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The Effectiveness of Giving Binahong (*Anredera cordifolia*) Leaf Extract on Granulation Tissue Thickness in Healing White Rat (*Rattus novergicus*) Cut Wounds

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ABSTRACT

Wounds result in loss of epithelial continuity with or without loss of underlying connective tissue. The dynamic and complex wound-healing process restores tissue integrity and balance. Medicinal plants in herbal medicine, namely Binahong, help heal wounds. This study tested binahong (*Anredera cardifolia*) leaf extract ointment on the thickness of granulation tissue in white rat (*Rattus novergicus*) incision wounds. Binahong leaf extract was applied at 15%, 25%, and 35%, then the wound was examined macroscopically and histopathologically. The type of research was a true experiment with a sample of 24 male Wistar rats, six per group, in four groups (control, treatment 1, treatment 2, treatment 3). The histopathological image of granulation tissue thickness shows that the P0 group (blue) has less collagen density. Treatment group 3 (P3) with 35% binahong leaf extract showed thick collagen. Analysis of the Shapiro-Wilk normality test and significant paired t-test values greater than p>0.05 for all groups. The Shapiro-Wilk normality test shows that the data is regularly distributed. Paired T-test showed significant differences in incision wound healing between groups (p-value <0.05). The research concludes that binahong leaf extract has antiseptic, antibacterial, and ascorbic acid properties and protects against oxidation, so it is helpful for wound healing.

Keywords:

Binahong Leaf, Skin, Collagen, Wound Healing, Granulation Tissue

1. INTRODUCTION

A wound is any structural injury to the skin, such as a rip in the skin or the exposure of an internal organ, that can be produced by either an external force or an internal one [1], [2]. According to Farahani and Shafiee, when tissues are damaged, the body's natural healing process kicks into gear, which means wound healing. Pathogenic bacteria can enter the body through wounds and infect the affected area, leading to chronic wounds that take significantly longer or do not heal [3].

One of the most significant organs, the skin, accounts for over 16% of total body mass and has an average surface area of 1.91 m2 [4]. The epidermis, dermis, and hypodermis are the three layers that make up the skin. Each layer has its unique function and is capable of self-renewal. The skin's primary role is to act as a protective barrier, shielding the body's internal organs from harmful microbes, ultraviolet radiation, and temperature fluctuations. Another way the skin aids the body is in its sensory detection and immune system functions. Exfoliation removes dead skin cells from the skin's surface, allowing new cells to grow from deeper layers underneath [5].

Damage to the skin's protective role and other tissues may co-occur in wounds, where tissue continuity is disturbed due to damaged or missing tissue substance [6]. A wide variety of pathological disorders, including diabetes and blood vessel disease, as well as extrinsic factors like pressure, burns, and wounds, can lead to the development of wounds. Acute wounds heal quickly, while chronic wounds take longer and are more challenging to treat. Restoration of anatomical and functional integrity is a continuous process due to acute wounds ordered and exact healing. Chronic wounds, on the other hand, never heal to their full structural and functional potential [7].

This silent epidemic affects an estimated 1-2% of the world's population living in underdeveloped nations at some point in their lives. Due to the negative association between age and wound healing, prevalence rises with the aging population. An estimated 9.73% of Indonesians are 60 and above, and the country might reach its peak population age as early as 2035, according to statistics from the Republic of Indonesia's Ministry of Home Affairs [8].

Meanwhile, according to the 2018 North Sumatra Province Riskesdas Report, the overall injury rate in the province was 23.92% (stabs, cuts, and tears) across all demographics (age, education, gender, occupation, and location), with 21.27% of those injuries happening in urban areas and 26.93% in rural areas. In addition, among all characters investigated, burn injuries accounted for 1.04 percent of the total. The data reveals that compared to the national average of 20.01%, the injury proportion in North Sumatra Province is still relatively high [9].

A series of intricate biological processes, the body's defense mechanisms for speedy recovery, are necessary for wound healing. The risk of infection, complications, and financial burden can all be significantly diminished by shortening the time it takes for wounds to heal [3], [10]. When tissues are opened or ruptured due to a physical injury, this is known as a wound, and it can lead to structural and functional problems. Depending on the severity of the wound, the underlying connective tissue may or may not be lost along with the epithelial continuity. They restore tissue integrity, and homeostasis results from the intricate and ever-changing wound healing process. The healing process of a wound consists of several steps, including inflammation, re-epithelialization, granulation tissue creation, neovascularization, wound contraction, and remodeling of the extracellular matrix [11], [12].

One of the earliest medical records from 2200 B.C. describes wound healing as a science including "three healing movements." The three steps to healing a wound are washing, plastering, and bandaging. Wound healing appears to be a fundamentally unchanged field; however, there has been noticeable advancement. In their groundbreaking work, Louis Pasteur and Joseph Lister identified the sources of diseases and methods to avoid them. Bacteria were brought into the wound by an outside source, as Pasteur demonstrated. For a wound to heal, it is necessary to remove harmful germs using aseptic techniques. In the past, wrist dressings typically consisted of animal fats, honey, and herbs. The Middle Ages saw some incremental improvements to wound dressing, but the nineteenth century saw the most significant technological and therapeutic leaps forward [13].

In rare cases, wound healing could take longer than expected. Wound healing pathophysiology leads to harmful granulation tissue buildup and complicated chronic wounds. Infection at the wound site, which leads to an overabundance of inflammatory cell recruitment, is one possible cause [7], [14]. The production of biofilms, which are a consequence of bacterial toxins and lead to poor wound healing, and an increase in the release of reactive oxygen species, which harm tissue, are both outcomes of infection. Additional problems such as abscesses, cellulitis, osteomyelitis, and amputations might develop due to infection [15], [16].

An integral part of wound healing is granulation tissue. There are two ways in which wounds can be mended: primary intention, when the wound's edges are easily accessible, and secondary intention when they are inaccessible. With both goals in mind, the granulation tissue matrix will fill the wound as it heals [3], [14], [17]. This tissue type is also common in chronic wounds, the origins of which can vary greatly. New thin-walled capillaries, fibroblasts, keratinocytes, endothelial cells, and inflammatory cell infiltration of the extracellular matrix are histological hallmarks of granulation tissue, classified as contractile organs. Creating granulation tissue at a wound site is a complicated process involving complex interactions between different types of cells [18].

As a nation rich in natural resources, Indonesia is home to many plants, some of which have long been used as traditional remedies due to their high antioxidant content and other beneficial compounds. Due to the low cost, ease of preparation, and accessibility of plant-based substances, natural medicines made from plants are gaining popularity [19]–[22].

The binahong plant, scientifically known as (*Anredera cordifolia* (*Tenore*) *Steensis*), is one of several herbal medicinal plants that influence wound healing; this makes it a promising candidate for treating a wide range of ailments [23]. The plant is originally from the Chinese plains. It goes by several names: cheng shan chi in China, Madeira vine in England, heartleaf Madeira vine in Europe, and simply Madeira vine in South America. Binahong, also known as gondola, is a typically utilized hedge plant in Indonesia that has been there for quite some time; nevertheless, the exact date of its discovery is unknown [24], [25].

Anredera cordifolia, often known as the binahong plant, is medicinal. According to anecdotal evidence, the binahong plant can alleviate various physical ailments, including but not limited to rheumatism, aches and pains, bruises, swollen glands, post-operative skin sensitivity, and cuts and scrapes [25]. Among the compounds found in binahong leaves are alkaloids, polyphenols, triterpenoids, saponins, and essential oils. The presence of betalain chemicals is a defining feature of the order Caryophyllales, to which binahong leaves are known to belong. Betanidine, an alkaloid component in binahong leaves, is formed when betalain hydrolyzes [26].

The flavonoids found in binahong leaves have multiple uses, including analgesic, anti-inflammatory, and antioxidant. Binahong's high flavonoid content during wound healing contributes to cell proliferation by enhancing mitogenesis, cell contact, and molecular adhesion. In addition to triterpenoids, oleanolic acid and saponins are also present in binahong [27].

The bioactivity concentration of plants in Indonesia makes the country a potential source for wound treatments. Combining ointments prepared from binahong leaves is one alternative that can be employed. To apply ointment to the skin or mucous membranes is to employ a semi-solid substance topically. A topical preparation's base, a liquid or a solid, touches the skin and transports the active component in ointment formulations. In light of the preceding, the researchers set out to investigate the effects of a topical ointment containing extracts from the binahong leaves on the rate of wound healing in white rats (*Rattus novergicus*).

2. METHOD

This study is an example of a genuine experiment or laboratory experimental study [28]. This study examined the effects of binahong (*Anredera cardifolia*) leaf extract ointment on the thickness of granulation tissue in healing white rats (*Rattus novergicus*) cut wounds using a post-test with a control group design. The sample size was 24 male Wistar rats split into four groups. Six mice make up each group. A variable is a changeable quantity that can affect outcomes or study findings [29]. Components of this investigation Factor that is dependent: Independent variable and granulation tissue thickness: Extract from Binahong leaves.

Equipment for rat rearing (cage, feed, and water containers), as well as analytical balance, razor, gloves, tweezers, pH meter, mortar, evaporating cup, beaker* glass, erlenmeyer, measuring cup, stirring rod, dropper pipette, spatula, oven, filter paper, rotary evaporator, and blender for documentation purposes, are all utilized in this research. The components employed include Binahong leaves, distilled water, ether, 70% ethanol, vaseline albumin, and healthy, defect-free white mice.

Research Procedures include Acclimation of Test Animals, Preparation of Binahong (*Anredera Cardifolia*) Leaf Extract Ointment, Testing of Ointment Preparations, Phytochemical Tests, Treatment of Test Animals, Observation of Histopathological Preparations and Collagen Density Scoring. The study of the data made use of descriptive tests, normality, and homogeneity. Then, we checked for homogeneity of variances and normality of distribution using the Levene test. A One-Way ANOVA test was utilized for group comparisons due to the non-normal distribution of the data [30]. We utilized SPSS 25.0 for Windows for data processing.

3. RESULTS AND DISCUSSION

The male white Wistar rats (*Rattus Norvegicus*) were used as samples for this study. Researchers followed the 3R Principle, which stands for Replacement, Reduction, and Refinement, to decide how many samples to employ for their study [31]. All twenty-four male Wistar rats used in the study will be randomly assigned to one of four groups. Six mice make up each group.

The components employed include Binahong leaves, distilled water, ether, 70% ethanol, vaseline albumin, and healthy, defect-free white mice. All three ointment formulations tested here contained 35 grams of binahong leaf extract: 15%, 25%, and 35%. Binahong leaf extract ointment is made with the following ingredients:

Table 1. Binahong Leaf Extract Ointment Formulation

Substance Name	F1	F2	F3
Binahong Leaf ethanol extract concentration	15%	25%	35%
Ointment base (Vaseline album)	29,75 gr	26,25 gr	22,75 gr
	35 gr	35 gr	35 gr

The binahong ointment extract was odorless, brownish green, and had a homogenous pH of 6.38, which was still compliant with human skin, according to the organoleptic test. The ointment spreadability test showed 3.2–4.8 cm in diameter in 0–125 gr packages. Thus, the ointment is safe for experimentation.

According to the formulation, each group received binahong leaf extract ointment twice daily in the morning and evening. Macroscopic wound and incision inspection. Initial incision wound 2 cm long, 0.2 cm deep, or up to the back subcutis layer with a sterile knife. After treatment, the wound will be measured again using a ruler to measure the incision length every two days for 14 days.

Table 2. Observation of Wound Healing

Groups	Observation	H2	H4	H6	H8	H10	H12	H14
Control	1	1,41	1,06	0,72	0,66	0,52	0,49	0,45
	2	1,62	1,46	1,47	1,32	1,38	1,23	1,035
	3	1,63	1,37	1,34	1,32	1,3	1,27	1,07
	4	1,72	1,21	1,01	0,95	1,1	1,03	0,81
	5	1,66	1,36	0,72	0,58	0,55	0,53	0,41
	6	1,71	1,61	1,09	0,81	0,74	0,68	0,58
Treatment 1 (P1)	1	1,57	1,46	1,27	1,17	0,62	1,02	0,49
	2	1,95	1,31	0,96	1,11	0,74	0,56	0,36
	3	1,79	1,41	0,93	0,82	0,67	0,64	0,49
	4	1,82	1,51	1,22	1,02	0,93	0,92	0,77

Groups	Observation	H2	H4	H6	H8	H10	H12	H14
	5	1,91	1,42	1,2	0,8	0,71	0,66	0,5
	6	1,8	1,18	0,99	0,58	0,5	0,48	0,39
Treatment 2 (P2)	1	1,6	1,31	1,21	1,04	0,91	0,78	0,53
	2	1,67	1,44	1,19	0,93	0,71	0,69	0,57
	3	1,57	1,29	1,1	1,19	1,1	1,05	0,73
	4	1,76	1,29	0,83	0,86	0,57	0,51	0,39
	5	1,38	1,21	0,76	0,72	0,49	0,48	0,38
	6	1,67	1,34	0,85	0,7	1,18	0,95	0,71
Treatment 3 (P3)	1	1,33	1,24	1,15	1,06	0,97	0,88	0,65
	2	1,55	1,46	1,37	1,28	1,19	0,98	0,21
	3	1,43	1,34	1,25	1,16	0,89	0,29	0
	4	1,36	1,27	1,18	1,09	1	0,8	0,52
	5	1,41	1,32	1,23	1,20	0,99	0,32	0
	6	1,48	1,39	1,3	1,21	1,12	1,03	0,94

Table 2 of the wound observations above shows that in treatment group 3, sample 3 mice and mouse 5, the cut wounds had closed entirely on (0 cm) day 14 of treatment with binahong ointment extract, while sample 3 mice with control treatment had a 1.07 cm incision scar.

Table 3. Average Percentage of Wound Healing (%)

Day	Groups			
	Control	Treatment 1 (P1)	Treatment 2 (P2)	Treatment 3 (P3)
H2	19	9,5	19,5	28,5
H4	32,5	31	34,5	33
H6	47	45	50,5	37,5
H8	53	54	54,5	41,5
H10	53,5	65	58,5	48,5
H12	56,5	64,5	63	64
H14	63,5	75	72,5	80,5

Table 3 shows that all groups of Wistar white rats (Rattus norvegicus) recovered. Average healing percentages differed between groups. The control group had 63.5% wound healing on the last day, treatment group 1 75%, treatment group 2 72.5%, and treatment group 3 80.5%. On average, treatment group 3 healed faster than the control group, treatment 1, and treatment 2.

Table 4. Phytochemical Screening Results of Binahong Extract

Secondary Metabolites	Result
Alkaloid	+
Flavonoid	+
Saponin	+
Tanin	+
Steroid/Triterpenoid	+
Glikosida	-

Table 4 shows performing a phytochemical test using binahong leaf extract and seeing the color change in each test; the alkaloid, flavonoid, saponin, tannin, and steroid/triterpenoid results showed positive results, indicating secondary metabolite content from Binahong Leaf extract on this compound. Glycosides lack secondary metabolites. Secondary metabolites are not necessary for organism growth and vary by species. Thus, binahong leaf extract offers several wound-healing advantages.

Histological examinations performed in Image J using the area fraction method utilizing five fields of view at 400x magnification revealed that the control group (P0), which consisted of subjects exposed solely to UVB light and not treated in any way, exhibited the lowest percentage of collagen density when contrasted with the other groups.

Since the P0 group (blue) appears to have lower collagen density than the other groups, it was awarded a score of +2. Meanwhile, P3, the third treatment group, received a collagen density score of +4 due to the high content of binahonge leaf extract (35%). Histological examinations on patients undergoing treatment one revealed moderately dense collagen fibers (scoring +2), while patients undergoing treatment 3 showed dense collagen fiber density (score +3) in their skin.

Table 5. Histopathological Skin Tissue

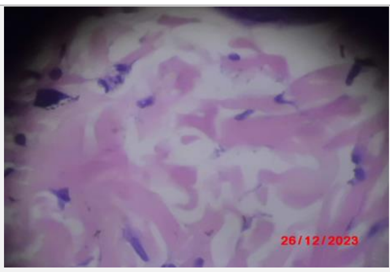
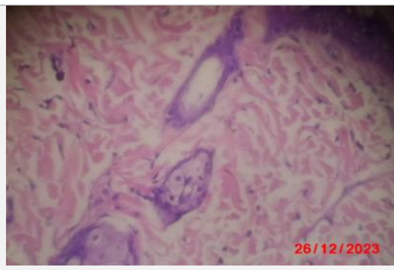
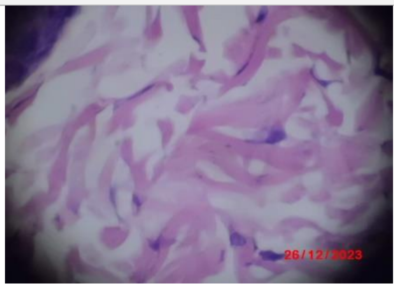
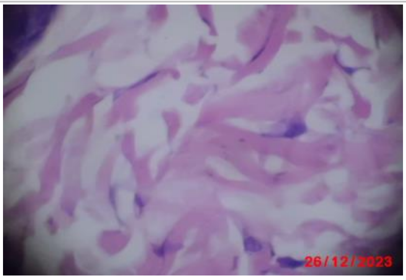
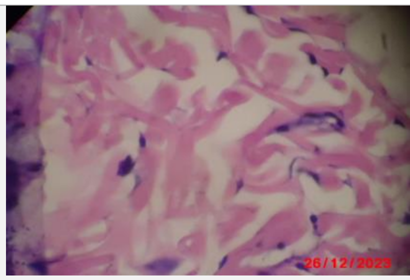
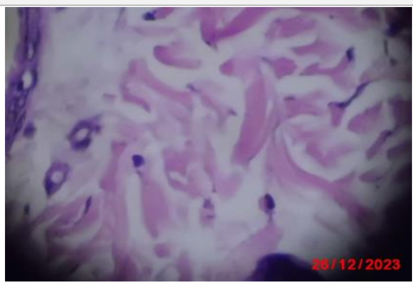
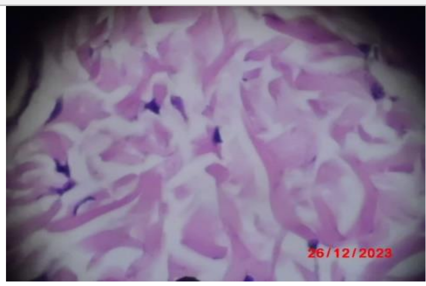
No	Group	Histopathological Figure of Skin Tissue	
1	Control Collagen density is moderate and still rare (score+2)		
2	Treatment 1 (P1) Collagen density is medium and is starting to become dense (score +2)		
3	Treatment 2 (P2) The density of collagen fibers in the skin is tight (score+3)		
4	Treatment 3 (P3) The collagen density in the skin is very dense (score+4)		

Table 6. Normality Test

Groups		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Results	Control	.184	6	.200*	.922	6	.518
	Treatment 1 (P1)	.136	6	.200*	.994	6	.997
	Treatment 2 (P2)	.277	6	.168	.918	6	.488
	Treatment 3 (P3)	.239	6	.200*	.915	6	.472

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

The normality test's Table 6 shows that from day 1 to day 14, the wound length variable had significant values for both the control and treatment groups. The p-value for Group K is 0.518, Group P1's is 0.997, Group P2's is 0.488, and Group P3's is 0.472; these values surpass the typical margin of p>0.05 in the Shapiro-Wilk Test [30]. Therefore, according to the Shapiro-Wilk normality test, the data follows a normal distribution.

Table 7. Paired T Test

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Result - Group	1.44917	1.13799	.23229	.96864	1.92970	6.239	23	.000

The Paired Sample T Test determines if the averages of two unpaired samples differ. Data must be regularly distributed and homogenous for the Paired Sample T Test. After passing normality and homogeneity tests and having customarily distributed and homogeneous variances, a Paired Sample T-Test tests the equality of two means with a t-test for two groups. The first and second assumptions are the initial wounds before treatment, and the results end after treatment with a significance level of 0.05 [30].

According to the results in Table 7, the t-test yields a probability value (sig.2-tailed) of 0.000. Because the calculated significance level was less than 0.05, we can say that the rates of incision wound healing in the two groups were significantly different.

Discussion

This study aimed to determine whether or not a topical ointment containing binahong leaf extract reduced the thickness of granulation tissue in white rats (*Rattus novergicus*) who were recovering from cuts. The most common cause of cuts is direct contact with sharp objects. When a sharp object pierces the skin or causes other tissue to bleed or become damaged, this is called a cut. The depth of the wound throughout the skin's layers determines how long it will take to heal. Burns, decubitus ulcers, and foot ulcers are examples of chronic wounds that do not heal in a set or predictable amount of time [13].

Bacterial biofilms, which are populations of microbes that produce mucus to evade human defenses and proliferate, are another component that might impede wound healing. Biofilms have the potential to create an oxygen-depleted, acidic wound environment. These biofilms can form A physical barrier, which inhibits cell movement and the entry of antibodies and antibiotics [32].

So, we used phytochemical assays to examine the concentration of active compounds in binahong (*Anredera cardifolia*) leaf extract as part of this research. The chemicals tested for alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids all came back positive, suggesting that the Binahong Leaf extract (Anrederacordifolia Ten. Steenis) contained secondary metabolites. On the other hand, glycosides are devoid of any secondary metabolites. Saponin, found in binahong leaves, has antiseptic qualities that help fight off germs that can grow in wounds, reducing the likelihood of infection.

Antiscorbute, one of the flavonoid chemicals found in binahong leaves, protects collagen synthesis against ascorbic acid and oxidation. Binahong leaves contain alkaloids, which have antibacterial properties. These compounds harm the peptidoglycan components in bacterial cells, preventing them from fully forming their cell wall and ultimately leading to cell death. This is only one of the many ways that binahong leaf extract helps wounds heal.

Using data from wound monitoring results and histopathological examinations of the wound area, this study also examined the thickness of granulation tissue in healing wounds from rats that did not receive binahong leaf extract. The rats in treatment group 3 had scars. On the fourteenth day of therapy with binahong ointment extract, the incision wound in rats 3 and 5 had fully closed (0 cm); however, in rat sample 3 with control treatment, the incision scar remained at 1.07 cm. Therefore, the most effective concentration of binahong leaf extract for speeding up the healing of cuts in mice is 35%.

At the end of the third day, the average wound healing percentage was 80.5% in treatment group 3, 72.5% in treatment group 2, and 63.5% in the control group. Treatment 3 outperformed the control group, treatment 1, and Treatment 2 regarding average healing rate.

Additionally, microscopic and histopathological examinations of the wound and scoring the thickness of granulation tissue in healing wounds were employed to assess the effects of binahong leaf extract on rats when administered doses of 15%, 25%, and 35% and studied white mice by analyzing histopathological findings. Based on the observation data, the P0 group (blue) has lower collagen density than the other groups. Collagen density was exceptionally high in treatment group 3 (P3), which received a 35% binahonge leaf extract concentration from Treatment 1 and Treatment 2 groups.

The normalcy test indicated that the data was normally distributed, with a significant value greater than 0.05 in both the control and treatment groups for the wound length variable from day 1 to day 14. Furthermore, the paired t-test yields a probability value (sig.2-tailed) 0.000. Because the calculated significance level was less than 0.05, we can say that the rates of incision wound healing in the two groups were significantly different.

4. CONCLUSION

According to the results of the phytochemical tests conducted on the binahong (*Anredera cardifolia*) leaf extract, its active substances are alkaloids, flavonoids, saponins, tannins, and steroids/triterperoids. The positive results indicated the presence of secondary metabolite content in the extract. Steenis) on these chemicals. On the other hand, glycosides are devoid of any secondary metabolites. This demonstrates that binahong leaf extract is an antioxidant and can heal damaged skin by forming collagen.

The incision wounds in the treatment group of 3 mice and five mice had fully healed by the 14th day of treatment with binahong ointment extract, while in the control group of 3 mice, incision scars measuring up to 1.07 cm were still visible. At the end of the third day, the average wound healing percentage was 80.5% in treatment group 3, 72.5% in treatment group 2, and 63.5% in the control group. Treatment 3 outperformed the control group, treatment 1, and Treatment 2 regarding average healing rate.

Using macroscopic and histological measurements of the wound region, this study determined that the thickness of the granulation tissue in the healing of rats that were not administered binahong leaf extract was significantly different. Compared to the other groups, the normal control group (P0), which received no therapy and was just exposed to UVB rays, exhibited the lowest percentage of collagen density. The P0 group (blue) appears to have lower collagen density in the photo than the other groups. Collagen density was exceptionally high in treatment group 3 (P3), which received a 35% binahong leaf extract concentration from Treatment 1 and Treatment 2 groups.

The control and treatment groups exhibited significant values for the wound length variable from day 1 to day 14. The data were found to be distributed according to the Shapiro-Wilk normality test, indicating that binahong (*anredera cardifolia*) leaf ointment was effective in treating wounds. The results of the Paired T-Test, which demonstrated significant differences in the healing of cut wounds in each group, were acquired with a significance value smaller than 0.05. It was recommended to use this test to determine whether the data was normal.

The results of this research have been replied to by using binahong, an ointment extract from the plant leaves. Specifically, it influences the thickness of granulation tissue in the healing of cut wounds in white rats (*Rattus novergicus*). Based on the results of this study, it can be inferred that the application of binahong leaf extract to the thickness of granulation tissue facilitated wound healing in mice.

The findings suggest that anyone interested in continuing the study could look into how healthy binahong (*anredera cardifolia*) leaf extract speeds up the healing process of human cut wounds. We hope this study shows how well binahong (*anredera cardifolia*) leaf extract speeds wound healing and promotes skin regeneration.

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