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Effectiveness Test of Red Betel Leaf Essential Oil Ointment on Wound Healing in Wistar Rats (Rattus Norvegicus)

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Abstract: **About 350,000 vascular plants (tracheophytes) have the potential to become medicinal plants, but about 10% of them, namely about half a million species, are used as medicinal plants. Red betel leaf is one of the medicinal plants that deserve to be considered as an herbal medicine to replace chemical drugs. This study was designed to determine the effectiveness of ointment from the essential oil of red betel leaves (piper crocatum linn.) in healing wounds in Wistar rats (Rattus norvegicus). This type of research is experimental, with a Pre-test and Post-test group only control design approach conducted from March to November 2022. This research was conducted at Herbarium Medanese FMIPA USU, Pharmacognosy Laboratory of Faculty of Pharmacy USU, and Pharmacology Laboratory of Pharmacy USU. The study sample was 25 white male rats randomly selected, which were divided into five groups (4 treatment groups and one control group) so that one treatment group consisted of 5 rats. Essential Oil Saleb Red Betel Leaf extract (piper crocatum linn.) has several bioactive compounds such as alkaloids, flavonoids, saponins, and tannins that play a role in wound healing. The optimum concentration of Essential Oil Saleb of Red Betel Leaf extract (piper crocatum linn.) that can heal cut wounds in white rats is 6%. The highest percentage of healing on day k-14 was in the positive control (Bioplacenton®), which was 95% and followed by 6% v/v extract with a 90% healing percentage. Essential Oil Saleb preparation of Red Betel Leaf extract (piper crocatum linn.) has an ability that is close to Bioplacenton® in the recovery of cut wounds in rats.**

Keywords: **Red Betel, Essential Oil, Wound Healing.**

I. INTRODUCTION

About 350,000 vascular plants (tracheophytes) have the potential to become medicinal plants, but about 10% of them, namely about half a million species, are used as medicinal plants. Red betel leaf is one of the medicinal plants that deserve to be considered as an herbal medicine to replace chemical drugs (1). The red betel plant contains chemical elements that are useful for treatment, but the part of the red betel plant most widely used as medicine is the leaf. Piper crocatum extract or red betel leaf extract contains antiseptic and antibacterial chemicals (2). The chemical content in red betel leaves phytochemical compounds, namely essential oils, alkaloids, saponins, tannins, and flavonoids (3). Antioxidants in red betel are known to overcome diseases caused by free radicals. Betel leaves contain 4.2% volatile oil, mainly betephenol, an isomer of Eugenol allypyrocatechine, Cineol methyl eugenol, and Caryophyllene (sesquiterpene), chavicol, kavibekol, estragole, and terpinene. In addition, essential oils found in red betel have an antibacterial activity that can help prevent infection in burn wounds. The flavonoid content is also effective as an anti-inflammatory. This is supported by research conducted by Syahrinastiti (2015), which states that red betel leaf extract (Piper crocatum) has a better inhibitory effect on the growth of Escherichia coli than green betel leaf extract (Piper betel L.) (4). Essential oils have properties easily soluble in absolute ethanol, ether, kerosene ether, and chloroform, otherwise microscopic in water. This study was designed to determine the effectiveness of ointment from the essential oil of red betel leaves (piper crocatum linn.) in healing wounds in Wistar rats (Rattus norvegicus).

II. LITERATURE REVIEW

In Indonesia, betel nut is a popular plant for medicinal purposes, including as an antimicrobial, antihypertensive, antiallergic, anti-inflammatory, hepatoprotective and, antioxidant, antidiabetic (5). Tomagola et al. (2016). stated that natural antioxidants generally come from spices, herbaceous plants, fruits, vegetables, and grains. One of the herbaceous plants in Indonesia that are utilized as a natural antioxidant is betel leaf. The herbaceous plant that has recently been widely used is red betel (Piper crocatum Ruiz & Pav.) (6); (7). The red betel plant contains chemical elements that are useful for treatment, but the part of the red betel plant most widely used as medicine is the leaves (8). The chemical content in red betel leaves phytochemical compounds, namely essential oils, alkaloids, saponins, tannins, and flavonoids (9). The natural wound healing mechanism will undergo several phases: inflammation, proliferation, and maturation or remodeling. The inflammatory phase includes hemostasis, a tool to stop bleeding naturally starting when there is a wound, releasing histamine and other mediators to damaged cells such as platelets, thrombin, and fibrin to clot blood. The proliferative phase is when new blood vessels are formed, collagen tissue deposition, fibroblast and epithelial cells infiltration, and granulation tissue formation occur. The remodeling phase starts around 2-3 weeks after the wound and granulation becomes scar tissue. Then, recapitalization occurs, where the connected cell tissue's arrangement and the new epithelium's strength determine the scar (10).

III. RESEARCH METHODS

This type of research is experimental, with a Pre-test and Post-test group only control design approach conducted from March to November 2022. This research was conducted at Herbarium Medanese FMIPA USU, Pharmacognosy Laboratory of Faculty of Pharmacy USU, and Pharmacology Laboratory of Pharmacy USU. The study sample was 25 white male rats randomly selected, which were divided into five groups (4 treatment groups and one control group) so that one treatment group consisted of 5 rats.

Materials and Tools

The materials used are alcohol, aluminum foil, distilled water, red betel, 96% ethanol, rat test animals (mus musculus), sterile gauze, Whatman filter paper, methylparaben, petroleum ether, plaster, propylene glycol, gloves, triethanolamine. The tools used included glassware (pyrex®), an autoclave, a maceration vessel, a blender (Maspion®), a porcelain cup, a caliper (Tricle brand®), oven, tweezers, rotavapor (Heidolf®), iron spoon, analytical balance (Precisa®), and water bath.

Sample Processing

Samples of Red Betel Leaf (piper crocatum linn.) used in this study were obtained from one of the traditional markets in Medan City. Then the pieces of Red Betel Leaf (piper crocatum Linn.) were identified at Herbarium Medanense FMIPA USU. Red Betel Leaves (piper crocatum linn.) that have been placed are washed thoroughly with running water, drained, and then spread on morning paper until the water is absorbed, after which the sample of Red Betel Leaves (piper crocatum Linn.) is weighed. Then the material was dried by aerating. Next, the weight of the dried material was considered. Finally, the dry cloth of Red Betel Leaf (piper crocatum linn.) was pulverized into powder and formed (11).

Preparation of Essential Oil of Red Betel Leaf (piper crocatum Linn.)

Red Betel Leaves (piper crocatum linn.) used in this study were obtained from one of the traditional markets in Medan city. Then the Red Betel Leaf (piper crocatum linn.) was identified at Herbarium Medanense FMIPA USU. Red Betel Leaves (piper crocatum linn.) as much as (200 grams) were distilled by Hydro-distillation process for 4 hours at 80oC; the remaining water residue in the distillation results was removed by adding anhydrous sodium sulfate, which was then filtered to obtain the oil (12).

Calculation of Red Betel Leaf Oil Yield (piper crocatum linn.)

The calculation of the yield of Red Betel Leaf Oil (piper crocatum linn.) (13).

$$\text{Yield (\%)} = \frac{\text{Red Betel Leaf Essential Oil x 100\%}}{\text{Sample Period of Red Betel Leaf}}$$

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Uji Fitokimia (Identifikasi golongan senyawa)

Essential oil of Red Betel Leaf (piper crocatum linn.) identified several compounds such as flavonoids, tannins, alkaloids, phenols, steroids/triterpenoids, terpenoids, and saponins. The phytochemical test used the modified Farnsworth method.

Preparation of Ointment from Essential Oil of Red Betel Leaf (piper crocatum linn.)

Table 1: A topical formulation of each ointment

Material Name	Formula/concentration (%, v/v)			
	I	II	III	IV
Red Betel Leaf Oil	1	3	5	7
Lanolin	2.5g	2.5g	2.5g	2.5g
Solid paraffin	2.5g	2.5g	2.5g	2.5g
Cetostearyl alcochol	2.5g	2.5g	2.5g	2.5g
White Vaseline	42.5g	42.5g	42.5g	42.5g

Wounding

A total of 25 rats that have been prepared, anesthetized using liquid ether, then shaved rat fur enough on the back area. Next, each rat was given an incision on its own shaved back. How to provide an incision for a rat, first put the mat under the rat's body, then wash your hands, use gloves, then disinfect the skin area to be given an incision using a sterile scalpel, make a 2 cm long incision with a depth of 1 mm from the surface of the skin of the white rat's back.

Antioxidant Test

An antioxidant test was conducted to see the antioxidant activity of Red Betel Leaf extract (piper crocatum Linn.). The method used is the reduction of diphenylpicrylhydrazyl (DPPH) concentration. EC50 (extract concentration in reducing 50% DPPH) is the parameter used. First, the DPPH test was carried out by preparing 50 ppm DPPH in ethanol. Then a control solution was prepared by adding 2 ml of 96% ethanol to 1 ml of 50 ppm DPPH. The sample solution was prepared by making a 100 ppm mother solution and then diluted with a concentration variation of 3, 5, 7, and 9 ppm. The resolution of each concentration was taken at 2 ml, and 2 ml of DPPH was added. All solutions were incubated for 30 minutes in a dark room at room temperature. UV-vis measured the capture capacity of the sample against DPPH at a wavelength of 517 nm.

Processing Techniques and Data Analysis

Observations were made regarding changes in the wound and the size of the damage in the treated area. Data analysis using statistical data tests including;

a. Normality Test

b. ANOVA test to determine the effectiveness of ethanol extract of Red Betel Leaf (piper crocatum linn.) and Bioplacenton® on rat back incision wound healing.

IV. RESULTS AND DISCUSSION

Tabel 2: Phytochemical Screening of Red Betel Leaf (piper crocatum linn.).

Test	Result	Description
Alkaloid	Red-brown precipitate	(+)
	White precipitate	(+)
	Brown precipitate	(+)
Flavonoid	The red color in the amyl alcohol layer	(+)
Saponin	Permanent foam	(+)
Tanin	Blackish green color	(+)

Table 2 shows that the Red Betel Leaf extract (piper crocatum linn.) contains alkaloid, flavonoid, saponin, and tannin chemical compounds. In the alkaloid test, a red-brown precipitate was found to form for the Dragendorff reagent, a white deposit resulting from adding the Mayer reagent, and a brown precipitate for the Bouchardt test (9); (14).

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Table 3: Inhibition percentage data of Red Betel Leaf extract (piper crocatum linn.) against DPPH.

Extract Concentration (ppm)	Absorbance Extract	Absorbance Control	Inhibisi (%)
2	0.221	0,536	57.73
4	0.218	0,536	58.48
6	0.213	0,536	58.86
8	0.162	0,536	67.22

Based on table 3. it can be seen that the absorbance of DPPH by the extract of Red Betel Leaf extract (piper crocatum linn.) shows a decrease along with the increase in extract concentration. The inhibition value of the extract also increases as the concentration of the extract increases with the largest inhibition value is 67.22% at a concentration of 9 ppm. The optimum concentration of Red Betel Leaf (piper crocatum linn.) extract was at 6% v/v with the wound remaining 0.2 cm long. For more details, it can be seen in the table 4:

Table 4: Changes in wound length with various concentrations of Red Betel Leaf extract (piper crocatum linn.)

Days	Perubahan Panjang Luka (cm)				
	Concentration 2%	Concentration 4%	Concentration 6%	Concentration 8%	Bioplacenton
1	2	2	2	2	2
3	1.8	1.6	1.6	1.6	1.6
5	1.6	1.4	1.4	1.4	1.3
7	1.4	1.1	1.1	1.2	0.9
9	1.2	0.8	0.8	1	0.6
11	1	0.5	0.5	0.8	0.4
14	0.8	0.3	0.2	0.5	0.1

Based on Table 4, it can be seen that Bioplacenton®, as the positive control, experienced faster wound healing. On day 3, the wound length was already reduced, and on day 14, the incision wound treated with Bioplacenton® had the highest percentage of the recovery. This is because the Bioplacenton® composition has active ingredients of placenta extract and neomycin sulfate, which effectively trigger new tissue formation and prevent infection in the wound area. When viewed from the wound healing rate per day, from day 1 to day seven, the wound healing rate was still linear, but from day 9 to day 14, there was a decrease in the healing rate at 6% concentration compared to the treatment at 4% concentration and far behind when compared to those treated with Bioplacenton®.

When compared, the wound length in the 4% concentration treatment of Red Betel Leaf (piper crocatum linn.) was only 0.1 cm different from Bioplacenton® on day 14. Therefore, it can be concluded that Red Betel Leaf extract (piper crocatum Linn.) can heal wounds, although the healing speed is not as fast as Bioplacenton® when seen from the reduction in wound length from day to day. The compounds such as flavonoids, alkaloids, saponins, and tannins may influence this wound-healing ability in the extract.

Efektifitas Ekstrak ekstrak Daun Sirih Merah (piper crocatum linn.) Terhadap Penyembuhan Luka Sayat

The effectiveness of all concentrations of Red Betel Leaf extract (piper crocatum linn.) on wound healing can be seen with analysis of variance (ANOVA). The ANOVA test is significant to know the difference in administering Red Betel Leaf extract (piper crocatum linn.) on wound healing. The results of the test can be seen in the following table:

Table 5: Test Results of the Effect of Red Betel Leaf extract (piper crocatum linn.) on Wound Healing

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Red Betel Leaf Extract	Between Groups	6.833	3	2.280	7.226	.004
	Within Groups	38.387	136	.280		
	Total	45.156	139			

Table 5. shows the f-count value of 7.998. To find the value in the f-table for df = 3/136 with a probability (α) of 0.05, the fable value is 2.67. So that the value of f-count> f-table means that overall there is a real influence on the administration of

Red Betel Leaf extract (piper crocatum linn.) on wound healing. To emphasize this hypothesis test, it can be seen in the Sig. The count value of 0.004 while the Sig (α) value is 0.05, which means the Sig. Calculate value < Sig (α). This means that the administration of Red Betel Leaf extract (piper crocatum linn.) has a natural effect on wound healing in rats.

Effectiveness of Bioplacenton® on Incision Wound Healing

The Anova test can review the effect or effectiveness of Bioplacenton® on wound healing. The results of the test can be seen in the following table:

Table 6: Test Results of the Effect of Bioplacenton® (positive control) on Wound Length

ANOVA					
	Sum of Squares	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	11.927	6	1.982	34.142	.022
Within Groups	1.604	28	.060		
Total	13.631	34			

Based on the table above, it can be seen that the f-count value is 34,142 while the f-table value is 2.45, which means f-count ≥ f-table. When viewed from the significance value, the calculated significance value is 0.022, which is smaller than the alpha value of 0.05 or p < 0.05. From this data, it can be concluded that there is a real influence in the administration of Bioplacenton® on wound healing. The research results support this study by Ulviani (2016), where variations in extract concentration affect the speed of wound healing. Gel with 3% red betel leaf extract concentration has the greatest healing effect with a healing percentage of 85.81% compared to 1% and 2% extract gels with percentages of 65.32% and 76.58%, respectively (3). The existence of anti-inflammatory and antibacterial activity of red betel leaves is thought to be due to the activity of secondary metabolite compounds contained in red betel, namely essential oils, saponins, tannins and flavonoids. This is evidenced by the phytochemical screening carried out which shows that red betel leaf extract is positive for flavonoids, alkaloids, tannins and polyphenols. The anti-inflammatory activity of red betel leaf extract is thought to be due to the presence of flavonoids, saponins and tannins. The mechanism of flavonoids in inhibiting the inflammatory process is through two ways, namely by inhibiting capillary permeability and inhibiting arachidonic acid metabolism so that prostaglandin production is reduced. Flavonoids also inhibit the secretion of lysosomal enzymes, which are inflammatory mediators. Inhibition of these inflammatory mediators can inhibit the proliferation of the inflammatory process. Meanwhile, the anti-inflammatory mechanism of saponins is to inhibit the increase in vascular permeability.

V. CONCLUSION

Based on the results of research and data analysis on the effectiveness of the administration of Essential Oil Saleb Red Betel Leaf extract (piper crocatum Linn.) and Bioplacenton® on wound healing in white rats, it can be concluded that Essential Oil Saleb Red Betel Leaf extract (piper crocatum linn.) has several bioactive compounds such as alkaloids, flavonoids, saponins, and tannins that play a role in wound healing. The optimum concentration of Essential Oil Saleb of Red Betel Leaf extract (piper crocatum linn.) that can heal cut wounds in white rats is 6%. The highest percentage of healing on day k-14 was in the positive control (Bioplacenton®), which was 95% and followed by 6% v/v extract with a 90% healing percentage. Essential Oil Saleb preparation of Red Betel Leaf extract (piper crocatum linn.) has an ability that is close to Bioplacenton® in the recovery of cut wounds in rats.

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