Jurnal Fitofarmaka Indonesia, 2026; 13(1) 29-38
https://journal-umi.com/jffi/index

Wound Healing Potential of *Artocarpus lanceifolius* Roxb. Methanolic Extracts in Hyperglycemic Wounds

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Article info
History
Submission: 22-08-2026
Review: 22-08-2026
Accepted: 25-09-2026

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DOI:
10.33096/jffi.v13i1.natkgp66

Keywords:
Artocarpus lanceifolius; methanol extracts; hyperglycemic wound; wound healing; in vivo

Abstract

The bark of Artocarpus lanceifolius Roxb. *has potential as a natural wound-healing agent because it contains secondary metabolites with various biological activities. This study aimed to evaluate the wound-healing activity of a methanol extract of A. lanceifolius bark in a hyperglycemic animal model. An in vivo study was conducted using hyperglycemic experimental animals that were subsequently induced with wounds. The animals were divided into a negative control group (Na-CMC), a positive control group (povidone-iodine), and treatment groups that received methanol extract of A. lanceifolius bark at concentrations of 10%, 20%, and 30%. Observations were made on days 0, 3, 7, 11, 12, and 14. The primary parameters evaluated were changes in wound length and the percentage of wound healing. All treatment groups showed a progressive decrease in wound length throughout the observation period. The negative control group exhibited the slowest healing response, with wound length decreasing from 1 cm on day 0 to 0.36 cm on day 14. Treatment with the methanol extract resulted in a progressive increase in the percentage of wound healing over time. On day 14, the wound healing percentage reached 77% at a 10% methanol extract concentration, 89% at 20%, and 100% at 30%. The 30% methanol extract demonstrated the highest healing response, reaching complete wound closure on day 12. The wound-healing activity of the methanol extract may be associated with its secondary metabolites, particularly flavonoids, phenolic compounds, and tannins, which may contribute through antioxidant and anti-inflammatory activities that support tissue repair under hyperglycemic conditions. The methanol extract of A. lanceifolius* Roxb. *bark demonstrated potential wound-healing activity in a hyperglycemic animal model, with the 30% concentration exhibiting the most optimal activity compared with the 10% and 20% concentrations.*

I. Introduction

Diabetes mellitus is a chronic metabolic disease with a continuously increasing global prevalence. The rising prevalence of diabetes mellitus is accompanied by an increase in diabetes-related complications, particularly impaired wound healing (Yadav *et al.*, 2025). Diabetic wounds are among the major chronic complications of diabetes mellitus and are characterized by delayed wound healing due to oxidative stress, persistent inflammation, impaired angiogenesis, and an increased risk of infection. Diabetic wound healing remains a challenging clinical problem because its underlying pathophysiological mechanisms are not yet fully understood (Deng *et al.*, 2021). It is estimated that approximately 700 million people, corresponding to 7.8% of the global population, will be affected by diabetes by 2045, and approximately 15–25% of diabetic patients are expected to develop chronic wounds that are difficult to heal and may ultimately lead to amputation (Chen *et al.*, 2021; Zhang *et al.*, 2023). According to Burgess et al. (2021) moreover, one estimate suggests that between one in three and one in five patients with DM will develop a chronic non-healing wound in their lifetime, such as a diabetic foot ulcer (DFU), with an alarming recurrence rate (40% within one year and 65% within five years) and no reliable methods available to predict its occurrence.

The current use of topical antibiotics for diabetic wounds may cause allergic reactions and contribute to the development of antimicrobial resistance, particularly among microorganisms that commonly colonize wounds, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Valentino *et al.*, 2023). In addition, commonly used antiseptic agents for wound management, including hydrogen peroxide, povidone–iodine, and silver sulfadiazine, may exert cytotoxic effects on the major cell types involved in

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the wound healing process (Yudaev *et al.*, 2023). Systemic antibiotics are generally not routinely used for wound treatment because their penetration into poorly perfused wound tissues may be inadequate (Sychrová *et al.*, 2022). The high morbidity associated with diabetic wounds highlights the need to identify novel therapeutic agents that are more effective, safe, and capable of addressing the complex pathological conditions associated with diabetic wound healing. According to Amparo et al. (2020), an ideal natural compound for the treatment of infected wounds and soft-tissue injuries should possess four important properties: antioxidant, antibacterial, anti-inflammatory, and wound-healing activities.

Various natural products have been investigated as potential therapeutic candidates for diabetic wounds; however, most studies have focused on plant species that have already been extensively investigated. One promising source of natural therapeutic agents for diabetic wound management is the genus *Artocarpus*. This genus is known to be rich in secondary metabolites, particularly prenylated flavonoids and phenolic compounds, which have demonstrated antioxidant, anti-inflammatory, antimicrobial, and antihyperglycemic activities. *A. lanceifolius* Roxb. is one of the species within the genus *Artocarpus* with potential for the treatment of diabetic wounds. According to Hasan et al. (2019), the stem bark extract of *A. lanceifolius* exhibits antibacterial and antioxidant activities and demonstrates toxicity against *Artemia salina* larvae.

Although *A. lanceifolius* Roxb. is known to contain secondary metabolites with potential pharmacological activities, information regarding the use of its stem bark as a wound-healing agent remains limited. Previous studies have primarily explored its biological activities through *in vitro* approaches, particularly antioxidant and anti-inflammatory activities, whereas evidence regarding the effectiveness of specific stem bark fractions of *A. lanceifolius* in promoting wound healing *in vivo* remains insufficient. Furthermore, the ability of the methanolic fraction of *A. lanceifolius* stem bark to accelerate wound healing under hyperglycemic conditions has not been clearly established. Hyperglycemia is associated with persistent oxidative stress and inflammation, both of which can impair tissue regeneration and delay the wound healing process.

Thus, a knowledge gap remains regarding the relationship between the bioactive potential of the methanolic fraction of *A. lanceifolius* stem bark and its effectiveness in improving wound healing under hyperglycemic conditions. To date, the wound-healing activity of the methanolic fraction of *A. lanceifolius* stem bark, particularly in a hyperglycemic wound model, has not been adequately evaluated *in vivo*. Moreover, the use of a methanolic fraction may enrich polar bioactive compounds, such as flavonoids and phenolic compounds, which are presumed to contribute to its biological activity, thereby providing more specific information than studies using crude extracts.

Therefore, this study aimed to evaluate the wound-healing activity of the methanolic extracts of Kaledang (*A. lanceifolius* Roxb.) stem bark in a hyperglycemic wound model *in vivo*.

II. Material and Method

II.1 Materials and Equipment

Ingredients: alloxan (Sigma-Aldrich), methanol 70% (Merck), sodium CMC (Sigma), glucose sticks (Nesco®), *A. lanceifolius* Roxb. stem bark, povidone iodine (OneMed), *n*-hexane (Merck), chloroform (Merck), ethyl acetate (Merck), mice, Mg powder, hydrochloric acid, Liebermann Bouchardat, Dragendorff's, Wagner's reagents, Meyer's reagents, $FeCl_3$, sodium hydroxide, Salkowski's,

Equipment: stirring rod, maceration vessel, disposable (OneMed®), Erlenmeyer flask (Pyrex®), chemical glass (Pyrex®), filter paper, UV lamp 366 nm and 254 nm (CAMAG®), Multhicheck (Nesco®), analytical balance (OSUKA®), dropper pipette, oral probe, spatula, stopwatch, reaction tubes, mouse weighing scale, vials, observation containers, rotary evaporator, 5 cc disposable, blender (Philip).

II.2 Extraction of Kaledang (*A. lanceifolius* Roxb.) Stem Bark

The extraction procedure was performed according to Hasan et al. (2023), with appropriate modifications to the present study. The dried stem bark of *A. lanceifolius* Roxb. was powdered and subjected to sequential maceration using solvents of increasing polarity. The extraction was initiated with *n*-hexane (5,000 mL), followed successively by chloroform (3,000 mL), ethyl acetate (5,000 mL), and methanol (6,000 mL). Each extraction step was performed by maceration for 3 × 24 h. The filtrates obtained from each solvent were collected separately and concentrated under reduced pressure using a rotary evaporator at 40 °C.

The sequential extraction procedure was intended to facilitate the differential extraction of phytochemical constituents according to their solubility and affinity toward solvents of different polarities. The resulting materials were therefore designated as *n*-hexane, chloroform, ethyl acetate, and methanolic extracts.

II.3 Phytochemical Screening of Sequential Extracts

Preliminary phytochemical screening was performed on all extracts obtained from the sequential maceration procedure, namely the *n*-hexane, chloroform, ethyl acetate, and methanolic extracts of *A. lanceifolius* stem bark. The screening was conducted to determine the distribution of major

classes of secondary metabolites across extracts obtained using solvents of different polarities.

Qualitative tests were performed to detect alkaloids, flavonoids, tannins, saponins, steroids, and triterpenoids using standard phytochemical procedures. Each extract was tested separately using the appropriate reagents and reaction conditions. In the flavonoids test, a small amount of dilute NaOH solution was added to a test tube containing 1 mL of the stock solution, resulting in the development of a dark yellow color. The presence of flavonoids was indicated by the gradual addition of a weak acid, which caused the solution to become colorless (Hossain *et al.*, 2013). The flavonoid test was performed by dissolving 1 g of the extract in an appropriate solvent, followed by the addition of 0.1 g of magnesium powder and five drops of concentrated HCl. A color change to red or orange indicated a positive result (Reiza, Rijai and Mahmudah, 2019). The alkaloid test was performed by adding 1 mL of 2 N HCl to 1 g of the extract, followed by the addition of Dragendorff's, Mayer's, or Wagner's reagent to the filtrate. The formation of an orange-brown precipitate with Dragendorff's reagent, a cream-white precipitate with Mayer's reagent, or a yellowish-brown to reddish-brown precipitate with Wagner's reagent indicated the presence of alkaloids (Godlewska *et al.*, 2022).

The tannin test was performed by placing 1 g of the extract in a test tube and adding 10 mL of hot water, followed by heating for 5 min. The filtrate was then treated with 3–4 drops of $FeCl_3$. The development of a greenish-black or bluish-black color indicated the presence of tannins (Sutoyo *et al.*, 2021). Saponins were identified by adding 5 mL of hot distilled water to 1 g of the extract and shaking the mixture for 1 min. The formation of foam was considered indicative of saponins. Subsequently, 2 drops of 2 N HCl were added, and the formation of stable foam measuring 1–3 cm in height that persisted for 10 min confirmed the presence of saponins (Sutoyo *et al.*, 2021; Isa *et al.*, 2025). The presence of a particular phytochemical group was determined based on characteristic color changes, precipitate formation, foam formation, or other specific reactions. The results were recorded qualitatively as positive (+) or negative (−) for each phytochemical group and are presented for comparison among the four sequential extracts.

II.4 Evaluation of the Methanolic Extracts of *A. lanceifolius Roxb*. Stem Bark in Diabetic Mice

Male mice (*Mus musculus*) were used as experimental animals in this study. Mice were selected based on their availability, relatively low maintenance costs, and ease of handling under laboratory conditions. The animals were 2–3 months old and weighed 20–30 g. A total of 15 mice were randomly divided into five experimental groups, with three mice in each group: a negative control group treated with sodium carboxymethyl cellulose (NaCMC), a positive control group treated with povidone–iodine, and three treatment groups receiving the methanolic extracts of *A. lanceifolius* Roxb. at concentrations of 10%, 20%, and 30%, respectively. Each group consisted of three animals as biological replicates. All experimental procedures involving animals were reviewed and approved by the Animal Research Ethics Committee of Universitas Negeri Gorontalo, Indonesia, under protocol number 00902275713111132025111700013, dated 28 November 2025. The animals were housed under standard laboratory conditions with free access to food and water and were acclimatized for one week before the experiment. All procedures were performed in accordance with institutional guidelines for the care and use of laboratory animals, and every effort was made to minimize animal suffering and stress throughout the study.

II.5 Preparation of the Topical Formulation and Treatment

The methanol extracts of *A. lanceifolius* Roxb stem bark were formulated using freshly prepared 1% sodium carboxymethyl cellulose (Na CMC) as the topical vehicle. Three concentrations of the methanolic fraction were prepared, namely 10%, 20%, and 30%. The formulations were applied directly to the excision wounds at a volume of two drops per application, twice daily.

II.6 Induction of Diabetes

Before diabetes induction, the mice were fasted for 8–12 h, with free access to drinking water. Diabetes was induced by intraperitoneal administration of alloxan at a dose of 180 mg/kg body weight (BW). Alloxan powder was weighed according to the calculated dose for each animal and dissolved in 25 mL of distilled water immediately before administration.

Following alloxan administration, blood glucose levels were monitored using a glucometer (Nesco). Mice were considered diabetic when their blood glucose levels reached ≥200 mg/dL, whereas early hyperglycemia was indicated by blood glucose levels above 176 mg/dL. The reported normal blood glucose range in mice is approximately 62.8–176 mg/dL.

II.7 Excision Wound Induction in Mice

The wound induction procedure began by shaving the hair from the entire dorsal surface of the mice until the area was completely clean. A circular excision wound with a diameter of approximately 1 cm was then created using a surgical scalpel. Prior to wound induction, the mice were anesthetized by intramuscular injection of 0.2–0.3 mL of lidocaine.

II.8 Treatment Groups

The experimental animals were divided into five groups, each consisting of three mice: Negative control: 1% NaCMC vehicle, Positive control:

povidone–iodine 10%, Methanolic fraction 10% (D1), Methanolic fraction 20% (D2), Methanolic fraction 30% (D3). The 10% methanolic fraction formulation was prepared by dispersing 10 g of the methanolic fraction in freshly prepared 1% NaCMC solution to a final volume of 100 mL. Similarly, the 20% and 30% formulations were prepared by dispersing 20 g and 30 g of the methanolic fraction, respectively, in 1% NaCMC solution to a final volume of 100 mL. The topical formulations were applied directly to the excision wounds at a volume of two drops per application, twice daily. The treatment and wound-healing observation were conducted for a maximum of 14 days or until complete wound closure was achieved.

II.9 Treatment Groups

The experimental animals were divided into five groups, each consisting of three mice: Negative control: 1% NaCMC vehicle, Positive control: povidone–iodine 10%, Methanolic fraction 10% (D1), Methanolic fraction 20% (D2), Methanolic fraction 30% (D3). The 10% methanolic fraction formulation was prepared by dispersing 10 g of the methanolic fraction in freshly prepared 1% NaCMC solution to a final volume of 100 mL. Similarly, the 20% and 30% formulations were prepared by dispersing 20 g and 30 g of the methanolic fraction, respectively, in 1% NaCMC solution to a final volume of 100 mL. The topical formulations were applied directly to the excision wounds at a volume of two drops per application, twice daily. The treatment and wound-healing observation were conducted for a maximum of 14 days or until complete wound closure was achieved.

II.10 Measurement of Wound Healing Percentage

The methanolic fraction was applied topically to the wound twice daily until day 14. The percentage of wound closure was calculated based on measurements of the wound area beginning on the first day after excision.

$$\% \text{ Wound Closure} = \frac{A_0 - A_t}{A_0}$$

Where:

- A_0 = Initial Wound Area
- A_t = Wound area on the day of observation

III. Results and Discussion

The extraction process was performed using a sequential maceration method with four solvents of different polarities, namely hexane, chloroform, ethyl acetate, and methanol. This sequential extraction approach was employed to separate groups of compounds according to their polarity characteristics, thereby producing fractions with relatively different phytochemical compositions. Maceration was carried out for 3 × 24 h with each solvent sequentially. The extraction yield obtained from each solvent was calculated and is presented in Table 1.

Table 1. Yield of Kaledang (*A. lanceifolius* Roxb.) stem bark extracts

Solvent	Initial Sample Weight (g)	Extracts Weight (g)	Percentage Yield (%)
n-hexane	1000	51.4	5.11
Cloroform	987.55	99.75	10.1
Ethyl acetate	971.35	134.55	13.85
Methanol	918.80	145.4	15.81

Based on Table 1, the methanolic extract exhibited the highest yield (15.81%), followed by ethyl acetate (13.85%), chloroform (10.10%), and n-hexane (5.11%). The higher yield obtained with methanol suggests that the stem bark contains a greater proportion of constituents with moderate-to-high polarity. However, extract yield alone does not necessarily reflect bioactive compound abundance or biological potency. Therefore, the selection of the methanolic extract for subsequent wound-healing evaluation was based on the combined consideration of extraction yield, phytochemical profile, and the reported biological relevance of polar secondary metabolites, particularly flavonoids and phenolic compounds. The phytochemical screening results of the methanol extract are shown in Table 2.

Table 2. Phytochemical screening results of each extract

Phytochemical Test	Reagent	Extact			
		n-hexane	Chloroform	Ethyl Acetat	Methanol
Flavonoids	Mg powder + concentrated HCl	-	+	+	++
	NaOH 10 %	-	+	++	+++
Terpenoids	Salkowsky	+	-	-	-
Steroids	Liebermann Bouchardat	+	-	-	-
Alkaloids	Dragendorf	-	-	-	+
	Wagners	-	-	+	+
	Mayers	-	-	+	+

Phenol	$FeCl_3$ + Ethanol 70%		+	+	++
Saponin	Hot water + HCl	-	-	-	+
Tannin	$FeCl_3$ 1%	-	-	+	++

Note: The symbols (+), (++), and (+++) indicate increasing intensity of the observed color or positive reaction, with (+++) representing the strongest reaction intensity. These scores are qualitative and should not be interpreted as quantitative measurements of phytochemical content.

Phytochemical screening revealed differences in the distribution and reaction intensity of secondary metabolites among the extracts of *A. lanceifolius* Roxb. stem bark. Flavonoids were not detected in the *n*-hexane extract but were detected in the chloroform, ethyl acetate, and methanol extracts, with the strongest reaction observed in the ethanolic extract, particularly in the NaOH test (+++). A positive color reaction with magnesium powder and HCl indicates the presence of a benzopyran nucleus characteristic of flavonoids, as demonstrated by the formation of red or orange flavilium salts (Reiza, Rijai and Mahmudah, 2019). The alkaline reaction with NaOH is related to the ionization of phenolic hydroxyl groups in flavonoid structures. The stronger reaction observed in the methanolic extract suggests that polar flavonoid constituents were preferentially extracted by methanol. Phenolic compounds were detected in the chloroform, ethyl acetate, and methanol extracts, with the strongest reaction observed in the methanolic extract (++). The positive reaction with $FeCl_3$ is attributed to the formation of colored complexes between Fe^{3+} ions and hydroxyl groups of phenolic compounds. Phenolic compounds were detected in the chloroform, ethyl acetate, and methanol extracts, with the strongest reaction in the methanolic extract (++). Tannins were detected in the ethyl acetate (+) and methanol (++) extracts, while alkaloids and saponins were detected in the ethyl acetate and methanol extracts. The stronger reaction in the methanolic extract suggests a greater qualitative detection of tannin-related constituents in this extract. Alkaloids were detected in the ethyl acetate and methanol extracts, as indicated by positive reactions with Dragendorff's and Wagner's reagents. The absence of alkaloid reactions in the n-hexane and chloroform extracts suggests that these constituents were preferentially recovered in the more polar extracts. In the alkaloid tests, acidification with HCl facilitates protonation and solubilization of alkaloids, whereas specific reagents such as Dragendorff's and Wagner's reagents produce characteristic precipitates through ionic or complexation reactions. Saponins were detected in the ethyl acetate (+) and methanol (++) extracts. The positive test was characterized by the formation of persistent foam, which is associated with the amphiphilic structure of saponins containing hydrophilic sugar moieties and hydrophobic aglycones. This structure allows saponins to reduce surface tension and stabilize foam.

The positive reactions observed in these tests are consistent with the presence of the respective secondary metabolites. Overall, the methanolic extract showed the broadest distribution and relatively stronger reaction intensities of the detected secondary metabolites, particularly flavonoids, phenolic compounds, and tannins. Therefore, the methanolic extract was selected for further wound-healing evaluation, as its phytochemical profile indicated a broader distribution and stronger qualitative reactions of polar secondary metabolites potentially relevant to the biological activity under investigation. These findings are broadly consistent with our previous phytochemical screening conducted by Hasan et al. (2019), which also detected similar secondary metabolites in the methanol and other extracts of *A. lanceifolius* stem bark. However, tannins, saponins, and phenolic compounds were not evaluated in the previous study; therefore, their detection in the present study provides additional information on the phytochemical profile of this plant material.

The results of the activity test of the methanol extracts of the kaledang (*A. lancefolius* Roxb.) bark as a treatment for diabetic wounds are shown in Table 3.

Table 3. Wound healing measurements in mice

Treatment	Wound Diameter (cm)					
	Day 0	Day 3	Day 7	Day 11	Day 12	Day 14
Negative Control (Na-CMC) 1 %	1	0.92	0.63	0.42	0.39	0.36
Positive Control (Povidone-iodine)	1	0.65	0.41	-	-	-
Methanolic Extracts of Kaledang Stem Bark (10%)	1	0.81	0.61	0.40	0.35	0.23
Methanolic Extracts of Kaledang Stem Bark (20%)	1	0.75	0.57	0.36	0.29	0.11
Methanolic Extracts of Kaledang Stem Bark (30%)	1	0.70	0.44	0.16	-	-

Note: – = complete wound closure

Based on Table 3, all treatment groups showed a gradual reduction in wound length throughout the observation period, indicating progressive wound closure over time. The reduction

in wound length was subsequently converted into the percentage of wound healing, as presented in Table 4. Thus, a greater reduction in wound length relative to the initial wound size resulted in a higher percentage of wound healing. Overall, a consistent relationship was observed between the reduction in wound length in Table 3 and the increase in wound healing percentage in Table 4. Groups with a greater reduction in wound size exhibited higher wound healing percentages. Among the methanol extract-treated groups, the 20% concentration showed a better healing response than the 10% concentration, whereas the 30% methanol fraction demonstrated the highest healing response, reaching 100% wound closure on day 12. These findings indicate a tendency toward increased wound-healing activity with increasing concentrations of the methanol fraction. Results of the Percentage of Wound Healing in the Hyperglycemic Wound Model Treated with the Methanolic extracts of Kaledang *A. lanceifolius* Roxb. are shown in Table 4.

Table 4. Percentage of wound healing following treatment with the methanolic extract of kaledang (*A. lanceifolius* Roxb.) in a hyperglycemic wound model

Treatment	Percentage of Wound Healing (%)					
	Day 0	Day 3	Day 7	Day 11	Day 12	Day 14
Negative Control (Na-CMC) 1 %	0	8	37	58	61	64
Positve Control (Povidon-iodine)	0	35	59	100	100	100
Methanolic Extract of Kaledang Stem Bark (10%) W/V	0	19	39	60	65	77
Methanolic Extract of Kaledang Stem Bark (20%) W/V	0	25	43	64	71	89
Methanolic Extract of Kaledang Stem Bark (30%) W/V	0	30	56	84	100	100

The observations showed that all treatment groups experienced a reduction in wound length throughout the observation period. The negative control group treated with Na-CMC exhibited the slowest wound closure, with wound length decreasing from 1 cm on day 0 to 0.36 cm on day 14. This finding indicates that the wound healing process continued physiologically but was relatively slower than that observed in the groups receiving active treatments, as shown in Figure 1.

Wound healing percentage increased progressively in all groups throughout the observation period. The negative control group showed the slowest healing response, whereas the positive control group achieved 100% wound healing by day 7. Treatment with methanol fractions at concentrations of 10%, 20%, and 30% resulted in an increasing healing response over time. The 30% methanol extract produced the highest wound healing percentage among the extract-treated groups, reaching 100% on days 12 and 14, thereby demonstrating a response that most closely approached that of the positive control. These findings indicate that the 30% methanol extract has the greatest potential to accelerate the wound healing process compared with the other concentrations. The greater wound healing activity observed at the 30% concentration may be related to the greater amount of secondary metabolites delivered to the wound site.

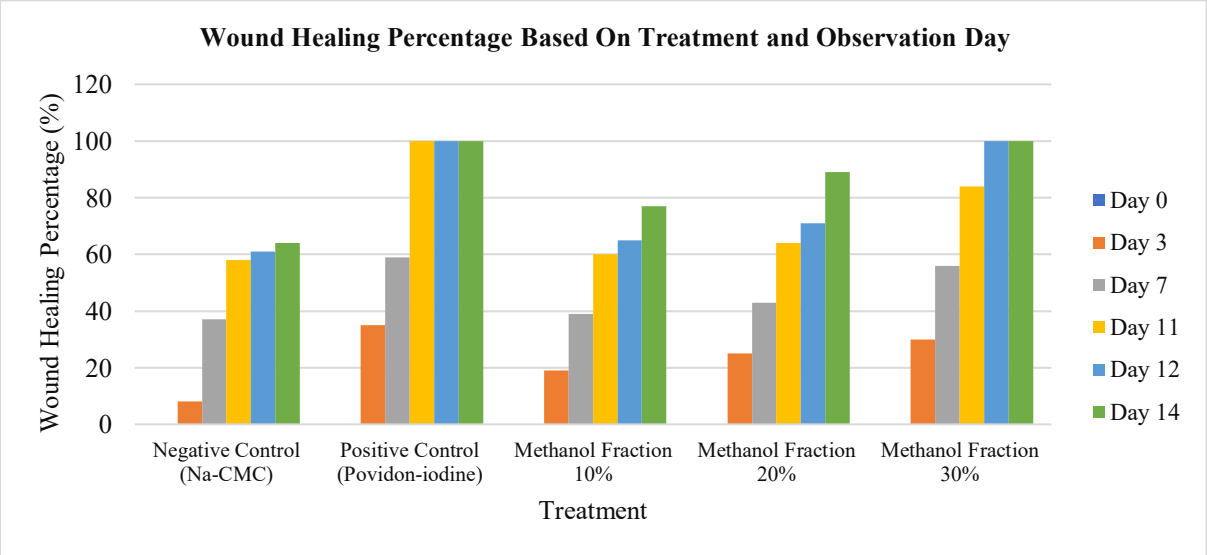

Figure 1. Percentage of wound healing according to observation day

Based on Figure 1, Wound healing percentage increased progressively in all groups throughout the observation period. The negative control group showed the slowest healing response, whereas the positive control group achieved 100% wound healing by day 7. Treatment with methanol fractions at concentrations of 10%, 20%, and 30% resulted in an increasing healing response over time. The 30% methanol fraction produced the highest wound healing percentage among the fraction-treated groups, reaching 100% on days 12 and 14, thereby demonstrating a response that most closely approached that of the positive control. These findings indicate that the 30% methanol fraction has the greatest potential to accelerate the wound healing process compared with the other concentrations.

The present study demonstrated that the methanolic extract significantly enhanced wound closure compared with the negative control. A progressive increase in wound closure was observed throughout the observation period, although the magnitude and rate of healing differed among treatment groups. One-way ANOVA revealed significant differences among groups on days 3, 7, and 14 ($p < 0.001$), indicating that treatment significantly influenced the wound-healing response.

The most pronounced differences were observed during the early and intermediate stages of healing. On day 3, all methanolic fraction groups differed significantly from the Na-CMC group, indicating that the treatment effect was already apparent during the early phase of tissue repair. Notably, the 30% methanolic fraction did not differ significantly from povidone iodine ($p = 0.271$), suggesting a comparable wound-healing response at this stage. A similar pattern was observed on day 7, when the 30% fraction remained statistically comparable with povidone iodine ($p = 0.340$), whereas the 10% fraction did not differ significantly from Na-CMC ($p = 0.411$). These findings suggest that the higher concentration may accelerate wound healing more effectively during the early-to-intermediate stages of repair. At day 14, wound closure was markedly increased in all treatment groups. Although the 30% fraction showed the highest descriptive wound closure, approximately 100%, the differences among the methanolic fraction concentrations were no longer statistically significant. Moreover, the methanolic fraction groups did not differ significantly from povidone iodine, whereas they remained significantly different from the Na-CMC group. This finding is important because it indicates that increasing the concentration did not produce a proportional improvement in the final wound-closure outcome. Rather, concentration appeared to influence the rate of healing, particularly during the earlier stages. Therefore, the 30% fraction may be considered the most promising concentration based on its earlier and more pronounced response, but it should not be described as statistically superior to the lower concentrations at the end of the observation period.

The observed wound-healing activity may be associated with bioactive secondary metabolites present in the methanolic extract, particularly phenolic and flavonoid constituents, tannins, alkaloids, and saponins, if confirmed by the phytochemical analysis. Flavonoids have been reported to promote wound repair through antioxidant and anti-inflammatory effects (Chen *et al.*, 2025), as well as modulation of angiogenesis, fibroblast activity, extracellular matrix formation, and re-epithelialization (Zulkefli *et al.*, 2023). Oxidative stress and inflammation are closely interconnected during wound repair; excessive or prolonged oxidative and inflammatory responses may delay tissue regeneration. Consequently, antioxidant and inflammation-modulating activities of plant-derived polyphenols may create a more favorable microenvironment for progression from the inflammatory phase toward proliferation and tissue remodeling (Sanchez *et al.*, 2018).

From a wound-healing perspective, tannins promote healing through multiple mechanisms: they precipitate proteins at the wound surface to form a protective layer, exhibit antimicrobial activity against common wound pathogens, scavenge reactive oxygen species that delay healing, and stimulate fibroblast proliferation and collagen synthesis (Samavati *et al.*, 2025). These properties may be relevant to wound healing by limiting oxidative damage, supporting tissue contraction, and potentially reducing microbial burden at the wound site.

The presence of alkaloids in the extract may also contribute to wound healing activity through their antibacterial effects. Alkaloids exhibit anti-inflammatory effects by modulating signaling pathways critical for cellular responses in damaged tissue (Esad *et al.*, 2025). In addition, exhibit antibacterial activity against a broad range of Gram-positive and Gram-negative bacteria through several mechanisms, including disruption of bacterial cell wall and membrane integrity, thereby disrupting intracellular homeostasis and inducing cell death (Yan *et al.*, 2021; Zhang *et al.*, 2026). This inhibits microbial growth and viability (Cushnie, Cushnie and Lamb, 2014). In the wound healing process, controlling bacterial growth is important because excessive microbial colonization can sustain the inflammatory response and impair subsequent tissue proliferation and new tissue formation. Therefore, the antibacterial activity of alkaloids may help create a more favorable wound environment for re-epithelialization, granulation tissue formation, and wound contraction. This contribution may be particularly relevant under hyperglycemic conditions, in which wound healing is generally

delayed and more susceptible to complications associated with bacterial infection.

The presence of phenolics in the extract may also contribute to wound healing. According to Yazarlu et al. (2021), phenolics are among the most explored natural products from plants and have been reported for their medicinal properties, including wound healing. The wound-healing properties of phenolics are attributed to their potent antioxidant effects and their ability to modulate metabolic activities associated with inflammation, energy dysmetabolism, and insulin resistance (Chen *et al.*, 2020).

The present saponin may contribute to wound healing through antioxidant, anti-inflammatory, antimicrobial, and tissue repair-related activities, although the specific contribution of these compounds requires further investigation. According to Shaikh (2026), Saponins, a diverse class of naturally occurring plant glycosides, have gained increasing attention for their therapeutic potential in skin regeneration and wound healing. Saponins inhibit and downregulate pro-inflammatory cyclooxygenase (COX) and lipoxygenase (LOX) pathways.

Overall, the present findings indicate that the methanolic extract has potential as a wound-healing agent, particularly because its higher concentration produced an early response comparable with the reference treatment. Nevertheless, the mechanism underlying this activity cannot be established solely from macroscopic wound closure. Further investigations using histopathological evaluation and molecular markers related to inflammation, oxidative stress, collagen deposition, angiogenesis, and re-epithelialization are warranted to confirm the biological mechanisms responsible for the observed effect. For wound-healing development, however, further studies involving histopathological and molecular markers are required to confirm the underlying mechanisms.

IV. Conclusion

The methanol extract of *A. lanceifolius* Roxb. bark demonstrated potential wound-healing activity in a hyperglycemic animal model. The 30% concentration exhibited the most optimal wound-healing activity compared with the 10% and 20% concentrations.

V. Conflict of Interest

The authors declare no conflict of interest.

VI. Acknowledgments

We would like to thank the Rector and the head of LP2M Gorontalo State University for the financial support for this research.

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