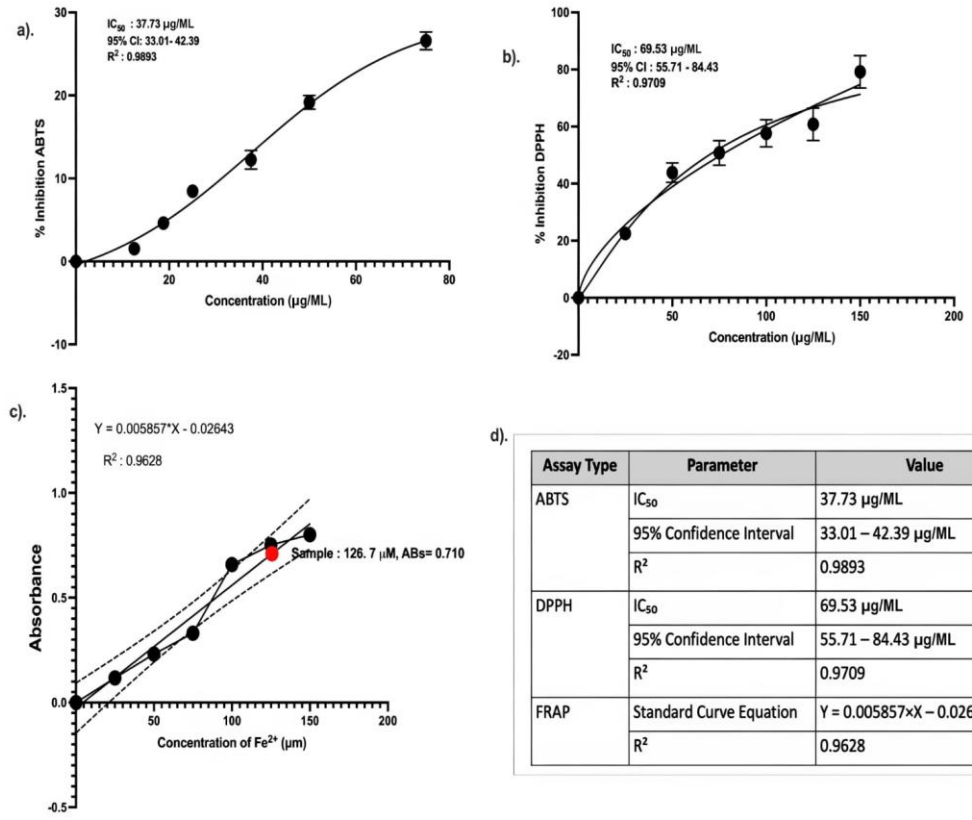
Figure 2. Bar chart representation of the top ten chemical constituents identified in the ethanolic leaf extract of *G. procumbens* based on GC-MS analysis.

The compounds are arranged according to the relative abundance (% area). The most predominant compound was n-hexadecanoic acid (palmitic acid) at 26.357%, followed by octadecanoic acid (stearic acid) and o-xylene. The chart shows the chemical diversity and the dominance of fatty acids in the extract profile.

Other significant detected compounds comprised o-xylene (2.742%), p-xylene (1.369%), dl-alanyl-l-alanine (1.973%), as well as benzenethanamine derivatives and eucalyptol in lesser quantities. The recognized chemical categories included fatty acids (such as palmitic acid, stearic acid, eicosanoic acid), amino acid derivatives (dl-alanyl-l-alanine, l-guanidinosuccinimide), hydrocarbons and alkaloid analogs, as well as phenolic or aromatic esters. Fig. 1 shows a chromatogram with distinct and well-defined peaks, signifying superior separation quality and consistent extract composition.

Antioxidant activity (ABTS, DPPH, and FRAP assays)

The antioxidant activity assay of the extract using the ABTS method presented a significant capacity to scavenge $ABTS^{+}$ radicals in a concentration-dependent manner. The graph shows a rise in the percentage inhibition value corresponding to elevated extract concentration, signifying a positive linear correlation between concentration and antioxidant activity (Fig. 3). The determined IC_{50} value from the curve signified the concentration of extract necessary to block 50% of $ABTS^{+}$ radicals.



Assay Type	Parameter	Value
ABTS	IC_{50}	37.73 µg/ML
	95% Confidence Interval	33.01 – 42.39 µg/ML
	R^2	0.9893
DPPH	IC_{50}	69.53 µg/ML
	95% Confidence Interval	55.71 – 84.43 µg/ML
	R^2	0.9709
FRAP	Standard Curve Equation	Y = 0.005857×X – 0.02643
	R^2	0.9628

Figure 3. Antioxidant efficacy of *G. procumbens.*

(a) ABTS radical scavenging activity, **(b)** DPPH radical scavenging activity, **(c)** Ferric Reducing Antioxidant Power (FRAP) assay, and **(d)** Antioxidant values of ABTS, DPPH, and FRAP. The results are expressed as mean ± standard deviation (n = 3).

The IC_{50} value of *G. procumbens* extract was below 100 µg/mL, representing modest antioxidant activity (Fig. 3a). This activity could be attributed to the presence of phenolic and flavonoid chemicals, including quercetin, kaempferol, and chlorogenic acid, which served as electron donors in the neutralization of free radicals. The DPPH method for assessing antioxidant activity showed that the extract significantly captured DPPH radicals. The efficacy of antioxidant activity was signified by the IC_{50} value, representing the extract concentration required to block 50% of DPPH free radicals. A lower IC_{50} value signifies the greater antioxidant activity of a sample. The IC_{50} value of *G. procumbens* was less than 100 µg/mL; therefore, it was classified as a potent antioxidant (Fig. 3b). This activity correlated with the presence of phenolic and flavonoid chemicals, recognized for the active hydroxyl groups capable of donating hydrogen atoms to neutralize free radicals. The antioxidant activity assessment using the FRAP method found that *G. procumbens* extract possessed a commendable capacity to convert Fe^{3+} ions to Fe^{2+}. The graph shows that absorbance values rise with increasing extract content, signifying an enhancement in the reducing capacity of the antioxidant chemicals in the sample (Fig. 3c). In the ABTS assay, the extract caused significant inhibition of $ABTS^{+}$ radicals, as evidenced by a marked increase in the percentage inhibition with rising concentration. The DPPH assay showed a comparable pattern, representing the capacity of the extract to donate hydrogen atoms for the neutralization of DPPH radicals, as signified by the low IC_{50} value. Simultaneously, the FRAP method found that the extract possessed a significant capacity for Fe^{3+} ion reduction, suggesting its potential as an effective electron donor. The uniformity of the results from all three methods suggests that *G. procumbens* comprises bioactive constituents, including flavonoids, phenolics, and other natural antioxidants, which operate by diverse mechanisms such as free radical scavenging, electron donation, and hydrogen donation (Fig. 3d). *G. procumbens* is a promising prospect for development as a source of natural antioxidants in both the pharmaceutical and functional food sectors.

Wound-closure kinetics and histomorphometry

The macroscopic morphology presented in Fig. 4 showed that on day 3 of treatment, all groups had a reduction in wound size, signifying the proliferative phase. On day 3, the C- and C groups had larger

wound sizes with persistent signs of inflammation, while the C+ and D groups experienced a reduction in wound size under similar conditions. Drying and crust formation were more pronounced by day 5, with smaller wound sizes, except for the C- group, which still had a larger wound with ongoing crust formation. Observations on day 14 showed wound samples in the C+ and D groups increasingly covered with scar tissue, which represented the remodeling phase of healing. The diabetic wound healing process entering the remodeling phase after treatment was found in other studies, such as with phosphorylated chitosan treatment on day 14.

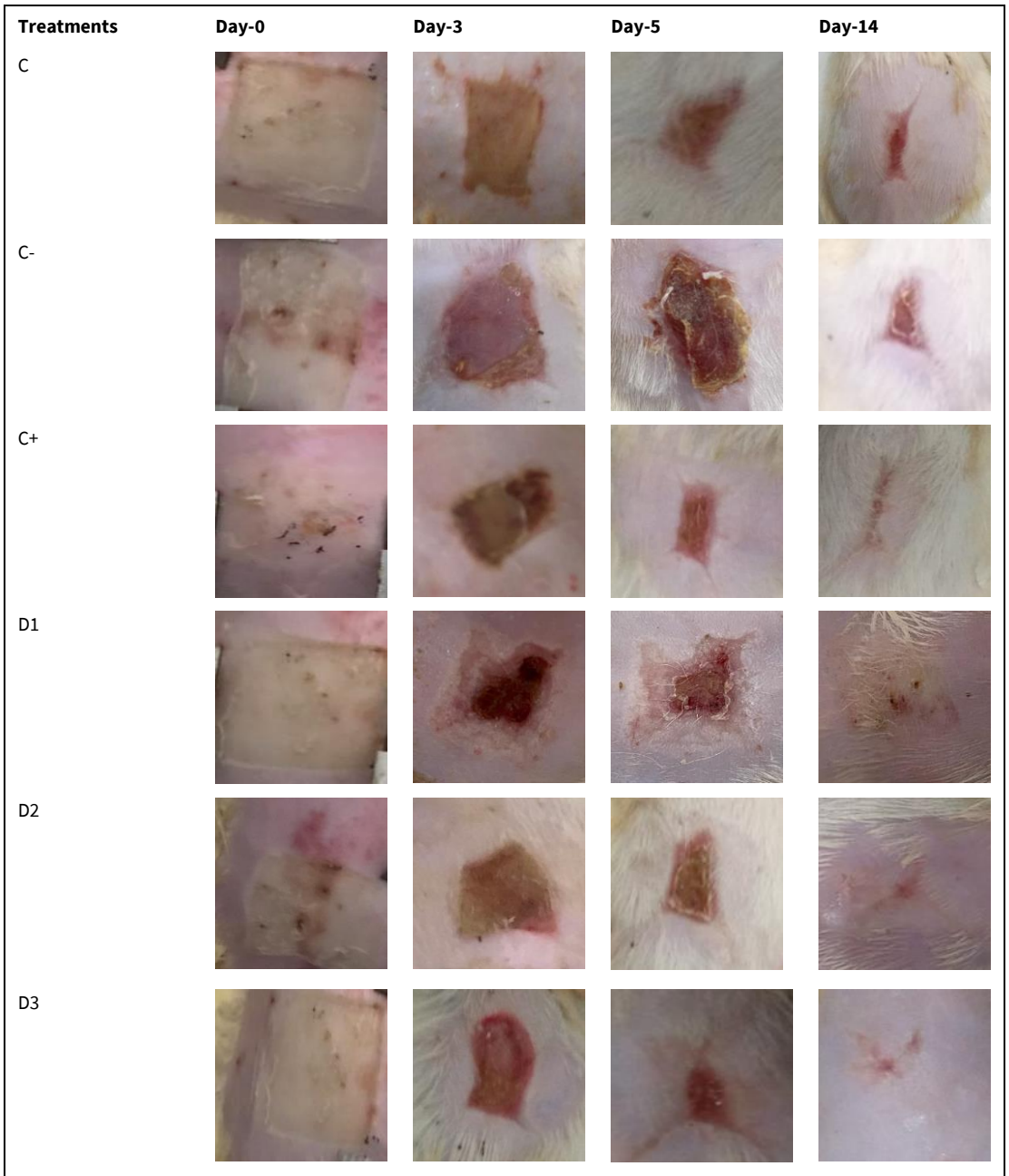


Figure 4. Macroscopic observation of wound healing progression in diabetic rats treated with *G. procumbens* ethanolic extract compared to MEBO ointment.

C: normal control; C−: diabetic control without treatment; C+: diabetic rats treated with MEBO; D1–D3: diabetic rats treated with *G. procumbens* extract at 100, 200, and 300 mg/kg BW, respectively. Photographs were taken on days 0, 3, 5, and 14 after wound induction.

Table 2. Wound area and percentage of wound contraction on days 3, 5, and 14 after treatment.

Treatment	Day 0	Day 3	Wound contraction day 3 (%)	Day 5	Wound contraction day 5 (%)	Day 14	Wound contraction day 14 (%)
C	4.00 ± 0.00^{a}	3.04 ± 0.39^{b}	24.00 ± 3.20^{b}	1.76 ± 0.52^{b}	56.00 ± 4.22^{b}	0.25 ± 0.20^{b}	93.75 ± 2.98^{b}
C-	4.00 ± 0.00^{a}	2.91 ± 0.49^{b}	27.25 ± 2.81^{b}	1.60 ± 0.47^{c}	60.00 ± 3.23^{c}	0.12 ± 0.13^{ab}	97.00 ± 2.10^{ab}
C+	4.00 ± 0.00^{a}	2.95 ± 0.37^{b}	26.25 ± 3.52^{b}	0.94 ± 0.42^{bc}	76.50 ± 3.89^{bc}	0.06 ± 0.47^{a}	98.50 ± 2.19^{a}
D1	4.00 ± 0.00^{a}	2.66 ± 0.37^{bc}	33.50 ± 3.21^{bc}	0.89 ± 0.68^{bc}	77.75 ± 3.78^{bc}	0.02 ± 0.01^{a}	99.50 ± 1.39^{a}
D2	4.00 ± 0.00^{a}	2.10 ± 0.16^{a}	47.50 ± 2.90^{a}	0.89 ± 0.50^{bc}	77.75 ± 2.89^{bc}	0.01 ± 0.01^{a}	99.75 ± 1.00^{a}
D3	4.00 ± 0.00^{a}	2.06 ± 0.55^{a}	48.50 ± 2.97^{a}	0.65 ± 0.51^{a}	83.75 ± 2.87^{a}	0.01 ± 0.00^{a}	99.75 ± 1.23^{a}

Note: Values represent the mean wound area and contraction for each group (n = 6 per group). C: normal control; C−: diabetic control without treatment; C+: diabetic group treated with metformin; D1–D3: diabetic rats treated with *G. procumbens* extract at doses of 100, 150, and 200 mg/kg BW, respectively. Different superscript letters ($^{a, b, c, ab, bc}$) in the same column imply significant differences between groups ($p < 0.05$, Duncan's post hoc test). Wound contraction was calculated as ((Area Day 0 – Area Day X) / Area Day 0) × 100%.

Table 3. Analysis of tissue from diabetic wounds using hematoxylin-eosin staining.

Treatment	Epithelial tissue thickness (µm)	Number of fibroblast cells
C	32.36 ± 8.04^{a}	62.10 ± 14.79^{a}
C-	49.81 ± 7.03^{b}	76.20 ± 8.43^{b}
C+	50.59 ± 4.51^{b}	64.90 ± 17.49^{a}
D1	52.76 ± 1.80^{b}	77.60 ± 12.0^{b}
D2	56.42 ± 0.48^{bc}	72.75 ± 11.07^{b}
D3	63.46 ± 3.17^{c}	69.25 ± 10.75^{a}

Note: C: Untreated rats, C-: Only induced by alloxan, C+: induced by alloxan + MEBO ointment. D1 + Induced by alloxan GB leaves at a dose of 100 mg/kg BW, D2: induced by alloxan GB leaves at a dose of 200 mg/kg BW, D3: induced by alloxan GB leaves at a dose of 300 mg/kg BW. The results are expressed as mean ± standard deviation (n = 6). Different superscript letters ($^{a, b, c, bc}$) in the same column represent significant differences between groups ($p<0.05$, Duncan's post hoc test).

Healing advancement was quantitatively evaluated by measuring wound area and calculating the percentage of wound contraction on days 3, 5, and 14 (Table 2). All treatment groups showed a decrease in wound area on day 3, with contraction varying from 24.00 ± 3.20% in the normal control (C) to 48.50 ± 2.97% in the D3 group (*G. procumbens* 200 mg/kg). The diabetes control (C−) group had a contraction of 27.25 ± 2.81%, signifying compromised healing relative to the treatment groups. By day 5, wound contraction markedly escalated in all groups. The most significant shrinkage occurred in the D3 group (83.75 ± 2.87%), followed by D1 and D2 (77.75 ± 3.78% and 77.75 ± 2.89%, respectively), which signified a dose-dependent effectiveness of *G. procumbens*. The metformin-treated group (C+) had a significant contraction (76.50 ± 3.89%), while the diabetic control (C−) had a lower contraction rate of 60.00 ± 3.23%. On day 14, near-total wound closure was observed in the D2 and D3 groups, with contraction rates of 99.75 ± 1.00% and 99.75 ± 1.23%, respectively. The normal control group achieved 93.75 ± 2.98%, the metformin group (C+) attained 98.50 ± 2.19%, and the diabetes control (C−) recorded 97.00 ± 2.10%. The data showed that *G. procumbens* extract enhanced wound healing in a dose-dependent manner, as signified by markedly elevated contraction percentages during the observation period compared to both diabetic and normal controls.

Mean epithelial thickness of burn wound in rats on day 14 was highest at a size of 63.46 µm in the treatment group receiving an ethanol extract of *G. procumbens* leaves at 200 mg/kg body weight (D3), while the lowest was in the normal treatment group (C) at 32.36 µm (Table 3). Based on statistical testing, the burn wound group in the normal treatment (C) showed significant differences compared to the treatment groups. The diabetic burn wound group treated with *G. procumbens* leaves at 200 mg/kg body weight (D3) differed considerably from the other groups, but not significantly from the diabetic burn wound group treated with 150 mg/kg body weight (D2). The diabetic burn wound group treated with *G. procumbens* showed an increase in epithelial tissue thickness during the healing process, with higher concentrations yielding the most significant results (Fig. 5).

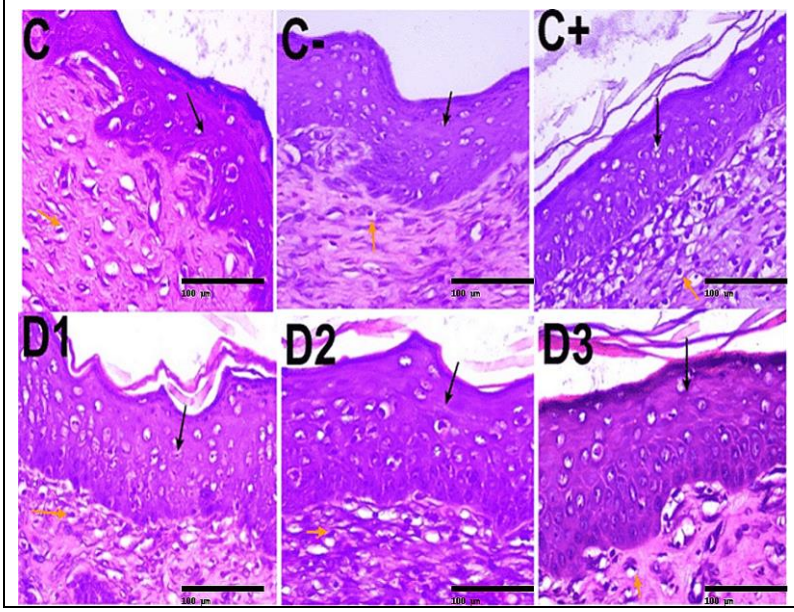


Figure 5. Histological features of diabetic wound treated with hematoxylin-eosin staining.

C: Untreated rats, C-: Only induced by alloxan, C+: induced by alloxan + MEBO ointment. D1 + Induced by alloxan GB leaves at a dose of 100 mg/kg BW, D2: induced by alloxan GB leaves at a dose of 200 mg/kg BW, D3: induced by alloxan GB leaves at a dose of 300 mg/kg BW. Black arrows: epithelial tissue, orange arrows: fibroblast cells. Scale bar: 100 µm. Magnification: 400×.

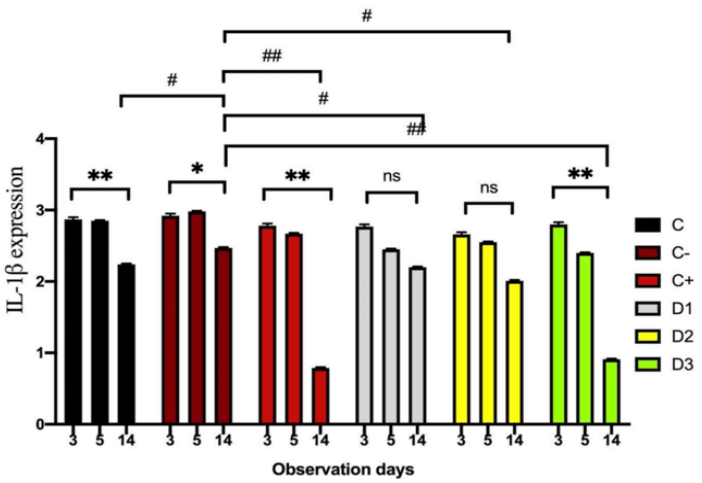


Figure 6. The expression of IL-1β in burn-injured rats after the administration of *G. procumbens* following alloxan injection.

C: Untreated rats, C-: Only induced by alloxan, C+: induced by alloxan + MEBO ointment. D1 + Induced by alloxan GB leaves at a dose of 100 mg/kg BW, D2: induced by alloxan GB leaves at a dose of 200 mg/kg BW, D3: induced by alloxan GB leaves at a dose of 300 mg/kg BW. The results are expressed as mean ± standard deviation (n = 6). ($^{\#}p<0.05$ vs C-, $^{\#\#}p<0.01$ vs C-, $^{*}p<0.05$; $^{**}p<0.01$ compared day 3 with day 14 in the same group).

In contrast, the untreated group's potential for infection in the wound could potentially slow down the healing process. Epithelial cell proliferation often leads to thickening of the epidermal layer. The untreated burn wound group (C-) had a longer healing process, with the lowest mean epithelial tissue thickness. This is attributed to the absence of topical treatment, leading to a continuous risk of infection and a greater potential for recovery failure during the healing process.

The impact of *G. procumbens* (GB) on IL-1β expression in diabetic skin tissue

A significant inhibition ($p<0.001$) of IL-1β expression by *G. procumbens* at 200 and 300 mg/kg body weight was observed on day 14. There was continued instability in the gene expression on days 3 and 5, which might be due to the prolonged process of cell proliferation. The graph shows that this plant can inhibit the synthesis of IL-1β, even at the highest dose. Based on nonparametric data, IL-1β expression of the groups varied significantly, as identified by the Kruskal-Wallis Test (Fig. 6). Group A+ and IL-1β expression differed significantly ($p<0.01$, $p=0.001$), according to the Mann-Whitney test. The *G. procumbens* doses D1 (100 mg/kg body weight) and D2 (200 mg/kg body weight) were both below the threshold for statistical significance.

Histological alterations caused by IL-1β expression in skin tissue supported the results presented in Fig. 7. On Day 14, the *G. procumbens* group and the C+ group showed the lowest levels of IL-1β expression at a dose of 300 mg/kg body weight, while the C- group presented the highest levels. Prolonged inflammation requires more time for healing, as wound leads to an overproduction of pro-inflammatory cytokines, including IL-1β. Therefore, compounds or drugs with immunoregulatory properties are needed to counter-

act this excessive production. IL-1β stimulates the development of new skin tissue, suggesting that *G. procumbens* and other herbal medicines may help promote the formation of new skin tissue while balancing excessive IL-1β production.

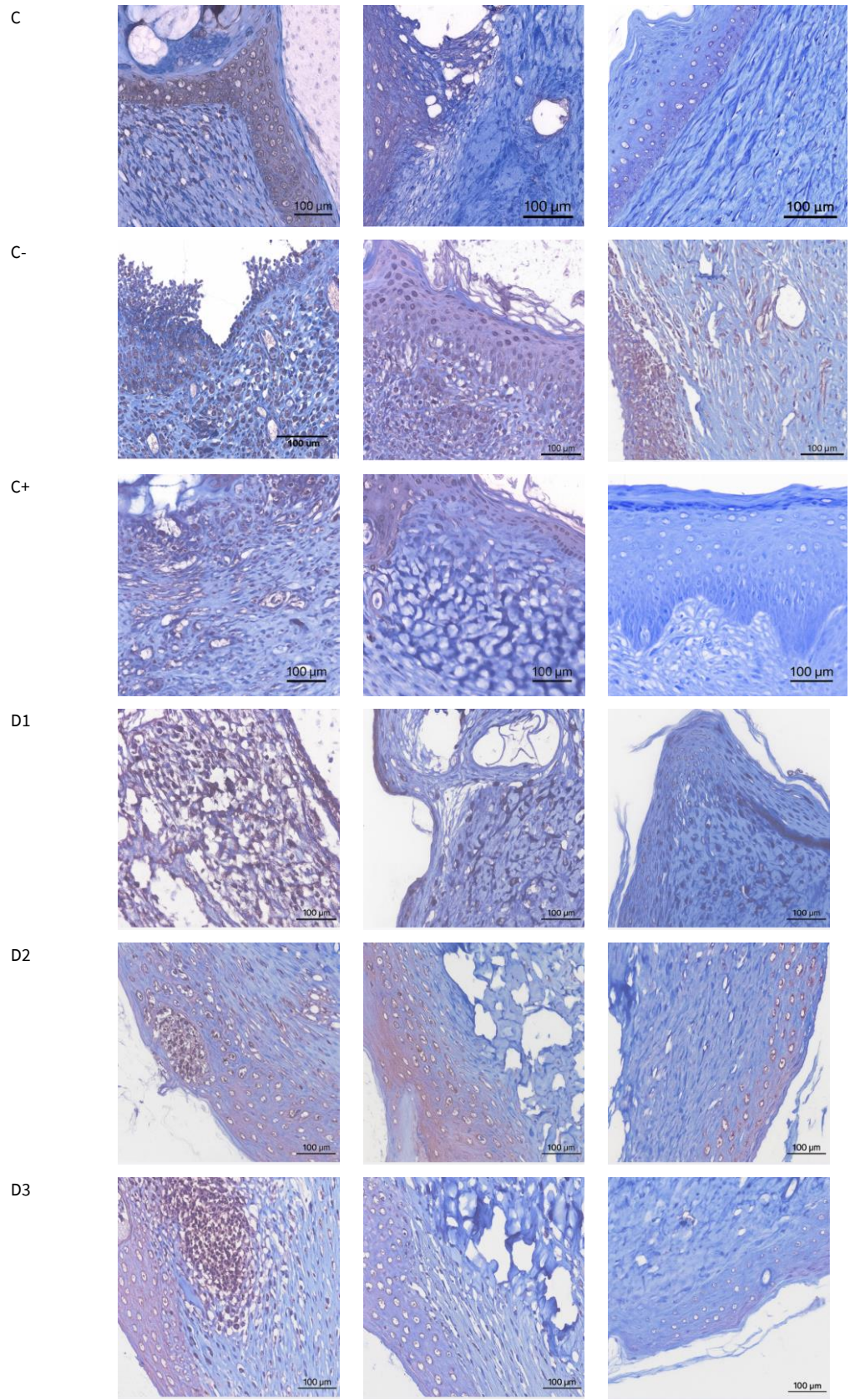


Figure 7. Immunohistochemical staining of IL-1β expression in diabetic wound tissue treated with *G. procumbens* ethanolic extract.

C: normal untreated rats; C−: diabetic rats without treatment; C+: diabetic rats treated with MEBO®; D1–D3: diabetic rats treated with *G. procumbens* extract at 100, 200, and 300 mg/kg BW, respectively. Positive IL-1β expression is represented by brown chromogenic deposits in the cytoplasm of dermal and epithelial cells. Scale bar: 100 µm. Magnification: 400×.

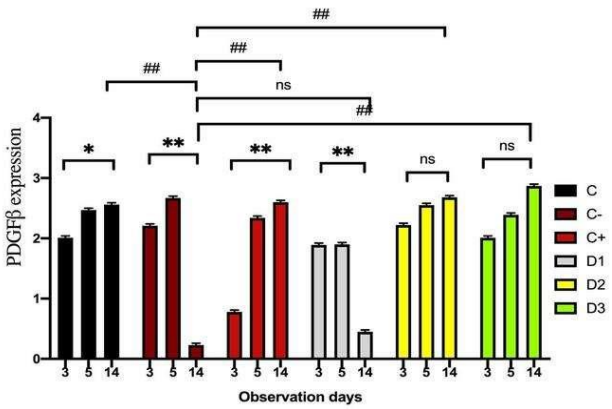


Figure 8. The expression of PDGFβ in burn-injured rats after the administration of *G. procumbens* injected with alloxan.

C: Untreated rats, C-: Only induced by alloxan, C+: induced by alloxan + MEBO ointment. D1 + Induced by alloxan *G. procumbens* leaves at a dose of 100 mg/kg BW, D2: induced by alloxan GB leaves at a dose of 200 mg/kg BW, D3: induced by alloxan GB leaves at a dose of 300 mg/kg BW. The results are expressed as mean ± standard deviation (n = 6). (#p<0.05 vs C-, ##p<0.01 vs. C-, *p<0.05; **p<0.01 compared day 3 with day 14 in the same group).

The impact of *G. procumbens* on PDGFβ expression in diabetic skin tissue

The group with the highest dose (D3) expressed the most PDGFβ, followed by the group treated with MEBO as a comparator (C+) and the D2 group. On Day 14, diabetic animals induced by alloxan (C-) showed reduced expression of PDGFβ. *G. procumbens* inhibited the synthesis of this protein even at the lowest dosage (D1). PDGF often promotes the production of collagen in skin fibroblasts by enhancing fibroblast cell migration and proliferation in the epithelium. PDGFβ expression was modulated at 200 and 300 mg/kg BW, while the mean values were nearly the same as the values of the C+ comparison group. The expression levels varied significantly ($p<0.01$) between groups when compared to C-, but did not differ significantly in the same group on different days (Fig. 8). The expression of PDGFβ in the tissue caused structural alterations in the epithelium and triggered the migration of endothelial cells (Fig. 9). The plant components restored the structure to resemble the normal group structure, suggesting that *G. procumbens* regulated PDGFβ levels in the tissue and played a crucial role in both fibrosis and wound healing.

The impact of *G. procumbens* on TGFβ expression in diabetic skin tissue

Fig. 10 shows a significant increase in TGFβ expression at the highest dose in the group receiving MEBO (C+) and at 300 mg/kg BW (D3). The expression of TGFβ varied significantly ($p<0.01$) among the groups (Fig. 10). The Mann-Whitney U test showed a statistically significant difference in TGFβ expression between C- and the other groups ($p<0.01$, $p=0.001$). This change was not statistically significant when *G. procumbens* was administered at a dose of 100 mg/kg BW. The C- dose and the recommended doses of 200–300 mg/kg BW showed significant differences. Groups D2 and D3, as well as the control group (A+), had the highest TGFβ expression, while C- and D1 had the lowest expression. The constituents of *G. procumbens* may play a role in balancing TGFβ expression and stimulating new tissue formation (Fig. 11). TGFβ is crucial for epithelialization and collagen production during wound healing. Moreover, TGFβ collectively belongs to a group of growth factors, cytokines, and chemokines classified as secretory proteins.

DISCUSSION

GC-MS profiling of *G. procumbens* ethanolic extract showed the dominance of fatty acids, particularly n-hexadecanoic acid (palmitic acid) and octadecanoic acid (stearic acid), which were previously reported to have antimicrobial, anti-inflammatory, and pro-healing properties. The presence of other constituents such as amino acid derivatives and aromatic esters suggests a broad spectrum of biological activities. The chemical complexity of the extract signified the potential for multitargeted modulation in wound environments. *G. procumbens* showed potential for treating diabetic wounds in addition to the antioxidant qualities.

Numerous studies reported that extracts from different portions of the *G. procumbens* plant had potent antioxidant capabilities. The leaves are abundant in bioactive phytochemicals, including phenolic acids, flavonoids, and other polyphenolic substances. These antioxidant-rich chemicals are thought to enhance the capacity of the plant to efficiently scavenge free radicals, chelate metal ions, and impede lipid peroxidation (Akowuah et al., 2012; Chandradevan et al., 2020; Olszewska et al., 2021)

The extract presented strong antioxidant activity as confirmed by DPPH, ABTS, and FRAP assays. The IC_{50} values were consistently below 100 µg/mL, signifying potent radical scavenging and reducing capacity. Oxidative stress is a key barrier in diabetic wound healing; therefore, the presence of antioxidant-rich phytochemicals such as palmitic acid may contribute to the protective effects observed *in vivo*.

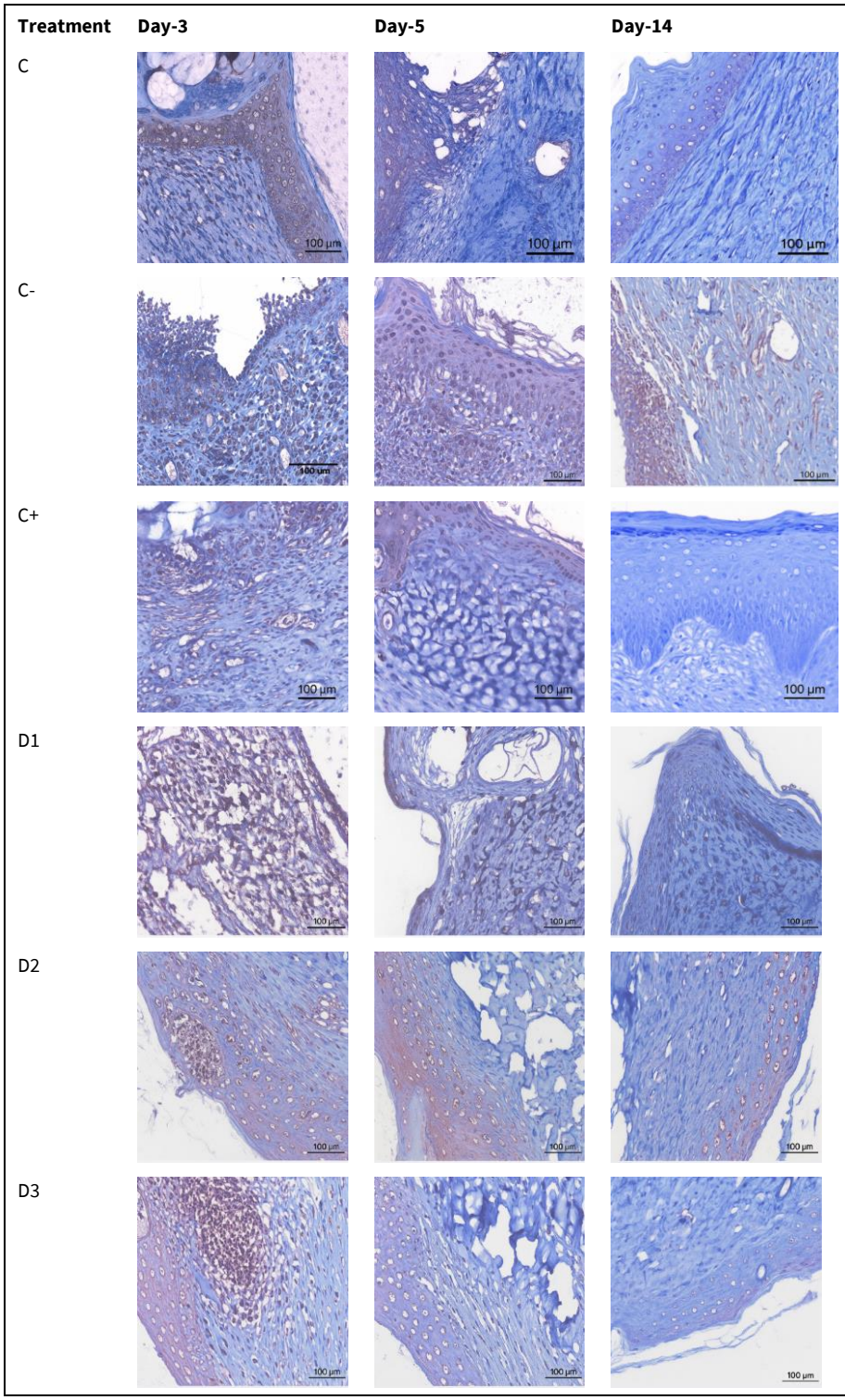


Figure 9. Immunohistochemical staining of PDGFβ expression in diabetic wound tissue treated with *G. procumbens* ethanolic extract.

C: normal untreated rats; C−: diabetic rats without treatment; C+: diabetic rats treated with MEBO; D1–D3: diabetic rats treated with *G. procumbens* extract at 100, 200, and 300 mg/kg BW, respectively. Positive PDGFβ expression is represented by brown chromogenic deposits in the cytoplasm of dermal and epithelial cells. Scale bar: 100 µm. Magnification: 400×.

Antioxidants help preserve cell viability and support the function of repair-related cells, including fibroblasts and keratinocytes. *G. procumbens* extract showed significant ferric-reducing, metal-chelating, and DPPH radical-scavenging properties *in vitro*, signifying the potential as a beneficial natural antioxidant source (Anggraini et al., 2018; Chandradevan et al., 2020). Studies reported that the leaves possessed a greater antioxidant capacity than stems and blooms, rendering the leaves a compelling subject for further investigation and potential medicinal applications. Besides the antioxidant capabilities, *G. procumbens* showed potential in wound healing, specifically for diabetic ulcers. Studies using animal models reported that the topical application of *G. procumbens* leaf extract markedly enhanced wound healing, augmented collagen deposition, and facilitated angiogenesis in rats with alloxan-induced diabetes (Anggraini et al.,

2018; Arulselvan et al., 2014). Moreover, new investigations show that *G. procumbens* has potent anti-inflammatory characteristics. A study suggests that the plant extract can markedly influence the expression of critical inflammatory mediators, such as platelet-derived growth factor (PDGF-β), transforming growth factor-beta (TGF-β), and interleukin-1 (IL-1β) (Anggraini et al., 2018). The abundant phytochemical constituents of *G. procumbens*, particularly n-hexadecanoic acid and several bioactive chemicals, are thought to facilitate the anti-inflammatory effects. These chemicals tend to impede the synthesis and secretion of pro-inflammatory cytokines, diminishing inflammation and facilitating tissue repair (Olszewska et al., 2021).

Uncontrolled inflammation is a hallmark of chronic diabetic wounds. In this study, *G. procumbens* significantly downregulated IL-1β expression, an early pro-inflammatory cytokine participating in neutrophil recruitment and tissue damage. The immunohistochemical results suggested that the extract contributed to inflammation resolution, corresponding with previous reports of anti-inflammatory effects mediated by phenolic compounds and fatty acids, thereby supporting the traditional use of *G. procumbens* in inflammatory conditions. In alloxan-induced diabetic rats, the effects of *G. procumbens* leaf extract on wound healing were investigated. The results showed that the extract markedly improved angiogenesis, augmented collagen deposition, and expedited wound closure, suggesting its potential as an agent for the treatment of diabetic wounds (Arulselvan et al., 2014; Rethinam and Venkatanarasimhan, 2020; Shrivastav et al., 2020).

The capacity of *G. procumbens* to modulate many biological processes, including inflammation, cellular proliferation, and tissue remodeling, has been associated with wound-healing attributes. The antioxidant components in the extract are thought to be crucial in mitigating oxidative stress and fostering a conducive environment for healing (Akmar and Mahanem, 2016; Arulselvan et al., 2014). Effective wound healing requires a balance between inflammation and regeneration. The extract increases the expression of PDGFβ to recruit fibroblasts and supports angiogenesis, as well as TGF-β, which regulates ECM deposition and tissue remodeling. Moreover, *G. procumbens* extract significantly alters the production of key mediators associated with inflammation and wound healing, such as TGF-β, PDGFβ, and IL-1β. The results provide essential data for the potential future applications of *G. procumbens* in the pharmaceutical sector while serving as an effective instrument for the rapid identification of promising antioxidant, anti-inflammatory, and wound-healing compounds from natural sources. The phytochemical contents of *G. procumbens*, specifically n-hexadecanoic acid and other bioactive chemicals, contribute to the significant antioxidant properties and efficacy of the plant in wound healing (Le et al., 2020). The modulation of critical inflammatory and tissue regeneration pathways by the extract shows the treatment potential for diverse wound-related diseases, including those associated with diabetes. These results establish a foundation for further investigations and possible application of *G. procumbens* in the formulation of natural, plant-derived healthcare and pharmaceutical products.

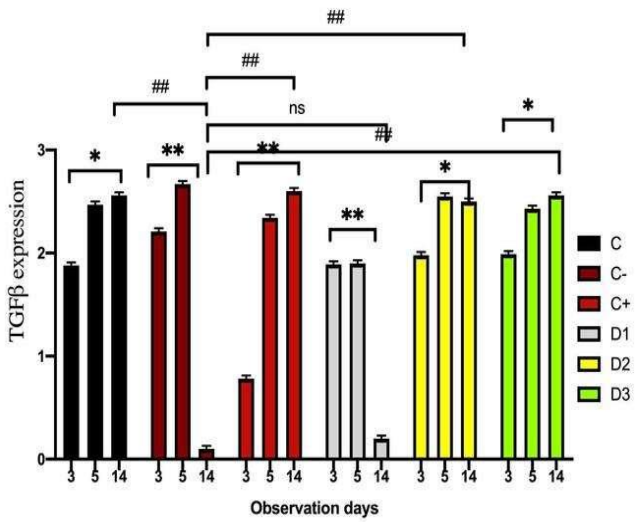


Figure 10. The expression of TGFβ in burn-injured rats after the administration of *G. procumbens* injected with alloxan.

C: Untreated rats, C-: Only induced by alloxan, C+: induced by alloxan + MEBO ointment. D1 + Induced by alloxan GB leaves at a dose of 100 mg/kg BW, D2: induced by alloxan GB leaves at a dose of 200 mg/kg BW, D3: induced by alloxan GB leaves at a dose of 300 mg/kg BW. The results are expressed as mean ± standard deviation (n = 6). (#p<0.05 vs C-, ##p<0.01 vs C-, *p<0.05; **p<0.01 compared day 3 with day 14 in the same group).

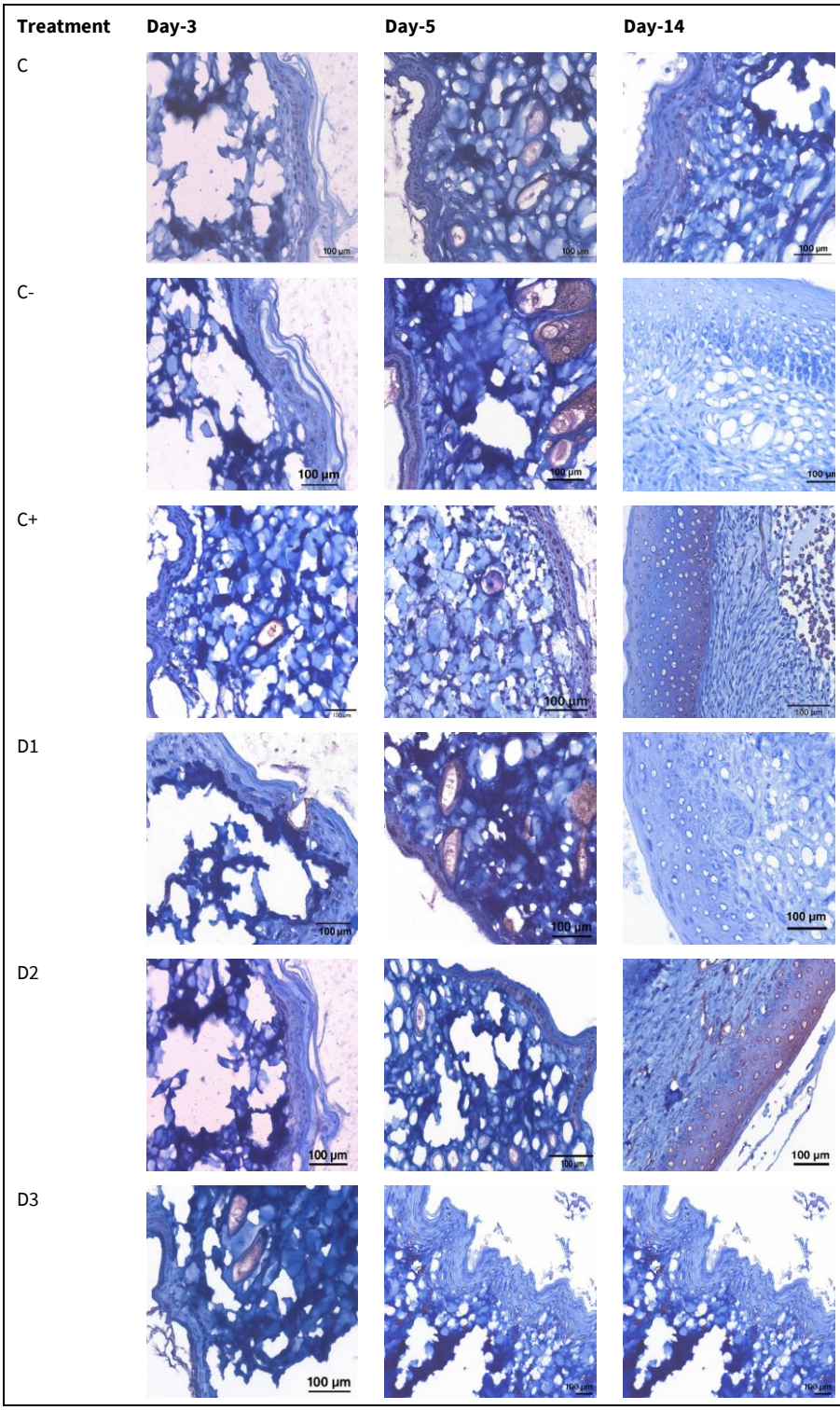


Figure 11. Immunohistochemical staining of TGF-β expression in diabetic wound tissue treated with *G. procumbens* ethanolic extract.

C: normal untreated rats; C−: diabetic rats without treatment; C+: diabetic rats treated with MEBO; D1–D3: diabetic rats treated with *G. procumbens* extract at 100, 200, and 300 mg/kg BW, respectively. Positive TGF-β expression is represented by brown chromogenic deposits in the cytoplasm of dermal and epithelial cells. Scale bar: 100 µm. Magnification: 400×.

Historically, natural sources including plants such as ginseng, turmeric, castor oil, neem bark, and rosemary, have been significant for wound healing. Approximately 70%, 20%, and 10% of active chemicals in commercial wound healing medications are sourced from plants, minerals, and animals, respectively. Over 13,000 chemical compounds were meticulously examined for the potential to enhance wound healing. Plant components that facilitate healing include flavonoids, glycosides, steroids, saponins, resins, and mucilages. *G. procumbens* is a plant possessing numerous advantageous bioactive chemicals (Patel et al., 2019). Multiple studies reported that *Ligusticum wallichii* could diminish inflammatory reactions, IL-1β-induced apoptosis, and the degradation of the ECM in rat chondrocytes. The GC-MS metabolic profile of the chondrocytes showed that *L. wallichii* therapy might alleviate the consequences of IL-1β activation. Compounds such as cholesterol, linoleic acid, hexadecanoic acid, proline, L-valine, L-leucine, pyruvate, and

palmitic acid have been found in this plant (Wei et al., 2020). The expression of the TGF-β gene is modulated by particular natural substances. The application of aloe vera gel promotes TGF-β gene expression, thereby facilitating the wound-healing process (Takzaree et al., 2016). A multitude of growth factors, matrix metalloproteinases (MMPs), cytokines, inflammatory cells, keratinocytes, fibroblasts, and endothelial cells effectively coordinate and synchronize the normal wound healing process.

Growth factors in the form of biologically active polypeptides are essential for all stages of the healing process. In the granulation phase of tissue formation, the initial phase of inflammation is amplified. Deficiencies in growth factors, regarding both type and quantity, are commonly observed in injured wounds due to altered expression, reduced production, diminished release, entrapment, or excessive degradation. Platelets secrete PDGF, a crucial serum mitogen that stimulates fibroblast proliferation, matrix synthesis, and connective tissue development. Macrophages, crucial in the late inflammatory phase of wound healing, continuously produce this growth factor. PDGF functions as a chemoattractant for fibroblasts and inflammatory cells, enhancing the synthesis of glycosaminoglycans, proteoglycans, and collagen. During the healing process, fibroblast migration and proliferation, granulation tissue protein synthesis, provisional ECM formation, and angiogenesis are necessary. The reduced expression of PDGF and the receptors in diabetic wounds shows the significance of these proteins in wound healing. Multiple clinical studies reported that PDGF utilization could enhance healing outcomes (Pastar et al., 2018). Both humans and diabetic animals show reduced TGF-β levels in wound tissue, perhaps leading to impaired wound healing. TGF-β is essential for the recruitment and activation of inflammatory cells, such as fibroblasts, keratinocytes, neutrophils, macrophages, and lymphocytes, in addition to facilitating the production of growth factors. This system aids in preserving the integrity of the ECM while facilitating vascularization, angiogenesis, and matrix synthesis. Decreased TGF-β levels have been associated with compromised wound healing in people experiencing diabetes (Hozzein et al., 2015).

Multiple studies show the promoter region of genes encoding MMPs harbors a TGF-β1-dependent inhibitory element that diminishes the expression of these genes. Growth factors are significantly degraded due to elevated matrix metalloproteinase expression and reduced TGF-β levels. Transcription factors, including Smad-2, Smad-3, and Smad-4, control TGF-β target genes. TGF-β1 induces collagen production through the activation of Smad-2 and Smad-3. Reduced TGF-β1 levels impede the transition from the inflammatory to the proliferative phase in diabetic wound healing (Chen et al., 2018). Diabetic patients with reduced levels of TGF-β1 possess elevated concentrations of TGF-β3, which enhances macrophage activity and diminishes collagen formation. Elevated macrophage activity in diabetes mellitus leads to an extended inflammatory phase and augmented production of reactive oxygen species (ROS). Consequently, people with diabetes show compromised and protracted wound healing attributable to diminished levels and expression of the growth factors (Elbialy et al., 2021). Natural substances possess the capacity to concurrently address fibrosis in many organs while suppressing fibrosis in specific organs, thereby providing new opportunities for the advancement of antifibrotic treatments (Chen et al., 2018; Situmorang and Ilyas, 2018). Extracts from medicinal plants and the extracted active components show significant potential as wound healing agents in many *in vivo* and clinical trials, due to the different mechanisms of action, antibacterial capabilities, and excellent safety profiles. Innovative wound dressing formulations have numerous benefits compared to traditional dressings and effectively mitigate certain drawbacks associated with natural materials, such as diminished efficacy at the wound area and solubility concerns (Yazarlu et al., 2021). *Spirulina platensis* has been reported to downregulate fibrotic genes (TGF-β1 and α-SMA) and upregulate angiogenic genes (bFGF and VEGF). When used topically, spirulina may function as a biomedical intervention for diverse skin lesions and enhance the healing process (Elbialy et al., 2021).

Several studies have comprehensively shown the medicinal flexibility of *G. procumbens*. The high antioxidant characteristics, capacity to facilitate wound healing, and propensity to diminish inflammation make *G. procumbens* a promising candidate for the formulation of natural, plant-derived pharmaceutical and healthcare products (Tan et al., 2016). Wound healing is a multifaceted biological process comprising three overlapping phases, including (1) inflammation, defined by immune cell recruitment and cytokine secretion, (2) proliferation, distinguished by fibroblast activation, angiogenesis, and re-epithelialization, and (3) remodeling, where collagen is reorganized and tissue strength is reinstated. The progression of these phases is governed by signaling molecules, such as cytokines and growth hormones, which the imbalance in diabetes leads to a persistent, non-healing wound. A disadvantage of this study is the lack of a vehicle control group using nano-chitosan devoid of the *G. procumbens* extract. The nanoencapsulation method was primarily developed to improve extract stability and bioavailability, but

the potential biological activity of chitosan cannot be entirely dismissed. Subsequent investigations should incorporate a chitosan-only group to identify and clarify the role of the delivery matrix in enhancing wound healing. Immunohistochemistry (IHC) offers spatial and semi-quantitative insights into cytokine expression in wound tissues, but there is a necessity for more accurate quantification.

A significant drawback of this study is the lack of further mechanistic experiments, including collagen quantification and examination of angiogenesis markers such as VEGF expression or CD31 immunostaining. Although this study focused on cytokine expression and histological enhancement as main indications of wound healing, direct assessment of ECM remodeling and vascularization may yield more robust mechanistic insights. Future investigations should incorporate hydroxyproline tests to measure collagen deposition and angiogenesis-related assays to thoroughly evaluate the functional effects of *G. procumbens* on tissue regeneration. Other investigations need to use biochemical tests, including ELISA and quantitative real-time polymerase chain reaction (qPCR), for the confirmation of the modification of IL-1β, PDGFβ, and TGF-β at the molecular level. The suggested methods will enhance the mechanistic comprehension and translational significance of the results. Despite showing the effectiveness of *G. procumbens* nano-chitosan extract in regulating crucial cytokines associated with diabetic wound healing, additional exploration is necessary. More studies should incorporate *in vitro* mechanistic experiments, including fibroblast proliferation and migration assays, for direct validation of cellular responses to the extract. Pharmacokinetic analysis and dose-dependent safety evaluations are crucial for identifying appropriate treatment ranges and ensuring translational relevance. Defining these criteria will provide a more robust foundation for the clinical advancement of *G. procumbens*-based formulations in chronic wound treatment.

Fig. 12 shows the potential process by which *G. procumbens* facilitates diabetic wound healing. The phytochemicals in the ethanolic extract, specifically antioxidant-rich components such as n-hexadecanoic acid, enhance cellular defense by reducing oxidative stress. The antioxidant activity is essential in inhibiting the development of the pro-inflammatory cytokine IL-1β, which is frequently increased in chronic diabetic wounds. The decrease in IL-1β expression facilitates the reinstatement of essential growth factors, particularly PDGFβ and TGF-β. PDGFβ promotes the recruitment and proliferation of fibroblasts, while TGF-β is crucial for collagen synthesis and ECM remodeling. The elevation of both growth factors leads to increased fibroblast activation, augmented collagen deposition, and a higher rate of re-epithelialization, which are crucial processes in wound closure and tissue regeneration. These molecular events collectively underscore the treatment potential of *G. procumbens* as a multifunctional agent for managing diabetic wounds.

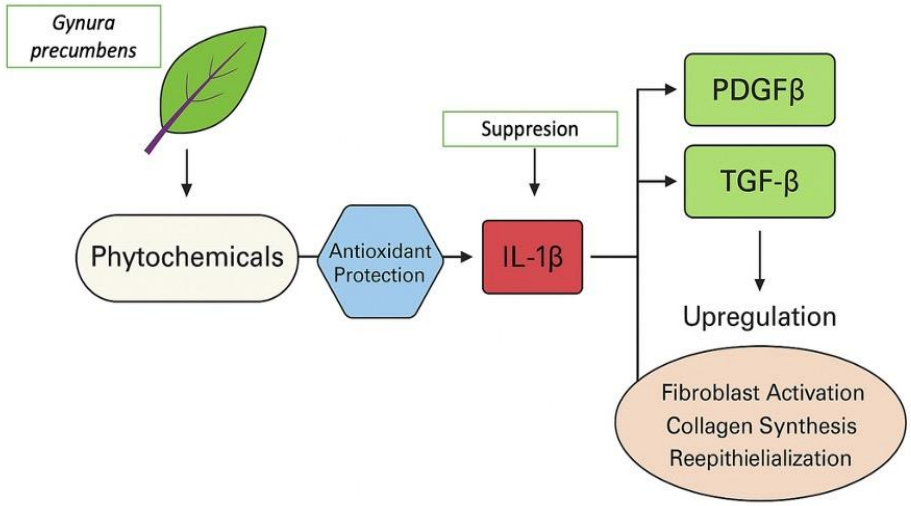


Figure 12. The mechanistic pathway of *G. procumbens* in promoting diabetic wound healing.

Phytochemicals from the ethanolic extract exert antioxidant protection, leading to suppression of the pro-inflammatory cytokine IL-1β. This downregulation facilitates the upregulation of PDGFβ and TGF-β, which are key growth factors included in fibroblast activation, collagen synthesis, and re-epithelialization. These molecular events collectively contribute to enhanced tissue regeneration in diabetic wounds.

CONCLUSION

This study showed that the ethanolic extract of *G. procumbens* significantly enhanced diabetic wound healing by modulating key cytokines involved in inflammation and tissue regeneration. Specifically, the extract reduced the expression of pro-inflammatory IL-1β while upregulating PDGFβ and TGF-β associated with fibroblast proliferation and ECM remodeling. These therapeutic effects could be attributed to the antioxidant-rich phytochemical profile of the extract, particularly the presence of n-hexadecanoic acid (palmitic acid) as the major constituent. The results provided a scientific basis for the traditional use of *G. procumbens* in wound care. Further studies should isolate and characterize the bioactive compounds as well as validate the results through well-designed clinical trials.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Concepts or ideas	x		x		
Design	x	x	x		
Definition of intellectual content	x		x		
Literature search	x	x		x	
Experimental studies	x	x	x		
Data acquisition					x
Data analysis	x	x			
Statistical analysis			x		
Manuscript preparation	x			x	x
Manuscript editing	x			x	
Manuscript review	x	x	x	x	x

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