

The Effectiveness of Kaffir Lime Leaf Extract (*Citrus hystrix* DC) on Incised Wound in Wistar Rats (*Rattus novergicus*)

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ABSTRACT

Incised wounds are injuries caused by cuts from sharp instruments, characterized by open wounds, pain, and a wound length greater than its depth. The extract of kaffir lime leaf (*Citrus hystrix* DC) is believed to have antibacterial and anti-inflammatory properties that can accelerate the wound healing process. This study aims to determine the effectiveness of kaffir lime leaf extract on the healing of incised wounds in Wistar rats. This research used a true experimental method with a control group design. A total of 25 rats were divided into five groups: a negative control group (P0) treated with 0.9% NaCl, a positive control group (P4) treated with 0.1% gentamicin, and three treatment groups (P1, P2, P3) treated with kaffir lime leaf extract at concentrations of 10%, 20%, and 30%. Observations were carried out for 14 days. The results showed macroscopic changes, particularly in the average wound length and wound condition characterized by erythema, redness, dry open wounds, and dry closed wounds. Histopathological observations revealed the highest number of fibroblasts and collagen fibers in group P1. ANOVA test showed no significant difference between groups ($p > 0.05$), although the 10% extract proved to be more effective in healing incised wounds.

Keywords: *incised wound; kaffir lime; extract; fibroblast*

INTRODUCTION

The skin performs several crucial functions, including serving as the body's primary defense barrier against environmental insults, regulating thermoregulation, maintaining fluid and electrolyte homeostasis, and facilitating sensory perception (e.g., touch, pain, pressure) (Laksmi et al., 2025). Its continuous exposure to external elements renders it highly susceptible to injury and subsequent wound formation (Aulia, 2023).

A wound is a disruption of skin integrity that affects its normal function, potentially involving superficial to deep tissues and caused by various internal or external factors. Incised wounds, often resulting from sharp objects, are characterized by open cuts longer than they are deep (Dewi, 2020; Pangondian et al., 2024). In Indonesia, abrasions and contusions are the most common (64.1%), followed by incised, lacerated, and puncture wounds (20.1%) (Ali Akbar et al., 2023; Firdaus et al., 2020;

Kemenkes, 2019). Wound healing occurs in three phases: inflammation (day 0–3), proliferation (day 3–14), and remodeling (up to a year) (Primadina et al., 2019). This natural process may be impaired by age, nutrition, infection, or treatment efficacy (Aulia, 2023). While topical antibiotics are commonly used, inappropriate application can cause bacterial resistance and irritation, emphasizing the need for safe, accessible natural alternatives.

Kaffir lime leaf (*Citrus hystrix* DC) is a potential natural agent for wound healing. Traditionally used in Indonesian medicine, its application remains largely empirical and lacks scientific standardization. Phytochemical analysis is essential to identify active compounds and ensure safety, consistency, and efficacy in modern use (Adlini & Umaroh, 2021; Wahyuni et al., 2023). The leaf contains flavonoids, alkaloids, saponins, tannins, and essential oils with antimicrobial, anti-inflammatory, and antioxidant properties (Nasution, 2023). Antioxidant activity, though lower than *Citrus sinensis*, has been confirmed via DPPH (Mutia, Maya Sari; Sihotang, 2024). Antimicrobial effects against *E. coli*, *S. aureus*, and *C. albicans* further support its therapeutic potential (Astriani et al., 2021; Sophia et al., 2021).

Research on the efficacy of topically applied kaffir lime leaf extract cream for treating incised wounds in animal models is limited. This study investigated the wound-healing efficacy of kaffir lime leaf extract cream at three concentrations (10%, 20%, and 30%) in a Wistar rat model of incised wounds. This study contributes to the scientific basis for using locally sourced plants in wound care.

METHODS

This research applied a true experimental design with a pre-test and post-test control group approach to assess the effectiveness of kaffir lime leaf extract (*Citrus hystrix* DC) in promoting healing of incised wounds in male Wistar rats (*Rattus norvegicus*). The extraction process of kaffir lime leaves was performed at the Cosmetology Laboratory, Faculty of Pharmacy, University of North Sumatera. Meanwhile, the *in vivo* experiments using animal models were conducted at the Pharmacology Laboratory within the same university. The study period spanned from November 2024 to January 2025. The subjects were male white Wistar rats (*Rattus norvegicus*) weighing 160–200 grams and aged between 2 to 3 months. A total of 25 rats were randomly assigned into five groups: one negative control group (P0), one positive control group (P4), and three treatment groups (P1, P2, and P3).

Kaffir lime (*Citrus hystrix* DC) leaves were oven-dried at 40°C, pulverized into fine powder, and subjected to two-stage maceration using 96% ethanol. The first maceration was performed with 1 L ethanol, followed by a second maceration using 0.5 L ethanol, each lasting 72 hours. The combined filtrates were then concentrated using a rotary evaporator at 40°C. Qualitative phytochemical screening was conducted to detect the presence of flavonoids, alkaloids, saponins, and tannins based on standard procedures (Girsang et al., 2019).

The cream base was formulated using a two-phase emulsification method. The oil phase consisted of stearic acid, cetyl alcohol, and propyl paraben. The aqueous phase included *triethanolamine* (TEA), glycerin, methyl paraben, and distilled water. Both phases were separately heated to 70°C, and the aqueous phase was gradually added to the oil phase under constant stirring until a stable emulsion formed. The concentrated extract was incorporated into the base cream at concentrations of 10%, 20%, and 30%. The resulting creams were cooled to room temperature and evaluated for organoleptic properties (color, odor, texture, homogeneity), pH, viscosity, and spreadability (Dominika, 2023)

Twenty-five adult male Wistar rats (*Rattus norvegicus*) were acclimatized for 7 days and randomly assigned to five groups (n=5/group): P0 (0.9% NaCl solution); P1 (10% extract

cream); P2 (20% extract cream); P3 (30% extract cream); P4 (0.1% gentamicin cream). Rats were anesthetized (ketamine, 70 mg/kg, i.p.). A 2 cm × 1 mm standardized incisional wound was created on the dorsal region and cleaned with 0.9% NaCl solution. Topical treatment was applied twice daily for 14 days (KEPALA BADAN PENGAWAS OBAT DAN MAKANAN, 2023).

Assessments were conducted on days 4, 7, and 14 to determine the average incision wound length and its standard deviation, along with a quantitative evaluation of wound characteristics across four healing stages: swollen and erythematous (MB), erythematous (M), dry and open (KT), and dry and closed (KM). Additionally, wound lengths were measured every two days from day 2 to day 14, with mean values calculated for analysis. On day 14, wound tissue samples were collected for histopathological examination. The samples were fixed in 10% buffered formalin, processed, sliced into 5 µm sections, and stained with hematoxylin and eosin (HE). Fibroblast proliferation and collagen fiber density were evaluated using a four-point scale (0–3), where 0 represented absence of collagen and fibroblasts; 1 indicated sparse, immature collagen with few fibroblasts; 2 denoted moderate collagen with mixed maturity and fibroblast presence; and 3 corresponded to abundant, mature, well-organized collagen and high fibroblast density. The mean scores for each group were then calculated.

Data analysis was performed using SPSS version 25. The dependent variable was the rate of wound healing in male Wistar rats. Normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene's tests, respectively. The results of these tests are presented in the Results section. One-way ANOVA was used to compare the means of the treatment groups, followed by a post-hoc Tukey test if the ANOVA result was significant ($p < 0.05$). If the assumptions of normality or homogeneity of variance were not met, the Kruskal-Wallis test was performed. The results of all statistical analyses are presented in the Results section.

RESULTS

Table 1. Result of Phytochemical Screening Examination

Secondary Metabolite Compounds	Test Reagent	Result	Information
Alkaloids	Bouchardart	Brown-colored precipitate	Positive
Flavonoids	Mg, concnecrated HCL	Dark red color	Positive
Saponin	Aquadest + Whisk 1 min	Absence of foam	Positive
Tannins	FeCl ₃	There is a change in color to blackish green	positive

Phytochemical screening of kaffir lime leaf extract, confirmed the presence of four secondary metabolites: flavonoids, alkaloids, saponins, and tannins.

After formulating the extract into creams at specified concentrations, comprehensive physical evaluations were performed to assess organoleptic characteristics (such as aroma, color, texture, and uniformity), pH, viscosity, and spreadability. These assessments are critical to verify the cream's quality, stability during storage, and ease of application, ensuring both effectiveness and user comfort. Physical evaluation is especially vital for topical products, as they must comply with standards that support therapeutic efficacy and patient adherence. The outcomes of these evaluations are summarized in Table 2.

Table 2. Result of Cream evaluation

Parameters	F1 (10%)	F2 (20%)	F3 (30%)
Organoleptic properties			
1. Smell	Characteristic odor	Characteristic odor	Characteristic odor
2. Color	Greenish white	Green	Dark green
3. Form	Cream	Cream	Cream
4. Homogeneity	Homogeneous	Homogeneous	Homogeneous
pH (pH unit)	6,37±0,01	6,34±0,01	6,32±0,00
Spreadability (cm)	4,87 ±0,37	4,33±0,21	4,33±0,21
Viscosity (cps)	9765,55 ± 0,55	9772,25 ± 0,55	9764,60 ± 1,20

The kaffir lime leaf extract creams (F1-F3) exhibited acceptable organoleptic properties and consistent semi-solid, homogenous texture across all formulations. Color deepened with increasing extract concentration (greenish-white to dark green), pH values remained within the normal physiological range for skin. Spreadability and viscosity were consistent across formulations and stable over time.

Wound length (mm) was measured in the treatment (F1-F3) and control groups from days 4, 7 and 14 post-treatment.

Table 3. Average Incision Wound Healing Rate (mm)

Kelompok	Hari ke -4	P	Hari ke - 7	P	Hari ke - 14	P
P0	16,72 ±1,63		10,04 ± 2,43		0 ± 0	
P1	15,9 ±0,83		11,86 ±1,73		0 ±0	
P2	16,74 ±1,26	0,072	12,56 ±2,33	0,194	1,86 ± 4,16	0,701
P3	16,46 ±0,83		12,78 ±1,43		0,96 ±2,14	
P4	14,8 ±0,57		11,42 ±1,03		0,96 ±2,14	

On the fourth and seventh days, the assumptions of normality (as assessed by the Shapiro-Wilk test) and homogeneity of variances (as evaluated by Levene's test) were upheld ($p > 0.05$), thereby permitting the application of the One-Way ANOVA analysis. Conversely, on the fourteenth day, the Shapiro-Wilk test did not meet the requisite criteria, necessitating the utilization of the Kruskal-Wallis test, which produced a p-value of 0.701. In light of the outcomes derived from the statistical analyses, it can be inferred that no statistically significant differences exist among the various treatment groups.

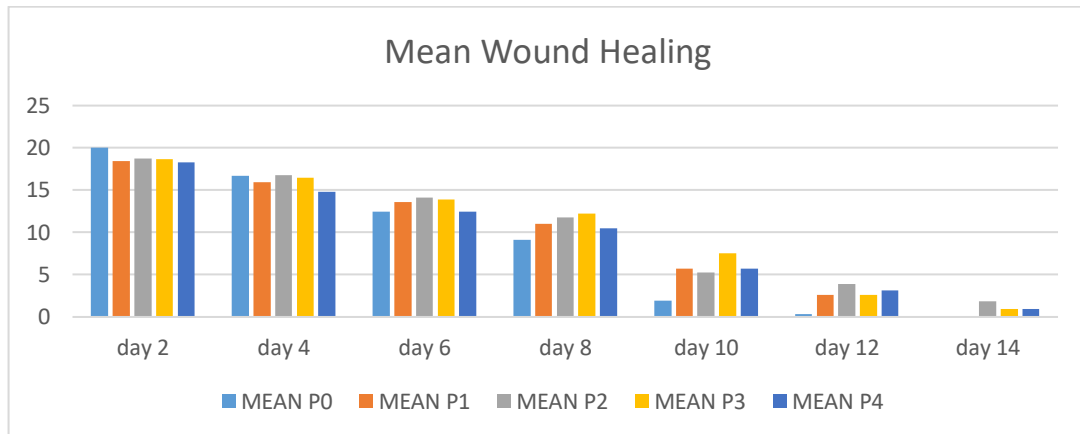


Figure 1. Mean Wound Healing

A comparison of mean incision wound lengths, recorded bi-daily from day 2 to day 14, indicated that the P0 group exhibited the most effective healing, followed by P1, P4, P3 and P2 in descending order.

Table 4. Macroscopic Observation of Incisional Wound Healing

Kelompok tikus	Tikus ke-	Hari ke-		
		4	7	14
P0 (kontrol negatif)	1	MB	M	KM
	2	MB	M	KM
	3	MB	M	KM
	4	MB	M	KM
	5	MB	MB	KM
P1 (10%)	1	MB	M	KM
	2	MB	KT	KM
	3	MB	M	KM
	4	M	M	KM
	5	MB	M	KM
P2 (20%)	1	MB	M	KT
	2	MB	M	KM
	3	MB	M	KM
	4	MB	MB	KM
	5	MB	M	KM
P3 (30%)	1	MB	M	KM
	2	MB	M	KM
	3	MB	M	KM
	4	MB	MB	KT
	5	MB	M	KM

P4 (Kontrol positif)	1	M	KT	KM
	2	MB	M	KM
	3	MB	M	KM
	4	MB	M	KT
	5	MB	M	KM

Note: 1. MB (red and swollen), 2. M (red), 3. KT (dry and open), 4. KM (close wound)

Macroscopic wound observation was performed on days 4, 7, and 14. On day 4, most rats in all groups exhibited stage 1 wounds (red and swollen), indicating an active inflammatory phase. However, one rat each in the P1 (10% extract) and P4 (gentamicin) groups had progressed to stage 2 (red), suggesting a potentially earlier transition to the proliferative phase. By day 7, one rat in both P1 and P4 groups showed stage 3 wounds (dry and open), consistent with the initiation of granulation tissue formation. Meanwhile, several rats in groups P0, P2, and P3 remained at stage 1, indicating slower healing progress. On day 14, complete wound closure (stage 4: dry and closed) was observed in all rats from the P0 and P1 groups, while one rat each in the P2, P3, and P4 groups had not yet achieved full closure.

Histopathological evaluation of fibroblast proliferation and collagen fiber density—key indicators of connective tissue formation during wound healing—was conducted through microscopic analysis. Skin sections from 25 rats were stained with Hematoxylin and Eosin (HE) and examined at 400x magnification. This analysis aimed to correlate cellular responses with the macroscopic healing outcomes previously described. The findings are presented in the following figures and tables.

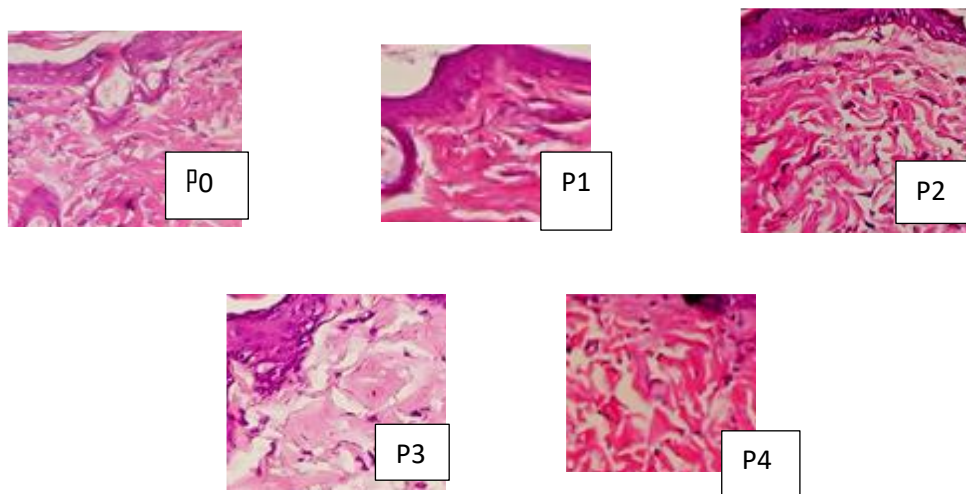


Figure 2. Representative images of histopatological features of wound Tissue on day 14

P0: 0,9% NaCl (negative control), P1: 10% extract. P2: 20% extract, P3: 30% extract, P4: 0,1% gentamicin (positive control). Note: Representative images stained with Hematoxylin & Eosin (400x magnification). Higher fibroblast proliferation and collagen density observed in P1.

Table 5. Microscopic Observation of Incisional Wound Healing

Group	Fibroblast and Collagen Scoring (M ± SD)	P-value
P0	1,4 ± 0,89	0,75
P1	1,8 ± 1,1	
P2	1,4 ± 0,89	
P3	1,4 ± 0,89	
P4	1,4 ± 0,89	

Histopathological analysis of collagen density revealed no statistically significant differences among the P0 (negative control), P2, P3, and P4 (positive control) groups ($p = 0.75$). However, the P1 group (10% extract) showed a relatively higher collagen density compared to the other groups, although the difference was not statistically significant.

DISCUSSION

Phytochemical analysis of kaffir lime leaf extract (*Citrus hystrix* DC) revealed the presence of flavonoids, alkaloids, saponins, and tannins, which play a significant role in wound healing. Alkaloids act as anti-inflammatory and antibacterial agents while supporting early vasoconstriction. Flavonoids exhibit antiseptic and antioxidant properties that aid the hemostasis and inflammatory phases. Tannins and saponins contribute to re-epithelialization and tissue maturation through antimicrobial activity and stimulation of wound contraction (Nasution, 2023; Rante, 2020). These findings are consistent with (Mutia, 2021), who reported the pharmacological potential of *Citrus* species as anti-inflammatory and antioxidant agents.

Although statistical analyses on days 4 and 7 (One-Way ANOVA) and day 14 (Kruskal-Wallis test) showed no significant differences among treatment groups, the use of appropriate methods based on data characteristics enhances the validity of the results. The absence of statistical significance suggests minimal variation in wound healing across groups during the observation period. However, the wound length trend from day 2 to day 14 indicates that groups P0 and P1 (10% extract) demonstrated more effective healing compared to others. These findings highlight the potential of kaffir lime leaf extract at specific concentrations to promote tissue regeneration, likely through anti-inflammatory and antioxidant mechanisms, and provide a valuable basis for further research on its optimal dosage and mode of action in wound healing.

Macroscopic wound assessment further supported the superior healing progression observed in P1. On day 4, one rat each in P1 and P4 already showed red wounds (stage 2), while other groups predominantly remained in the red and swollen phase (stage 1), suggesting P1 and P4 had entered the proliferative phase earlier. On day 7, one rat in both P1 and P4 advanced to the dry and open wound stage (stage 3), indicative of granulation tissue formation. Meanwhile, most rats in P0, P2, and P3 remained in stages 1 or 2. By day 14, complete wound closure (stage 4: dry and closed) was observed in all rats in P0 and P1, while one rat in each of the P2, P3, and P4 groups still showed incomplete closure, indicating delayed transition to the maturation phase.

Histopathological examination revealed that collagen density and fibroblast proliferation in P0, P2, P3, and P4 were relatively similar, while P1 exhibited a higher score, indicating superior connective tissue regeneration in this group.

Although P1 showed better wound healing outcomes, statistical analysis revealed no significant difference. Interestingly, P3 (30% extract) displayed slower healing compared to P1, despite its higher concentration. This non-linear dose-response may be attributed to reduced diffusion of active compounds at higher concentrations. As extract concentration increases, it may reach a saturation point where excessive density hinders diffusion, limiting the optimal absorption of active constituents into the tissue. Other factors may have also contributed to the absence of statistical significance, such as the relatively small sample size, which increases the risk of generalization error. Intrinsic biological factors, such as animal stress and elevated cortisol levels, are also known to impair wound healing (Djuddawi et al, 2019)

These findings differ from those of Astriani and Sophia (2021), who reported that higher concentrations of the extract exhibited greater antimicrobial activity in vitro against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. This discrepancy suggests that the efficacy of a plant extract is not solely determined by its concentration, but also by factors such as the formulation type, route of application, and the biological characteristics of the target tissue.

Overall, P1 (10%) outperformed both the negative control (P0) and the positive control (P4) in promoting wound healing. While NaCl 0.9% functions primarily as a wound cleanser and gentamicin (an aminoglycoside antibiotic) acts to inhibit bacterial growth, the 10% kaffir lime extract offers additional therapeutic benefits through its bioactive phytochemicals. These compounds, with their anti-inflammatory, antibacterial, and antioxidant properties, likely contribute to enhanced tissue regeneration and more optimal wound healing (Rante, 2020).

CONCLUSION

Kaffir lime leaf (*Citrus hystrix* DC) extract cream demonstrated variable effects on incisional wound healing in Wistar rats. Phytochemical analysis confirmed the presence of flavonoids, alkaloids, saponins, and tannins with known anti-inflammatory, antibacterial, and antioxidant activities. The 10% extract cream (P1) showed the most favorable wound healing outcomes among the groups, although the differences were not statistically significant. This may be attributed to factors such as limited sample size, diffusion barriers at higher concentrations, and biological variability.

These findings support the potential of kaffir lime leaf extract as a natural wound healing agent. Further studies involving larger sample sizes, broader concentration ranges, extended observation periods, and toxicity profiling are recommended to validate its therapeutic efficacy and ensure safety for future clinical application.

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