

The Effectiveness of Moringa Leaf Extract Ointment (*Moringa oleifera lam*) In the Healing Process of Incision Wounds in Mice (*Mus musculus*)

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ABSTRACT

Background: Wounds are a form of damage or loss of tissue in the body. The use of both modern and traditional medicines in wound healing itself aims to speed up the healing process. One of the traditional plants that can be used as medicine is moringa leaf (*moringa oleifera lam*).

Objective: The aim of this study is to knowing the effectiveness of moringa leaf extract ointment (*moringa oleifera lam*) in the healing process of incision wounds in mice (*mus musculus*).

Methods: This study uses a true experiment design research design with a post test control group. The sampling technique is using purposive quota sampling and sample grouping by randomization. Samples divided into 4 groups consisted of aquades group, moringa leaf extract ointment group 5%, 10%, and 15%. The wound healing was valuation by measuring the length of the wound using a caliper and macroscopic observation of fibroblast growth. The study analysed in univariate and bivariate by Kruskal-Wallis test and continued with post hoc LSD test.

Result: Bivariate analysis result shows there is a significant difference of giving moringa leaf extract ointment (*moringa oleifera lam*) to the length of closing of the mice incision wound (*Mus musculus*) with significance value $p < 0,05$ on the 3rd day ($p = 0,001$) and 7th ($p = 0,000$). The results of the stung number of fibroblasts showed that Moringa leaf extract ointment with a concentration of 5% had the largest increase in the number of fibroblasts compared to other groups.

Conclusion: There is a significant difference effect of giving moringa leaf extract ointment (*moringa oleifera lam*) on the healing process of incision wounds in mice (*mus musculus*) with a concentration of 5%.

KEYWORDS: Moringa leaf extract, Mice, Ointment, Incision Wound, Fibroblast Growth.

INTRODUCTION

A wound is defined as tissue damage or loss that may result from various factors disrupting the body's protective mechanisms, such as mechanical trauma, electrical trauma, thermal trauma, and chemical trauma. A wound causes impairment of both anatomical structure and body function.¹ An incised wound (*vulnus scissum/incisivum*) is trauma caused by sharp objects, either accidentally or for medical purposes, leading to tissue damage. Incised wounds are typically elongated with smooth and well-defined edges.² This type of wound is classified as an open wound, which directly communicates with the external environment. Based on depth, incised wounds of the skin are divided into stage I, stage II, stage III, and stage IV.³ Data from the Ministry of Health of the Republic of Indonesia (2008) reported that the prevalence of open wound injuries in Indonesia was 25.4%, with the prevalence being highest (32.0%) in the age group of 25–34 years.⁴

Following tissue damage, the body initiates the wound healing process. In this process, the body exhibits a physiological response characterized by a series of complex mechanisms aimed at restoring tissue integrity. Wound healing is divided into three phases: inflammation, proliferation, and maturation. The inflammatory phase occurs as a response to soft tissue injury, involving vascular and cellular reactions that serve to stop bleeding and clear the wound of foreign materials, bacteria, and necrotic cells, thereby allowing the healing process to begin. Fibroblasts are responsible for the proliferative phase in the wound repair process.

Fibroblasts are spindle-shaped or flattened connective tissue cells with elongated, ovoid nuclei and finely granular cytoplasm. They synthesize structural protein products such as mucopolysaccharides, and the amino acids glycine and proline, which serve as the basic components for collagen formation. Collagen functions to approximate and stabilize the wound, while fibroblasts also influence the process of re-epithelialization, which leads to wound closure and tissue reconstruction. Collagen provides strength and integrity to all wounds that heal properly. Therefore, fibroblasts play a crucial role in determining the final outcome of wound healing. The last phase, maturation, aims to remodel and strengthen the newly formed tissue into a more durable and functional structure.³ In addition, several factors may influence the wound healing process, including age, nutrition, infection, circulation and oxygenation, wound condition, and medications.

The use of medication in wound healing aims to accelerate the healing process. Medications can originate from modern drugs or from natural remedies derived from traditional sources such as plants and spices. According to the World Health Organization (WHO), countries in Africa, Asia, and Latin America have utilized traditional medicine as a complement to their primary healthcare. In fact, up to 80% of the population in Africa relies on herbal medicine as their primary treatment (WHO, 2003).⁶ Data from the Central Bureau of Statistics (2014) in Indonesia reported that 90.54% of the population self-medicated with modern drugs, while 20.99% used traditional medicine. In East Nusa Tenggara, the prevalence of self-medication with traditional medicine was 30.44%.⁵ Traditional medicine refers to formulations prepared from natural materials, including plants, animals, minerals, galenic preparations, or their mixtures, which have been used as therapeutic sources based on empirical knowledge.⁷ Although traditional medicine is highly favored by the community due to its accessibility and relatively minimal side effects, its use should not be arbitrary. Each ingredient must first be examined for its content and therapeutic properties to avoid potential adverse effects on health.⁶

One of the plants that can be used as a traditional remedy is moringa (*Moringa oleifera* Lam). Moringa (*Moringa oleifera* Lam) is an indigenous plant from Indonesia that has applications as both medicine and an antioxidant.⁸ Moringa is known to contain more than 90 types of nutrients, including essential vitamins, minerals, amino acids, anti-aging, and anti-inflammatory compounds. It also contains flavonoids, saponins, alkaloids, tannins, and phenols.⁹ A study conducted by Hendri Poernomo and Setiawan in 2019 on the effect of moringa (*Moringa oleifera* Lam) leaves in accelerating the reduction of inflammatory signs (erythema) in sterile wounds of guinea pigs (*Cavia porcellus*) demonstrated that moringa leaves were effective in accelerating the wound healing process, as observed from the reduction of erythema. The results of this study revealed that 15% moringa leaf gel effectively increased collagen density and shortened bleeding time.¹⁰

Ointment is a semisolid dosage form used as a topical medication that can be applied to the skin and mucous membranes. The selection of the ointment base significantly influences its therapeutic effect, as it determines the optimal release of the active substance. The release of the active substance itself may be affected by the physicochemical properties of the ointment base and the drug, including solubility, viscosity, particle size, homogeneity, and formulation. Ointment bases are generally classified into four types: hydrocarbon bases, absorption bases, water-removable bases, and water-soluble bases.^{11,12} A study conducted by Rahmat Nasution in 2019 on the antibacterial activity test of moringa (*Moringa oleifera* Lam) leaf methanol extract ointment against *Staphylococcus epidermidis* in incised wound healing of mice revealed that the ointment containing a 10% concentration was able to heal incised wounds in mice within five days.¹³

Based on the aforementioned description, the author aims to further investigate the benefits of moringa (*Moringa oleifera* Lam) leaves in the medical field, particularly in the healing process of incised wounds in mice (*Mus musculus*) through the administration of moringa leaf extract ointment (*Moringa oleifera* Lam) prepared using ethanol as a solvent.

METHOD

The research began with the preparation of moringa (*Moringa oleifera* Lam) leaf extract ointment which was carried out at the Bioscience Laboratory of Nusa Cendana University, Kupang. The moringa leaf extract was then tested for its chemical compounds at the Chemistry Laboratory of Widya Mandira Catholic University, Kupang. The extract was subsequently formulated into moringa leaf extract ointment preparations with three variations, namely 5%, 10%, and 15%, which was conducted at the Pharmacy Laboratory of Poltekkes Kemenkes Kupang. Furthermore, the research was carried out at the Laboratory of the Faculty of Medicine and Veterinary Medicine, Nusa Cendana University, Kupang, and the preparation of specimens was performed at the Anatomical Pathology Laboratory of Prof. Dr. W.Z. Johannes General Hospital. This study is a laboratory experimental research

with a true experimental design using a post-test with control group. In this study, the samples were divided into four groups, namely the aquadest group, the 5% moringa leaf extract ointment group, the 10% moringa leaf extract ointment group, and the 15% moringa leaf extract ointment group. The assessment of the study was carried out in two ways, namely macroscopically and microscopically. Macroscopic assessment was performed by measuring the wound length from the first day of incision until the wound completely closed, using a caliper (cm). Microscopic assessment was performed by observing the increase in the number of fibroblasts using an Amscope microscope at 40 x 10 magnification in four fields of view. The wound closure data examined were obtained from the wound length of mice on day 3, day 7, day 10, and day 14. The fibroblast proliferation data were obtained from experimental animals that were terminated on day 3 (two mice), day 7 (two mice), day 10 (one mouse), and day 14 (one mouse) from each group. The data analysis results were considered homogeneous if $p > 0.05$ and normally distributed according to the Shapiro–Wilk test if $p > 0.05$. Bivariate analysis was performed to assess the effect of moringa leaf extract ointment administration; if the requirements for parametric tests were met, One-Way ANOVA was used, whereas if not, the Kruskal–Wallis test was applied, followed by a Post Hoc test. The Post Hoc test used was the LSD (Least Significant Difference) test.

RESULT

The concentrated extract of moringa (*Moringa oleifera* Lam) leaves obtained through maceration and evaporation using a rotary evaporator yielded 144.455 g of thick extract. Phytochemical screening of the moringa leaf extract revealed the presence of alkaloids, tannins, steroids, flavonoids, terpenoids, and saponins.

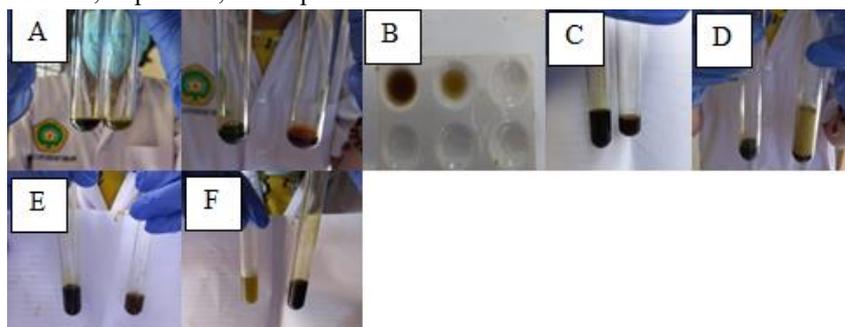


Figure 1. Phytochemical Test

Description: A. Alkaloids, B. Tannins, C. Steroids, D. Flavonoids, E. Terpenoids, F. Tannins

The moringa (*Moringa oleifera* Lam) leaf extract ointments with concentrations of 5%, 10%, and 15% were prepared in semisolid form with a characteristic odor of moringa leaf extract, free of granules, normal pH levels, and good spreading ability. The 5% ointment variant exhibited a pale green color, the 10% variant showed a brownish-green color, and the 15% variant appeared dark green.



Figure 2. Results of Moringa Leaf Extract Ointments

Description: Moringa leaf extract ointment 5% (F1), Moringa leaf extract ointment 10% (F2), Moringa leaf extract ointment 15% (F3)

The measurement of incised wound length and the observation of fibroblast proliferation in mice were carried out to determine the effect of moringa (*Moringa oleifera* Lam) leaf extract ointment on the healing of incised wounds in mice.

Table 1. Average Wound Length

Group of Mice	Day of Wound Length Observation (cm)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Aquadest	1	0,95	0,88	0,80	0,76	0,71	0,66	0,51	0,43	0,32	0,20	0	0	0
5% Ointment	1	0,80	0,74	0,64	0,62	0,59	0,54	0,52	0,51	0,49	0	0	0	0
10% Ointment	1	0,80	0,75	0,68	0,66	0,61	0,58	0,58	0,55	0,53	0,43	0,32	0,22	0
15% Ointment	1	0,87	0,82	0,74	0,70	0,65	0,62	0,61	0,56	0,48	0,41	0,33	0	0

The mean wound length table demonstrates that moringa leaf extract ointment is effective in accelerating the healing process of incised wounds in mice, as observed in the 5% ointment group with complete wound closure on day 11, followed by the aquadest group with complete wound closure on day 12, then the 15% ointment group with complete wound closure on day 13, and finally the 10% ointment group with complete wound closure on day 14.

Table 1. Increase in the Number of Fibroblasts

Group	Fibroblast count (day)			
	Day 3	Day 7	Day 10	Day 14
Aquadest Group	29	30	50	30
5% Extract Ointment Group	34	54	76	44
10% Extract Ointment Group	41	44	36	38
15% Extract Ointment Group	42	49	53	37

Based on microscopic observations, the increase in the number of fibroblasts in each group varied. In the aquadest group, the highest increase in fibroblast count was observed on day 10, followed by a decrease on day 14. In the 5% ointment group, an increase in fibroblast count was observed on day 10, with the number of fibroblasts being markedly higher compared to the other groups. In the 10% ointment group, an increase in fibroblast count occurred on day 7, followed by a decrease on day 10. In the 15% ointment group, an increase in fibroblast count was observed on day 10, followed by a decrease on day 14.

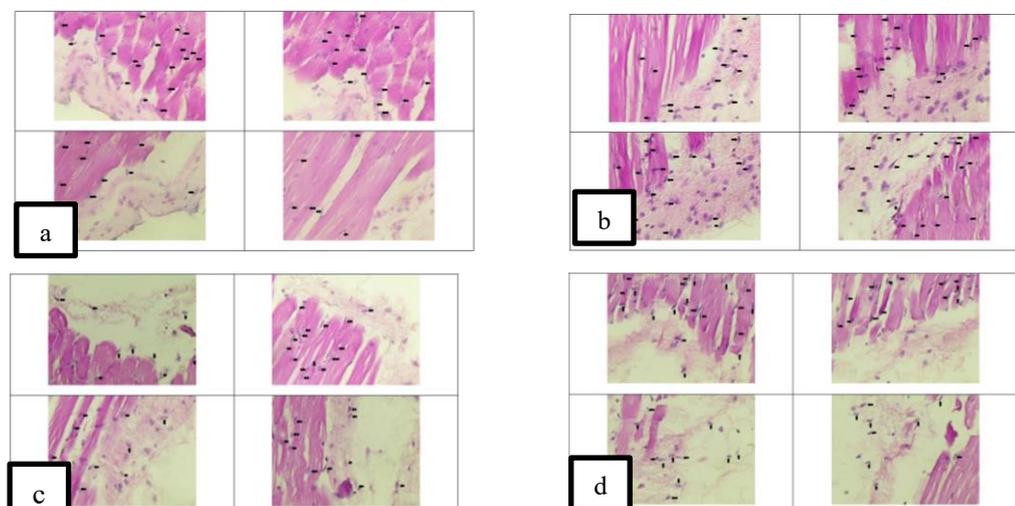


Figure 3. Histological Features of Incision Wounds



Description: a. Aquadest Group, Day 10; b. Moringa Leaf Extract Ointment 5% Group, Day 10; c. Moringa Leaf Extract Ointment 10% Group, Day 7; d. Moringa Leaf Extract Ointment 15% Group, Day 10.

The results of the bivariate analysis showed that there was a significant effect on wound closure length between the aquadest group and the 5% Moringa leaf extract ointment group on day 3 with a p-value < 0.005 (0.007), and on day 7 with a p-value < 0.005 (0.002).

Subsequently, an LSD test was performed by comparing the aquadest group with the three groups receiving Moringa leaf extract ointment on day 3 and day 7.

Table 2. LSD Test of Wound Length on Day 3

(I) Group	(J) Group	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Lower Bound
Aquadest	5% Ointment	0,13667*	0,001	0,0623	0,2110
	10% Ointment	0,12167*	0,003	0,0473	0,1960
	15% Ointment	0,05333	0,150	-0,0210	0,1277

The results of the LSD test for wound length on Day 3 showed a significant difference between the aquadest group and the 5% moringa leaf extract ointment group, with a p-value < 0.05 (0.001).

Table 3. LSD Test of Wound Length on Day 7

(I) Group	(J) Group	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Lower Bound
Aquadest	5% Ointment	0,12750*	0,000	0,0712	0,1838
	10% Ointment	0,08500*	0,006	0,0287	0,1413
	15% Ointment	0,04500	0,107	-0,0113	0,1013

The LSD test results for wound length on day 7 showed a significant difference between the aquadest group and the 5% moringa leaf extract ointment group, with a p-value < 0.05 (0.000). Data analysis was also performed on the increase in the number of fibroblasts using the Kruskal-Wallis test, and the results showed a p-value = 0.201, indicating that there was no significant effect among the compared groups in the increase in the number of fibroblasts.

DISCUSSION

Wounds, as a manifestation of tissue damage in the skin, can be classified into several types, one of which is an incised wound.¹ An incised wound is a trauma caused by a sharp object, either accidentally or for medical purposes, resulting in tissue damage. Incised wounds are characterized by an elongated shape with regular edges.² In the wound healing process, the body exhibits a physiological response to the injury, consisting of various complex mechanisms to restore tissue integrity. The wound healing process is divided into three phases: inflammation, proliferation, and maturation.

The inflammatory phase occurs as a result of injury to soft tissue, leading to vascular and cellular responses aimed at stopping bleeding and clearing the wound of foreign bodies, bacteria, and necrotic cells, thereby allowing the healing process to begin. Fibroblasts play a crucial role in the proliferative phase of repair. Fibroblasts are spindle-shaped or flattened cells within connective tissue, with elongated ovoid nuclei and finely granular cytoplasm. Fibroblasts synthesize structural protein products, including mucopolysaccharides, and the amino acids glycine and proline, which serve as the fundamental components for collagen formation. Collagen, in turn, binds the wound, influences the process of re-epithelialization that closes the wound during tissue reconstruction, and provides strength and integrity to all wounds that heal properly. Therefore, fibroblasts are essential in determining the final outcome of wound healing. Finally, the maturation phase serves to refine the newly formed tissue into strong and high-quality tissue.³



In this study, ointments of moringa leaf extract at concentrations of 5%, 10%, and 15% were used, with aquadest as the control group. The results of this study showed that moringa leaf extract ointment exerted an effective wound-healing effect, occurring between days 7–14 across the four groups. Macroscopic observations were conducted by measuring the wound closure length for 14 days using a caliper. Based on the observations, the wound healing time varied among the groups. In the aquadest group, wound healing was observed on day 12. In the 5% moringa leaf extract ointment group, wound healing occurred on day 11. In the 10% moringa leaf extract ointment group, wound healing was observed on day 14. In the 15% moringa leaf extract ointment group, wound healing occurred on day 13. From the obtained data, it can be concluded that the 5% moringa leaf extract ointment group demonstrated a faster healing time compared to the other groups, whereas the 10% moringa leaf extract ointment group demonstrated a slower healing time compared to the other groups.

This is due to the fact that the moringa plant (*Moringa oleifera* Lam) is a nutrient-rich plant that is beneficial for the body.²⁴ The results of the chemical test on the macerated moringa leaf extract revealed the presence of alkaloids, flavonoids, saponins, tannins, and steroids. This finding is consistent with the study conducted by Putra et al. in 2006, who performed phytochemical screening of moringa leaves (*Moringa oleifera* Lam) by macerating moringa leaf powder with ethanol solvent, which demonstrated the presence of flavonoids, alkaloids, saponins, terpenoids, tannins, and steroids.⁹

The inflammatory process begins at the time of injury and lasts for three days. When tissue injury occurs, vasoconstriction of arteries and capillaries takes place with the aim of stopping bleeding. The saponin content in moringa leaf extract has a mechanism that can trigger vascular endothelial growth factor (VEGF) and increase the number of macrophages migrating to the wound area.⁴² Neutrophils, macrophages, mast cells, endothelial cells, and fibroblasts are stimulated by inflammatory cells through Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF), Transforming Growth Factor α (TGF- α), Transforming Growth Factor β (TGF- β), and Platelet-Derived Growth Factor (PDGF). The alkaloid and saponin content can stimulate an increase in Epidermal Growth Factor (EGF), which plays a role in stimulating the growth of epidermal and epithelial cells, thereby accelerating the wound healing process. EGF exerts a proliferative effect on cells of ectodermal origin, particularly keratinocytes and fibroblasts. Saponins can also stimulate an increase in Fibroblast Growth Factor (FGF), which promotes cell proliferation. Alkaloids can also increase Transforming Growth Factor α (TGF- α), which plays a role in stimulating the growth of epidermal and epithelial cells.⁹ ⁴² Tannins have the ability to induce TGF- β , which functions in cell differentiation and regulates the expression of genes involved in tissue repair.⁴³ During the inflammatory process, Reactive Oxygen Species (ROS) are also generated, which are free radicals produced to accelerate wound cleansing from bacterial invasion. However, in low amounts, ROS can inhibit the migration and proliferation of various cell types, including skin cells (keratinocytes), whereas in high amounts they can cause severe tissue damage and potentially progress to neoplasia. Steroids, as anti-inflammatory and antioxidant agents contained in moringa leaf extract, may play a role in neutralizing these free radical compounds during the inflammatory phase.³

The inflammatory phase that has been successfully completed will then proceed to the proliferative phase. This phase occurs between the third and fifth day, during which there is a reduction in the number of cells, a decrease in signs of inflammation, and the emergence of proliferated fibroblasts, new blood vessel formation, epithelialization, and wound contraction.³ The flavonoid and terpenoid content in moringa leaf extract, acting as antimicrobial and astringent agents, can aid in wound contraction and enhance the rate of epithelialization. Flavonoids may also promote fibroblast proliferation, thereby increasing collagen synthesis and enhancing oxygen diffusion into the cells. Terpenoids play a role in reducing lipid peroxidation by preventing cell necrosis and increasing the rate of vascularization.²⁹ In addition, tannins have the ability to induce TGF- β , which plays a role in fibroblast proliferation.⁴³ The increase in fibroblast count was observed microscopically on day three in each group, showing that the increase in fibroblast count in the 5%, 10%, and 15% moringa leaf extract ointment groups was markedly higher compared to the aquadest group. Observations also revealed that the increase in fibroblast numbers varied across the groups. In the aquadest group, the highest fibroblast count was observed on day 10, followed by a decline on day 14. In the 5% ointment group, fibroblast proliferation peaked on day 10 with a markedly higher count compared to the other groups, followed by a decrease on day 14. In the 10% ointment group, the fibroblast count peaked on day 7 and declined on day 10. In the 15% ointment group, fibroblast proliferation peaked on day 10 and decreased on day 14.

“Macroscopic and microscopic observations based on wound healing time and the increase in fibroblast count showed a proportional relationship, in which the 5% moringa leaf extract ointment group demonstrated a faster wound closure time along with a higher increase in fibroblast count compared to the other groups. This was followed by the aquadest group, and subsequently



the 15% ointment group. In contrast, the 10% moringa leaf extract ointment group exhibited a slower wound closure time, with a decrease in fibroblast proliferation starting on day 10, whereas the other groups showed a decrease in fibroblast count on day 14. The results of this study are consistent with the findings of Niswah (2013), which demonstrated differences in wound healing effectiveness at each concentration.³ The 5% moringa leaf extract ointment concentration provided a more effective wound healing effect, whereas the 10% and 15% concentrations also exerted wound healing effects.

The final phase is the maturation phase, which aims to refine the newly formed tissue into strong and well-structured tissue. This phase begins in the third week after injury and lasts for approximately 12 months. During this phase, fibroblasts gradually leave the granulation tissue, blood vessels undergo regression so that the reddish color of the tissue fades, and collagen and fibrin fibers increase in number to strengthen the scar tissue.

CONCLUSION

Based on this study, several conclusions can be drawn :

1. There is a significant effect of the administration of moringa leaf (*Moringa oleifera* Lam) extract ointment at a concentration of 5% on the healing process of incised wounds in mice (*Mus musculus*).
2. The 5% moringa leaf extract ointment formulation demonstrated a more optimal effect on wound healing, as evidenced by wound closure length, wound closure time, and the increase in fibroblast count compared to the aquadest group.

SUGGESTIONS

1. Further research is needed regarding the comparison among different formulations of moringa leaf (*Moringa oleifera* Lam) extract.
2. Additional studies are required on moringa leaf (*Moringa oleifera* Lam), which contains various bioactive compounds and therefore has the potential to be developed as another natural-based therapeutic agent.

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