Effectiveness Of Red Betel Leaf Ointment (*Piper Crocatum, Ruiz & Pav*) On Slash Wounds In White Mice

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Abstract.
Normal wounds may heal on their own in some cases, but if there are obstructions in the way, the wound won't heal, making them more difficult to treat. The purpose of this study was to evaluate the phytochemical makeup of red betel plant leaves and the effectiveness of red betel extract in accelerating wound healing in white mice. 25 male white mice made up the animal population, which was divided into 5 groups, including 3 treatment groups. using red betel extract percentages of 15%, 30%, 45%, and 10%. a pair of control groups, one positive and the other negative. The control utilized a gel-based solution and 10% povidone iodine. The white mice's backs were cut into using a 1 cm^2 incision. The data was gathered by observing the entire wound healing procedure from the inflammatory phase (redness, edema, and pus presence) through the proliferative phase. The current data were then assessed using a method called the Test of Homogeneity of Variances. The One Way Anova test is useful for determining whether or not there are meaningful changes in the data variance if the prerequisites for the parametric test are met. If the p value is less than or equal to 0.05, this test is significant. The LSD post hoc test also determines the most significant set of data. When testing the distribution, if abnormal data are discovered, another alternative method is using the Kruskal-Wallis test, which is followed by the Mann-Whitney test with a significant value of 0.05. Between the positive and negative control groups, redness (p=0.003), granulation tissue (p=0.038) on the third day, and wound area reduction (p=0.048) on the ninth day were all significantly different when red betel extract concentrations of 15%, 30%, and 45% were used. It may be concluded that giving white mice betel extract at a concentration of 46% accelerates the healing of wounds. According to phytochemical analysis, red betel extract contains alkaloids, flavonoids, saponins, and tannins, making it a potential natural antioxidant.

Keywords: *Red Betel, White Mice and Phytochemicals.*

I. INTRODUCTION

The skin is one of the largest organs in the body and makes up 15% of an adult's body weight. The main function of the skin is to protect deep tissue from trauma, UV damage, extreme temperatures, toxins, and bacteria. The wound basically healed on its own. If there were obstacles, it would be quite difficult to heal normal wounds[1].According to Kartika (2015), wounds that do not heal properly can have an impact on patient health and result in high wound care costs. Red betel is one example of an herbal plant that is the right choice for the treatment of wound healing (Piper crocatum) [2]. The skin has an extraordinary ability to repair itself after injury . The biological process of wound healing occurs in the body and requires a number of complex, complicated and possibly distorted processes. Consequently, ideal conditions are needed for successful healing. According to clinical studies, a number of things can prevent wound healing, including hypoxia, infection, tumors, metabolic diseases such as diabetes mellitus, presence of debris and necrotic tissue, certain medications, and poor diet. [3]. According to Palumpun and Wiraguna, the goals of wound care include minimizing tissue damage, oxygenation, proper nutrition, reducing risk factors that inhibit wound healing, accelerating the healing process, and reducing the incidence of wound infection [4]. In the field of wound care, wounds can be treated with drugs both locally (topically) and systemically (orally), or a combination of both.

These drugs can be used to eliminate and control bacteria, manage wound exudate, and keep the wound surface moist and protected. In the clinical setting, antiseptics including hydrogen peroxide, chlorhexidine, and povidone-iodine are frequently used for wound care.Today, highly effective herbal or conventional medicines are used to create new wound care techniques. The improvement of health services

is best served by this traditional medicine. This is a national heritage site that must be investigated, researched, and developed so that it can be exploited (Dewi, Anthara, & Dharmayudha, 2014). Regarding natural or herbal compounds that may be good for health, several studies have been conducted. Binahong extract accelerates the healing of diabetic ulcers, according to Rosarian Firdaus's 2015 study. The Effects of Binahong Ethanol Extract (Anredera cordifolia), Hispathological Profile of Alloxan Wound Healing in Male Wistar Rats [5].Furthermore, second-degree burns on the skin of rabbits with long-degree burns treated with crushed red betel leaves proved to have a significant impact in the Betel Leaf Extract (Piper crocatum) study by Noor Fithriyah, Syamsul Arifin, and Eka Santi (2013). The second-degree burn on the rabbit's skin healed.

II. METHODS

The tools used in this study were animal cages, gram scales, scissors, scalpels/surgical scissors, gloves, a 1 cc syringe, a ruler, a cutton butt, a transparent dressing, sterile gauze, plaster, tweezers, a blander, a filter, a measuring cup, ovens, and digital cameras.The materials used in this study were red betel extract, a solvent in the red betel extraction process (96% ethanol), an ingredient for making ointments (Vaseline), normal saline (0.9% NaCL), a drug to anesthetize white rats (ketamine), alcohol swabs, povidone iodine for wound care for the positive control group, clean water for the white rats to drink, pellets for the white rats to eat, and rice husks for the cage mat..

Sample Preparation

Mice treated at the Laboratory of Biochemistry, Faculty of Medicine, University of North Sumatra They are white mice (Mus musculus). During the laboratory experiments, animals were placed in cages lined with rice husks and fed pellets while being sprayed with clean water twice a day, once in the morning and once in the evening. One week before treatment, the mice were adapted to a laboratory environment to reduce the metabolic effects of stress. Next, the white rat's head should be cut off at a location that is one-third the length of the animal's total length. After the spot was found, the hair on the white rat's back was sheared with scissors. After that, cotton soaked in alcohol was used to sterilize the backs of white mice. After that, an injection of ketamine (50 mg/kg body weight) was used to euthanize the guinea pig. Then make a box-shaped wound measuring 1 cm^2. After securing the skin in place, the dermis and underlying connective tissue are removed with a scalpel.

Parameters in Test

Processing of Red Betel Extract

Crushed red betel leaves Then, add 1000 ml of 96% ethanol to the soaking flask together with 100 g of red betel powder until the powder is completely submerged. Stir it, let it stabilize for 24 hours, then filter to get the filtrate. The evaporator receives an immersion output. The water tank is filled with water by the evaporator, which is connected to it. Every appliance installed, including the water heater (set at 70–80°C), is plugged into a power source. Wait between 1.5 and 2 hours per flask for the ethanol solution to stop flowing into the vessel.

Using the following recipe, red betel extract is combined with Vaseline [6].

$$L = - X\ 100\%$$

Information :

L = concentration of solution (%)

α = mass of solute

b = mass of solution (mg)

Using the following procedure, 50 mg of Vaseline is added to the red betel extract to make a concentrated form, resulting in:

1. Concentration 15%.

50 mg of Vaseline combined with 7.5 mg of red betel extract.

2. Concentration 30%.

50 mg of Vaseline combined with 15 mg of red betel extract.

3. Concentration 45%.

50 mg of Vaseline combined with 22.5 mg of red betel extract

Phytochemical analysis

The findings of red betel leaf extract without aroma were tested in methanol (an ester compound). Red betel leaf extract passed the methanol test and was proven to contain no methanol. When methanol in red betel leaf extract reacts with palmitic acid in vegetable oil, esters are formed. This reaction is accelerated by sulfuric acid. In palmitic acid, the O atom is more electronegative than the C atom, so it attracts electrons. As a result, the carbon-oxygen bond becomes unstable, dissociating into C+ and OH ions. The oxygen atom in methanol withdraws electrons because it is more electronegative than the hydrogen atom. It is due to the electronegativity difference between the two atoms involved. When the O-H bonds become unstable under these conditions, they dissociate into O- and H+ dissociative radicals. Methyl palmitate (H2O) is formed when O-ions from methanol combine with C+ ions from palmitic acid-containing compounds. Red betel leaf extract in ethanol has a density of 0.69 g/mL, according to a density meter. Molecules with a lower density can diffuse into the bloodstream and interact with receptors more quickly, speeding up the healing process.

Wound Care Process

After the wound was treated, treatment for both the treatment group and the control group began immediately. All research samples underwent wound care. Red betel extract (Piper crocatum) was applied to the wound with a concentration of 15%, 30%, or 45% in the treatment group after washing with physiological saline solution. After that, a clear bandage is applied to the wound. Those serving as negative controls were given a base gel and a transparent bandage. Povidone iodine and transparent bandages were used in the active control group. The wound is treated every three days until the granulation grows, there is no edema (swelling), and the redness around the wound decreases.

Wound Healing Process Analysis

Animals were observed for redness around the wound, edema (swelling) around the wound, pus (present or absent), granulation tissue (present or absent), and the wound area. By measuring the edges of the wound, the size of the damage is determined. Until day 14, the wound was observed every three days.

III. RESULT AND DISCUSSION

Healing Process in the Inflammatory Phase

a) Redness around the wound.

Based on the results of the study on the third day, it was found that in the test treatment group 1 (15%), there were four samples that still experienced redness around the wound area, whereas all samples in the test treatment group 2 (30%) and test 3 (45%) did not experience redness around the wound. Meanwhile, for all samples in the negative control group, they still experienced redness around the wound, and for the positive control group, there were four samples where there was no redness around the wound.The redness in the study samples from the three treatment groups decreased gradually, and on the sixth day there was no more redness around the wound area. While the control group experienced a reduction in the area of redness around the wound, it was not statistically significant, on the sixth day, three samples in the negative control group and one in the positive control group still experienced redness around the wound.

Based on statistical tests, all three treatment groups that used red betel extract (Piper crocatum) had a significant effect on accelerating redness around the wound, but the most effective group in accelerating redness around the wound was the red betel extract (Piper crocatum) treatment group with a concentration of 45%; this can be seen from macroscopic observations, where on the third day, all samples in the group no longer experienced redness. The acceleration of redness around the wound in the treatment group is thought to be due to the effect of the active compound content derived from red betel leaf extract. The anti-inflammatory activity of betel leaf extract is due to the presence of compounds belonging to the classes of flavonoids, saponins, tannins, and essential oils. The presence of flavonoids serves to limit the release of inflammatory mediators (Fithriyah et al., 2013). That red betel extract provides a promising anti-inflammatory effect. The anti-inflammatory activity of flavonoids is carried out through the development of cyclooxygenation and lipoxygenation, so that there is a limit to the number of inflammatory cells that migrate to injured tissue. Furthermore, the inflammatory reaction lasted shorter, and the ability to proliferate was not too late.

Table 1. Results of observation of redness on the 6th day

No	Group	n	Assessment Score			
			0		1	
			f (x)	%	f (x)	%
1.	Negative control	5	2	80%	3	60%
2.	Positive control	5	4	80%	1	20%
3.	Test 1 (15%)	5	5	80%	0	20%
4.	Test 2 (30%)	5	5	100%	0	0%
5.	Test 3 (45%)	5	5	100%	0	0%
Info : 0 = No redness 1 = There is redness						

Based on table 1, the results of observations of redness around the wound on the sixth day, it was found that in the negative control group there were 3 samples with redness around the wound, in the positive control there were 4 samples without redness around the wound, and in all samples in the first treatment group (15%), test 2 (30%), and test 3 (45%), there was no redness.Four samples (15% of the total) in the first experimental treatment group showed persistent redness around the wound area on the third day, whereas none of the samples in the second group showed persistent redness. Similarly, the wound in Test 3 (45%) showed no redness. While redness persisted around the incisions in every sample from the negative control group, four samples from the positive control group did not.On the sixth day, there was no more redness around the wound area in the study samples from the three treatment groups. Redness around the wound also decreased, although not significantly, in the control group, whereas 3 out of 6 samples in the negative control group still showed redness on day 6. In the positive control group, the redness around the wound was still reduced.

b) Edema

On the third day, one sample in the negative control group showed edema around the wound. In contrast, neither the treatment group nor the positive control sample experienced periwound edema. The area of edema around the wound in the negative control group gradually decreased. All samples from the negative control group showed no edema around the site from days 6 to 14 of observation. The findings of the analysis are consistent with Vagashiya et al. (2007), who found that 300 mg of red betel extract significantly reduced acute and chronic edema in white rats (mus musculus), with slight edema in the first and third 1.2 hours. have inflammatory properties.

c) Pus

Analysis of samples from all five groups revealed that by day six, all wounds were clear of pus. On day 9, one sample from the control group was negative, including pus; all samples from the control group tested positive; and neither the first nor the second test in the treatment group yielded positive findings (15% and 30%, respectively). (45%) Third Trial.%) There was no pus in the wound. All samples in the negative control group were free of pus on days 12–14 (when the study was conducted). In the untreated group, the amount of pus continued to decrease. In the treatment group, pus formation was prevented thanks to red betel (Piper crocatum) alkaloids, tannins, saponins, and essential oils. Alkaloids have several uses, one of which is as an antibacterial agent [7]. stated that disruption of peptidoglycan causes bacterial cell death by preventing the formation of a full layer of the cell wall.

The wound healing process in the proliferative phase

a) Granulation tissue

We tracked the development of granulation tissue starting on the third day and provided data showing that there was no granulation tissue in the negative control sample. Three specimens from the positive control group had no granulation tissue, whereas all specimens from tests 1 and 3 (45%) had granulation rates of 15%, 30%, and 30%, respectively.These results indicated that granulation tissue developed faster in the three treatment groups given red betel extract (Piper crocatum) compared to the second control group. All treatment groups significantly affected the growth rate of granulation tissue in the wounds of white rats (Mus musculus), but the 45%, 30%, and 15% groups had the greatest impact.

Table 2. Observation results for granulation on the 9th day

No	Group	n	Assessment score					
			0		1		2	
			f (x)	%	f (x)	%	f (x)	%
1.	Negative control	5	0	80%	4	80%	1	20%
2.	Positive control	5	0	60%	2	40%	3	60%
3.	Test 1 (15%)	5	0	0%	1	20%	4	80%
4.	Test 2 (30%)	5	0	0%	1	20%	4	80%
5.	Test 3 (45%)	5	0	0%	0	0%	5	100%
Info : 0 = No granulation 1 = Partial wound granulation 2 = Granulation occurs all over the wound.								

All treatment groups significantly affected the growth rate of granulation tissue in the wounds of white rats (Mus musculus), but the 45%, 30%, and 15% groups had the greatest impact. Observations have corroborated this as well. Macroscopic examinations revealed that on the ninth day, granulation had occurred in all wounds in the four samples given 15% and 30% red betel extract, but not in all samples given 45% red betel extract. occurs in all injuries.

b) Narrowing of the wound area

The wound area began to narrow on the third day after the incision, but changes in the wound area were not seen until the ninth day. Based on the data, the positive control group had three specimens with a wound area of 0.28–0.53 cm^2, the negative control group had a wound area of 1 cm^2, the treatment group 1 had two specimens with a wound area of 0.28–0.53 cm^2, the treatment group 2 had two specimens with a wound area of 0.28–0.53 cm^2, and the treatment group 3 had two specimens.

Table 3. Observation results of the wound area on the 12th day

No	Group	n	Assessment score											
			0		1		2		3		4		5	
			f(x)	%	f(x)	%	f(x)	%	f(x)	%	f(x)	%	f(x)	%
1	Negative control	5	0	60%	3	60%	0	0%	1	20%	1	20%	0	0%
2	Positive control	5	0	0%	1	20%	0	0%	3	20%	3	60%	0	0%
3	Test 1 (15%)	5	0	20%	0	0%	1	20%	1	60%	1	20%	0	0%
4	Test 2 (30%)	5	0	0%	0	20%	2	20%	1	20%	1	20%	1	20%
5	Test 3 (45%)	5	0	0%	0	0%	0	0%	3	0%	3	60%	2	60%
Info : 0 = 1 cm^2 4 = 0,01 cm^2 – 0,27 cm^2 1 = 0,8 cm^2 – 0,99 cm^2 5 = 0 cm^2 2 = 0,54 cm^2 – 0,79 cm^2 3 = 0,28 cm^2 – 0,53 cm^2														

The wound area ranged from 0.01 to 0.27 cm^2.On the twelfth day, 3 samples were obtained from the negative control group with a wound area of 1 cm2 and 3 samples from the positive control group with a wound area of 0.01 cm2–0.27 cm^2.

Table 4. Kruskal-Wallis results of the wound area on day 12

Variable	Group	n	(mean)	P (asymp.Sig)
Wound area	Negative control	5	6,90	0,048
	Positive Control	5	14,00	
	Test 1 (15%)	5	11,00	
	Test 2 (30%)	5	12,70	
	Test 3 (45%)	5	20,40	
Total		25		

According to table 4.32, the Kruskal-Wallis test results between the five groups yielded a p-value of 0.048 0.05, indicating that there is a difference in the narrowing of the incision wound area in white mice (Mus musculus).The results of the Kruskal-Wallis test only showed an effect on the narrowing of the incision wound area in white mice (mus musculus), but did not show which group was more significant. Therefore, the Mann-Whitney test was carried out to see the difference in the effect of the treatment group on the narrowing of the wound area in white mice (mus musculus).

Table 5. Results of the Mann Whitney test for the wound area on the 12th day

Kelompok		Asymp.Sig
Kontrol negatif	Kontrol positif	0,151
	Uji 1 (15%)	0,310
	Uji 2 (30%)	0,222
	Uji 3 (45%)	0,016
Kontrol positif	Uji 1 (15%)	0,421
	Uji 2 (30%)	0,841
	Uji 3 (45%)	0,095
Uji 1 (15%)	Uji 2 (30%)	1,000
	Uji 3 (45%)	0,016
Uji 2 (30%)	Uji 3 (45%)	1,151

According to table 4.33, the Mann-Whitney test results in the negative control group were not significantly different from the positive control group and the treatment group for tests 1 (15%) and 2 (30%), while test 3 (45%) differed significantly from the significance value of 0.016 0.05 (p-value). With a significance value of p 0.05, there was no significant difference between the positive group and the three treatment groups. In the treatment group, test 1 (15%) did not differ significantly from test 2 (30%), but test 3 (45%) did, with a significance value of 0.016 0.05 (p-value). The treatment group for test 2 (30%) was not significantly different from test 3 (45%).Three samples from treatment group 1 (15%) had a wound area between 0.28 cm^2 and 0.53 cm2, two samples from treatment group 2 (30%) had a wound area between 0.54 cm2 and 0.79 cm^2, and two samples from treatment group 3. (45%) of the wound area is between 0.01 cm^2 and 0.27 cm^2.A betel extract (Piper crocatum) concentration of 15% used in the treatment group did not shrink the wound surface of white mice, in accordance with the findings of statistical tests (Mus musculus). To accelerate wound healing in white mice (Mus musculus), the treatment group used red betel extract (Piper crocatum) with concentrations of 45% and 30%, but 45% red betel extract had the greatest effect. This is also evident from the findings of the macroscopic observations: on the 14th day, 2 samples of healing wounds measuring 0 cm^2 were obtained in the treatment group and red in the treatment group containing 30% red betel extract. The wound area of the four samples in the group with 45% betel extract content was 0 cm^2 (healed).

IV. CONCLUSION

Red betel leaf extract prepared by maceration using ethanol as a solvent has a boiling point of 43 °C and a density of 0.69 g/mL. The contents of plant compounds found in the extracts were alkaloids, flavonoids, saponins, and tannins. This shows that red betel leaf extract has significant levels of antioxidant chemicals, making it suitable for medicinal use. It is also possible to conclude that red betel leaf extract contains phytochemicals that can be used as wound healing drugs. According to statistical analysis and macroscopic observations, the effect of red betel extract at concentrations of 15%, 30%, and 45% is different in the wound healing process. Compared to concentrations of 30% and 15%, red betel extract with a concentration of 45% had the greatest effect on wound healing.

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