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The Topical Effect of Binahong Fraction Leaves [*Anredera Cordifolia* (Ten.) *Steenis*] on Increased Epithelization and Hydroxyproline Level at Incision Wound in Rats

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Abstract

Binahong leaves [*Anredera cordifolia* (Ten.) *Steenis*] are easily found in Indonesia, efficacious and had been used as a wound-healing herbal medicine. This research aimed to assess the effectiveness of acetate ethyl fraction in binahong leaves [*Anredera cordifolia* (Ten.) *Steenis*] as a topical agent to the histopathology changes including increased epithelization and histochemistry examination by assessing hydroxyproline level in the acute incision wound healing process in Sprague Dawley rats. An in vivo laboratory experiment, using the Pre and Post Test Only Control Group Design. The subject was Sprague Dawley white male rats, randomly divided into 5 (five) treatment groups, a negative control group (gel-based), positive control group (Bioplacenton), 1% concentration of acetate ethyl fraction from binahong leaves group (FADB 1%), 2% concentration of acetate ethyl fraction from binahong leaves (FADB 2%) and 4% concentration of acetate ethyl fraction from binahong leaves (FADB 4%). The collected data was analyzed using SPSS programme version 21.0 with homogeneity test, normality test, Wilcoxon test, Mann Whitney test, Independent T-test, and Post Hoc confirmation using LSD test. In 4th-day skin tissue examination, the effectiveness of 4% concentration of acetate ethyl fraction from binahong leaves was not significantly different from Bioplacenton effectiveness in increasing epithelization and hydroxyproline level in the 10th-day skin tissue examination.

Keywords: Binahong leaves, epithelization, hydroxyproline, acetate ethyl fraction, *In vivo*

Introduction

Injury is a condition where continuity of tissue is damaged due to trauma from sharp or blunt objects, changes in temperature, chemical, electricity, radiation, or animal bites[1]. Cut wounds are types of wounds caused by incisions from sharp objects, can be metal or wood and so on with the type of wound usually thin. The body will try to repair damaged tissue through the mechanism of wound healing in response to damage that occurs in the skin tissue. Wound healing is a response to injury and is an attempt to maintain normal structure and function[2].

Wound healing is needed to get back intact body tissue. Time for wound healing usually ranges from no more than 30 days[3]. There are many ways to treat wounds, among others by administering drugs such as antibiotics (one of the examples is neomicin sulfate) and or by using modern medical techniques. However, this is not without obstacles, it is also possible to arise resistance due to the use of antibiotics or cost problems which of course are not cheap. Treatment using nutritious plants as medicine can be an option for now because the costs are relatively inexpensive and easy to use. This has become one of the motivations for the authors to try to develop and optimize the use of nutritious plants as wound healing drugs, namely binahong plants [Anredera cordifolia (Ten.) Steenis]. Binahong [Anredera cordifolia (Ten.) Steenis] is one of the plants that is easily found in Indonesia. The efficacy of this plant is already known by the community and has been used for generations. China is the home country of the Binahong [Anredera cordifolia (Ten.) Steenis] plant, both in Europe and America, this plant is well known, but researchers there are not yet interested in further researching about this plant. South East Asia region, especially in Vietnam, this plant is consumed by local residents as a medicine in curing various diseases both mild and severe. And during the war in Vietnam against America, this plant was used as a wound healing medicine. Part of the binahong plant [Anredera cordifolia (Ten.) Steenis] used in herbal therapy is the tubers, stems, flowers and leaves [4]. Various studies on this binahong plant have been carried out, among others regarding its activity as an anti-bacterial and its secondary metabolite content. Binahong simplisia [Anredera cordifolia (Ten.) Steenis] contains alkaloids, polyphenols and saponins[5]. The chemical content of binahong [Anredera cordifolia (Ten.) Steenis] that can be used in the treatment of wounds is flavonoids, oleanolic acid, proteins, saponins and ascorbic acid. The content of ascorbic acid in this plant functions as a cofactor at the hydroxylation stage to activate the prolactive hydroxylase enzyme which will convert proline to hydroxyproline to play a role in the formation of collagen[6], thus accelerating wound healing[7]. Based on the description above, it can be seen that the plant Binahong [Anredera cordifolia (Ten.) Steenis] especially its leaf parts, has anti-bacterial properties and its ascorbic acid content can support the formation of collagen which plays an important role in the wound healing process. Collagen is one of the parameters for the formation of skin regeneration tissue composed of hydroxycillin and hydroxyproline. As the development of previous studies on binahong leaf extract [Anredera cordifolia (Ten.) Steenis], the authors felt the need to conduct further research to determine the benefits of the binahong leaf fraction [Anredera cordifolia (Ten.) Steenis] as a wound healer that was tested based on histopathological features of epithelial tissue enhancement and biochemical observation by measuring hydroxyproline levels in the

wound tissue on the skin of test animals compared with the use of bioplacenton. The aim of this study was to evaluate the effectiveness of topah ethyl acetate fraction of binahong leaves [Anredera cordifolia (Ten.) Steenis] on histopathological changes in this case increasing epithelialization and histochemical examination by looking at hydroxyproline levels in the process of healing new cuts in Sprague Dawley rat skin.

Methods

This research is an in vivo laboratory experimental study, with the Pre and Post Test Only Control Group Design to assess the effectiveness of the administration of topical ethyl acetate fraction of binahong leaves [Anredera cordifolia (Ten.) Steenic] to histopathological changes with parameters increasing epithelialization and hydroxyproline levels in the process healing of new incision wounds on the skin of Sprague Dawley white rats has been carried out in January - June 2019 in the Animal House laboratory and Biomolecular Laboratory, Biochemistry lab of the Faculty of Medicine, Sriwijaya University. The research subjects were Sprague Dawley white rats with male sex, aged 8-10 weeks with body weight 150-265 grams.

The rats were divided into 5 (five) randomized treatment groups, namely the negative control group (gel base), the positive control group (Bioplacenton), the binahong ethyl acetate fraction group 1% concentration (FADB 1%), the ethyl acetate fraction binahong leaf group concentration of 2% (FADB 2%) and the ethyl acetate fraction group of binahong leaves concentration of 4% (FADB 4%). All treatment groups were examined for an increase in epithelialization and hydroxyproline levels after the intervention of the ethyl acetate fraction of binahong leaves.

Assessment of the increase in epithelialization in this study was done by the method score (9) score of 0 means Absent which describes the overall damage to the epidermis. A score of 1 means Starting, which describes the formation of the epidermis layer. A score of 2 means an Incomplete representing the epidermal layer has formed, but there is still thickening. A score of 3 means Complete, which depicts the epidermis layer completely formed and no thickening was found in the epidermis layer. The score assessment is carried out at 40x magnification.

In this study weighing the rats of each group before the intervention. The data obtained were then analyzed statistically using the SPSS version 21.0 program. Homogeneity test with Levene test. The testing process was carried out by comparing the data obtained between the ethyl acetate fraction group binahong concentration of 1%, the ethyl acetate fraction group binahong concentration 2% and the binahong leaf ethyl acetate fraction group concentration 4% with the negative control group (gel base) and the positive control group (bioplacenton). To

see the effectiveness of the binahong leaf ethyl acetate fraction on the increase in epithelialization, a histopathological examination was done to increase the epithelialization of each group on day 0 and day 4 after treatment. With Shapiro-Wilk normality test. To see the comparison of the effectiveness of the binahong leaf ethyl acetate fraction against the increase in epithelialization in each of the two groups, we used the Mann Whitney Test. The Post Hoc confirmation test uses the LSD Test.

Results

In the process of epithelialization, epithelial cells merge in the middle of the wound, then the wound will be completely closed and free from contamination of the environment outside the body so that the process of maturation of the underlying tissue will proceed better (10), as shown in Figure 1 where rat skin begins to be injured (day to 1) and the wound begins to close (day 4).

Figure 1. On the left: rat skin appearance on day 1, right: rat skin appearance on day 4 of the rat group treated with ethah acetate fraction gel of binahong leaves 4%.

Table 1. Effectiveness of the Binahong Leaf Ethyl Acetate Fraction on Increased epithelialization

Group	Increased Epitelization Day 0 Before Treatment	Increased Epitelization Day 4th After Treatment	Difference	P
Basis Gel	0,000 ± 0,000	1,333 ± 0,516	1,333 ± 0,516	0,023
Bioplacenton	0,000 ± 0,000	2,500 ± 0.548	2,500 ± 0.548	0,024
FADB 1%	0,000 ± 0,000	2,333 ± 0.516	2,333 ± 0.516	0,023
FADB 2%	0,000 ± 0,000	2,333 ± 0.516	2,333 ± 0.516	0,023
FADB 4%	0,000 ± 0,000	2,500 ± 0.548	2,500 ± 0.548	0,024

Wilcoxon, p = 0.05

Table 1 showed the mean increase in epithelialization of each group before and after treatment. All groups that initially did not experience an increase in epithelialization (absent) were found to have increased epithelialization starting from starting, incomplete to complete with a probability of 0.023 respectively; 0.024; 0.023, 0.023 and 0.024 ($p < 0.05$). It showed that there was an increase in epithelialization after administration of gel base, bioplacenton and ethyl acetate

fraction of binahong leaves of all concentrations but in the gel base group an increase in complete epithelialization did not occur.

Table 2. Comparison of the Effectiveness of the Binahong Leaf Ethyl Acetate Fraction Against Hydroxyproline Levels

Group	Mean ± SD	Group	Mean ± SD	*p value*
Basis Gel	0,075 ± 0,002	Bioplacenton	3,705 ± 0,032	0,000
		FADB 1%	1,169 ± 0,047	0,000
		FADB 2%	2,214 ± 0,037	0,000
		FADB 4%	3,670 ± 0,025	0,000
Bioplacenton	3,705 ± 0,032	FADB 1%	1,169 ± 0,047	0,000
		FADB 2%	2,214 ± 0,037	0,000
		FADB 4%	3,670 ± 0,025	0,061
FADB 1%	1,169 ± 0,047	FADB 2%	2,214 ± 0,037	0,000
		FADB 4%	3,670 ± 0,025	0,000
FADB 2%	2,214 ± 0,037	FADB 3%	3,670 ± 0,025	0,000

Independent T Test, p = 0.05

From the results of the Independent T Test. obtained the probability value between the ethyl acetate fraction of binahong leaves concentration of 4% with a positive control group that is bioplacenton of 0.061 (p> 0.05) so that it can be concluded that there is no difference in hydroxyproline levels between the ethyl acetate fraction of binahong leaves concentration of 4% with bioplacenton. However, the probability value obtained between the ethyl acetate fraction of binahong leaves concentration of 1% and 2% with bioplacenton <0.05, which means that there are differences in hydroxyproline levels between the ethyl acetate fraction group of binahong leaves concentration of 1% and 2% with bioplacenton.

Post Hoc confirmation test using LSD test, obtained the results of the probability value between the positive control group (bioplacenton) with the ethyl acetate fraction group binahong concentration of 4% by 0.082 (p> 0.05) so that it can be concluded that there is no difference in hydroxyproline levels after bioplacenton administration with an ethyl acetate fraction of binahong leaves at a concentration of 4%. However, a probability value was obtained between the positive control group (bioplacenton) and the binahong leaf ethyl acetate fraction concentration of 1% and 2% <0.05, which means that there were differences in hydroxyproline levels after bioplacenton administration and the ethyl acetate fraction of binahong leaves 1% and 2% concentration. . So that it can be concluded that the ethyl acetate fraction of binahong leaves with a concentration of 4% is as effective as bioplacenton on the levels of hydroxyproline of the skin tissue.

Discussion

Homogeneity test with Levene test obtained a probability value of 0.485 (P> 0.05) which shows that the body weight of all rats is homogeneous, so that the experimental test requirements are met. To see the effectiveness of the binahong leaf ethyl acetate fraction on the increase in epithelialization, a histopathological examination was done to increase the epithelialization of each group on day 0 and day 4 after treatment. With the Shapiro-Wilk normality test, the probability value of all groups <0.05, which means that the data distribution of each group is not normal, because the data distribution is not normal then to see the effectiveness of the ethyl acetate fraction of binahong leaves to increase epithelialization the Wilcoxon test is used. All groups initially did not experience an increase in epithelialization (absent) found to have increased epithelialization starting from incomplete to complete with a probability of 0.023 respectively; 0.024; 0.023, 0.023 and 0.024 (p <0.05). This shows that there is an increase in epithelialization after administration of gel base, bioplacenton and ethyl acetate fraction of binahong leaves of all concentrations but in the gel base group an increase in complete epithelialization does not occur. Increased epithelialization of each group was then compared. With the Shapiro-Wilk normality test (obtained the probability value of all groups <0.05, which means that the data distribution of each group is not normal, because the data distribution is not normal then to see the comparison of the effectiveness of the ethyl acetate fraction of binahong leaves to the increase in epithelialization of each two groups, we use the Test Mann Whitney.From the Mann Whitney test results obtained probability values between the ethyl acetate fraction of binahong leaves of all concentrations and bioplacenton with a gel base <0.05 namely 0.011, 0.014, 0.014, 0.011 which means that there is a difference in the increase in epithelialization between the ethyl acetate fraction of the binahong leaves of all concentrations and bioplacenton with a gel base. However, the probability values obtained between the ethyl acetate fraction binahong leaves of all concentrations with a positive control group are bioplacenton> 0.05 where bioplacenton to ethyl acetate fraction 1% p = 0.575, to ethyl acetate fraction 2% p = 0.575, and to ethyl fraction acetate 4% p = 1,000, so it can be concluded that there is no difference in the increase in epithelialization between the ethyl acetate fraction of binahong leaves of all concentrations with bioplacenton and especially for the ethyl acetate fraction of binahong leaves 4%, there is no significant difference with bioplacenton. Post Hoc confirmation test using LSD test, obtained the results of the probability value between the positive control group (bioplacenton) with the ethyl acetate fraction group binahong all concentrations of> 0.05 so it can be concluded that there is no difference in the

increase in epithelialization after bioplacenton administration with leaf ethyl acetate fraction Binahong all concentration. However, a probability value was obtained between the positive control group (bioplacenton) and the ethyl acetate fraction group binahong all concentrations on a gel basis <0.05 so that it could be concluded that there was a difference in the increase in epithelialization after bioplacenton administration and the ethyl acetate fraction of binahong leaves all concentrations with a gel base . So it can be concluded that the ethyl acetate fraction of binahong leaves in large doses (FADB 4%) is as effective as bioplacenton in the process of increasing epithelialization of rat skin tissue. Examination is carried out on the 10th day after injury, where in this phase the formation of fibroblasts occurs. Fibrolas are collagen fibers that play a role in wound healing where collagen is a parameter of tissue formation or skin regeneration. The effectiveness of wound healing in this study by determining the level of hydroxyproline. Hydroxyproline levels in tissues can be used as an index of parameters in the skin. The higher levels of hydroxyproline can be indicated that an increase in collagen synthesis is correlated in the wound healing process[11]. Through macroscopic examination (with visible) can be seen a very significant difference in the skin of mice that have been injured on the first day compared with rat skin on day 10. On the first day the wound still looks red, the cut still looks wet with a long incision more than 2 cm, and on the 10th day on the skin of rats treated with 4% ethyl acetate fraction gel, binahong leaves were no longer visible.If the data were analyzed statistically with the Shapiro-Wilk normality test it was obtained the probability value of all groups> 0.05, which means the data distribution of each group was normal, because the data distribution was normal then to see the comparison of the effectiveness of the ethyl acetate fraction of binahong leaves against the hydroxyproline levels of each of the two groups, we using the Independent T Test. From the results of the Independent T Test. obtained the probability value between groups of fractions Binhong leaf ethyl acetate concentration of 4% with a positive control group is bioplacenton of 0.061 (p> 0.05) so it can be concluded that there is no difference in hydroxyproline levels between the binahong leaf ethyl acetate fraction group concentration of 4% with bioplacenton. However, the probability value obtained between the ethyl acetate fraction of binahong leaves concentration of 1% and 2% with bioplacenton <0.05, which means that there are differences in hydroxyproline levels between the ethyl acetate fraction group of binahong leaves concentration of 1% and 2% with bioplacenton.With the Post Hoc confirmation test using the LSD test, it was obtained the probability value between the positive control group (bioplacenton) with the ethyl acetate fraction group binahong concentration of 4% by 0.082 (p> 0.05) so that it can be concluded that there were no differences in hydroxyproline levels after administration bioplacenton with a

binahong leaf ethyl acetate fraction of 4% concentration. However, a probability value was obtained between the positive control group (bioplacenton) and the binahong leaf ethyl acetate fraction concentration of 1% and 2% <0.05, which means that there were differences in hydroxyproline levels after bioplacenton administration and the ethyl acetate fraction of binahong leaves 1% and 2% concentration. . So that conclusions can be drawn from the ethyl acetate fraction of binahong leaves, the concentration of 4% is as effective as bioplacenton on the levels of hydroxyproline of skin tissue.

Conclusion

Application of topical concentration of ethyl acetate fraction of binahong leaves was able to provide an effect of increasing the formation of epithelial tissue in rat skin incision. On the examination of rat skin tissue on day 4, the effectiveness of the binahong leaf ethyl acetate fraction with a concentration of 4% was not significantly different from the effectiveness of bioplacenton in increasing epithelialization and topical administration of binahong leaf ethyl acetate fraction concentration 4% able to increase hydroxyproline levels which were not significantly different from bioplacenton in bioplacenton rat skin tissue examination on day 10.

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