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Activity Of 70% Ethanol Extract From Moringa Olefera Leaves (Moringa oleifera Lam.) On Wound Healing In Hyperglycemia Rats

Sarah Zaidan*, Novi Yantih, Diah Kartika Pratami, Aiy Diah Ayu Pengasih

Universitas Pancasila

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*Correspondence: Sarah Zaidan
Email:
sarah.zaidan@univpancasila.ac.id

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Abstract: This study aimed to evaluate the wound healing profile in hyperglycemic rats treated with 70% ethanolic extract of Moringa oleifera Lam. leaves compared with povidone iodine, to determine the most effective extract concentration, and to assess the quality of the leaf extract. The extract was prepared using 70% ethanol maceration and evaluated for yield, moisture content, pH, total flavonoid content, and quercetin levels. Twenty-five rats were divided into five groups: negative control, positive control (povidone iodine), and extract concentrations of 5%, 10%, and 15%. A 2-cm dorsal incised wound was created on each rat, followed by once-daily topical application for 14 days. Macroscopic observations included wound closure, scab formation, and hair growth. Quality evaluation showed a yield of 23.8% (≥ 9.2%), moisture content of 4.05% (< 10%), pH of 5.70 (4.5–6.0), total flavonoid content of 2.58 mg QE/g, and quercetin content of 0.69%. Wound-healing analysis demonstrated significant differences between groups, with a p-value of 0.001 ($p < 0.05$). The mean percentage of wound-area reduction was 95% for the 5% extract, 94.71% for the 10% extract, 95.87% for the 15% extract, and 90.37% for the positive control group. The 15% extract was the most effective in accelerating scab formation and hair growth. In conclusion, especially at the 15% concentration, the ethanol extract of Moringa oleifera leaves demonstrated good extract quality and successfully accelerated wound healing in hyperglycemic rats.

Keywords: Moringa Leaves, Extract, Diabetes, Wound Healing, Scabs, Mice

Introduction

The prevalence of diabetes mellitus, a serious worldwide health issue, keeps rising year. According to WHO forecasts, Indonesia's diabetes population would almost treble from 8.4 million to 21.3 million cases by 2030 (PERKENI, 2021). According to the International Diabetes Federation (IDF) 2024 study, Indonesia has 20.4 million diabetics between the ages of 20 and 79, placing it fifth in the world. By 2050, this number is predicted to rise to 28.6 million, of which 15 million are anticipated to go undiagnosed (IDF, 2025).

Diabetes-related wounds are a complicated clinical disease marked by decreased angiogenesis, which leads to poor oxygen and nutrition delivery, protracted inflammation, and extracellular matrix degradation that prevents tissue regeneration. In addition to posing a major financial burden, diabetic wounds significantly lower patients' quality of life and raise the chance of limb amputation (Soliman AM et al, 2018).

Appropriate wound-care techniques that speed up tissue repair and avoid delayed healing have a significant impact on the success of wound healing. Appropriate antiseptics are frequently necessary for effective wound care. Because of their broad-spectrum antibacterial activity, povidone iodine and normal saline are frequently used in conventional wound treatment. However, a number of studies have shown that povidone iodine may prevent neutrophil migration and has cytotoxic effects on leukocytes and fibroblasts. Particularly in diabetes circumstances where immunological and vascular disorders are already present, these effects may worsen wound healing (Lachapelle JM et al, 2016).

Saponins, tannins, alkaloids, and flavonoids are among the many secondary metabolites found in Moringa oleifera Lam. leaves, with quercetin being the main active ingredient. By reducing inflammation, boosting fibroblast proliferation, promoting collagen synthesis, and offering antimicrobial activity that promotes epithelialization, this phytochemical has been shown to aid in wound healing (Marhaeni LS, 2021).

According to research by Mirsa Herdiani et al, M. oleifera leaf extract, especially at a concentration of 15%, has a beneficial effect on wound healing (Herdiani et al, 2022). Comparable outcomes were also shown in a diabetic rat model by Nurhikmah Awaluddin et al. (Awaluddin et al, 2024).

Based on these findings, the current investigation tests the ethanol extract of Moringa oleifera leaves' capacity to accelerate the healing of incised wounds in vivo using Sprague Dawley rats.

Methodology

Three groups that received 5%, 10%, and 15% doses of Moringa oleifera ethanol extract, a negative control group (wounds left untreated), and a positive control group (wounds treated with 10% povidone iodine). The dependent variables that were measured were hair growth, scab formation, and the percentage of wound-area decrease. After the extract was given, these parameters were selected to evaluate the wound-healing response. This study was conducted over four months at several cooperating labs and establishments, such as the Herbarium Depokensis (UIDEP), Department of Biology, Universitas Indonesia; the Indonesian Spice and Medicinal Crops Research Institute (BALITRO), Bogor; the School of Veterinary Medicine and Biomedical Sciences, Bogor Agricultural University; the Pharmacology Laboratory, Faculty of Pharmacy, Pancasila University; and Bogor's Q-LAB Testing and Research Laboratory.

Tools and Materials

The tools used in this study included a TLC Scanner 3 (CAMAG, Switzerland), vacuum pan evaporator (Ogawa, Japan), 870 KF Titrino Plus (Metrohm, Switzerland), food dehydrator (IRASTAR, China), maserator (IKA), pH meter, glucometer and test strips (NESCO Multicheck), analytical balance, maceration chamber, TLC plates (Merck, Germany), capillary tubes, shaving tools, scalpels, filter paper (Whatman No. 1, UK), rat cages, rat weighing scales, scissors, alcohol swabs, volumetric flasks, vials, measuring cylinders, beaker glasses, and drop pipettes.

The ingredients used included *Moringa oleifera* leaves, 70% ethanol (PT. Pancasakti Putra Kencana, Indonesia), 10% povidone iodine, ketamine HCl (PT. Ganesha Agrofarma, Indonesia), alloxan monohydrate (Sigma Aldrich, Singapore), quercetin standard (Sigma Aldrich, Singapore), 0.9% NaCl, toluene, ethyl acetate, methanol, formic acid, sodium acetate, $AlCl_3$, distilled water, male white rats (*Rattus norvegicus*), and standard feed and drinking water.

Research Procedure

1. Plant Determination: The Herbarium Depokensis (UIDEP), Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, was used to determine the leaves of Moringa oleifera.
2. Ingredient Gathering and Simplisia Powder Production: The Indonesian Spice and Medicinal Crops Research Institute (BALITRO), Bogor, provided the M. oleifera leaves used in this investigation as dried leaf powder that had been sieved through mesh No. 60.
3. Extract Making: The powdered leaves were placed into maceration jars and extracted with 70% ethanol at a simplicia-to-solvent ratio of 1:10. Maceration was carried out for 3 × 24 hours with occasional stirring, followed by filtration. The filtrate was concentrated using a vacuum drying system at temperatures not exceeding 60°C to obtain a thick extract.
4. Determine the Quality Parameters
   a) Organoleptic: Moringa oleifera leaf extract's form, color, and scent are examined.
   b) pH: The pH of Moringa oleifera extract is measured using a pH meter calibrated with pH buffers of 4.0 and 7.0. One gram of extract is diluted with 100 milliliters of aquadest, and the probe is dipped until it shows a constant pH value (Chandra et al, 2019).
   c) The moisture content of Moringa oleifera leaf extract is determined using the Karl Fischer Titrator (Metrohm 870 KF Titrino Plus). After ±50 mg of samples were put in a titration chamber with CombiMethanol-5 solvent, the moisture content readings were automatically recorded as a percentage (% w/w) of the sample weight (Aktivitas T et al, 2023).
5. Testing for Phytochemical Screening
   a) Two drops of Dragendorff reagent were added after 0.5 g of extract had been dissolved in 1 mL of HCl 2 N and 9 mL of aquades. The mixture was then allowed to sit for half an hour. The presence of orange deposits indicates that alkaloids are effective.
   b) Identification of Flavonoids: 5 mL of boiling water is used to dissolve up to 0.5 g of the extract, which is then heated for 5 minutes before being filtered. The mixture is stirred after 0.1 g of magnesium powder and 1 mL of concentrated HCl are added to 2 mL of filtrate. The appearance of red, yellow, or orange tones indicates the effectiveness of flavonoids.

c) Saponin identification involves dissolving one gram of the extract in ten milliliters of hot water, heating it for five minutes, and then filtering it. For ten seconds, five milliliters of cold filtrate are shaken quickly. One drop of HCl does not dissolve the stable foam, which is 1–10 cm high. When 2 N is introduced, saponins are functioning.

d) 10 mL of aqueducts are used to dissolve 0.5 g of extract, which is then filtered and diluted until it is colorless. One or two drops of 1% $FeCl_3$ are then added to a total of two milliliters of solution. A shift in color to green, blue, or blackish indicates positive tannin findings.

e) Polyphenol Identification: Ten milliliters of filtered and distilled water are used to dissolve 0.5 grams of extract. After the filter is combined with 10 mL of heated aquades and allowed to cool, three to four drops of 10% NaCl and a $FeCl_3$ solution are added. When polyphenol chemicals are present, the hue changes from bluish-green to black, indicating favorable outcomes.

f) Identification of Steroid/Triterpenoid: 10 mL of n-hexane was combined with a spatulaful of the extract sample, and the mixture was left to stand for 15 minutes. After filtering the combination, the filtrate was dried by evaporating it on a hot plate in an evaporating dish. Along the dish's inner wall, five drops of Liebermann-Burchard reagent were applied. Steroids were identified by the development of a green coloring, whereas terpenoids were indicated by the appearance of a purple coloring.

g) Identification of essential oil: Ten milliliters of the sample were evaporated in an evaporating dish over a water bath, and then ten milliliters of petroleum ether were added. A funnel containing wet cotton was positioned at the top of an Erlenmeyer flask containing the mixture. After ten minutes of heating and cooling, the solution was filtered via filter paper. After the filtrate was evaporated in an evaporating dish, the residue was dissolved in five milliliters of alcohol and then filtered once more. The resulting filtrate was evaporated, and an aromatic-smelling residue indicated the presence of essential oils (Wulandari et al, 2023).

6. Each year, 10 milligrams of the extract were diluted in methanol to determine the total flavonoid content. in a 10 mL volumetric flask and agitated for approximately 10 minutes, or until completely dissolved. A 10 mL volumetric flask was then filled with 1 mL of the stock solution, 1 mL of methanol, 1 mL of a 10% aluminum chloride solution, and 1 mL of a 1 M sodium acetate solution. Using pure water, the volume was adjusted to the proper level and properly blended. After the solution was allowed to stand for the specified optimal incubation period, the absorbance at the previously established maximum wavelength was measured using a UV-Vis spectrophotometer (Nurul Hidayati et al, 2023). Total flavonoid content was calculated using the method and expressed as milligrams of quercetin equivalent (QE) per gram of extract:

$$\%Total\ Flavonoids = \frac{Volume(L)\ x\ Concentration\left(\frac{mg}{L}\right)\ x\ Dilution\ Factor}{Sample\ Weight\ (g)}$$

7. Determination of Quercetin Content Using TLC–Densitometry: A silica gel GF254 plate (10 × 10 cm) was prepared and activated by heating in an oven at 100°C for 30 minutes. A spotting line was drawn 1 cm from the bottom edge and 0.5 cm from the top edge of the plate. Standard quercetin solutions and triplicate sample solutions were applied onto the spotting line using a standard capillary tube at a volume of 20 μL for each spot, with a spacing of 1 cm between spots. After the spots were allowed to air dry, the plate was raised to a height of 1 cm in a chromatography chamber that had already been saturated with the mobile phase. The mobile phase was permitted to ascend until it reached the upper boundary line after the chamber was sealed. After development, the chamber was opened and the plate was dried, then observed under UV light at 254 nm. The separated spots were subsequently scanned using a TLC–densitometer at 312 nm to obtain peak area values. The quercetin content was calculated using the linear regression equation $y = ax + b$ (Fatmawati et al, 2021).
8. 8. Twenty-five male Sprague Dawley strain white rats (Rattus norvegicus) served as test subjects. Based on visual assessments, they appeared healthy, active, and free of any structural abnormalities. The rats weigh between 170 and 200 grams and are 3 to 4 months old. Federer's formula was used to split the study into five treatment groups, each consisting of five mice (Ginting et al, 2022).

Information:

t = The quantity of therapies

n = Required number of repetitions

So $(n-1)\ (t-1) \geq 15$

$(n-1)\ (5-1) \geq 15$

$4(n - 1) \geq 15$

$4n \geq 19$

$N \geq 4.75 \sim 5$

The mice were acclimated to the cage setting for seven days in order to reduce stress and allow them to exhibit their normal behavior. Each group of rats was housed in five cages, each of which was an 80 cm² by 15 cm high plastic cage (BPOM, 2014) with a hardwood shaving base that had been sun-dried and cleaned with 0.5% NaCl to absorb dirt. Every day, boiled water and BR-2 standard feed are provided without restriction (Ollu et al, 2019).

9. Diabetes Induction: To induce diabetes mellitus in the experimental animals, 150 mg/kg body weight of alloxan monohydrate was administered intraperitoneally. To guarantee chemical stability prior to injection, alloxan was freshly synthesized in sterile normal saline. Following induction, the animals were monitored for clinical signs of hyperglycemia, and fasting blood glucose levels were reassessed on day 7 to verify successful induction. Rats were considered hyperglycemic when their fasting blood glucose concentration reached ≥200 mg/dL, which conforms to the accepted criteria for chemically induced diabetes models (Swastini Dewa et al, 2018).
10. Cutting Experimental Animals' Wounds: The rat's back fur is shaved using an animal fur cleaner the day before the incision is made. Rats were given an intraperitoneal

injection of 80 mg/kg BB of ketamine HCl to induce anesthesia (Noor et al, 2022). After the rat lost consciousness, the scalpel number 11 was sterilized with 70% ethanol, and an alcohol swab was used to clean the rat's back. A sterilized scalpel was used to make a 2 cm incision on a rat's back.

11. Wound Care: For 14 days, 0.5 mL of povidone iodine and Moringa oleifera leaf ethanol extract were given once daily (Mirsa Herdiani et al, 2022). The treatment groups were Group I (negative control, wound left untreated), Group II (positive control, 10% povidone iodine therapy), Group III (5% Moringa oleifera ethanol extract), Group IV (10% Moringa oleifera ethanol extract), and Group V (15 percent Moringa oleifera ethanol extract).
12. Macroscopic Examination: Macroscopic observation was performed visually each day for 14 days. Wound area was measured by determining the horizontal and vertical lengths using a digital caliper. To monitor wound healing, the wound area was measured at predetermined time points. The wound area was calculated using the ellipse formula:

$$L = \pi \times \frac{p}{2} \times \frac{l}{2}$$

Description:
L = wound area
p = wound length
l = wound width
π (phi) = 3,14 or $\frac{22}{7}$

The percentage of wound area reduction was calculated using the following formula:

$$\left(\frac{L0 - Ln}{L0}\right) \times 100\%$$

where L0 is the wound area on day 0 and Ln is the wound area on day n.
Daily wound photographs were taken using a digital camera to document scab formation and hair regrowth around the wound. Observations were recorded using the symbols “+” for the presence of scabs or new hair and “–” for their absence (Dita Primandari, 2019).

13. Data Analysis: The percentage of wound area decrease, scab development, and new hair growth at the wound site were among the data gathered. Kolmogorov-Smirnov and Levene's tests were used to check for normality and homogeneity. One-Way ANOVA and Bonferroni post hoc testing were used to investigate normally distributed and homogeneous data, whereas the Kruskal–Wallis test and Mann–Whitney test were used to analyze non-normal or non-homogeneous data.

Results and Discussion

Determination of Moringa oleifera

Plant determination was conducted prior to the study to confirm the authenticity and accuracy of the species used (Ismaurasi et al, 2024). Identification was performed at the Herbarium Depokensis (UIDEP), Department of Biology, Faculty of Mathematics and Natural Sciences, University of Indonesia. The specimen was classified under the family Moringaceae and identified as *Moringa oleifera* Lam.

Moringa oleifera Extraction

The extract was made by macerating 70% ethanol for three by twenty-four hours. Maceration was chosen because it preserves thermolabile bioactive substances like quercetin and phenolic components better because it is carried out at room temperature without heating (Sholikhati et al, 2023). Because 70% ethanol has a high water content, it promotes excellent cell penetration and increases extraction efficiency (Krisyanella et al, 2022).

The extraction yielded 23.8%, meeting the minimum requirement (≥9.2%) stated in the Indonesian Herbal Pharmacopoeia, Edition II (2017). This result indicates that the extraction process was optimal, influenced by solvent characteristics, active compound content, and extraction technique (Alfauzi et al, 2022).

Table 1. Yield Results of *Moringa oleifera* Leaves Extract

Name Simplisia	Weight of Thick Extract (grams)	Rendemen (%)	Kesimpulan
***Moringa oleifera* Lam.**	357	23,8	Meet the requirements (≥ 9,2%, FHI II)

Extract Quality Parameters

a. Organoleptic: According to the findings of organoleptic testing, Moringa oleifera's thick extract has a distinct aromatic odor, a thick consistency, and a blackish-brown hue (Kusriani et al, 2023).
b. Moisture Content: The extract exhibited a moisture content of 4.05%, remaining below the maximum allowable limit (<10%) according to the Indonesian Herbal Pharmacopoeia Edition II. Low moisture content indicates good extract stability, reduced microbial growth risk, and adequate storage quality (Nugraha et al, 2025).
c. pH: The pH of the extract was 5.70, within the ideal range for topical preparations (4.5–6.0). This pH supports wound healing, as alkaline environments may inhibit tissue regeneration and increase infection risk. pH variation is influenced by organic acids such as chlorogenic and acetic acids in the extract (Lukić et al, 2021).

Screening Phytochemistry

Phytochemical screening (Table 2) confirmed the presence of alkaloids, flavonoids, saponins, tannins, and polyphenols in the ethanolic extract of *Moringa oleifera* (Fauziah Matul et al, 2023). These metabolites contribute to wound healing via antimicrobial, antioxidant, anti-inflammatory, and collagen-stimulating activities (Satriyani Dpp, 2021; Singh, 2018).

Table 2. *Moringa oleifera* Extract Phytochemical Screening Test Results

Metabolite Seconds	Reagents	Parameter	Test Results
Alkaloid	HCl 2 N + Dragendorff	Orange deposits are formed	Positive (+)
	HCl 2 N + Wagner	Discoloration from red to orange	Positive (+)
Flavonoid	Mg Powder + amil alcohol + Concentrated HCl	Stable foam is formed 1–10 cm high that lasts a long time	Positive (+)
Saponins	Shake + HCl 2 N	Color change from green to blackish	Positive (+)
Tanins	$FeCl_3$	Blackish-green discoloration	Positive (+)
Polifenols	NaCl + $FeCl_3$	Orange deposits are formed	Positive (+)
Steroids/Triterpenoids	Lieberman-Burchard	Reddish ring (triterpenoid)	Positive (+)
Essential Oils	Petroleum Eter	No aromatic residue	Negative (-)

Determination of Total Flavonoid

Using UV-Vis spectrophotometry, absorbance was determined at 430 nm after 20 minutes of incubation. Results are shown in Table 3.

Table 3. Total Flavonoid Content of Extract

Replicate	Sample Weight (g)	Abs (A)	Concentration (ppm)	Flavonoid (mg QE/g)
1	0,01178	0,2717	0,0303	2,5797
2		0,2719	0,0304	2,5829
3		0,2714	0,0303	2,5747

Mean ± SD: 2.5791 ± 0.00415 mg QE/g

The analysis showed that the total flavonoid content of the ethanolic extract of *Moringa oleifera* leaves ranged from 2.5747 to 2.5829 mg QE/g extract, with a mean value of 2.5791 ± 0.00415 mg QE/g extract. The very low standard deviation indicates that the quantification method demonstrated high precision and reproducibility, thereby supporting the validity of UV–Vis spectrophotometry for determining flavonoid levels based on the aluminum chloride complexation reaction.

A flavonoid content of 2.58 mg QE/g suggests promising biological activity. Numerous studies have reported that flavonoids play key roles as antioxidants, anti-inflammatory agents, and protectors against free-radical-induced cellular damage. Therefore, this concentration indicates that *Moringa oleifera* leaf extract has potential to be developed as a natural raw material with pharmacological activity (Nurul Hidayati et al, 2023).

Quercetin Determination by TLC–Densitometry

Table 4. Quercetin Content in *Moringa oleifera* Extract

Area	Conc. (ppm)	% in 250 mg	Quercetin (g) in 357 g	Quercetin content (% w/w) per 100 g extract
1459,6	33,4217	0,17	0,5966	0,60
1987,9	48,4807	0,24	0,8654	0,87
2181	53,950	0,27	0,9636	0,60

Mean quercetin content: 0.69% ± 0.16 per 100 g extract

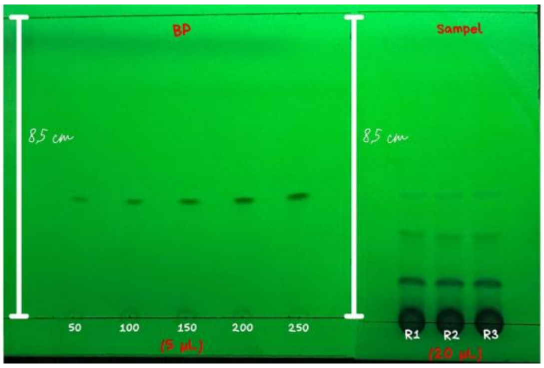

Figure 1. Chromatographic profile for quantitative determination using the quercetin standard and the ethanolic extract of *Moringa oleifera* leaves, eluted with the mobile phase toluene:ethyl acetate:formic acid (5:4:0.2) at a migration distance of 8.5 cm.

the The analysis showed that the samples exhibited varying quercetin levels, with concentrations of 0.17%, 0.24%, and 0.27% in 250 mg of extract. After standardization to a per-100-gram basis, the values were 0.60%, 0.87%, and 0.60%, yielding a mean quercetin content of 0.69% with a standard deviation (SD) of 0.16.

In the chromatogram (Figure 1), the spots obtained from the extract (in three replicates: R1, R2, R3) demonstrated comparable positions and intensities to those of the quercetin standard (BP). The similarity in spot location and size confirms the presence of quercetin in sample and indicates that the chromatographic conditions achieved optimal separation.

Potential of *Moringa oleifera* Ethanolic Extract in Incision Wound Healing

Over the course of a 14-day observation period, this study sought to determine whether the ethanolic extract of Moringa oleifera leaves could aid in the healing of incision wounds in male Sprague Dawley rats. The Health Research Ethics Committee, Faculty of Pharmacy, Universitas Pancasila, granted ethical permission for the study (No. 148/KEPK-FFUP/VII/2025). To protect the welfare and dignity of the test subjects, all procedures involving experimental animals were carried out in compliance with accepted ethical norms (Dewi et al, 2022).

Macroscopic Evaluation of Incision Wounds

Hemostasis, inflammation, proliferation, and remodeling (maturation) are the four different stages of the wound-healing process. The development of a blood clot that halts bleeding and shields the wound surface is what defines the hemostasis phase (days 0–1) (Ricardo et al, 2024). Redness, mild swelling, and the presence of exudate are characteristics of the inflammatory phase (days 1-3) (Sukma et al, 2025). In the groups treated with the ethanolic extract of *Moringa oleifera* leaves, wounds dried more rapidly and developed thicker scabs, indicating the onset of the proliferation phase (days 4–10), which involves granulation tissue formation, epithelialization, and angiogenesis (Aulia et al, 2024; Malaha et al, 2023). The remodeling phase (days 10–14) is characterized by scab detachment, formation of a new epidermal layer, and collagen reorganization. In the 15% extract group, scab detachment occurred earlier than in the other treatment groups, suggesting a more optimal wound-healing response (Salim et al, 2024).

Based on Figure 2, by day 14, the 15% *Moringa* extract group exhibited the greatest reduction in wound area (95.87%), followed by the 5% (95.00%) and 10% (94.71%) groups. All *Moringa* extract groups demonstrated better outcomes compared to the positive control (90.37%) and negative control (78.71%). These findings indicate that povidone–iodine was not significantly more effective than the *Moringa oleifera* ethanolic extract in accelerating wound closure.

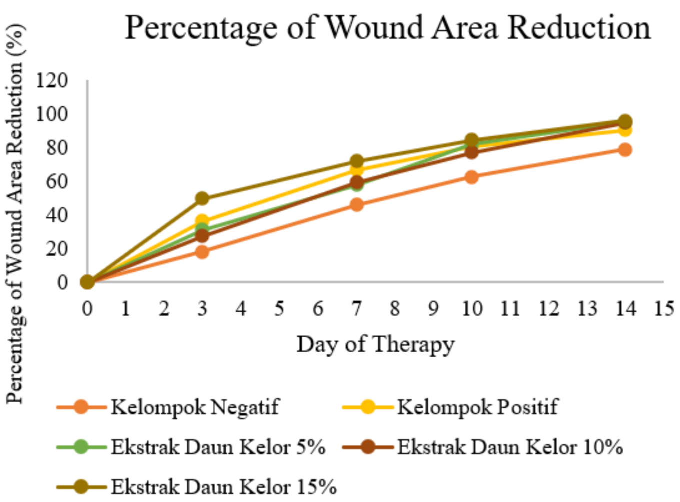

Figure 2. Mean Percentage Reduction in Wound Area

The Kruskal–Wallis analysis showed significant differences among the treatment groups ($p < 0.001$), and the Mann–Whitney post-hoc test confirmed that the 15% concentration differed significantly from the negative control, positive control, and the 5% extract group ($p = 0.021$). The 15% ethanolic extract of *Moringa oleifera* was the most effective in accelerating wound healing, likely due to its content of quercetin, tannins, and phenolic acids, which exhibit antioxidant, anti-inflammatory, and antibacterial properties that promote tissue regeneration and prevent infection (Al-Ghanayem Aa et al, 2022).

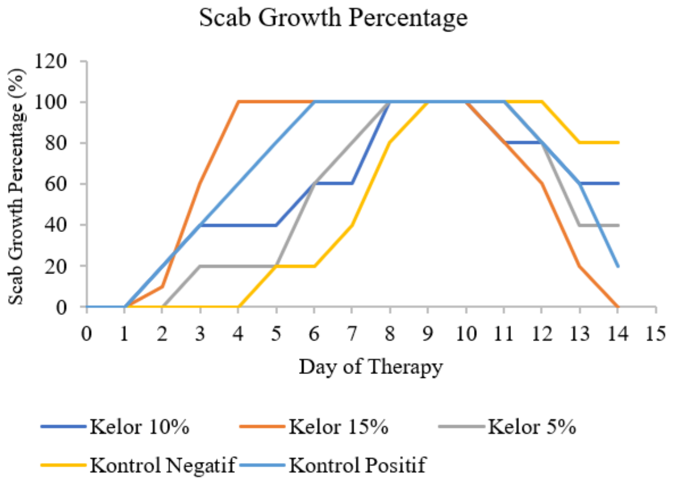

Figure 3. Percentage of Scab Formation

Based on the macroscopic observations of scab formation shown in Figure 3, the group treated with the 15% *Moringa oleifera* extract demonstrated the earliest scab detachment, with all rats showing complete scab removal by day 14. The 10% extract group followed, with partial scab detachment beginning on day 11. This indicates efficient resolution of inflammation and an optimal transition into the proliferative phase. In contrast, the negative control group showed delayed scab formation and detachment, with scabs remaining visible until day 12 and reaching 100% occurrence. These findings suggest that without treatment, the wound-healing process proceeds more slowly and less efficiently.

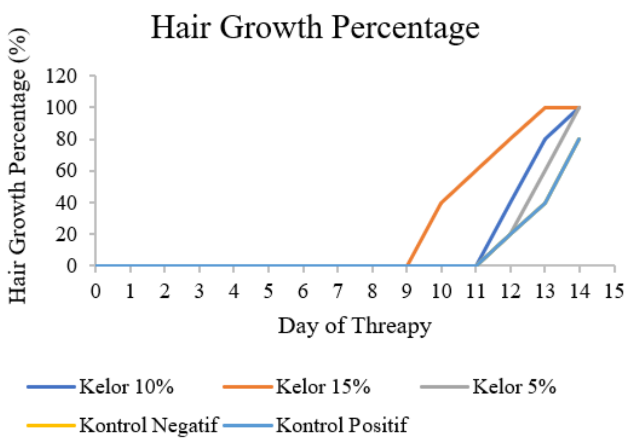

Figure 4. Percentage of Hair Regrowth

Based on the macroscopic observations of hair regrowth shown in Figure 4, the group treated with the 15% *Moringa oleifera* extract exhibited the earliest onset of hair growth, beginning on day 10 and reaching 100% regrowth by day 14. This indicates a rapid and uniform tissue regeneration process. The 10% extract group showed noticeable hair regrowth starting on day 13, achieving 100% by day 14, while the 5% extract group demonstrated initial hair growth on day 12. Compared with the control groups, clear differences were observed: the positive control group reached 80% hair regrowth on day 14, whereas the negative control showed a marked delay, with only 20% hair growth observed on day 13 as fine new hairs began to emerge. These findings indicate that *Moringa oleifera* extract effectively accelerates and enhances the quality of tissue regeneration during wound healing.

Conclusion

The ethanolic extract of Moringa oleifera leaves fulfilled the quality requirements according to the Indonesian Herbal Pharmacopoeia (FHI II), with an extraction yield of 23.8% (≥9.2%), moisture content of 4.05% (<10%), and pH of 5.70 (within the ideal topical range of 4.5–6.0). Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, polyphenols, and triterpenoids. Quantitative analysis demonstrated a total flavonoid content of 2.5791 ± 0.00415 mg QE/g extract and a mean quercetin level of 0.69% ± 0.16 (w/w) per 100 g extract, supporting its potential antioxidant and anti-inflammatory activity. In vivo evaluation on incision wounds in hyperglycemic male Sprague Dawley rats showed that the 15% extract concentration produced the highest wound area reduction (95.87%), exceeding the 5% (95.00%), 10% (94.71%), positive control (90.37%), and negative control (78.71%) groups, with statistically significant differences ($p < 0.001$; $p = 0.021$). The 15% group also demonstrated the earliest scab detachment and achieved 100% hair regrowth by day 14, indicating accelerated and more uniform tissue regeneration. Therefore, the 15% ethanolic extract of Moringa oleifera leaves can be considered the most effective concentration for promoting incision wound healing under hyperglycemic conditions. For future research, it is recommended to conduct histopathological and molecular analyses (e.g., collagen density, VEGF, TGF-β expression), perform dermal toxicity and stability studies, and develop standardized topical formulations to ensure consistent therapeutic efficacy and safety in potential clinical applications.

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