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The Effectiveness of Binahong (*Anredera cordifolia*) Leaf Extract Ointment in Stimulating Collagen Regeneration in the Dorsal of Mice

Noura Ramadhani Putri Okven, Safa Alzena, Umi Nadifah
Madrasah Aliyah Negeri Insan Cendekia OKI
Teluk Gelam, Ogan Komering Ilir, Sumatera Selatan 30673, Indonesia. Telp (0721) 321486.

Corresponding author
nourarara26@gmail.com

Abstract: Improperly treated incision wounds have the potential to cause infection and slow down the healing process. One approach to accelerating tissue regeneration is the use of bioactive compounds found in medicinal plants. Binahong leaves (*Anredera cordifolia*) are known to contain flavonoid compounds that play an important role in increasing collagen fiber production, thereby greatly contributing to the wound healing process. This study aims to determine the effectiveness of binahong leaf extract ointment by comparing control variables and the treatment of binahong leaf extract ointment on collagen fiber density after wounding on the dorsal of the mice. This study uses a test-only control group experimental design with 16 mice divided into 4 groups, with each group consisting of 4 mice. Two groups were given binahong leaf extract ointment (P1, P2) for different durations, namely 3 days and 4 days, with an extract concentration of 15% along with two control groups for durations of 3 days and 5 days (P3, P4). Incision wound were made 1,2 cm long and 0,5 cm deep on the dorsal of the mice. They were then observed macroscopically (measuring wound size) and microscopically (assessing collagen fiber density through scoring by an anatomical pathology specialist). The results showed that the treatment groups given binahong leaf extract ointment experienced an increase in collagen fiber density with an average score of 3,375 and a reduction in wound size 0,1 x 0,06 cm. In contrast, the control groups only reached an average score of 2 with wound size reduced to 0,6 x 0,06 cm. These data indicate that binahong leaf extract ointment is effective in accelerating the healing process of incision wound through the mechanism of increasing collagen fiber density and reducing wound size.

Keywords: Binahong leaves; collagen density; wounds.

Introduction

Incision wounds are a common type of injury that can cause serious complications if not treated properly. Improper treatment may allow the entry of pathogenic microorganisms into the wound, leading to local infections that can progress into systemic infections. This condition not only delays the healing process but also increases the risk of abscess formation and may even become life-threatening (Song et al. 2023). Furthermore, incision wounds can develop into chronic wounds when the migration of skin cells fails or healing does not occur over an extended period (Moenadjat 2023). Chronic wounds often require longer treatment durations, higher medical costs, and can significantly reduce the patient's quality of life.

Wound healing is a complex and dynamic biological process consisting of four main phases: hemostasis, inflammation, proliferation, and maturation (Sorg and Sorg 2023). During these stages, collagen plays a crucial role in this process as a component of the extracellular matrix. Collagen types I, II, and III are involved in tissue re-generation and strengthening of the damaged area, supporting the formation of granulation tissue, and promoting new skin formation. The deposition of collagen fibers is considered an important indicator of successful wound healing, as it reflects the degree of tissue re-generation and wound closure (Mathew-Steiner, Roy, and Sen

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2021). The accelerate healing process, various substances with antiseptic and regenerative properties have been explored, including natural compounds derived from medicinal plants. Among these, flavonoids have shown significant potential in supporting wound healing through several mechanisms. They can reduce inflammation, enhance angiogenesis (formation of new blood vessels), accelerate re-epithelialization, and decrease oxidative stress (Sartika, Rahmi, and Noorfajriwianti 2021). Flavonoids also modulate the activity of macrophages, fibroblasts, and endothelial cells through signaling pathways such as TGF -β, VEGF, NF-Kb and PI3K/Akt, while suppressing pro-inflammatory cytokines and reactive oxygen species (ROS). Moreover, flavonoids containing anthocyanins contribute to increased collagen synthesis cause their antioxidant properties (Venugopal et al. 2022).

One of the herbal plants known to contain flavonoids and other bioactive compounds beneficial for wound healing is *Anredera cordifilia* (Ten) Steenis, commonly known as binahong. Binahong leaves possess antibacterial, anti-inflammatory, analgesic, vasodilatory, and tissue-regenerating effects (Mengga, Rampe, and Sangande 2022). The extract of binahong leaves has been developed into ointments to enhance ease of application and prolong the retention of active compounds, allowing better absorption and therapeutic effectiveness. Additionally, ointments do not contain alcohol, thereby minimazing irritation risk.

Before being applied to humans, the effectiveness of new herbal formulations is commonly evaluated using animal models such as *Mus musculus* (mice). Mice are widely used in biomedical research because of their genetic and physiological similarities for human including comparable organ structures. These similarities make mice reliable models for studying the effects of natural compounds on wound healing (Mutiarahmi, Hartady, and Lesmana 2021).

Although several studies have reported the healing effects of binahong leaves based on physical wound observations, there is limited research has focused on its effect on collagen fiber thickness; a crucial parameter indicating the quality of tissue regeneration. Therefore, this study aims to evaluate the effectiveness of *Anredera cordifolia* leaf extract ointment on collagen fiber thickness in incision wound of mice *(Mus musculus*). The results of this study are expected to provide additional scientific evidence supporting the potential of *A. cordifolia* as natural agents that promotes wound healing.

Materials And Methods

Materials and Tools

The tools used are mouse cage, sample food and water container, handscoop and mask, surgical scissors, measuring cup, mortar and pestle, light microscope, ointment container, tweezers, cotton swabs, volumetric flasks, object glass. Then the materials are binahong leaves, 96% ethanol, methanol, methylparaben, aquadest, hematoxylin and eosin, ketamine, formalin, xylol, glycerin, vaseline, adeps lanae.

Procedures

Preparation of Experimental Animals

The preparation of the experimental animals involved a three-day adaptation period to the environment. During the adaptation period, the mice were fed pellets and water ad libitum. The mice were divided into four groups in different cages. The mice were cared for according to animal welfare standards, including proper ventilation, temperature, humidity, and lighting cycles.

Binahong Leaf Extract Preparation

The binahong leaves used were fresh, bright green, smooth-surfaced, and free from damage. First, the leaves were washed throughly, then cut into smaller pieces and dried in an oven at 45℃ for several days until completely dry. The leaves were ground using a blender until a fine powder was obtained.

The extraction of binahong leaves was carried out using the maceration method, as it is simple and does not damage the active compounds contained in the leaves (Dewi 2021). The stages of binahong leaf extraction are as follows:

1. The powdered binahong leaves were placed in an airtight container, then 96% ethanol was added until all the powder was completely submerged (approximately 2-3 cm above the surface of the powder). The container was then tightly closed and stored in a dark room for 48 hours.
2. The mixture was filtered to separate the filtrate from the residue. The obtained filtrate was transferred into a beaker covered with aluminum foil and then placed in an oven
3. The filtrate was left in the oven at a temperature of 45-50℃ until a concentrated extract was obtained.

Ointment Preparation

According to Alfilaili et al., (2022), ointment preparation uses vaseline and adeps lanae. Adeps lanae was used as a supporting ingredient because of its ability to absorb water, which helps increase the spread ability of the ointment. Meanwhile, vaseline was used to reduce the consistency of the ointment, making it softer and enhancing its spread ability. The steps for preparing the ointment were as follows:

1. Each ingredient (extract, vaseline, and adeps lanae) was accurately weighed.
2. The extract was ground using a mortar and pestle until it became smooth.
3. Adeps lanae and vaseline were gradually added, and the mixture was stirred continuously until a homogeneous ointment was obtained.

Treatment on the Mice's Back

Before wounding, the dorsal of the mice was shaved using scissors and cleaned with alcohol to encure sterility. Afterward, an incision measuring approximately 1.2 cm in length and 0.5 cm in depth was made on the back, followed by re-sterilization of the wound area. The mice were then given either control treatment or no treatment (Nabila et al. 2023). The wounding procedure was performed by trained professionals in accordance with the established standard operating procedures (SOP).

Post-Wounding Treatment Application

In this study, binahong leaf extract was administered to groups P1 and P2, and untreated to groups P3 and P4. The ointment was applied twice within 24 hours.

Figure 1. Injuries on the backs of mice

Euthanasia

Euthanasia of the mice was performed using the cervical dislocation method. This method was chosen because it is considered to minimize pain and distress during the process of death. Groups 1 and 3 were euthanized on day 5. The cervical dislocation procedure was carried out by trained professionals in accordance with standart operating procedures (SOP) and with attention to animal welfare.

Tissue Fixation and Processing

After euthanasia, tissue samples were collected by excising the wounded area on the mice's back using surgical scissors, According to Sitohistoteknologi by Khristian and Inderiati (2017), the tissue fixation and processing stages were carried out as follows: The wound tissue was cut and placed into an embedding cassette. The process began with fixation using 10% formalin solution for 12-18 minutes. This was followed by a gradual dehydration process using 70% alcohol for 15 minutes, 80% alcohol for 1 hour, 96% alcohol for 2 hours, and 100% alcohol for 3 hours.

Next, the clearing process was performed using xylene three times, each for 1-2 hours. After clearing, the tissue was embedded in paraffin.

Once the paraffin solidified, the tissue block were sectioned at a thickness of 4-6 nm using a microtome. The tissue sections were then mounted on glass slides using as water bath at 37°C. The slides were subsequently heated on a hotplate at 60°C for 30 minutes to melt any remaining paraffin. Finally, the tissue sections were stained with Hematoxylin-Eosin to enhance visualization of tissue structures under a microscope.

Tissue Observation
The tissue samples were observed under a light microscope. The primary structure examined was the collagen fibers in the treated wound tissue on the mice's back.

Phytochemical Screening Test

1. Alkaloid Test
   The extract was treated with 2% HCl and stirred until homogeneous. The mixture was then divided into three different test tubes, each treated with a different reagent and positive response standard. The first tube was treated with Dragendorff's reagent, producing an orange red precipitate; the second tube was treated with Mayer's reagent, producing a white to yellowish precipitate. If the addition of these reagents produced the corresponding characteristic precipitates, the extract was indicated to contain alkaloids (Maheshwaran et al. 2024).
2. Flavonoid Test
   The extract was dissolved in methanol and mixed with 0.5 g of magnesium metal and five drops of concentrated HCl, then heated. A color change of the extract to red indicated the presence of flavonoids (Salim et al. 2024).
3. Triterpenoid and Steroid Test
   A mixture consisting of 0.5 mL of chloroform and 0.5 mL of acetic anhydride was added to the extract. Concentrated sulfuric acid was then carefully added along the wall of the test tube. The formation of a brownish or violet ring indicated the presence of triterpenoids, whereas the appearance of a bluish ring indicated the presence of steroids (Herawati et al. 2021).
4. Saponin Test
   The extract was mixed with hot water and allowed to cool to room temperature. Once cooled, the test tube was tightly closed and shaken vigorously for 10 seconds. The height of the foam produces was measured, and the mixture was left to stand for 10 minutes before measuring the height of the secondary foam. If the foam height remained between 1-10 cm after standing, the extract was indicated to contain saponins (Nhon Hoang et al. 2023).
5. Tannin Test
   The extract was mixed with 2 mL of 1% $FeCl_3$ solution. The formation of a dark blue or blackish color indicated the presence of tannins in the extract (Gurning et al. 2021).
6. Quinone Test
   The extract placed in a test tube was treated with 1 N NaOH. The appearance of a red coloration indicated that the extract contained quinones (Dulo et al. 2021).

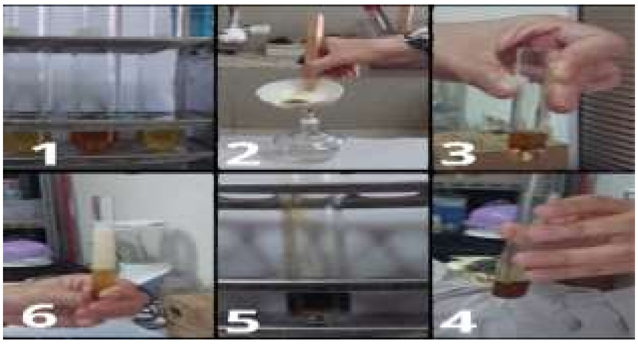

Figure 2. Alkaloid Test (1); Flavonoid Test (2); Triterpenoid and steroid Test (3); Saponin Test (6); Tannin Test (5); Quinone Test (4).

Microbiological Test
The microbiological test was conducted by BBLK Palembang using the following methods:

1. Total Plate Count (TPC)
   According to Hasibuan et al., (2021), this method begins by adding the sample into a petri dish, followed by the addition of agar medium. The observed parameter was the number of microbial colonies growing at different heating durations 1 hour, 2 hours, and 3 hours. Test result below 1.0×10^4 CFU/gram indicate that the sample is safe and acceptable for use.

2. Fungal Culture
   Based on Tjampakasari and Rosalina (2023), a sterile Petri dish was prepared with cotton and an L-shaped glass spreader inside as support for a glass slide placed on top. Fungal colonies were collected using an inoculating needle and streaked on the four sides of the agar. The plate was then covered with a cover glass. Sterile distilled water was added under the slide until it soaked the cotton, and fungal growth was subsequently observed.

Pathogenic Bacterial Culture (*E. coli, Salmonella, S. aureus,* and *P. aeruginosa*)

1. *Escherichia coli*, the sample was inoculated on EMBA medium using the spread plate method and incubated for 24 hours at 37°C. Colonies exhibiting a metallic green sheen were identified as *E. coli* (Zheng et al. 2022).
2. *Salmonella*, a 10 µL loop of the sample was inoculated on XLD agar and incubated for 24 hours. *Salmonella colonies* appeared as pink colonies without a black center (Geletu, Usmael, and Ibrahim 2022).
3. *Staphylococcus aureus*, the sample was inoculated on MSA medium sing the spread plate method. Discrete colonies formed on MSA plates were incubated in an inverted position for 24 hours at 37°C (Olawale et al. 2020).
4. *Pseudomonas aeruginosa*, the sample was inoculated on several types of agar media and incubated for 24 hours at 37°C (Ezeador et al. 2020).

Data analysis

The data obtained from this study were first tested for normality using the *Shapiro-Wilk* test, as the sample size was fewer than 50. Homogeneity was then tested using *Levene's* test to determine whether the data wwere normaally distributed. An independent *t*-test was conducted to compare collagen fiber density between the extract-treated groups on days 3 and 5.

Subsequently, a parametic analysis using *Two-way* ANOVA was performed to compare the collagen fiber density between the binahong leaf extract treatment groups and the control groups on each observation day. This was followed by a *post hoc* test to determine the significance of differences between the control and treatment groups.

Results And Discussion

Phytochemical Screening Result

Table 1. Overall results of phytochemical content analysis.

No	**Type of leaf extract**	**Active ingredient content**							
		Alkaloid			Flavonoid	Triterpenoid	Saponin	Tanin	Kuinon
		Mayer	Dragendroff	Wagner					
1.	Binahong	+	+	+	+	+	+	+	+

Note: (+) = presence of phytochemical compound; (–) = absence of phytochemical compound.

Phytochemical contents such as alkaloids, flavonoids, triterpenoids, saponins, tannins, and quinones have been proven to be present in binahong leaf extract. The use of Dragendorff, Mayer, and Wagner reagents in testing for alkaloid compounds showed changes consistent with standard characteristics to indicate that the extract contains these compounds, forming a red-orange precipitate, a white precipitate and brown precipitate, respectively. Furthermore, the results of flavonoid, triterpenoid, saponin, tannin, and quinone tests indicate the presence of these compounds, with the respective changes showing red color; brown tings; froth reaching 5,5 cm after shaking, and 5 cm after resting for 10 minutes; dark blue or black color; and red color.

Microbiological Test Result

From the microbiological test, including ALT, fungal culture, and identification of pathogenic microorganisms, all results were negative; the ALT value was 180, and the others were completely negative. Therefore, binahong ointment can be declared safe from microbial pathogens and meets BPOM standards and it is likely safe for use by both animals and humans.

Wound Healing Observation After Three Days of Treatment

Table 2. Collagen Fiber Density After Three Days of Treatment.

Sample	P1 (Binahong, Day 3)	P5 (Control, Day 3)
1	4	1
2	3	1
3	3	2
4	4	2
Mean ± SD	**3.50 ± 0.58**	**1.50 ± 0.58**

On day 3, the group treated with binahong ointment (P1) showed a mean score of 3.50 ± 0.59, which was higher compared to the control group (P5), both of which had a mean score of 1.50 ± 0.58. This finding indicates that, in the early phase of wound healing, treatment with binahong ointment tended to promote greater collagen fiber density compared to the control (Table 2). Images are represented of four independent samples per group. Consistent with the histological findings, wound width reduction was also more pronounced in the binahong group compare to control group (Figure 1).

Wound Healing Observation After Five Days of Treatment

Table 3. Collagen Fiber Density After Five Days of Treatment.

Sample	P2 (Binahong, day 5)	P6 (Control, day5)
1	3	3
2	4	2
3	4	2
4	2	2
Mean ± SD	**3.25 ± 0.83**	**2.50 ± 0.58**

On day 5, binahong ointment group (P2) had the highest mean score of 3.25 ± 0.83, followed by the control group (P6) with 2.50 ± 0.58. These results suggest that binahong ointment continued to demonstrate greater collagen fiber density compared to the control group at this stage of wound healing (Table 3). Similar to day 3, wound width measurements also confirmed better healing progression in the binahong group (Table 2).

Collagen Fiber Density Result

To ensure evidence of the ointment's effectiveness, an intergroup variance test was conducted. Two-Way ANOVA analysis showed that there was a significant effect of treatment on the collagen fiber density score ($F = 6,909$; $p = 0.006$). This is supported by the result of the homogeneity of variances test using Levene's Test, which showed a significance value of 0.214, indicating that the data variance among groups was homogenous and the

ANOVA assumption was met. Furthermore, the Tukey HSD test showed that the difference in mean scored between the binahong group and control groups was significant ($p < 0,05$). The binahong group's score was significantly higher than both the control group and the betel leaf group. These results indicate that binahong had the strongest effect in increasing collagen fiber density.

Discussion

By the phytochemical test, it revealed that binahong contains alkaloid, flavonoid, triterpenoid, saponin, tanin, and quinone. These compounds such as flavonoids, alkaloids, tannins, and saponins play role as antimicrobials, anti-inflammatories, analgesics, anticancer agents, antifungals, antiparasitic, and pathogen-resistant agents due to their antioxidant activity (Nortjie et al. 2022). Other compounds, such as triterpenoids, show significant pharmacological activities, including antibacterial, antimalarial, anti-inflammatory, and anticancer effects (Agidew 2022). Quinones also contribute to cell protection through their antifungal, antioxidant, anticancer, anti-inflammatory, and anti-allergic properties (Dulo et al. 2021). Based on test results showing a high antioxidant content, the ointment can alleviate oxidative stress, which is the cause of damage to body fats and proteins (Souhoka, Kapelle, and Sihasale 2021).

Supported by other properties, binahong leaf extract greatly aids the healing of incision wounds on the back of mice. Which proof in the results after three- and five-day treatments.

After 3 days of the treatment, the thickest collagen fibers were observed in the P1 group, which achieved an average collagen fiber thickness score of 3,5, indicating that collagen fibers had accumulated densely in the post-injury tissue (Table 6). In contrast, healing in P3 had an average collagen fiber thickness score of 1,5, suggesting that wound healing in the control group occurred more slowly compared to the binahong leaf extract group, with collagen fibers still appearing dispersed and thin. The microscopic visuals of collagen fibers for each treatment group are presented in the following images.

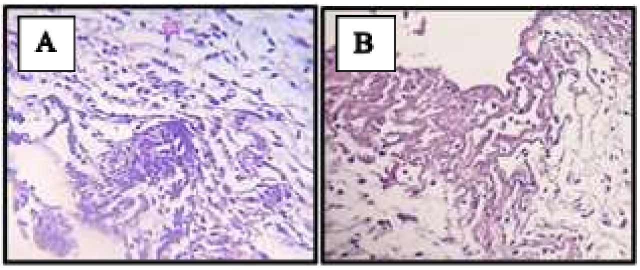

Figure 3. Microscopic appearance of collagen fibers in group P1, and P5. (A) Microscopic image showing collagen fiber thickness in sample 2 of group P1 with a score of 3, (B) Microscopic image showing collagen fiber thickness in sample 1 of group P3 with a score of 1.

Based on the data, it can be revealed that wound healing after three days of treatment was dominated by the group treated with binahong leaf extract ointment, which experienced healing twice as fast compared to the control treatment group. This is due to the activity of compounds in binahong leaves that can accelerate wound healing, such as triterpenoids, polyphenols, flavonoids, alkaloids, and saponins, which act as antioxidants and are highly effective in inhibiting the development of viruses, bacteria, and fungi in wounds (Ginting et al. 2025; Nasution, Ramadhani, and Marianti 2024).

Table 4. Wound length and width after three days of treatment.

Group	Wound Length (Mean ± SD)	Wound Width (Mean ± SD)
P1 (Binahong, day 3)	0.70 ± 0.10	0.07 ± 0.06
P5 (Kontrol, day 3)	0.73 ± 0.06	0.17 ± 0.12

Having the same trend as the microscopic results, the wound reduction in the P1 group showed only slightly more positive results compared to the P2 group. The respective calculated wound sized for the binahong leaf extract ointment treatment and the control were 0,7 x 0,067 cm and 0,7 x 0,167 cm. This indicated that evaluating wound healing solely by visual observation (macroscopically) cannot guarantee the actual healing occuring in the tissue.

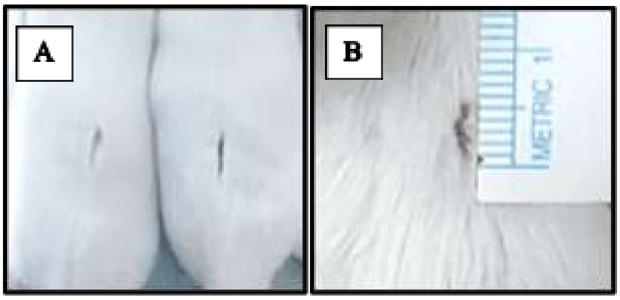

Figure 4. Wound width results after three days of treatment. (A) Wound width in P1 after treatment with binahong leaf extract ointment, (B) Wound width of group P3 after control treatment.

In the day five, like the previous euthanasia results, the treatment group with binahong leaf extract ointment (P2) experienced a higher thickening of fibers on the fifth day of euthanasia, reaching an average score of 3.25, indicating visually thickened collagen fiber accumulation. Meanwhile, the control group showed consistent development with a score of 2,5, evidenced by an increase of 1 point compared to the third day (Table 8).

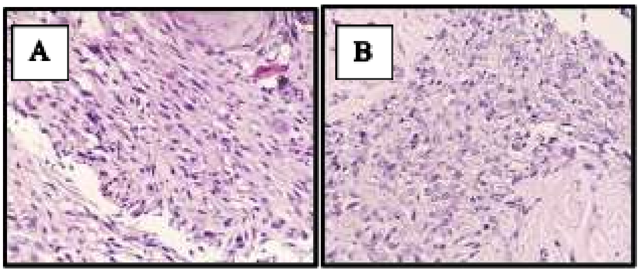

Figure 5. Microscopic appearance of collagen fibers in group P2, and P6. . (A) Microscopic image showing collagen fiber thickness in sample 2 of group P2 with a score of 4, (B) Microscopic image showing collagen fiber thickness in sample 2 of group P6 with a score of 3.

Collagen fibers have densely covered the wound, which indicates that the wound healing process is proceeding well and quickly. On the other hand, the control group experienced slow healing, with a score of 1,5 on the third day and reaching a score of 2,5 on the fifth day.

Table 5. Wound length and width after five days of treatment.

Group	Wound length (Mean ± SD)	Wound width (Mean ± SD)
P2 (Binahong, day 5)	0.10 ± 0.17	0.07 ± 0.12
P6 (Kontrol, day 5)	0.60 ± 0.10	0.07 ± 0.06

The smallest wound length was achieved by binahong leaf extract ointment group (P2), which had an average of 0,1 cm, with two of it is samples having fully healed, resulting in wounds that were covered and difficult to recognize during post-treatment observation. Similarly, microscopic observations showed that the reduction in wound size also progressed in the control group, which previously had an average size of 0,7 x 0,16 cm, decreasing to 0,6 x 0,06 cm on the fifth day (Table 9).

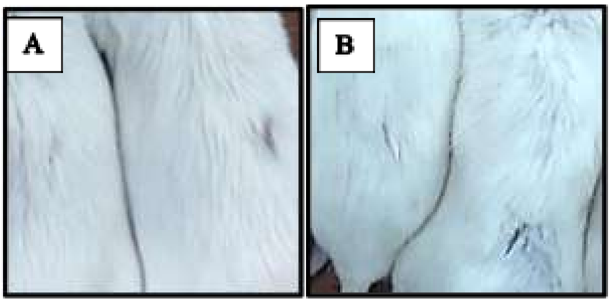

Figure 6. Wound width results after five days of treatment. (A) Wound width in P2 after treatment with binahong leaf extract ointment, (B) Wound width of group P4 after control treatment.

To ensure the results of the study, several statistical tests were conducted to support the statements of the results provided. Normally distributed before further analysis. Based on the Shapiro-Wilk test, a significance value of 0,083 (>0,05) was obtained, indicating that the data were normally distributed. This is important because Shapiro-Wilk test is more suitable for small sample sizes (n < 50), thus fulfilling the normality assumption. Next, Levene's Test for homogeneity was conducted to examine the equality of variance among groups. The test results showed a significance value 0f 0,214 (>0,05), indicating that the variances among groups were homogeneous. With both tests satisfied, the data were deemed suitable for analysis using ANOVA.

The results of the Two-Way ANOVA test showed that the research model was overall significant, with F = 6,909 and p = 0,006. Supported by a coefficient of determination (R^2) OF 0,633, this indicated that the treatment had a significant effect on the observed parameters. To determine whether there were significant differences between the treatment with binahong leaf extract ointment and the control group, a follow-up test using Post Hoc

Tukey HSD was conducted, yielding significant results ($p < 0,05$). This indicated that the use of binahong ointment can accelerate the wound healing process compared to the control group. This statement can be explained by the numerous bioactive compounds in binahong leaves, such as flavonoids, saponins, and alkaloids, which play a role in accelerating fibroblast proliferation, stimulating collagen synthesis, and reducing inflammation (Wang et al. 2021).

The results showed that the average trend in the binahong leaf extract ointment group remained higher compared to the control group, indicating that there is a significant difference between treatments. On the other hand, no contrasting difference was found between the duration of ointment application, as shown by the insignificant differences among the binahong leaf extract groups on the 3rd and 5th days. The reinforces that the binahong leaf extract ointment provides better healing effects in supporting tissue formation. The results of the Homogeneous Subsets test further strengthen these findings. The control group on the 3rd day was in the lowest subset with an average value of 1,5, while the binahong group on the 3rd and 5th days were classified in higher-value subsets.

Meanwhile, the day-5 control group (2,75) began to increase, although it was still at a lower level compared to the binahong group. Thus, it can be stated that the application of binahong leaf extract ointment can heal wounds faster and more effectively than the control group, especially in the early phase (day 3).

According to the statement by the anatomical pathology specialist, sample 2 in P2 has shown better healing compared to the other samples This is evidenced by the abundance of collagen fibers that have developed and begun to enter remodeling/maturation phase. The reason why sample 2 has a lower collagen fibers thickness score compared to the other sample is due to the collagen fibers having developed from type III collagen into stronger and more densely arranged type I collagen. Therefore, the sample can be considered to have reached the end of the proliferation phase, where the collagen is starting to become tidy, dense, and no longer resembles the characteristics of tissue at that stage; such as decreased fibroblast activity and wound tissue beginning to resemble normal pre-injury skin. Thus, the average score of groups P2, which is quantitatively (depending on the magnitude of the score) lower than P1, does not mean that the application of binahong leaf extract ointment is unaffected by the duration of treatment. Rather, it indicates that the healing process has progressed further and serves as a positive indicator of the effectiveness of the binahong leaf extract ointment.

Conclusions

The ointment binahong leaf extract demonstrated effective wound healing properties in mice, as shown by the increased collagen fibers thickness with an average score 3.375, compared to the control group with a score of 2.0. The treated group also showed a consistent decrease in wound size from 1.2 cm x 0.5 cm at the start to 0.1 cm x 0.006 cm, while the control group's wound measured 0.006 cm x 0.007 cm at the end of treatment. These findings indicate that the active compounds in binahong leaves significantly support tissue regeneration and accelerate skin repair.

Future research is recommended to apply more precise measurement methods and advanced histological staining techniques, such as Masson's Trichrome, for clearer collagen fiber analysis. Furthermore, the potential of binahong leaf extract can be explored in alternative topical formulations such as gels, creams, or pastes to enhance effectiveness, stability, and user comfort.

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Conflict of Interest: The authors declare that there are no conflicts of interest concerning the publication of this article.

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