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ORIGINAL ARTICLE

The effect of binahong leaf extract on hair growth and histopathological examination of skin tissue in wound healing

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Abstract

Binahong leaves are often used as traditional medicine to cure wounds, rheumatism, gout, lack of appetite, nosebleeds, kidney inflammation, intestinal inflammation, and cancer. And not many people know that binahong leaves can also accelerate the growth of hair follicles, therefore this study was conducted, namely to analyze and test the effect of giving binahong leaf extract (*Anderera cardifolia*) in collagenization to accelerate the healing process of incision wounds on the back of white rats (*Rattus norvegicus*) wistar strain and see the histopathological picture on rat skin tissue. This study used 24 male rats (Rattus norvegicus) wistar strains as samples, then divided into 4 groups with each group of 6 rats, and treated for 14 days. for 14 days On the last day the hair length of the control group/P0 was 0.45 and became the lowest group in hair growth in this treatment, while in the treatment of groups PI, P2 and P3 it was known that the P3 group experienced the fastest hair follicle extension with a result of 1.24 cm. This is also in line with the results of hispatology images for the treatment of treatment group 3 (P3) which is the fastest group of incision wound healing in skin tissue because based on these observations the skin epithelial tissue is seen to begin to thicken and almost perfect.

Keywords: binahong leaf, hair growth, incision wound

Introduction

Health is believed to be influenced by environmental and individual characteristics.[1] The skin serves as the main barrier between the body and the environment, providing a biological shield against various chemical and physical pollutants (e.g., ultraviolet rays, ozone, etc.).[2] Its layered structure makes it a mechanically complex tissue. From the outer surface downward, the main layers are the stratum corneum (10–15 µm), the viable epidermis (100–150 µm), the dermis (further divided into papillary and reticular dermis, 2 mm), and the hypodermis. Although the stratum corneum is part of the epidermis, it is often considered a separate layer due to its specific barrier properties. It consists of non-living, robust yet flexible and wrinkled cells. The epidermis primarily comprises cells migrating to the skin surface. As cells approach the stratum corneum, they become more keratinized. The viable epidermis has a wavy geometry that diminishes with age. The dermis mainly consists of densely packed collagen and elastin fibers, dominating the skin's overall mechanical behavior. The deepest skin layer, the hypodermis or subcutaneous adipose tissue, consists of loose fatty connective tissue. The dermis contains microstructures such as blood vessels, lymphatic vessels, nerve endings, sweat glands, hair follicles, and various cell types. The influence of different layers on mechanical properties is often overlooked, as the focus is primarily on the overall mechanical behavior dominated by the main skin components.[3]

The skin is an essential organ for the human body, and its health must be maintained to prevent disorders. One such disorder is a wound. A wound is damage to the epithelial surface and underlying connective tissue that may be complicated by excessive tissue damage, underlying pathology, and poor

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tissue perfusion and oxygenation. Wounds can be categorized into two main types: acute and chronic wounds. Acute wounds heal normally through an optimal hemostatic and inflammatory cascade with tissue repair and regeneration. Chronic wounds do not heal within the normal timeframe due to disturbances in these phases and persistent underlying pathology, mainly infection.[4]

Wounds can occur during daily activities due to various causes such as trauma from sharp or blunt objects, temperature changes, chemicals, explosions, electric shocks, or animal bites. Wounds are classified based on their underlying cause into open and closed wounds. Open wounds include lacerations, abrasions, punctures, penetrations, and incisions. Following a wound, the natural wound healing process occurs.[5] Wound healing is a natural process leading to the restoration of the injured tissue's structural and functional integrity. It involves several biochemical and cellular pathways to repair the lesion and restore physiological conditions. The human body has an innate capacity to support this repair process through three phases: inflammation, proliferation, and remodeling.[5] Efficient wound response and repair are crucial for preventing infections.[6]

Wounds heal through repair, not regeneration. Currently, when wounds heal, open wounds on adult mouse skin will form new hair follicles.[7] Hair is a distinctive mammalian feature functioning as a unique mini-organ. In humans, hair serves various functions, such as protection against external factors, sebum, apocrine sweat, and pheromone production, and thermoregulation. Hair also plays a vital role in individual social and sexual interactions.[8] The hair structure comprises concentric layers forming the hair follicle, with the medulla at the center, surrounded by the cortex and cuticle, and further enclosed by inner and outer root sheaths, with the entire mini-organ encased in connective tissue.[9] Hair originates from the epidermis and consists of two distinct parts: the follicle and the hair shaft. The follicle is crucial for hair formation. Humans have nearly 5 million hair follicles, with 100,000 on the scalp. Hair follicles can alter their type and density during seasonal shedding.[10] The hair shaft consists of cortical and cuticular cells, and the medulla in some hair types.[8] Hair follicles undergo a regular growth cycle throughout life, with changes primarily in the morphology and structure of the dermal papilla at the follicle base, the formation of new hair shafts, and the release of old hair shafts. This cycle is divided into three stages: anagen, catagen, and telogen.[11] Under certain conditions, the cycle's timeframe remains relatively constant and precise. One compound that can accelerate wound healing and hair growth is vitamin C.

Vitamin C is a potent antioxidant. Environmental factors, such as sun radiation, pollution, and smoking, can accelerate skin damage through oxidative stress. Vitamin C is one of the most potent antioxidants in the skin, neutralizing oxidative stress through electron transfer and/or donation.[12] Humans are among the few species requiring vitamin C supplementation for survival.[13] Tissue repair and regeneration in the body are influenced by vitamin C. Vitamin C's crucial role is in connective tissue synthesis, particularly collagen. It provides tensile strength to newly formed collagen that cannot stretch without tearing. Additionally, vitamin C is an essential antioxidant that can scavenge and neutralize oxidants in the body, which is vital in the epidermis.[14] Along with its role in collagen synthesis, evidence shows that vitamin C enhances dermal fibroblast proliferation, crucial for wound healing.[15] One plant containing vitamin C is the binahong plant.

Binahong (*Anredera cordifolia*) is a species from the Basellaceae family with fleshy leaves and thick aerial tubers. This plant is widely used as a medicinal plant in Indonesia. Binahong leaves are used for wound treatment, refreshing the body, headaches, and lowering blood pressure. The ether fraction of binahong leaf extract shows antioxidant activity measured by DPPH (1,1-diphenyl-2-picrylhydrazyl).[16] Binahong leaves are often used in traditional medicine to heal wounds, rheumatism, gout, lack of appetite, nosebleeds, kidney inflammation, intestinal inflammation, and cancer. However, the benefits of the binahong plant are not widely known among Indonesians. Binahong leaves contain saponins, tannins, flavonoids, alkaloids, and polyphenols with antioxidant properties.[17] This study aims to analyze and test the effect of binahong leaf extract on accelerating the collagenization process of incised wound healing on the back of Wistar rats (*Rattus norvegicus*) and to observe the histopathological features of the rats' skin tissue.

Method

This study employs a post-test only group design to determine and analyze the effect of binahong leaf extract on accelerating hair growth and incised wound healing on the backs of Wistar rats. The researchers used 24 Wistar rats for each experimental group, divided randomly into 4 groups. Rats were treated with binahong leaf extract at different concentrations for 14 days. The control group (K-1) was treated with 0.9% NaCl, treatment group 1 (K-2) with 10% binahong leaf extract, treatment group 2 (K-3) with 20% binahong leaf extract, and treatment group 3 (K-4) with 30% binahong leaf extract.

The equipment used included rat cages, drinking containers, shavers, digital scales, surgical tools, rulers, calipers, cameras, a set of maceration tools, filters, a rotary evaporator, evaporation dishes, and a water bath. Equipment for in vitro testing included 10 ml, 25 ml, 100 ml volumetric flasks, test tubes, test tube racks, BioHit 1000µL micropipettes, measuring pipettes, spatulas, vials, incubators, pH meters, cuvettes, centrifuges, centrifuge tubes, UV-Vis spectrophotometers, and beaker glasses. The materials used were binahong leaf simplicia and chemicals including trichloroacetic acid (TCA), ethanol p.a (Brataco), and distilled water.

Fresh binahong leaves were washed and dried for four days before being powdered. The powder was extracted by maceration using 96% ethanol and filtered using filter paper. The maceration result was evaporated using a rotary evaporator until a thick, pourable extract was obtained. The binahong leaf extract was applied to the shaved skin of rats subjected to incised wounds.

The hair growth observation process in this study was based on research conducted by Sulastri et al.[18] and modified by the researchers. Hair length was observed at the end of all treatments by plucking the 6 longest hairs from the rats, straightening them, and attaching them to tape. The hair length was measured using calipers. Hair weight was measured on day 21 by shaving the hair that grew in the test area and weighing it, and the results were statistically analyzed.

Sample collection involved anesthetizing the rats (ketamine-xylazine combination dose 0.1mg/200g body weight). Skin samples were taken from healed wounds from each rat and placed in 10% formalin buffer for histopathological studies. The healed skin samples were cut into 5 µm thick sections and stained with hematoxylin and eosin (H&E). Samples were placed in 40% PFA pots, stored at room temperature, and sent to the histopathology laboratory at the University of North Sumatra for histopathology preparation. Histopathological observations were based on reepithelialization scores: 1) 0-25% reepithelialization of the wound area; 2) 25-50% reepithelialization of the wound area; 3) 50-75% reepithelialization of the wound area; and 4) >75% reepithelialization of the wound area. The research data were analyzed using SPSS 25.0. Normality tests were conducted using the Kolmogorov-Smirnov test. The independent sample t-test was used to determine significant differences or comparisons between experimental groups.

Results

The main test was the hair growth length in rats subjected to different treatments. The control group (P0) was treated with 0.9% NaCl for 14 days. Treatment group 1 (P1) was treated with 10% binahong leaf extract for 14 days. Treatment group 2 (P2) was treated with 20% binahong leaf extract for 14 days. Treatment group 3 (P3) was treated with 30% binahong leaf extract for 14 days.

Based on observations conducted across all groups, hair growth was evident in all groups. To determine which treatment group exhibited the fastest hair growth, the researchers calculated the average hair growth for each group.

Table 1. Rat hair growth

Group	Repetition	Length of rat hair (cm)		
		Day 3	Day 7	Day 14
Control/P0	1	0	0.20	0.43
	2	0	0.24	0.47
	3	0	0.26	0.48
	4	0	0.29	0.51
	5	0	0.21	0.42
	6	0	0.18	0.38
P1	1	0.39	0.53	0.72
	2	0.34	0.57	0.75
	3	0.44	0.62	0.81
	4	0.49	0.66	0.82
	5	0.41	0.63	0.79
	6	0.47	0.67	0.83
P2	1	0.51	0.77	0.91
	2	0.53	0.79	0.93
	3	0.60	0.82	1.08
	4	0.59	0.78	0.96
	5	0.62	0.84	0.99
	6	0.67	0.88	1.02
P3	1	0.66	0.94	1.15
	2	0.72	1.37	1.44
	3	0.87	1.09	1.14
	4	0.53	1.03	1.20
	5	0.56	1.22	1.33
	6	0.52	1.11	1.20

Table 2. Average hair growth

Group	Day 3	Day 7	Day 14
Control /P0	0,00	0,23	0,45
P1	0,42	0,61	0,79
P2	0,59	0,81	0,98
P3	0,64	1,13	1,24

Table 3. Independent sample test

t	df	Sig. (2 tailed)
18.035	10	.000

The average hair length of the rats indicated hair growth in each group over 14 days. On the final day, the control group (P0) had the shortest hair length, averaging 0.45 cm. This was the lowest hair growth observed among the groups. In contrast, treatment group 3 (P3) exhibited the fastest hair follicle elongation, with an average hair length of 1.24 cm over 14 days. Treatment group 2 (P2) had an average hair length of 0.98 cm, and treatment group 1 (P1) had an average hair length of 0.79 cm. The control group (P0) was treated with 0.9% NaCl for 14 days. Treatment group 1 (P1) was treated with 10% binahong leaf extract for 14 days. Treatment group 2 (P2) was treated with 20% binahong leaf extract for 14 days, and treatment group 3 (P3) was treated with 30% binahong leaf extract for 14 days.

This study tested whether the average hair growth differed significantly after applying binahong leaf extract. To test for significant differences, the researchers compared the control group (P0) with treatment group 3 (P3). The results were evaluated by comparing the Sig. (2-tailed) value with the alpha value used in the analysis. From the table above, it can be seen that the significant value of 2 tailed is 0.00 < 0.05, so it can be concluded that the growth in each comparison group, namely the control group and treatment 3, is significantly different.

Epithelial tissue consists of homogeneous cells that cover or wrap the outer and inner surfaces of tubular (channels) and cavum (cavities) organs. Epithelial cells are also known to proliferate and grow glandular follicles, such as hair follicles. From the histopathological images, treatment group 3 (P3) showed the fastest healing of incisional wounds in the skin tissue. This is evident from the thickening of the skin epithelium and almost complete coverage of the white area after applying 30% binahong leaf extract for 14 days.

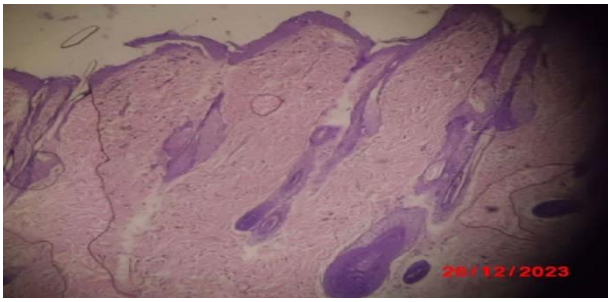
Discussion

Open wounds include lacerations, abrasions or superficial wounds, puncture wounds, penetration wounds, and incisions.[5] The natural wound healing process begins once a wound occurs. Wound healing is a natural process leading to the restoration of the structural and functional integrity of the injured tissue. It involves several biochemical and cellular pathways to repair lesions and restore physiological conditions. All organisms have a certain ability to respond to injuries and repair tissues. Efficient wound response and repair are essential to prevent infection.[6] Wounds heal through repair, not regeneration. Currently, when these wounds heal, open wounds on adult mouse skin form new hair follicles.[7]

This study utilized binahong plants. It is widely known that binahong contains various substances, including alkaloids, flavonoids, tannins, polyphenols, triterpenoids, and saponins.[19] Binahong leaves are often used as traditional medicine to heal burns, rheumatism, gout, poor appetite, nosebleeds, kidney inflammation, intestinal inflammation, and cancer.

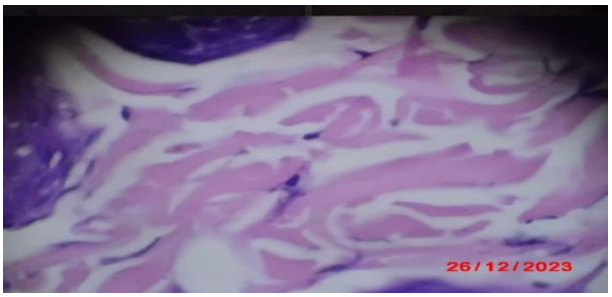
This research aimed to examine the effect of binahong leaf extract on hair growth and the histopathological features of skin tissue during the healing process of incisional wounds in Wistar rats. The control group (P0) was only treated with 0.9% NaCl for 14 days. Treatment group 1 (P1) was treated with 10% binahong leaf extract for 14 days. Treatment group 2 (P2) was treated with 20% binahong leaf extract for 14 days, and treatment group 3 (P3) was treated with 30% binahong leaf extract for 14 days. Phytochemical tests for alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids showed positive results, indicating the presence of secondary metabolites in the binahong leaf extract. Glycosides were not detected, possibly due to differences in the growing conditions of the plants. Therefore, the secondary metabolites in binahong leaf extract can stimulate hair growth and act as antioxidants for the skin.

From the study results, the average hair length of the rats indicated hair growth in each group over 14 days. On the last day, the control group (P0) had the shortest hair length, averaging 0.45 cm, making it the lowest hair growth observed. In contrast, treatment group 3 (P3) exhibited the fastest hair follicle elong-



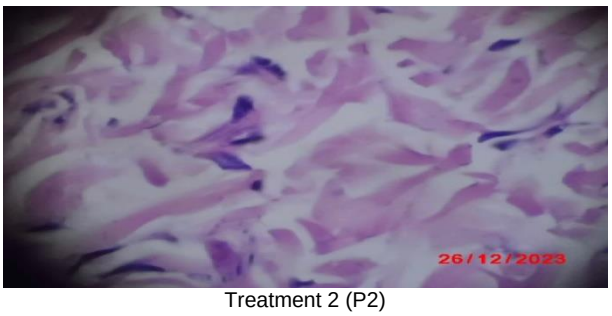
Control (P0)

In the P0 group, the skin epithelial tissue was still hollow so that the skin layer had not fused. The results of the observation of hispatolgi in the P0 group were reepithelialization of 0-25% of the wound from normal.



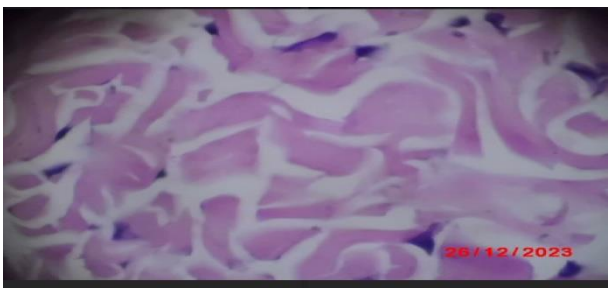
Treatment 1 (P1)

In the P1 group, the skin epithelial tissue was seen to begin to thicken but had not yet experienced wound closure that was close to normal, which could be seen in the white part that was still stretched out, and skin collagen had formed so that the observation of hispatolgi in this group was reepithelialization of 25-50% of the wound part from normal.



Treatment 2 (P2)

In the P2 group, the skin epithelial tissue is seen to begin to thicken and the white area begins to look covered. so that the observation of hispatolgi in this group is reepithelialization of 50-75% of the wound part from normal.



Treatment 3 (P3)

In the P3 group, the skin epithelial tissue is seen to begin to thicken and almost completely the white area begins to be covered. so that the observation of hispatolgi in this group is reepithelialization >75% of the wound part is from normal.

Figure 1. Overview of hispathology of healing cut wounds in rats

ation, with an average hair length of 1.24 cm over 14 days. Treatment group 2 (P2) had an average hair length of 0.98 cm, and treatment group 1 (P1) had an average hair length of 0.79 cm. The control group (P0) was only treated with 0.9% NaCl for 14 days, while treatment group 1 (P1) was treated with 10% binahong leaf extract, treatment group 2 (P2) with 20% binahong leaf extract, and treatment group 3 (P3) with 30% binahong leaf extract for 14 days. This aligns with the histopathological images showing that treatment group 3 (P3) exhibited the fastest wound healing in the skin tissue, with the skin epithelium thickening and nearly complete coverage of the white area after 14 days of treatment with 30% binahong leaf extract, resulting in reepithelialization of more than 75% of the wound area compared to normal.

The normality test using the One-Sample Kolmogorov-Smirnov Test showed a significance of 0.996 > 0.05, indicating that the data were normally distributed. This was followed by independent samples test to compare the mean differences between the variables tested. In this study, the variables tested were hair growth after applying binahong leaf extract to see if there were significant differences. The test results showed a significance of 0.00 < 0.05, indicating that hair growth in the control group and treatment group 3 was significantly different.

Conclusion

Based on the observations and research, binahong leaf extract (*Anredera cordifolia*) can accelerate hair growth and provide antioxidant benefits for the skin. Phytochemical screening results showed the presence of secondary metabolites such as flavonoids, saponins, tannins, and steroids/ triterpenoids, indicating the presence of these compounds in the binahong leaf extract. Data observations showed hair growth progression in all groups over 14 days, with the P3 group experiencing the fastest hair follicle elongation, reaching 1.24 cm in 14 days.

Histopathological images for the P3 treatment group indicated the fastest wound healing in the skin tissue. The epithelial tissue

of the skin began thickening and almost completely covered the white area after being treated with 30% binahong leaf extract for 14 days. Normality testing using the One-Sample Kolmogorov-Smirnov Test indicated that the data were normally distributed, and an independent sample test showed that wound healing between the control group and the P3 treatment group was significantly different.

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