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Therapeutic Potential of *Passiflora edulis* Sims Seed Extract on Molecular and Histological Markers of Wound Healing in a Diabetic Rat Model

Dimas Ning Pangesti[1,7,*], Ambar Mudigdo[1,2], Tatar Sumandjar[1,3], Eti Poncorini Pamungkasari[1,4], Paramasari Dirgahayu[1], Ratih Puspita Febrinasari[5] and Widyanti Soewoto[6]

[1]*Doctoral Program in Medical Sciences, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia*
[2]*Department of Anatomic Pathology Laboratory, Sebelas Maret University, Surakarta, Indonesia*
[3]*Department of Internal Medicine, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia*
[4]*Department of Public Health, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia*
[5]*Department of Pharmacology, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia*
[6]*Department of Surgical Oncology, Dr. Moewardi Regional General Hospital, Surakarta Solo, Indonesia*
[7]*Department of Health Sciences Institute Baitul Hikmah Lampung, Indonesia*

([*]Corresponding author's e-mail: dimasnp1305@gmail.com)

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Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia and oxidative stress, which contribute to vascular dysfunction, tissue damage, hypoxia, and an increased risk of foot ulceration and amputation. Uncontrolled hyperglycemia elevates reactive oxygen species (ROS), increases malondialdehyde (MDA) production, and activates the NF-κB signaling pathway, leading to the release of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6. This cascade promotes caspase-3 activation and inflammatory cell damage in keratinocytes and fibroblasts, impairing extracellular matrix formation and re-epithelialization. Although antioxidant defenses play a critical role in wound healing, scientific validation of natural topical agents remains limited. Seeds of Passiflora edulis Sims, rich in flavonoids, gallic acid derivatives, and antioxidant compounds with anti-inflammatory and antimicrobial properties, have been identified as promising candidates for wound therapy. This study investigated molecular pathways involving epidermal growth factor (EGF), MDA, TNF-α, collagen deposition, and epithelialization as key regulators of oxidative stress, inflammation, and tissue repair. A post-test-only experimental design was conducted using 25 male Wistar rats with streptozotocin-nicotinamide-induced diabetes, divided into 5 groups: Negative control (ointment base), positive control (Tribee salf), and 3 treatment groups receiving Passiflora edulis seed extract (PFSE) at doses of 50, 100, and 150 mg/g. Molecular markers were analyzed using ELISA (MDA, EGF, caspase-3), immunohistochemistry (TNF-α), and histological staining (Masson's Trichrome for collagen and hematoxylin-eosin for epithelialization). Data were statistically evaluated using ANOVA with Bonferroni post hoc, independent t-tests, and Kruskal-Wallis tests, with significance set at $p < 0.05$. The results demonstrated that PFSE significantly reduced MDA, TNF-α, and caspase-3 levels, while increasing EGF expression, collagen deposition, and epithelialization compared with the control groups ($p < 0.05$). These findings highlight the therapeutic potential of Passiflora edulis seed extract as an antioxidant, anti-inflammatory, and wound-healing agent for diabetic wounds.

Keywords: *Passiflora edulis* sims, *Passiflora edulis* sims seed extract (PFSE), Diabetes mellitus, Wound healing, Oxidative stress (MDA), Inflammation (TNF-α), Apoptosis (Caspase-3), Epidermal growth factor (EGF), Collagen deposition, Epithelialization

Introduction

Diabetes mellitus (DM) is a major global health concern [1]. In diabetic patients, the normal sequence of molecular and cellular events is disrupted, often resulting in delayed wound healing [2]. One of the most severe complications is diabetic foot ulcer (DFU), a condition with substantial worldwide impact [3]. It is estimated that 15% - 25% of individuals with diabetes will develop foot ulcers during their lifetime, with approximately 20% progressing to amputation [4]. The inflammatory phase, characterized by edema, pain, and swelling, occurs early in the healing process and is primarily mediated by inflammatory cells [5]. DFU represents the most critical form of diabetic wounds, frequently leading to lower-limb amputation or even death [6].

Under hyperglycemic conditions, increased levels of reactive oxygen species (ROS) activate the NF-κB pathway, resulting in elevated malondialdehyde (MDA) levels and the release of the pro-inflammatory cytokine TNF-α. Cell death receptors subsequently trigger caspase-3 signaling cascades [7]. Inflammatory resolution involves apoptosis through hyaluronan receptor CD44 expressed on cell surfaces, which becomes resistant to downregulation at high ligand concentrations [8]. Caspase-3, a key effector protease, executes the final steps of apoptosis and plays a central role in regulating programmed cell death [9].

Tissue regeneration is initiated by the proliferation of matrix-producing cells, driving repair through a process known as fibroplasia, which is marked by collagen deposition and angiogenesis [10]. Multiple cell types - including platelets, macrophages, fibroblasts, endothelial cells, and keratinocytes - release extracellular signals, such as epidermal growth factor (EGF), that activate fibroblasts [11]. Keratinocytes then migrate across the provisional clot matrix to close the wound, followed by proliferation at the wound edges [12]. The extracellular matrix (ECM) is crucial for re-epithelialization [10,13], a process further promoted by wound-associated mediators such as macrophage-derived nitric oxide, cytokines, and growth factors including EGF [14].

Delayed wound healing in diabetes mellitus is attributed to deficiencies in growth factors, abnormal cellular function, impaired angiogenesis and neovascularization, reduced collagen synthesis, and altered macrophage activity [15]. These impairments significantly increase morbidity and mortality, underscoring the urgent need for novel therapeutic strategies [16]. Previous studies have described the anti-inflammatory, antimicrobial, and wound-healing properties of Myrtus, with topical ointments derived from this plant shown to be effective against methicillin-resistant Staphylococcus aureus (MRSA) burn wound infections [17]. Many medicinal plants are traditionally used in local communities, such as *Hibiscus rosa-sinensis* L. leaves, which are applied to treat boils and skin inflammation [18]. Although synthetic drugs are widely used, their side effects have fueled growing interest in herbal alternatives, which are generally safer, more accessible, and more affordable [19]. A variety of alternative therapies - including honey, herbal extracts, and commercial ointments such as *Tribee salf* - have been traditionally utilized; however, most remain limited by insufficient clinical validation. A recent meta-analysis of topical herbal products indicated their therapeutic potential, though substantial heterogeneity across studies persists [20].

Passiflora edulis Sims, one of the most cultivated passion fruit varieties, has long been used in traditional medicine for its antibacterial, anti-inflammatory, and antioxidant activities [21]. In Indonesia, purple passion fruit (*Passiflora edulis* Sims) is predominantly grown in the highlands of North Sumatra, particularly in Berastagi, Karo Regency, a key horticultural production area with export potential [22]. According to the Central Bureau of Statistics, Indonesia produced 53,319 tons of passion fruit in 2020 [23]. The seeds are black, weighing 1.73 - 2.07 g per 100 seeds, with an average of 164.9 ± 17.98 seeds per fruit [24]. Reports have demonstrated that passion fruit seed extract (PSEE) possesses strong anti-inflammatory and antioxidant properties [25].

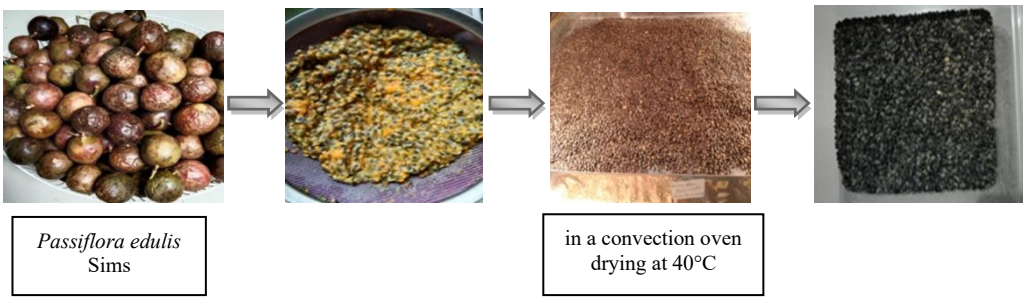
Materials and methods

Preparation and extraction of materials

Purple passion fruit (*Passiflora edulis* Sims) was obtained from Sidikalang, Dairi Regency, North Sumatra, Indonesia. From 52 kg of fruit, approximately 4,160 g of seeds were collected. Botanical identification and organoleptic evaluation were

performed by the Functional Service Unit for Research Support and Product Provision, RSUP Dr. Sardjito, Karanganyar Regency, Central Java. The specimen was authenticated as belonging to the family *Passifloraceae*, species *Passiflora edulis* Sims (*synonym Granadilla edulis* (Sims.) Ser.) under reference number TL.02.04/D.XI.6/22566.1062/2024.

(a) Preparation of purple passion fruit seed simplicia



(b) Extraction of purple passion fruit seed extract

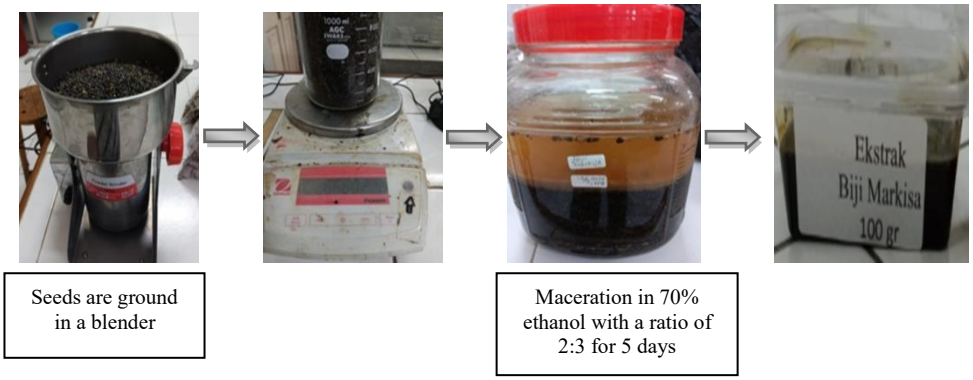


Figure 1 (a) Isolation and drying of purple passion fruit seeds at 40 °C. (b) Maceration of powdered seeds in 70% ethanol and concentration of the filtrate by evaporation at 40 - 50 °C to obtain the seed extract.

In **Figure 1(a)**, the simplicia preparation process involved separating the seeds from the fruit pulp, followed by drying in a cabinet dryer (or convection oven) at 40 °C, adapted from the method described by [26]. The extraction procedure was adapted from [21] with slight modifications (**Figure 1(b)**). Dried seeds were ground into a fine powder and subjected to maceration in 70% ethanol at a 2:3 (w/v) ratio for 5 days. The filtrate was concentrated using a rotary evaporator at 40 - 50 °C to obtain the seed extract. The extract was stored in aluminum foil–wrapped bottles at −20 °C for up to one month to maintain its stability and biomolecular integrity, according to [27].

Antioxidant activity assay of *Passiflora edulis* seed extract (DPPH method)

The antioxidant activity of *Passiflora edulis* seed extract (PFSE) was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, following the modified protocol of Handayani *et al.* [28]. A 0.15 mM DPPH stock solution was prepared by dissolving DPPH powder in absolute ethanol, and the maximum absorption wavelength (λ_max) was determined by scanning the absorbance of a DPPH-ethanol mixture in the 450 - 600 nm range using a UV-Vis spectrophotometer. The negative control consisted of 0.15 mM DPPH solution in ethanol. The PFSE sample was prepared by dissolving 20.16 mg of extract in ethanol, followed by sonication for 15 min, filtration, and dilution to obtain a clear stock solution. The optimal reaction time was determined by mixing the extract solution with the DPPH reagent and recording absorbance changes at λ_max over 0 - 60 min. The incubation time corresponding to stable absorbance was used for subsequent assays.

Serial dilutions of the extract stock solution were prepared to yield 6 different concentrations. Each concentration was reacted with an equal volume of DPPH solution and incubated at room temperature for the predetermined reaction time. Absorbance was then measured at λ_max, and the percentage of radical scavenging activity was calculated according to the equation:

$$\%\ Inhibition\ = \frac{A0 - As}{A0} \times 100$$

where $A0$ represents the absorbance of the negative control and As denotes the absorbance of the sample.

The half-maximal inhibitory concentration (IC_{50}) value, representing the extract concentration required to scavenge 50% of DPPH radicals, was determined by linear regression analysis of the concentration-inhibition curve. All measurements were performed in triplicate, and results were expressed as mean ± standard deviation (SD).

Preparation of *Passiflora edulis* Sims seed extract (PFSE) ointment

Topical ointment formulations containing Passiflora edulis seed extract have been studied in clinical and experimental settings [29] with slight modifications. PFSE was incorporated at concentrations of 5% (F1, 50 mg/g), 10% (F2, 100 mg/g), and 15% (F3, 150 mg/g) [30]. The formulation consisted of an ointment base (vaseline, lanolin, or a combination), nipasol as a preservative (0.01 g), and jasmine essential oil as a fragrance (0.06 g) [31,32]. The base ingredients were melted in a water bath at approximately 60 °C and transferred into a preheated mortar to prevent rapid solidification [33]. Subsequently, PFSE, nipasol, and jasmine essential oil were added to the molten base and mixed gently until a smooth and homogeneous consistency was achieved [32,34]. The final preparation was allowed to cool, filled into sterile ointment jars, and properly labeled [34]. Three formulations (50, 100 and 150 mg/g) were produced and evaluated for physical characteristics, stability, and wound-healing efficacy in a diabetic rat model.

Experimental animals and induction of the diabetic rat model

Male Wistar rats (10 weeks old, 180 - 200 g) were used to establish the diabetic model. The animals were housed under standard laboratory conditions with a BR-1 diet and unrestricted access to purified water. Prior to induction, rats were fasted for 12 h while water was provided ad *libitum* [35]. Diabetes was induced via intraperitoneal injection of streptozotocin (STZ, 45 mg/kg) combined with nicotinamide (NA, 110 mg/kg), both dissolved in sodium citrate buffer. Three days after STZ-NA administration, fasting blood glucose levels were measured from retro-orbital sinus samples using a glucometer [13]. Rats with fasting glucose levels exceeding 300 mg/dL were classified as diabetic [19]. All experimental procedures adhered to the principles of the 3Rs - Replacement, Reduction, and Refinement - to ensure compliance with high standards of laboratory animal research. This approach underscores the scientific validity of animal models in biomedical studies while maintaining animal welfare, laboratory safety, and environmental integrity [36]. Ethical approval for all experimental protocols was obtained from the Animal Ethics Committee, Faculty of Medicine, Sebelas Maret University, Surakarta (Approval No. 238/UN27.06.11/KEP/EC/2024).

Punch biopsy wound model and treatment protocol

Wound induction was performed after confirmation of persistent hyperglycemia, defined as a fasting blood glucose level exceeding 300 mg/dL. All invasive procedures were performed under an approved Institutional Animal Ethics and Use Committee protocol and in accordance with standard rodent survival surgery guidelines [37]. Prior to surgery, the researchers wore personal protective equipment, including laboratory coats, masks, head covers, and sterile gloves, throughout the procedure. All surgical instruments, such as the punch biopsy device, anatomical and surgical forceps, tissue scissors, calipers, and animal hair clippers, were sterilized or disinfected before use. Supporting materials included sterile gauze, cotton, 0.9% NaCl, 1 mL syringes, autoclaved surgical tools, and 70% ethanol to ensure aseptic conditions. The surgical procedure was carried

out in a clean and disinfected environment that maintained sterility throughout the process.

Animals were anesthetized by intraperitoneal injection of ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (5 mg/kg) [2]. The dorsal surface of each rat was shaved using an animal clipper [33], and any remaining hair was removed with a depilatory cream applied for 30 - 60 s. The skin was subsequently cleaned and disinfected with 70% ethanol [39]. Full-thickness excisional wounds were created using a 6 mm sterile punch biopsy tool to a depth of approximately 2 mm, involving the epidermis and dermis but avoiding penetration of the underlying muscle or deeper tissues.

Topical treatment protocol with *Passiflora edulis* seed extract ointment

The topical application of *Passiflora edulis* seed extract (PFSE) ointment was conducted under standardized laboratory conditions following the establishment of the diabetic wound model. Before treatment, researchers wore appropriate personal protective equipment, including laboratory coats, masks, head covers, and sterile gloves, to ensure biosafety and minimize contamination risks during animal handling. All procedures were approved by the Animal Ethics Committee of the Center for Food and Nutrition Studies, Universitas Gadjah Mada (Approval No. 293/UN.1/PS.24/Adm.PSPG/TA.00.03/2025) and were performed in accordance with the Guidelines for Rodent Survival Surgery [37].

Therapeutic intervention began 1 day after wound induction with methicillin-resistant Staphylococcus aureus (MRSA), following the infection stabilization phase. The animals were randomly divided into 5 groups: A negative control group treated with ointment base only, a positive control group treated with commercial Tribee Salf ointment, and 3 treatment groups receiving PFSE ointment at concentrations of 50, 100, and 150 mg/g, respectively. Approximately 0.1 g of the assigned formulation was applied topically to each wound once daily using a sterile applicator dedicated to each animal to prevent cross-contamination. The treatment continued for 14 days, during which wound progression and healing were carefully observed.

Macroscopic wound evaluation included measurement of wound diameter using a digital caliper, as well as observation of tissue coloration, wound morphology, and the degree of epithelialization [40]. Microscopic and molecular evaluations were subsequently performed to elucidate the mechanisms underlying the healing process. Histological examinations included hematoxylin-eosin (HE) staining to assess epithelialization, Masson's Trichrome (MT) staining to evaluate collagen fiber deposition, and immunohistochemistry (IHC) to detect TNF-α expression. Additionally, biochemical markers associated with oxidative stress and tissue repair - malondialdehyde (MDA), epidermal growth factor (EGF), and caspase-3 - were quantified from wound tissue homogenates using an enzyme-linked immunosorbent assay (ELISA).

Wound healing progression was monitored until day 14, and all observations were systematically documented using standardized observation sheets. Data obtained from macroscopic, histological, and molecular analyses were subsequently compared across groups to determine the efficacy of PFSE ointment in enhancing wound closure and tissue regeneration in diabetic conditions. Comparable methodological approaches for topical herbal formulations in diabetic wound models have been reported in previous studies, demonstrating similar treatment timelines and evaluation parameters [41,42].

Tissue sampling and molecular analyses

Following anesthesia induction with ketamine (100 mg/kg BW) and xylazine (10 mg/kg BW), wound tissues were collected for molecular and histological analyses. Tissue sampling included the entire wound bed beneath the crust to ensure a representative assessment of the healing process. Samples were divided for biochemical assays (MDA, EGF, and caspase-3), immunohistochemical evaluation (TNF-α), and histological staining (collagen and epithelialization).

Measurement of MDA, EGF, and caspase-3 levels (ELISA)

Biomarker levels of malondialdehyde (MDA), epidermal growth factor (EGF), and caspase-3 were quantified from tissue homogenates using a

quantitative sandwich ELISA kit (FineTest®, Wuhan Fine Biotech Co., Ltd., China), following the manufacturer's protocol. Wound tissues were weighed and homogenized in ice-cold phosphate-buffered saline (PBS; 50 mM, pH 7.3) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) at a ratio of 1 g tissue to 9 mL buffer. Homogenates were stored at –80 °C, processed by ultrasonication on ice or subjected to 2 freeze-thaw cycles, and centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was collected for ELISA assays.

The ELISA procedure involved antigen capture by plate-coated antibodies, detection with HRP-conjugated secondary antibodies, and colorimetric development using TMB substrate, followed by termination with stop solution. Absorbance was measured at 450 nm using a microplate reader, and biomarker concentrations were calculated using the regression equation $y = 0.00980x + 0.75556$ (where y = absorbance, x = concentration, ng/mL). Each parameter was analyzed in triplicate (3 biological replicates, 2 technical replicates per sample), following a workflow comparable to that described by [41] for biomarker quantification in tissue-based studies

Immunohistochemistry analysis of TNF-α expression

Immunohistochemical (IHC) staining was performed to assess tumor necrosis factor-alpha (TNF-α) expression in wound tissues. Paraffin-embedded sections were deparaffinized, rehydrated, and subjected to antigen retrieval using citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide, followed by incubation with primary anti–TNF-α antibody overnight at 4 °C. HRP-conjugated secondary antibody and DAB chromogen were used for visualization. Hematoxylin counterstaining was performed for nuclear contrast, and the slides were mounted with a coverslip for microscopic evaluation at 400× magnification. TNF-α–positive inflammatory cells were quantified as a percentage of total inflammatory cells in 5 random fields per sample, following the approach described by Fauzi *et al.* [43].

Histological analysis of collagen deposition (masson's trichrome)

Collagen fiber density and organization were evaluated using Masson's Trichrome (MT) staining. Sections were deparaffinized, stained sequentially with Weigert's hematoxylin, Biebrich scarlet-acid fuchsin, and aniline blue solutions, and then dehydrated and mounted. Collagen fibers appeared blue, while cytoplasm and muscle tissue appeared red. Image analysis was performed using ImageJ software to calculate collagen area percentage within the wound region. The method was adapted from [44,45]

Evaluation of re-epithelialization (hematoxylin-eosin staining)

Hematoxylin-Eosin (HE) staining was used to assess epithelialization and general tissue morphology. Paraffin sections were stained with hematoxylin and eosin, dehydrated, and mounted for microscopic evaluation. The percentage of re-epithelialization was calculated based on the proportion of the epithelial layer covering the wound area relative to the total wound width, using the formula described by Vyver *et al.* [46]. This quantitative assessment reflected the degree of epidermal regeneration and wound closure.

Statistical analysis

Data obtained from 5 experimental groups - negative control, positive control, and 3 treatment groups - were analyzed after 14 days of wound treatment. Data distribution was first evaluated using the *Shapiro-Wilk* test for normality and Levene's test for homogeneity of variances. For datasets meeting both assumptions, one-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test was employed to determine significant differences among groups in the molecular parameters (MDA, caspase-3, and EGF). For datasets violating the assumptions of normality or homogeneity, the Kruskal-Wallis test was applied as a nonparametric alternative. When the *Kruskal-Wallis* test indicated significant group effects, pairwise comparisons were conducted using the independent samples t-test to identify specific group differences.

A p-value < 0.05 was considered statistically significant for all analyses, including the evaluation of TNF-α expression, collagen deposition, and

epithelialization parameters. Statistical analyses were performed using IBM SPSS Statistics software (version 23.0; IBM Corp., Armonk, NY, USA)

Results and discussion

Results

Antioxidant activity of passiflora edulis seed extract (DPPH assay)

Results of the Antioxidant Activity of Purple Passion Fruit Seed Extract are illustrated in **Figure 2**.

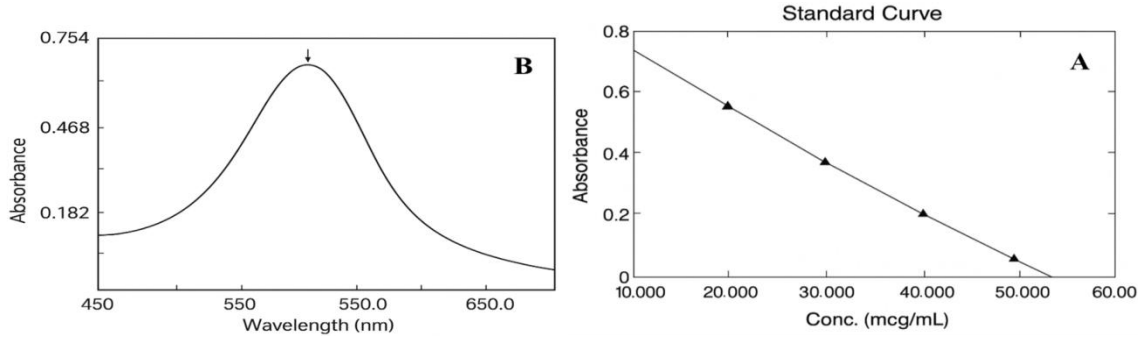


Figure 2 Representation of antioxidant activity based on the standard table report and spectrum peak report. (A) The standard calibration curve demonstrated a regression equation of $y = -0.00980\ x + 0.75556$ with a strong correlation coefficient ($r^2 = 0.99874$), indicating excellent linearity. (B) The UV-Vis spectrum was recorded within the wavelength range of 450 - 650 nm using a UV-1800 spectrophotometer in absorbance mode, with a slit width of 1.0 nm and fast scan speed.

Antioxidant activity was evaluated using the *2,2-diphenyl-1-picrylhydrazyl* (DPPH) assay. The *Passiflora edulis* Sims seed extract (PFSE) was dissolved in ethanol (p.a.) and prepared at various concentrations. Each solution was mixed with 0.15 mM DPPH solution, incubated for a predetermined operating time, and the absorbance was measured at the maximum wavelength of 516 nm. The percentage of free radical inhibition was calculated based on the decrease in DPPH absorbance, and the IC_{50} value was determined through linear regression analysis.

The results of the purple passion fruit seed antioxidant test were assessed using a standard curve and spectral analysis, with the results presented in **Table 1**.

Table 1 Antioxidant Activity of Purple Passion Fruit Seed Extract Expressed as $IC_{50} \pm$ SD (ppm).

No	Sample	$IC_{50} \pm$ SD (ppm) results
1	Purple passion fruit seed extract	41.017 ± 0.106 ppm

The findings demonstrated that purple passion fruit seed extract exhibited considerable antioxidant activity, with an IC_{50} value of 41.017 ± 0.106 ppm, indicating its capacity to neutralize free radicals. The mean IC_{50} of 41.017 ppm represents the concentration of extract required to scavenge 50% of DPPH radicals, while the associated variation (± 0.106 ppm) reflects the standard deviation (SD) or standard error (SE) calculated from experimental measurements. Previous studies have emphasized the importance of dose-response curves in determining IC_{50} values, underscoring that the reporting of error or uncertainty (variance or deviation) is essential for ensuring the reliability of such measurements [47].

Evaluation of wound healing in diabetic rats

After 14 days of treatment, clear differences in wound healing were observed among groups. Rats treated with *Passiflora edulis* seed extract (PFSE) ointment exhibited significantly improved healing

compared to the negative control, characterized by smaller wound diameters, reduced erythema, minimal exudate, and nearly complete epithelial coverage. The 100 and 150 mg/g PFSE group demonstrated the most pronounced wound contraction and tissue restoration, comparable to the positive control (*Tribee Salf*). In contrast, the negative control group showed persistent scab formation and delayed epithelialization. Statistical analysis confirmed that PFSE-treated groups had significantly greater wound closure ($p < 0.05$) in a dose-dependent manner. These results indicate that topical PFSE accelerates diabetic wound repair by enhancing epithelial regeneration and modulating inflammation.

Effect of PFSE on oxidative stress markers (MDA), apoptotic activity (caspase-3), and growth factors (EGF)

The Enzyme-linked Immunosorbent Assay (ELISA) was performed to measure MDA, EGF, and caspase-3 levels. The *Shapiro-Wilk* normality test applied to 5 groups (negative control, positive control, and 3 groups treated with PFSE at doses of 50, 100 and 150 mg/g) showed $p > 0.05$ for all parameters, indicating normally distributed data. *Levene's test* for homogeneity of variances yielded $p = 0.361$ (MDA), $p = 0.282$ (caspase-3), and $p = 0.932$ (EGF), suggesting no significant variance differences among groups. Accordingly, the data were considered homogeneous and fulfilled the assumptions for parametric analysis. Therefore, 1-way ANOVA followed by *Bonferroni post hoc tests* was conducted.

The quantitative results of MDA, caspase-3, and EGF measured by ELISA are presented in **Figures 3** - **5**. As shown in the figure, there were significant differences among the groups. The measurement of MDA, EGF and caspase-3 levels using ELISA revealed significant differences across treatment groups.

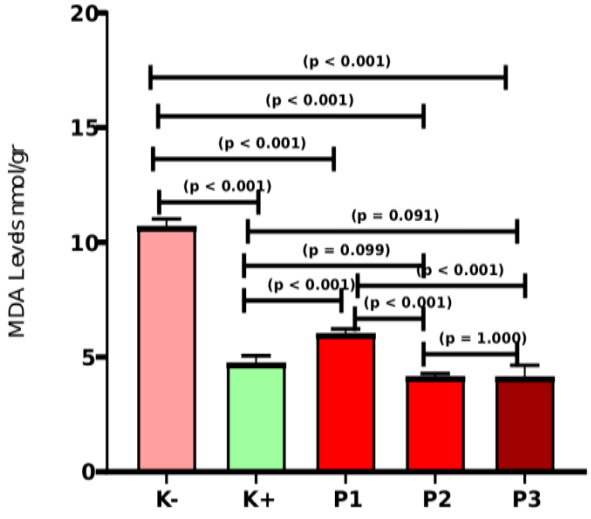


Figure 3 Comparison of treatment groups' levels of MDA. MDA (Malondialdehyde), K−: Negative control (ointment base), K+: Positive control (*Tribee Salf*), P1: Treatment 1 (50 mg/g extract), P2: Treatment 2 (100 mg/g extract), P3: Treatment 3 (150 mg/g extract).

The highest mean ± SD level of MDA was observed in the negative control group (10.69 ± 0.33 nmol/g), followed by the positive control (4.74 ± 0.33 nmol/g), treatment 1 (6.01 ± 0.22 nmol/g), treatment 2 (4.13 ± 0.15 nmol/g), and treatment 3 (4.13 ± 0.52 nmol/g). One-way ANOVA revealed a significant difference among the groups ($p < 0.05$). *Post hoc Bonferroni* analysis confirmed that treatments 2 and 3 significantly reduced MDA levels compared with the negative control, positive control, and treatment 1 ($p < 0.001$), whereas no significant difference was found between treatment 2 and treatment 3 ($p = 1.000$).

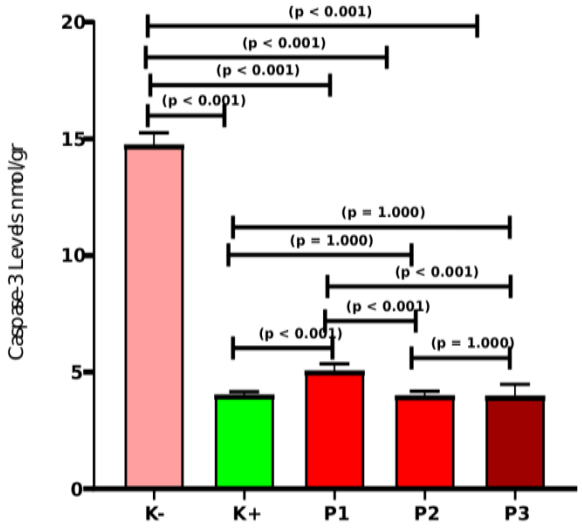


Figure 4 Comparison of treatment groups' levels of Caspase 3. K−: Negative control (ointment base), K+: Positive control (*Tribee Salf*), P1: Treatment 1 (50 mg/g extract), P2: Treatment 2 (100 mg/g extract), P3: Treatment 3 (150 mg/g extract).

For caspase-3, the highest mean level was observed in the negative control group (14.73 ± 0.52 nmol/g), followed by the positive control (4.02 ± 0.14 nmol/g), treatment 1 (5.04 ± 0.30 nmol/g), treatment 2 (3.98 ± 0.20 nmol/g), and the lowest in treatment 3 (3.96 ± 0.52 nmol/g). One-way ANOVA revealed significant differences among the groups ($p < 0.001$). *Post hoc Bonferroni* analysis showed that treatments 1 and 2 differed significantly from the negative control ($p < 0.001$), but not from treatment 3 ($p = 1.000$).

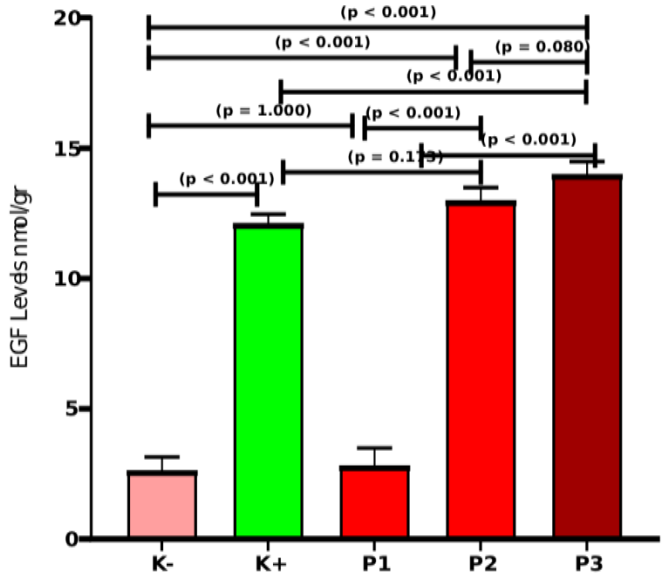


Figure 5 Comparison of treatment groups' levels of EGF. EGF (Epidermal Growth Factor), K−: Negative control (ointment base), K+: Positive control (*Tribee Salf*), P1: Treatment 1 (50 mg/g extract), P2: Treatment 2 (100 mg/g extract), P3: Treatment 3 (150 mg/g extract).

Conversely, EGF levels showed an increasing trend, with the lowest values observed in the negative control group (2.60 ± 0.55 nmol/g) and treatment 1 (2.80 ± 0.68 nmol/g), while higher levels were recorded in the positive control (12.09 ± 0.38 nmol/g), treatment 2 (12.97 ± 0.52 nmol/g), and treatment 3

(13.97 ± 0.52 nmol/g). One-way ANOVA revealed significant differences among the groups ($p < 0.001$). *Post hoc Bonferroni* analysis indicated that treatments 2 and 3 differed significantly from the negative control and treatment 1 ($p < 0.001$), whereas no significant difference was found between treatments 2 and 3 ($p = 0.080$).

These findings suggest that *Passiflora edulis* Sims seed extract (PFSE) has the potential to reduce oxidative stress and apoptosis while enhancing the expression of epidermal growth factor (EGF), thereby promoting wound healing in diabetes.

Effect of PFSE on inflammation (TNF-α), collagen deposition, and epithelialization

Microscopic analysis of TNF-α, collagen, and epithelialization levels showed that the data did not meet assumptions of normality and homogeneity. Therefore, the nonparametric *Kruskal-Wallis test* was applied as an alternative to 1-way ANOVA. The analysis revealed significant group differences, and subsequent independent samples t-tests were performed to evaluate pairwise comparisons for TNF-α, collagen deposition, and epithelialization. Data are presented as mean ranks with interquartile ranges in **Tables 2** - **4**, while the comparisons of TNF-α, collagen, and epithelialization are visualized in (**Figures 6**, **8** and **10**). TNF-α expression was converted into percentages (%), calculated by comparing positively stained inflammatory cells with the total inflammatory cells in each replicate, evaluated across 5 microscopic fields at 400× magnification [43]. Histopathological assessment of collagen density with Masson's Trichrome staining was performed using *ImageJ* software, where collagen deposition was quantified in blue-stained areas and expressed as a percentage [44,45]. For hematoxylin–eosin (HE) staining, the percentage of re-epithelialization was used as a measure of wound closure [46], with the re-epithelialization rate calculated using the following formula:

$$Re - epithelialization\ (\%) = \frac{st \times 100}{so}$$

Description: St - residual wound area at a given time and S0 - initial wound area [3].

Table 2 Results of TNF-α analysis (Median [IQR]).

Parameter	K− (n = 5)	K+ (n = 5)	P1 (n = 5)	P2 (n = 5)	P3 (n = 5)
TNF-α (pg/mL)	60.0 (20.0)	2.0 (0.10)	60.0 (40.0)	62.0 (constant)	62.0 (constant)

Table 3 Collagen analysis results (Median [IQR]).

Parameter	K− (n = 5)	K+ (n = 5)	P1 (n = 5)	P2 (n = 5)	P3 (n = 5)
Kolagen (µg/mL)	32.6 (8.55)	98.99 (0.66)	58.37 (4.81)	87.32 (6.97)	90.52 (14.68)

Table 4 Analysis of Epithelialization Results (Median [IQR]).

Parameter	K− (n = 5)	K+ (n = 5)	P1 (n = 5)	P2 (n = 5)	P3 (n = 5)
Epitelisasi (%)	9.47 (2.72)	12.0 (constant)	16.0 (3.44)	93.22 (5.59)	98.94 (6.17)

Data are presented as median (interquartile range); groups with constant values are presented as single values.

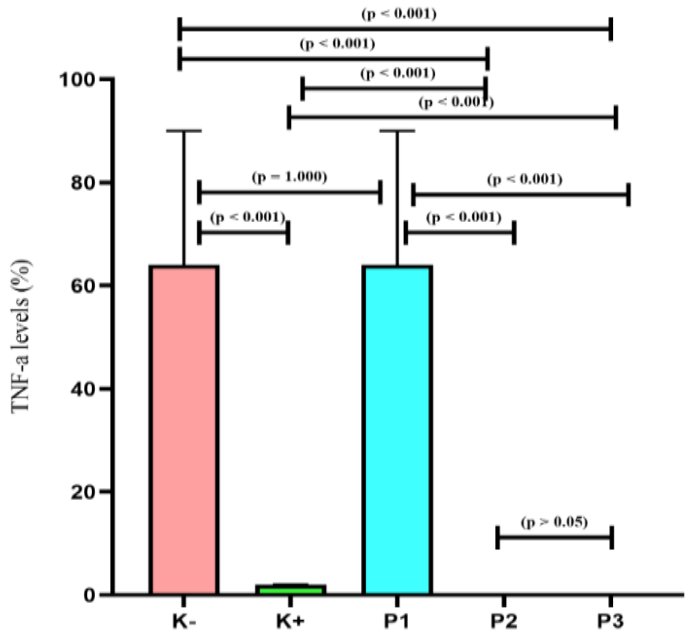


Figure 6 Comparison of TNF-α expression levels among treatment groups. TNF-α (tumor necrosis factor-α); K−: Negative control (ointment base), K+: Positive control (*Tribee Salf),* P1: Treatment 1 (50 mg/g extract), P2: Treatment 2 (100 mg/g extract), P3: Treatment 3 (150 mg/g extract).

Median TNF-α levels (**Table 2**) were 60.0 (20.0) in the negative control group and 60.0 (40.0) in Treatment 1 (P1). The *Kruskal-Wallis test* indicated no significant overall difference among groups ($p > 0.05$). *Independent t-test* analysis showed no significant difference between Treatments 2 and 3, suggesting comparable efficacy. Both treatments, however, significantly reduced TNF-α levels compared with the negative control, positive control, and Treatment 1 ($p < 0.001$; **Figure 6**). Immunohistochemical observations confirmed weaker TNF-α staining in Treatments 2 and 3, indicating suppression of the inflammatory response by *Passiflora edulis* seed extract (**Figure 7**).

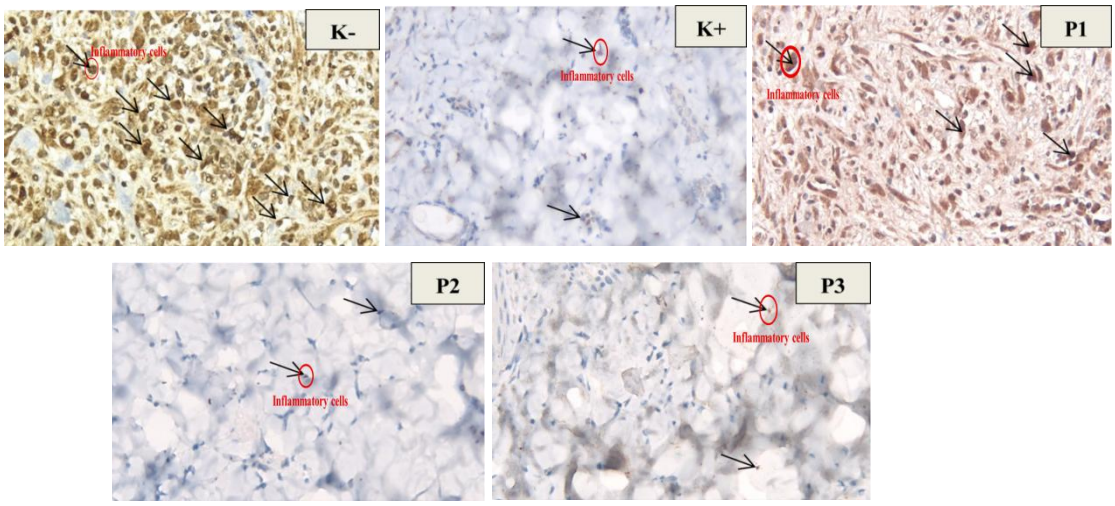


Figure 7 Immunohistochemical staining of TNF-α in dermis from diabetic rat wound tissue (400× magnification).

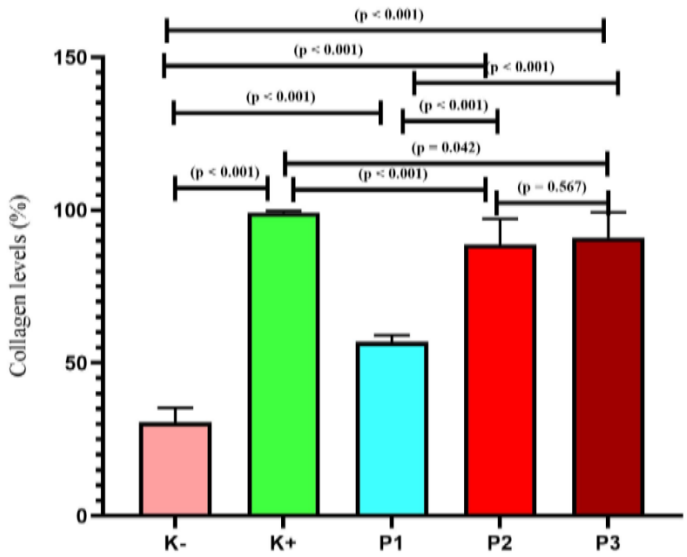


Figure 8 Comparison of collagen levels in the treatment groups. K−: Negative control (ointment base), K+: Positive control (*Tribee Salf*), P1: Treatment 1 (50 mg/g extract), P2: Treatment 2 (100 mg/g extract), P3: Treatment 3 (150 mg/g extract).

For the collagen parameter (**Table 3**), the negative control group exhibited the lowest median value of 32.6 (8.55), whereas the treatment groups showed markedly higher levels, with Treatment 2 at 87.32 (6.97) and Treatment 3 at 90.52 (14.68), approaching the positive control group at 98.99 (0.66). Statistical analysis using the *Kruskal-Wallis test* revealed a significant increase in collagen deposition in all treatment groups compared with the negative control ($p < 0.001$). Pairwise comparisons showed no significant difference between Treatment 2 and Treatment 3 ($p = 0.567$), indicating comparable efficacy of both doses in enhancing collagen deposition relative to the negative control and treatment 1 at 58.37 (4.81), although still slightly lower than the positive control ($p = 0.042$; **Figure 8**). Furthermore, Masson's Trichrome staining demonstrated denser and more organized collagen fibers in the treatment groups compared with the negative control, corroborating the extract's role in promoting fibroplasia [48] (**Figure 9**).

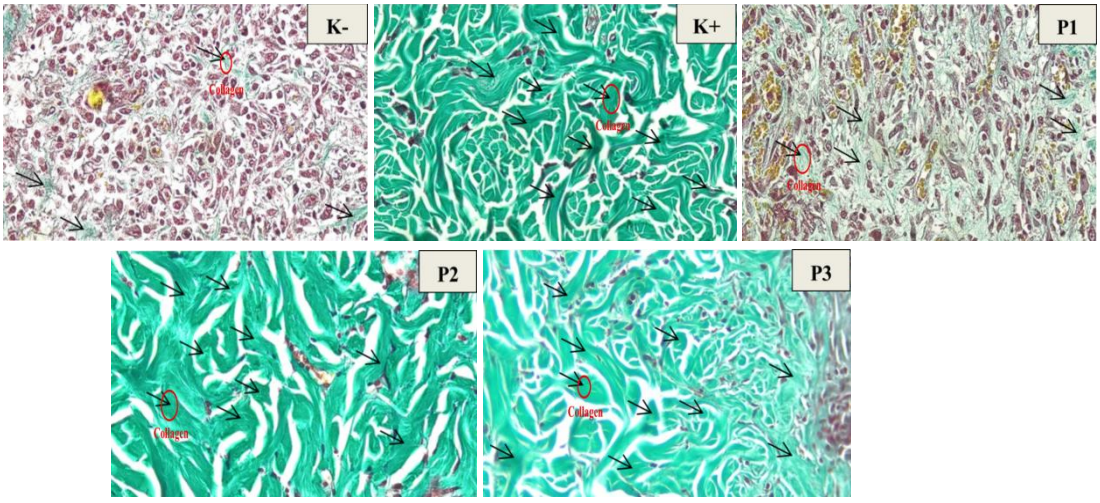


Figure 9 Photomicrograph depicting collagen deposition in the dermal layer of a diabetic rat wound biopsy, visualized using Masson's Trichrome (MT) staining at 400× magnification.

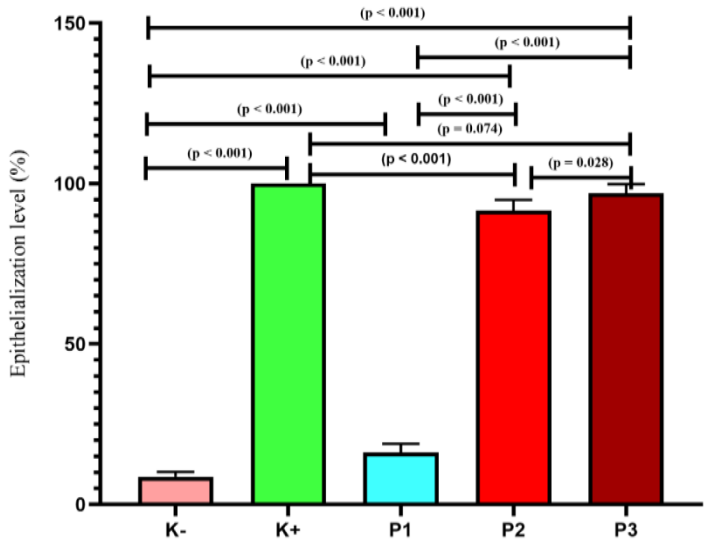


Figure 10 Comparison of epithelialization levels in the treatment groups. K−: Negative control (ointment base), K+: Positive control (*Tribee Salf*), P1: Treatment 1 (50 mg/g extract), P2: Treatment 2 (100 mg/g extract), P3: Treatment 3 (150 mg/g extract).

For the epithelialization parameter (**Table 4**), the negative control group exhibited the lowest median value of 9.47 (2.72), whereas Treatment 1 reached 16.0 (3.44). Treatment 2 and Treatment 3 showed markedly higher epithelialization levels of 93.22 (5.59) and 98.94 (6.17), respectively, approaching the positive control group (12.0). Statistical analysis using the *Kruskal-Wallis test* revealed significant differences among the groups ($p < 0.001$). Pairwise comparisons indicated that Treatments 2 and 3 significantly enhanced epithelialization compared with the negative control and Treatment 1 ($p < 0.001$). Moreover, a significant difference was observed between Treatment 2 and Treatment 3 ($p = 0.028$), while no significant difference was detected between the positive control and Treatment 3 ($p = 0.074$) in **Figure 10**. Histological examination with hematoxylin-eosin (HE) staining confirmed the formation of a continuous and mature epithelial layer in the treatment groups, comparable to that of the positive control. These findings indicate that Passiflora edulis seed extract effectively accelerates the re-epithelialization process in diabetic wounds (**Figure 11**)

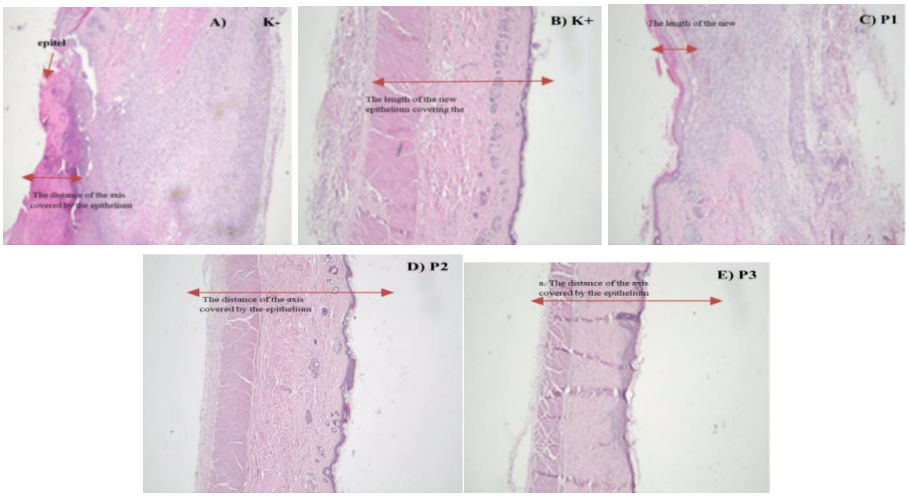


Figure 11 Photomicrograph of epithelialization in the dermis of a diabetic rat wound model stained with hematoxylin and eosin (HE) at 40× magnification.

Purple passion fruit seed extract exhibited significant potential in promoting wound healing in diabetic rats by downregulating TNF-α expression, enhancing collagen deposition, and accelerating epithelialization. The most pronounced therapeutic effects were observed at concentrations of 100 and 150 mg/g, which demonstrated comparable outcomes to the positive control.

Discussion

This study focused on the effects of *Passiflora edulis* Sims seed extract (PFSE) on wound healing in an infected diabetic rat model through molecular and histological approaches. The parameters analyzed included oxidative stress biomarkers (MDA), inflammatory mediators (TNF-α), apoptosis markers (Caspase-3), and tissue growth factors (EGF), combined with the evaluation of collagen deposition and the degree of epithelialization as indicators of tissue regeneration. The results demonstrated a reduction in MDA, TNF-α, and Caspase-3 levels, accompanied by an increase in EGF expression, collagen formation, and accelerated epithelialization, particularly at concentrations of 100 and 150 mg/g.

These findings confirm that PFSE exhibits antioxidant, anti-inflammatory, and cytoprotective activities that support diabetic wound healing. This is consistent with the report by Fazil *et al.* [5], which stated that the majority of topical wound healing agents are derived from natural sources, with 70 drugs documented in the reviewed literature. Furthermore, Sukketsiri *et al.* [25] demonstrated that Passiflora edulis seed extract possesses higher antioxidant and anti-inflammatory activity compared to extracts derived from the fruit. Therefore, this study not only reinforces the evidence that PFSE acts as an antioxidant and anti-inflammatory agent but also contributes to scientific knowledge regarding its regenerative effects on tissues, particularly epithelialization and collagen deposition, which may approximate the wound healing profile observed in the positive control.

The antioxidant activity assay using the *2,2-difenil-1-pikrilhidrazil* (DPPH) method provided empirical evidence that PFSE possesses the ability to neutralize free radicals. The principle of this method is based on the scavenging reaction of DPPH radicals, indicated by a color change from purple to yellow as a result of hydrogen atom or electron donation from antioxidant compounds [49]. This activity is reflected in the IC_{50} value, which serves as a quantitative indicator of antioxidant potential, where a lower IC_{50} corresponds to stronger radical scavenging capacity. These findings provide a scientific basis for further exploration of the role of PFSE in modulating oxidative stress in diabetic wound models.

Antioxidants function as the primary defense line against free radicals and play an essential role in supporting wound healing processes [49]. The topical administration of antioxidants to the skin is considered an effective strategy to prevent oxidative stress [50,51], while also exhibiting strong antibacterial properties [21]. Consistent with previous studies, the antioxidant activity of a compound can be classified based on its IC_{50} value [28]. Based on the results of this study, the IC_{50} value of *Passiflora edulis* Sims seed extract (PFSE) was recorded at 41.017 ± 0.106 ppm, thereby falling into the category of very strong antioxidant activity (IC_{50} < 50 μg/mL) according to the classification by Hartaman *et al.* [52]. This finding underscores that PPFSE possesses a high radical scavenging capacity, comparable to other potent antioxidants, and supports its potential in mitigating oxidative stress under diabetic wound conditions.

The levels of malondialdehid (MDA), caspase-3, and epidermal growth factor (EGF) were assessed using the enzyme-linked immunosorbent assay (ELISA), a method recognized for its high sensitivity and specificity in detecting biomarkers in biological tissues [53]. The results demonstrated that administration of PFSE at doses of 100 mg/g (treatment 2) and 150 mg/g (treatment 3) significantly modulated oxidative stress markers, apoptosis, growth factors, collagen deposition, and epithelialization in the diabetic wound model. The observed reduction in MDA levels in treatment groups 2 and 3 indicates an antioxidant effect capable of suppressing lipid peroxidation through the attenuation of oxidative stress [54]. This finding is consistent with Nakhate *et al.* [15], who reported that decreased MDA levels reflected reduced lipid peroxidation due to the antioxidant potential of A. catechu extract. Importantly, excessive

accumulation of MDA promotes pro-inflammatory mediators and enhances lymphocyte activation, thereby exacerbating wound complications in diabetes [55]

In line with these findings, the reduction of caspase-3 levels in the treatment groups indicates that PFSE plays a role in inhibiting the apoptotic pathway. Caspase-3 is recognized as a key protease responsible for executing the final stages of apoptosis during programmed cell death [9]. In diabetes, increased apoptosis renders wounds more susceptible to infection and delays the healing process. This is consistent with reports that impaired diabetic wound healing is associated with excessive apoptosis and reduced fibroblast viability [50]. Polyphenolic compounds present in passion fruit seeds, including anthocyanins, phenolic acids, flavones, and flavonols, exhibit strong antioxidant properties that neutralize free radicals [56]. Such activity supports a more balanced regulation of apoptosis, thereby preserving epithelial and fibroblast survival and promoting tissue regeneration. The consistent reduction of caspase-3, particularly in treatment groups 2 and 3, reinforces the potential of PFSE as an apoptosis-modulating agent in diabetic wound conditions. These observations are further supported by recent studies demonstrating that topical application of flavonoid-rich extracts exerts anti-inflammatory effects by blocking the production of reactive oxygen species (ROS), enhancing the expression of antioxidant enzymes such as superoxide dismutase (SOD), and reducing caspase-3 levels [57].

Conversely, the increased EGF levels observed in the treatment groups highlight the role of PFSE in stimulating growth factors associated with epithelial cell proliferation and differentiation. EGF levels are known to decline at wound sites due to protease activity and persistent infection [55]. With its bioactive compounds and antioxidant potential, PFSE may activate the PI3K/Akt and MAPK/ERK signaling pathways, which are critical for transducing signals that drive the synthesis of growth factors such as EGF. In particular, the PI3K/Akt pathway plays an essential role during the inflammatory and proliferative phases of wound healing, underscoring the beneficial effects of PFSE bioactive constituents as antioxidants, anti-inflammatory agents, and antimicrobials, including their therapeutic potential in diabetic wound repair [58]. Moreover, EGF regulates free radical production by keratinocytes, thereby stimulating cell proliferation and accelerating inflammatory resolution. It also enhances the expression of antioxidant defense enzymes by upregulating gene transcription, thus protecting cells from the deleterious effects of superoxide radicals [59]. The findings of this study demonstrate that treatment groups 2 and 3 elicited responses comparable to the positive control, strengthening the hypothesis that purple passion fruit seed extract could serve as a supportive therapeutic strategy in the management of diabetic wounds.

The findings of this study demonstrate that administration of *Passiflora edulis* Sims seed extract (PFSE) significantly suppressed TNF-α expression in treatment groups 2 and 3 compared with the negative control and treatment group 1. This reduction in TNF-α levels indicates modulation of the inflammatory response, given that TNF-α is a key pro-inflammatory cytokine responsible for prolonging the inflammatory phase and exacerbating tissue damage in diabetic wounds. These results are consistent with the report of Fauzi *et al.* [43], which showed that elevated TNF-α levels under diabetic conditions are associated with NF-κB pathway activation and increased inflammatory mediators that hinder the wound healing process.

Histologically, immunohistochemical analysis at 400× magnification revealed more intense TNF-α staining in the negative control group (**Figure 7**), reflecting persistent infiltration of inflammatory cells. The staining appeared as brownish deposits within the endothelial cell cytoplasm [60]. Conversely, treatment groups 2 and 3 exhibited reduced staining intensity, supporting the quantitative data that PFSE effectively suppressed pro-inflammatory cytokine production. The correlation between decreased TNF-α levels and the histological findings reinforces the hypothesis that bioactive compounds in PPFSE exert antioxidant and anti-inflammatory activities, thereby accelerating the transition from the inflammatory to the proliferative phase.

These observations are also in line with the findings of Luka *et al.* [10], who emphasized that resolution of the inflammatory response is a prerequisite for successful tissue regeneration. Furthermore, Didier *et al.* [61] in Antioxidants reported that passion fruit, which is rich in vitamins and

antioxidant compounds, is capable of counteracting free radicals that contribute to cellular damage.

In the collagen parameter, the positive control group as well as treatment groups 2 and 3 showed a significant increase in collagen deposition compared to the negative control and treatment group I, although the values remained slightly lower than those of the positive control. Histological analysis using Masson's Trichrome staining, assessed with *ImageJ* software at 400× magnification, demonstrated denser and more organized collagen fibers in the positive control, treatment group 2, and treatment group 3. Conversely, collagen deposition appeared thinner and more irregular in the negative control and treatment group 1. In this staining, collagen fibers were observed as greenish-blue structures. Masson's Trichrome is a well-established connective tissue staining technique widely employed to visualize supporting tissue elements, particularly collagen, in histopathological evaluation [62].

Collagen serves as the main component of the extracellular matrix, playing a pivotal role in tissue regeneration and wound strength. Our findings demonstrate that PFSE administration, particularly at 10% and 15%, enhanced collagen deposition compared to negative control, supporting fibroplasia and matrix remodeling. This aligns with [48], who reported that polyphenols, flavonoids, and anthocyanins stimulate fibroblast activity and extracellular matrix synthesis. Moreover, the transition from type III to type I collagen observed during wound repair [63] likely explains the improved tensile strength and structural organization in the treated groups. These results suggest that PFSE facilitates the proliferative and remodeling phases, ultimately accelerating wound repair, consistent with previous reports on natural antioxidants in tissue regeneration [64].

Re-epithelialization is a critical marker of wound healing, as keratinocytes migrate across granulation tissue to close the wound surface [65]. In this study, groups 2 and 3 demonstrated significantly enhanced epithelial coverage, closely resembling the positive control. Histological evaluation with hematoxylin-eosin staining confirmed broader epithelial layers in these groups, whereas the negative control and group I exhibited minimal or partial closure. These findings indicate that PPFSE facilitates keratinocyte proliferation and accelerates the transition from inflammation to tissue remodeling. Consistent with [46], the promotion of epithelialization reflects the extract's ability to provide a favorable antioxidant and growth factor-mediated environment essential for wound closure.

Overall, the findings of this study confirm that *Passiflora edulis* Sims seed extract (PFSE) is capable of modulating key pathways involved in diabetic wound healing. PFSE significantly reduced oxidative stress biomarkers (MDA), pro-inflammatory cytokines (TNF-α), and apoptotic markers (Caspase-3), while enhancing the expression of tissue growth factors (EGF). In addition, PPFSE promoted collagen deposition and accelerated re-epithelialization, as evidenced histologically by denser collagen fibers and broader epithelial coverage resembling the positive control group. The most optimal effects were observed at doses of 100 and 150 mg/g, demonstrating wound healing profiles comparable to the positive control.

These results suggest that PFSE holds promise as a natural therapeutic agent with antioxidant, anti-inflammatory, cytoprotective, and regenerative activities in supporting wound healing under diabetic conditions. Previous studies have also reported that purple passion fruit seeds contain flavonoids, phenols, tannins, and saponins, which contribute to the suppression of inflammation. Phenolic compounds, in particular, are known for their antioxidant activity in inhibiting lipid peroxidation and neutralizing lipid peroxyl radicals [66]. Thus, PFSE has potential to be developed as a natural adjuvant therapy in the management of diabetic wounds.

This study has several limitations. First, the research was conducted on an animal model, and therefore the findings cannot be directly generalized to humans. Second, the evaluated parameters focused primarily on selected biomarkers and histological analyses, without exploring more complex molecular pathways such as NF-κB, MAPK, or TGF-β regulation. In addition, the use of purple passion fruit seed extract did not involve the isolation of specific bioactive compounds, making it unclear which constituents play the most dominant role.

Conclusions

The purple passion fruit seed extract (*Passiflora edulis* Sims seed extract, PFSE) demonstrated significant potential in accelerating diabetic wound healing through antioxidant, anti-inflammatory, cytoprotective, and regenerative activities. These effects were evidenced by reductions in MDA, TNF-α, and caspase-3 levels, along with increased expression of EGF, collagen deposition, and re-epithelialization, particularly at optimal doses of 100 - 150 mg/g, which were comparable to the positive control. These findings underscore the therapeutic potential of PFSE as a natural adjuvant therapy for diabetic wound management. Further studies are warranted to isolate and characterize its active compounds, evaluate a broader dosage range, and elucidate the underlying molecular mechanisms. In addition, chronic toxicity testing and well-designed clinical trials are essential to confirm its long-term safety and efficacy before broader clinical application**.**

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Declaration of generative AI in scientific writing

The authors declare that generative artificial intelligence tools (e.g., QuillBot and ChatGPT) were used solely to improve the clarity of language and grammar during the preparation of this manuscript. The authors are solely responsible for the scientific content, data interpretation, and conclusions presented in this article

CRediT author statement

Dimas Ning Pangesti: Conceptualization, Methodology, Validation, Funding Acquisition, and Writing – original draft. **Ambar Mudigdo**: Supervision, Data curation, Formal analysis, Investigation, Validation, and Visualization. **Tatar Sumandjar**: Data curation, Formal analysis, Investigation, Validation, and Visualization. **Eti Poncorini Pamungkasari**: Data curation, Formal analysis, Investigation, Validation, and Visualization. **Paramasari Dirgahayu**: Data curation, Investigation, Validation, and Visualization. **Ratih Puspita Febrinasari**: Methodology, Project administration, Resources, Validation. **Widyanti Soewoto**: Validation and Visualization.

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