

The effect of the ethanolic extract of *Laportea decumana* (Roxb.) Wedd. on the inflammatory, proliferative and maturation stages of wound healing of an acute injury using a rat model

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ABSTRACT: *Laportea decumana* (Roxb.) Wedd. is a plant that is traditionally used for its analgesic, antipyretic, antioxidant, anti-inflammatory, and antibacterial purposes. This study aimed to determine the wound-healing effects of fractionated *L. decumana* ethanol extract ointment on the inflammatory, proliferation, and maturation phases in a rat model of acute injury. *L. decumana* leaves were extracted with 70% ethanol and then fractionated with n-hexane with a centrifuge. The polar fraction was used in the animal model. Acute injury was induced in four areas on male rats (n=15), which were assigned to receive either Vaseline, 2% *L. decumana* extract, 4% *L. decumana* extract, or Myrthax ointment (control). The wound histological assessments during the inflammatory, proliferation, and maturation phases were conducted on day 1, day 4, and day 9 after injury, respectively. The results show that the wound diameter on Day 9 was significantly lower with 4% *L. decumana* treatment than with Vaseline and 2% *L. decumana* treatment and was similar to the results of using Myrthax ointment. Histopathological examination showed that during the inflammatory phase, all wounds exhibited edema, leucocytes, and macrophages; however, during the proliferation phase, 4% *L. decumana* treatment resulted in significantly more granulation and fibroblasts, as well as thicker collagen and faster reepithelialization during the maturation phase compared to Vaseline-only treatment. In conclusion, 4% *L. decumana* demonstrated a potent wound-healing effect in the rat acute injury model, especially hastening the proliferation and maturation phases of wound healing.

KEYWORDS : *Laportea decumana*, wound healing, inflammation, proliferation, maturation

1. INTRODUCTION

Wounds, or disruptions to the skin resulting from the compromised integrity of skin tissue, result from various factors, including physical forces, chemicals, heat, and temperature fluctuations [1]. The reparative mechanisms that the body initiates in response to tissue damage involve intricate intracellular and extracellular biochemical processes. Wound healing occurs in sequential phases of inflammation, proliferation, and maturation [2]. A promising approach to enhancing the efficiency of wound healing and mitigating the risk of infection involves utilizing medicinal plants that are rich in phytochemicals that are known to possess wound-healing potential. These phytochemicals, including alkaloids, flavonoids, saponins, tannins, steroids, and phenolics, exhibit antimicrobial and antioxidant properties, offering a multifaceted approach to combating infections and expediting the overall wound-healing process [3]. This exploration of natural remedies underscores the potential of phytochemical-rich medicinal plants to advance therapeutic interventions for cutaneous injuries.

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An intriguing candidate for wound-healing applications is *Laportea decumana* (Roxb.) Wedd., commonly known as “itchy leaves”. This shrub belongs to the Urticaceae family and is distinctive for inducing a pronounced itching sensation when the leaves contact the skin [4]. This characteristic is attributed to the presence of formic acid, which, upon topical application, causes pore enlargement, promoting enhanced blood circulation. This physiological response is believed to contribute to the alleviation of muscle fatigue, body pain, and soreness [5]. Notably, *L. decumana* is recognized for the composition of alkaloids, flavonoids, saponins, and steroids, it contains; these compounds are postulated to possess wound-healing properties [5,6]. The unique attributes of *L. decumana*, both in terms of its distinctive sensory effect and phytochemical composition, emphasize its potential as a valuable resource in the search for novel wound-healing therapies.

Recent reports on *L. decumana* have demonstrated a spectrum of bioactive properties, including anti-inflammatory and analgesic activity [7], antibacterial properties, and cytotoxicity [8]. Given *L. decumana*'s diverse contents and benefits, this study aimed to investigate the wound-healing potential of ethanol extract fractions derived from the plant. The experimental focus involved the topical application of *L. decumana* extract fractions to rat models of acute injury to elucidate its effects on the phases of wound healing. Further investigation into the specific mechanisms and efficacy of *L. decumana* for wound repair could unveil promising opportunities for the development of botanical-based treatments.

2. RESULTS

2.1 Thin-Layer Chromatography Analysis of *L. decumana* Leaf Polar Fraction

Table 1 provides an overview of the thin-layer chromatography (TLC) analysis conducted on the polar fraction of ethanolic extract of *L. decumana*. The positive reaction indicated the presence of flavonoids. The chromatographic spots exhibited a distinctive dark yellow fluorescence upon the application of a citric-boric acid (CBA) mixture, and subsequent spraying with aluminum chloride (AlCl_3) resulted in a dark blue spot. Notably, the AlCl_3 -sprayed TLC revealed four distinct spots with R_f values of 0.36, 0.63, 0.72, and 0.8. Three spots were also detected upon spraying with CBA, with corresponding R_f values of 0.33, 0.63, and 0.8.

Table 1. Identified compounds in polar fraction of *L. decumana* ethanolic extract using a thin-layer chromatography

Reagent	Stain Color	R_f Value	Indication
AlCl_3	Dark Blue	0.36	Flavonoid
		0.63	
		0.72	
		0.8	
CBA	Dark Yellow	0.33	Flavonoid
		0.63	
		0.8	

AlCl_3 : aluminium chloride; CBA: citric-boric acid

Table 2. Wound diameter before (day-0) and after application of treatments (day-1 to 9)

Treatment	Diameters (mm)					
	Day-0	Day-1	Day-3	Day-5	Day-7	Day-9
Vaseline	6.0±0.00	6.0±0.00	5.0±0.00	5.0±0.00	4.4±0.49	3.4±0.49
<i>L. decumana</i> 2%	6.0±0.00	6.0±0.00	5.0±0.00	4.0±0.00*	3.4±0.49*	2.4±0.49*
<i>L. decumana</i> 4%	6.0±0.00	6.0±0.00	5.0±0.00	3.4±0.49*	3.0±0.63*	1.4±0.49*
Myrhax	6.0±0.00	6.0±0.00	5.0±0.00	3.4±0.49*	3.0±0.63*	1.6±0.80*
P Value	1.00	1.00	1.00	0.002	0.023	0.007

Values are expressed in mean ± SD. * indicates $P < 0.05$ compared to Vaseline (controls). No significant differences were found between the other groups.

2.2. The Effect of *L. decumana* Ointment Based on Wound Diameter

Table 2 and Figure 1 depict the progression of wound diameter post-treatment from day 1 to day 9. From day 0 to day 3 of treatment, the wound diameters were slightly decreased, which were similar across all treatment groups. However, starting on day 5, the 2% *L. decumana*, 4% *L. decumana*, and Myrhax ointment treatments led to significantly reduced wound diameters compared to the Vaseline control group ($P<0.05$). The Vaseline group exhibited a non-significant reduction in wound diameter from 6.0 ± 0.00 mm on day 0 to 3.4 ± 0.49 mm on day 9. In contrast, the 2% *L. decumana* and 4% *L. decumana* groups demonstrated progressive and statistically significant wound-healing improvements over the same period ($P<0.05$), with the 4% *L. decumana* group displaying the fastest healing, similar to that of the Myrhax group

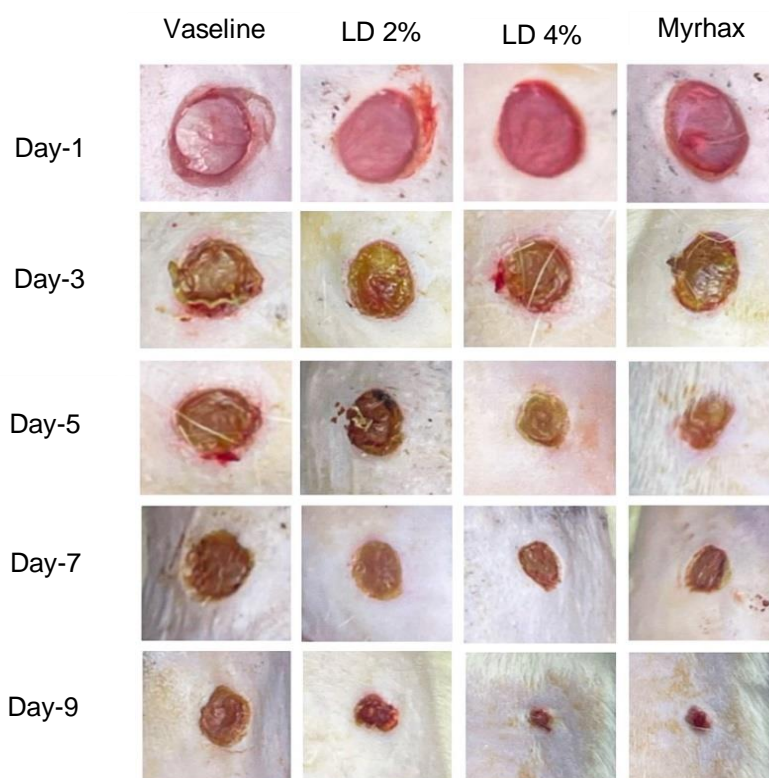


Figure 1. Wound healing process in all treatment groups. LD= *L. decumana*

2.3 Histopathological Examination During the Inflammatory Phase

In the inflammatory phase, the presence of edema, leukocytes, and macrophages was recorded and quantified by intensity, as shown in Table 3 and Figure 2. During the early inflammatory phase (day 1), treatment with Vaseline alone resulted in a significantly lower level of inflammation than in all other treatment groups ($P<0.05$). Interestingly, the application of 4% *L. decumana* or Myrhax ointment resulted in more pronounced edema and leukocyte infiltration in the acute wounds, indicative of an amplified inflammatory response compared to the response after Vaseline-only treatment.

Table 3. The scores of edema, leukocyte, and macrophage infiltration during the inflammatory phases

Treatments	Scores (mean \pm SD)			
	Edema	Leukocytes	Macrophages	Total score
Vaseline	0.2 ± 0.45	1.2 ± 0.45	2.0 ± 0.00	3.4 ± 0.80
<i>L. decumana</i> 2%	0.4 ± 0.55	2.4 ± 0.55	2.4 ± 0.55	$5.2 \pm 0.40^*$
<i>L. decumana</i> 4%	1.0 ± 0.00	2.8 ± 0.45	2.8 ± 0.45	$6.6 \pm 0.80^*$
Myrhax	0.8 ± 0.45	2.6 ± 0.55	2.6 ± 0.55	$6.0 \pm 0.89^*$

Values are expressed in mean \pm SD. * indicates $P<0.05$ compared to vaseline (controls). No significant differences were found between the other groups.

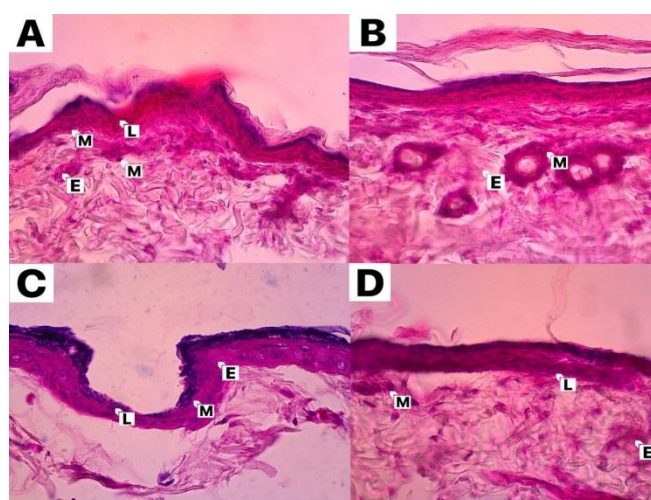


Figure 2. The photomicrographs of wound during the inflammatory phase. A: Vaseline, B: *L. decumana* 2%, C: *L. decumana* 4%, dan D: Myrhax Ointment (E: Edema, L: Leukocytes, M: Macrophages).

2.4 Histopathological Examination During the Proliferation Phase

During the proliferation phase, which was assessed on Day 4 of treatment, the intensity of the granulation tissue composition and fibroblast migration were systematically scored. Granulation tissue and fibroblast migration were more dominant in wounds treated with 2% and 4% *L. decumana* compared to those treated with Vaseline alone. Therefore, the cumulative scores reflecting the wound-healing progress during the proliferation phase were significantly higher in the *L. decumana* groups ($P < 0.05$) (see Table 4). The microscopic observations providing visual representations of the proliferation phase are depicted in Figure 3.

Table 4. The scores of granulation tissue and fibroblast migration during proliferation phase

Treatments	Scores (mean \pm SD)		Total Score
	Granulation Tissue	Fibroblasts	
Vaseline	1.4 \pm 0.55	1.8 \pm 0.45	3.2 \pm 0.75
<i>L. decumana</i> 2%	2.2 \pm 0.45	2.4 \pm 0.55	4.6 \pm 0.80
<i>L. decumana</i> 4%	2.6 \pm 0.55	2.8 \pm 0.45	5.4 \pm 0.49*
Myrhax	2.4 \pm 0.55	2.6 \pm 0.55	5.0 \pm 0.89*

Values are expressed in mean \pm SD. * indicates $P < 0.05$ compared to vaseline (controls). No significant differences were found between the other groups.

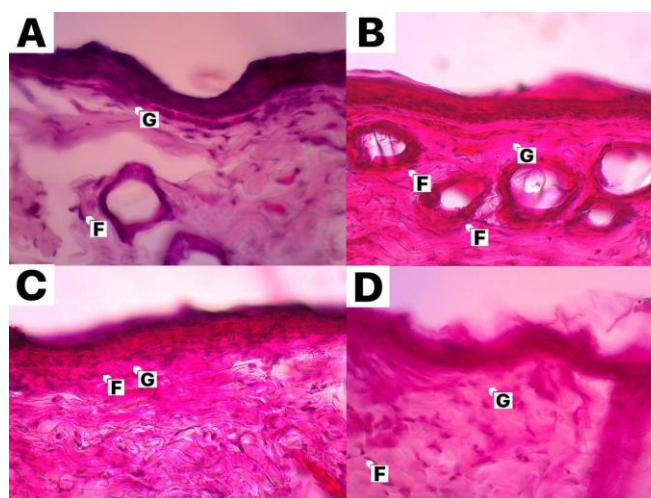


Figure 3. The photomicrographs of wound during the proliferative phase. A: Vaseline, B: *L. decumana* 2%, C: *L. decumana* 4%, dan D: Myrhax Ointment (G: Granulation Tissue, F: Fibroblast)

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2.5 Histopathological Examination of the Maturation Phase

As shown in Table 5, the amount of collagen and epithelialization during the maturation phase were significantly greater in wounds treated with 2% and 4% *L. decumana* compared to those treated with Vaseline ($P < 0.05$). The total scores for the maturation phase were not significantly different between the *L. decumana*-treated and Myrhax-treated wounds. To further elucidate the histological aspects of the maturation phase, Figure 4 provides a detailed microscopic examination. Epithelialization is notably more pronounced in wounds treated with 2% and 4% *L. decumana* than those treated with Vaseline

Table 5. The scores of collagen deposition and epithelization during maturation phase

Treatments	Scores (mean \pm SD)		Total Score
	Collagen	Epithelialization	
Vaseline	1.2 \pm 0.45	1.8 \pm 0.45	3.0 \pm 0.63
<i>L. decumana</i> 2%	2.6 \pm 0.55	2.4 \pm 0.55	5.0 \pm 0.89*
<i>L. decumana</i> 4%	2.8 \pm 0.45	2.6 \pm 0.55	5.4 \pm 0.80*
Myrhax	2.6 \pm 0.55	2.2 \pm 0.45	4.8 \pm 0.75*

Values are expressed in mean \pm SD. * indicates $P < 0.05$ compared to vaseline (controls). No significant differences were found between the other groups.

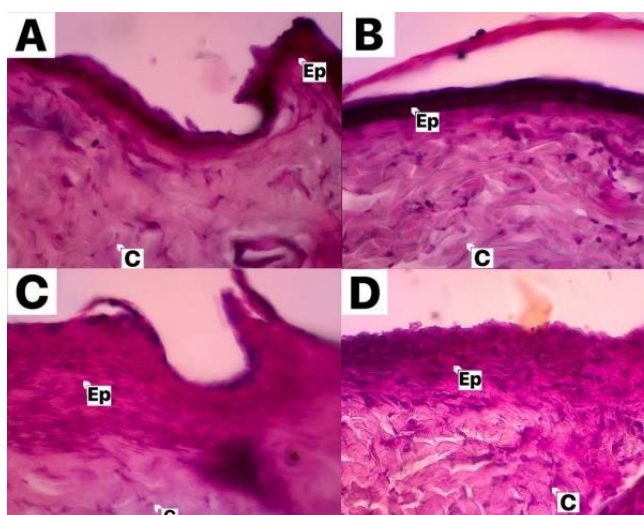


Figure 4. The photomicrographs of wound during the maturation phase. A: Vaseline, B: *L. decumana* 2%, C: *L. decumana* 4%, dan D: Myrhax Ointment (C: Collagen, Ep: Epithelialization).

3. DISCUSSION

Injuries such as cuts, bruises, burns, and other forms of trauma can compromise the integrity of the skin, interrupting its normal functions [9]. Effective wound care is imperative, as unsuccessful healing and extended recovery periods may escalate costs and adversely affect the well-being of patients and their families, causing effects that include prolonged pain and heightened anxiety [10]. Improper wound care practices can generate complications such as bleeding, infection, inflammation, tissue damage, and angiogenesis and regeneration, culminating in the formation of scars [11].

This study demonstrated that the topical application of *Laportea decumana* (Roxb.) Wedd. ointment significantly promoted wound healing, especially during the proliferation and maturation phases. The proliferation and maturation phases are pivotal in attaining wound closure and restoring normal skin integrity [12]. Previous studies have explored the analgesic potential of incorporating *L. decumana* leaf extract into ointment formulations [5]. A more recent study found that the anti-inflammatory and analgesic properties of *L. decumana* leaf extract in a cream formulation were pronounced at a 2% concentration [7]. Unlike in other studies, the ethanolic extract of *L. decumana* leaves in this study was divided into polar and non-polar fractions with n-hexane. It is found that the polar fraction of the *L. decumana* extract demonstrated

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a superior wound-healing effect in the pilot study. Therefore, only the polar fraction of the extract was used in this study at concentrations of 2% and 4%.

Wound size measurements are crucial for monitoring the wound-healing process and evaluating the effects of the experimental treatment [13]. Wound measurements were conducted daily and recorded in photos [14]. In Vaseline-treated wounds, wound closure was significantly slower than in wounds treated with either 2% or 4% *L. decumana* or Myrhax. Although the wound diameters in the 2% and 4% *L. decumana* groups were not statistically significant from day 1 to day 9, the mean diameter with 2% *L. decumana* treatment was 2.4 ± 0.49 mm on day 9, compared to 1.4 ± 0.49 mm with 4% *L. decumana* treatment, indicating a faster healing rate with the 4% extraction.

A closer look at the wound-healing effects was conducted through histopathological examination. Wound tissues were collected on day 1 of treatment to observe the inflammatory reactions that occurred within the first 24–72 hours after injury [15], including the presence of edema, leukocytes, and macrophages [16]. The results indicate that all wounds exhibited edema at the wound edge. This is caused by the release of neuropeptides, predominantly substance P (SP), which triggers mast cell degranulation, leading to histamine, serotonin, and protease release that increases blood vessel permeability around the wound and, later, contributes to swelling [17,18]. In addition, all wounds showed leukocyte and macrophage infiltration near the surface regardless of the treatment applied. When the skin is injured, intracellular signaling pathways are activated, leading to the release of chemokines, cytokines, and antimicrobial peptides as the inflammatory reaction is activated [19,20]. This activation of the innate immune system provides a defense mechanism against pathogens and facilitates dead tissue removal [21,22,23]. Neutrophils are mobilized from the bloodstream to the site of injury during the initial inflammatory phase, which amplifies the inflammatory response by generating cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6 [24]. However, prolonged inflammation can hinder the normal progression through the proliferation stage of wound healing [25]. About three days after injury, monocytes are attracted to the site of injury, where they undergo differentiation into macrophages. Unlike neutrophils, macrophages play a key role in facilitating the transition from the inflammatory to the proliferation phase in wound healing [21,25]. Interestingly, 2% and 4% *L. decumana* significantly augmented the inflammatory reaction, which was characterized by profuse leukocyte and macrophage infiltration. In contrast, the inflammatory reaction was less prominent in wounds treated with Vaseline. The increased inflammatory reaction soon after *L. decumana* topical application may have been triggered by the formic acid compound that also triggers the itching sensation on the skin with contact [26].

Although the inflammatory reaction was augmented, the wounds treated with *L. decumana* did not show persistent inflammation. The histopathological examination of the tissues on day 4 showed an active proliferation phase, characterized by the increased formation of granulation tissue and fibroblast proliferation, especially with 4% *L. decumana* treatment. This was significantly different from the Vaseline-treated wounds, which showed less granulation tissue and fewer fibroblasts, indicating slower wound repair activity. During wound healing, the proliferation phase normally initiates tissue granulation on days 2 to 5 after injury [27], which stimulates fibroblast proliferation and myofibroblast differentiation, leading to collagen deposition and the initiation of wound contraction. It also promotes the development of new blood vessels (angiogenesis) and extracellular matrix deposition [28]. During this phase, macrophages remain the most common inflammatory cells as they play important roles in the process of wound repair [19].

After granulation tissue development ends, the maturation phase begins. Impaired granulation tissue development can cause the formation of excessive scar tissue or delayed wound healing [29]. After the 9-day post-injury period, histopathological examination revealed significantly greater collagen intensity in wounds treated with 2% and 4% *L. decumana*, like those treated with Myrhax ointment. In contrast, treatment with Vaseline only failed to accelerate the healing process, as evidenced by thin collagen deposition and a lack of re-epithelialization, resulting in larger wound diameters ($P < 0.05$). Various wound-related signals, including nitric oxide (released by macrophages), cytokines, and growth factors, such as nerve growth factor (NGF), KGF, IGF-1, and epidermal growth factor (EGF), are secreted by diverse wound-related cell types [30, 31]. All of these elements interact to support the re-epithelialization process, which starts at the edges of the wound and is facilitated by neovascularization. As the wound heals, collagen synthesis increases as fibroblast proliferation gradually diminishes, maintaining a delicate balance between the formation and breakdown of the extracellular matrix [23].

4. CONCLUSION

In conclusion, the polar fraction of *L. decumana*, especially at a 4% concentration, demonstrates promise as a wound-healing therapy in rats. The wound repair effect was evident throughout the inflammation, proliferation, and maturation phases. This substantiates the scientific interest in exploring *L. decumana* as a viable bioactive component in topical wound-healing formulations. Further study is warranted to identify the chemical contents of *L. decumana* that are responsible for these healing effects

5. MATERIALS AND METHODS

5.1. Plant Material

L. decumana was obtained from the village of Wakal, Maluku Province, in eastern Indonesia. The leaves were dried and ground before extraction. The extraction was performed through maceration with 70% ethanol for 72 hours with occasional stirring. The filtrate was evaporated with a rotary evaporator to create a concentrated extract.

5.2. Drugs, Chemicals, and Cream Preparation

All drugs and chemicals, such as the commercial Myrhax ointment (3 g olibanum, 3 g viola, 3 g myrrh, and 11 g Vaseline as the carrier), were purchased from a local pharmacy in Makassar. The *L. decumana* fraction was formulated in a topical Vaseline-based ointment at concentrations of 2% and 4% w/w.

5.3. Animals

Male albino rats weighing 200–250 grams were used in this study. The animals were acclimated for 14 days in a well-ventilated laboratory and maintained under standard light, food, water, and temperature (25°C) conditions. Ethical clearance was obtained from the Faculty of Medicine, Hasanuddin University, with ethical clearance number 498/UN4.6.4.5.31/PP36/2023.

5.4. Leaf Extract Fraction of *L. decumana*

The fractionation of *L. decumana* leaf extract was performed by dissolving 10 grams of the ethanol extract of *L. decumana* in n-hexane as the solvent. The separation of compounds was achieved through centrifugation at 5,000 rpm for approximately 20 minutes, resulting in the formation of two layers: the hexane-soluble (non-polar) and insoluble (polar) fractions. This process was iteratively performed until the solvent separation became distinct. Then, the fractions were evaporated to yield concentrated fractions for further analysis.

5.5. Thin-Layer Chromatography Test of *L. decumana* Leaf Extract Fraction

The fractions obtained from the *L. decumana* leaf extract underwent thin-layer chromatography (TLC) analysis. The TLC profiles of the *L. decumana* extract, as well as its polar and non-polar fractions, were compared on TLC plates (F254) using an n-hexane:ethyl acetate solvent system (4:1). UV lamps at 254 nm and 366 nm were employed for initial observation, followed by spraying with 10% H₂SO₄. The plates were then heated and the observation was conducted under visible light. The polar fraction of the *L. Decumana* extract underwent further analysis using an n-hexane:ethyl acetate:acetic acid system (2:1:3 drops). Post-elution, the plate was air-dried and examined under UV lamps at 254 nm and 366 nm. Subsequent spraying with AlCl₃ and citric-boric acid reagents was performed to reveal the presence of flavonoids.

5.6. Acute Wound Induction in Rats

To induce acute wounds in the rats, albino male rats (*Rattus norvegicus*) were anesthetized with an intraperitoneal injection of 0.1 ml of ketamine per 100 g rat body weight (10 mg/kg). The back area of the rats was shaved and divided into left and right sides. Four 6-mm diameter wounds were created with a biopsy punch. The wounds were labeled A, B, C, and D.

5.7. Wound Treatment Protocol

In our pilot study with a limited sample size (n=3), the polar fraction of *L. decumana* exhibited superior efficacy for wound healing in rats. Therefore, the polar fraction was selected as the *L. decumana* treatment in this study. Fifteen animals were used, each with four designated wound areas subjected to distinct treatments: Wound A was treated with Vaseline, Wound B was treated with 2% *L. decumana* leaf

extract, Wound C was treated with 4% *L. decumana*, and Wound D was treated with Myrhax ointment. All wounds were daily treated in the morning, and then the diameters of the wounds were measured using calipers over nine consecutive days. Euthanasia was performed on day 1 (n=5), day 4 (n=5), and day 9 (n=5) to obtain wound tissues representing the inflammatory, proliferation, and maturation phases, respectively. The treatment protocol is depicted in Figure 5.

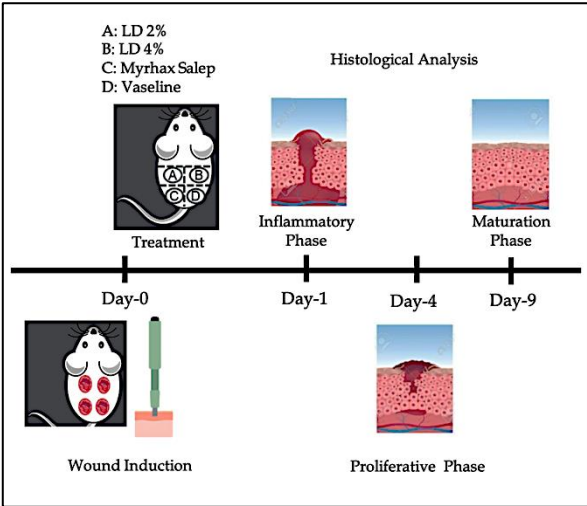


Figure 5. Treatment Protocol

5.8. Wound Diameter Measurement

Wound macroscopic appearance was recorded daily and the diameter were measured in 4 different directions (vertical, horizontal, diagonal left and diagonal right) with a caliper to obtain the average of wound diameter in each rat.

Table 6. The features and respective scores of histopathological changes in wound tissues modified from study of Nussbaum et al., 2009 [33]

Phases	Characteristic	Score	Features
Inflammation	Edema	0	No evidence
		1	Focal presence at the wound margins
		2	Present in <50% of the wound tissue examined
		3	Present in >50% of the wound tissue examined
	Leucocytes	0	No evidence
		1	Mild presence
		2	Moderate number of cells
		3	Prominent feature
	Macrophages	0	No evidence
		1	Mild presence
		2	Moderate number of cells
		3	Prominent feature
Proliferation	Granulation tissue	0	No evidence
		1	Present at the wound margins
		2	Present in <50% of the wound tissue examined
		3	Present in >50% of the wound tissue examined
	Fibroblast	0	No evidence
		1	Present only in the perivascular spaces
		2	Present in <50% of the wound tissue examined
		3	Present in >50% of the wound tissue examined
Maturation	Collagen	0	No evidence
		1	Focal presence in fibroblast around new capillaries
		2	Moderate amount in the repair tissue
		3	Dominant feature
	Epithelialization	0	No evidence
		1	Epidermal thickening and cell migration at wound margins
		2	>50% of wound epithelialized
		3	Epithelialization complete

5.9. Histopathological Examination

Wound tissue samples were fixed in 10% buffered formalin, processed, blocked with paraffin, and then cut into 5- μ m sections. Each wound had 4 sections that proceeded to staining process and analysis. Hematoxylin and eosin were applied to all sections for staining. Data analysis for the inflammation, proliferation, and maturation phases involved calculating the average scores of three observed fields under a light microscope at 100x and 400x magnifications. Images were captured using a camera, and the indicators of histopathological changes were categorized based on scoring. The scores were categorized by their respective phases [33].

5.10 Statistical Analysis:

The numerical and categorical data were collected and analyzed using SPSS 20 software. The wound diameter data were analyzed with the Shapiro-Wilk test for normality, followed by a repeated measures ANOVA. Significant differences between groups were defined with Tukey's HSD post-hoc test. Histopathology analysis scores were analyzed with a Kruskal-Wallis test, followed by a Mann-Whitney U test to assess the significance between pairs of treatment groups.

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Conflict of interest statement: The authors declare that they have no conflict of interest.

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