TEST EFFECT OF CREAM PREPARATIONS OF RED BETEL LEAF EXTRACT (Piper crocatum Linn.) IN ACCELERATING THE HEALING OF CUT WOUNDS IN MALE WISTAR RATS

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ABSTRACT

The wound healing process can be divided into three main phases, namely, the inflammatory phase, the proliferation phase, and the remodeling phase. In red betel leaves, phytochemical compounds are contained: alkaloids, saponins, tannins, and flavonoids. The content of flavonoids as immunostimulants and saponins can be a cleanser and antiseptic that kills or prevents the growth of microorganisms that usually arise in wounds so that wounds do not experience severe infections. The study aims to develop nano herbal red betel leaves as an active ingredient in preventing cut injuries on the skin. This type of research is experimental, with a Pre-test and Post-test group-only control design approach carried out in March 2022; the entire study was carried out at the USU Pharmaceutical Pharmacology Laboratory. Determination of sample size according to Frederer's formula, with a minimum sample number of 5 mice for each group. Red betel extract contains alkaloid chemical compounds, flavonoids, saponins, and tannins. The wound recovery rate per day, from day 1 to day 7, the wound recovery rate is still linear. Still, on the 9th to the 14th day, there is a decrease in the cure rate at a concentration of 7% compared to treatment at a concentration of 5% and far behind when compared to those given Bioplacenton®. The conclusion of the optimum concentration of red betel ethanol extract that can heal cut wounds in white rats is 5%. The highest recovery percentage on k-14 days was in a positive control (Bioplacenton®), which was 95%, followed by extracting 5% v /v with a cure percentage of 90%. Gel preparations of red betel ethanol extract have an ability close to Bioplacenton® in healing cut wounds in rats.

Keywords: *red betel, cream, wounds.*

1. INTRODUCTION

Routine wound healing is complex and dynamic (Dictara et al., 2018); the wound-healing process can be divided into three phases: inflammatory, proliferation, and remodeling (Hesketh et al., 2017). The primary cells involved in the wound-healing process are fibroblasts. Fibroblasts more actively synthesize matrix components in response to wounds by proliferating and increasing fibrinogenesis (Govindaraju et al., 2019). Therefore, fibroblasts are the leading agent in the wound healing process. Indonesia has a variety of natural potentials that can treat alternatives to existing diseases, one of which is red betel leaf. In red betel leaves, phytochemical compounds are contained: alkaloids, saponins, tannins, and flavonoids (Armansyah et al., 2022).

The content of flavonoids as immunostimulant substances, then the production of growth hormones such as EGF, TGFα, PDGF, VEGF, FGF, and TGFβ will also increase so that wound healing can be accelerated (Li et al., 2019). Saponins have the ability as a cleanser and antiseptic that functions to kill or prevent the growth of microorganisms that usually arise in wounds so that wounds do not experience severe infections. To the knowledge of researchers, there is currently very little information related to the biological activity of the red betel leaf nano herbal, especially in increasing the activity of antioxidants and immunostimulants associated with preventing or accelerating the

recovery of cut wounds on the skin. It is hoped that these data can support the development of red betel leaf nano herbal as an active ingredient in preventing cuts on the skin.

2. RESEARCH METHODS

This type of research is experimental, with a Pre-test and Post-test group-only control design approach carried out in November 2022; the entire study was carried out at the USU Pharmaceutical Pharmacology Laboratory. Determination of the sample size according to Frederer's formula, which is with a minimum sample number of 5 mice for each group. The materials used are alcohol, aluminum foil, aqua dest, red betel, ethanol 96%, rat test animals (mus musculus), sterile gauze, Whatman filter paper, methylparaben, petroleum ether, plaster, propylene glycol, gloves, triethanolamine. The tools used include glassware (pyrex®), autoclaves, maceration vessels, blenders (Maspion®), porcelain cups, calipers (Tricle brand®), ovens, tweezers, rotavapors (Heidolf®), iron spoons, analytical scales (Precisa®), and water baths.

Sample Extraction

Red betel Simplicia was weighed as much as 200 grams each, then extracted using a maceration technique with a 96% ethanol solvent. Allowed to stand for five days, the container should be protected from direct rays or light while frequently stirring, squeezing, wash the pulp with enough visceral liquid until 4 L is obtained. Then the simplistic is transferred into a closed vessel, left in a cool place, and protected from light for two days. Then this simplistic is filtered. The results are concentrated using a Rotary Evaporator tool until most of the solvent has evaporated. Then, the evaporation process continues on a water bath until a viscous extract is obtained (red betel ethanol extract / Piper crocatum Linn.). The red betel ethanol extract was then weighed and divided into four parts with successive concentrations of 1%, 3%, 5%, and 7% (v/v), which were then made in the dosage form of a cream.

Table 1. Cream Making Red Betel Leaf Ethanol Extract

No	Material	Formula/concentration (%, v/v)			
		I	II	III	IV
1	Red Betel Leaf Ethanol Extract	1	3	5	7
2	Setil alcohol	3	3	3	3
3	Stearic acid	5	5	5	5
4	Glycerin (ml)	15	15	15	15
5	Paraffin	5	5	5	5
6	Adeps lanae	5	5	5	5
7	Span 60	2	2	2	2
8	Tween 60	2	2	2	2
9	Methyl parabens	0,1	0,1	0,1	0,1
10	Propyl parabens	0,05	0,05	0,05	0,05
11	Vitamin E	0,05	0,05	0,05	0,05
12	Distilled water	57,8	57,6	55,8	55,6

Red Betel Extract Cream Making

The oil phase is made by fusing a mixture of stearic acid, cetyl alcohol, adeps lanae, and liquid paraffin, span 60. Then propyl parabens and vitamin E are added, then the temperature is maintained at 70º C thermostat in the waterbed. Next, the water phase is made by dissolving methyl parabens in part of the volume of water at a temperature of 90º C and adding glycerin. Next, Tween 60 is added and maintained at a temperature of 70ºC thermostat in the waterbody. Finally, the cream is made by mixing the oil and water phases while stirring tightly for 3 minutes. I then added ethanol extract from red betel leaves. Stirred until homogeneous, added part of the volume of water, and stirred until homogeneous. Then it is allowed to stand for 20 seconds, then stir again until a homogeneous cream is formed.

Treatment Methods in Rats

Rats were first adapted for seven days in the laboratory and fed with standard Vital Horse brand feed. Grouping was done by simple randomization. As many as 25 rats were divided into five groups. Group I was given regular meals and 1% red betel ethanol extract cream preparation as much as 1 g, which was applied every 24 hours.

Group II was given a standard meal and 3% red betel ethanol extract cream practice as much as 1 g, used every 24 hours. Group III was given a regular meal and 5% red betel ethanol extract cream practice as much as 1 g, used every 24 hours. Group IV was given an everyday meal and 7% red betel ethanol extract cream and practiced as much as 1 gram every 24 hours. Finally, group V positive control was given standard feed and used Bioplacenton® as much as 1 g every 24 hours. Bioplacenton® contains placenta extract and neomycin sulfate, which are very effective in wound care. Placenta extract is a biogenic stimulator that accelerates cell regeneration and wound healing, while neomycin sulfate is an antibiotic that kills various microbes. Observed changes that occur in the incision wound.

Wound Feeding

A total of 25 rats that have been prepared, and treated using liquid ether, then shaved the rat fur sufficiently in the back area. Next, each rat was given an incision on its back that had been shaved. How to provide an incision for a rat, first attach the lower base of 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 white rat's back skin.

Antioxidant Test

An antioxidant test was conducted to see the antioxidant activity of red betel extract. The method used is the reduction of diphenylpicrylhydrazyl (DPPH) concentration. The parameter used is EC50 (extract concentration in reducing 50% DPPH) (Molyneux, 2004). The DPPH test was performed by preparing 50 ppm DPPH in ethanol. Then a control solution was made by adding 2 ml of 96% ethanol to 1 ml of 50 ppm DPPH. The sample solution was made 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 dilution 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 (Tristantini, et al. 2016).

Data analysis

Data analysis using statistical data tests, among others, with;

a. Normality Test

ANOVA test to determine the effectiveness of red betel ethanol extract and Bioplacenton® on healing rat back cut wounds.

3. RESULTS AND DISCUSSION

Red Betel Extract Phytochemical Screening

Based on the results of the identification of chemical compounds group, it can be seen in the following table:

Table 2. Red betel phytochemical screening.

Uji	Result	Information
Alkaloids	Red-brown precipitate	(+)
	White precipitate	(+)
	Brown precipitate	(+)
Flavonoids	Red color on the amyl alcohol coating	(+)
Saponins	Permanent foam	(+)
Tannins	Blackish-green color	(+)

From the table 2, it can be seen that red betel extract contains alkaloid chemical compounds, flavonoids, saponins and tannins (Tandi et al., 2020). According to Calsum (2018), tannin compounds can act as astringent in wounds while saponins work to increase the speed of epithelialization. Flavonoid compounds also play a role in wound healing by stopping bleeding, namely through vasocontriction mechanisms in blood vessels, free radical antidotes, inhibitors of hydrolysis and oxidation of enzymes, and anti-inflammatory (Calsum et al., 2018).

Antioxidant testing of red betel extract

Antioxidant testing was carried out using the UV-Vis spectrophotometry method at a wavelength of 517 nm with 2-2-Diphenyl-1-picrylhydrazil (DPPH). The results of antioxidant testing of red betel extract can be seen in the following table:

Table 3. Data on the percentage of inhibition of red betel extract against DPPH.

Extract Concentration (ppm)	Absorbance Extract	Absorbance Extract	Inhibition (%)
3	0.221	0,527	57.22
5	0.217	0,527	58.41
7	0.215	0,527	58.67
9	0.163	0,527	68.97

Based on the table above, it can be seen that the absorption of DPPH by red betel extract shows a decrease along with the increase in extract concentration. The inhibition value of the extract also increases with the increase in the concentration of the extract with the greatest inhibition value being 68.97% at a concentration of 9 ppm.

Effectiveness of Extracts Against Cut Wounds

Table 4. Changes in wound length with varying concentrations of the extract

Day To	Changes in Wound Length (cm)				
	Concentration 1%	Concentration 3%	Concentration 5%	Concentration 7%	Bioplacenton
1	2	2	2	2	2
3	1.8	1.7	1.7	1.7	1.7
5	1.6	1.4	1.4	1.4	1.3
7	1.4	1.1	1.1	1.2	0.7
9	1.2	0.8	0.8	1	0.6
11	1	0.5	0.5	0.8	0.3
14	0.8	0.4	0.3	0.6	0.1

Based on the table above, it can be seen that Bioplacenton® as a positive control experiences faster wound healing. The length of the wound on day 3 has already experienced a reduction in the length of the wound and on day 14, the cut wound given Bioplacenton® has had the largest percentage of recovery. This is because the composition of Bioplacenton® has the active ingredients of placenta extract and neomicin sulfate which are efficacious in triggering the formation of new tissues and preventing infection of the wound área (Megawati et al., 2020). When viewed from the wound recovery rate per day, on day 1 to day 7 the wound recovery rate is still linear, but on the 9th to the 14th day there is a decrease in the cure rate at Concentration 7% compared to the treatment at Concentration 5% and far behind when compared to those given Bioplacenton®.

When compared, the wound length in the Concentration treatment of 5% red betel extract was only 0.1 cm different from Bioplacenton® on day 14. It can be concluded that red betel has the ability to heal wounds, although the speed of healing is not as fast as Bioplacenton® when viewed from the reduction in wound length from day to day. The ability to heal these wounds may be influenced by the content of compounds in the extract such as flavonoids, alkaloids, saponins, and tannins.

Results from the normality test in table 5. which uses the Kolmogorov–Smirnov method which shows an Absolute value of 0.080. The value of kolmogorov table for the number of samples of 140 is 0.115, then 0.80<0.115 or the value of kolmogorov counts < of the value of kolmogorov table. This means that the wound recovery data for the extract are normal >ly distributed. For treatment on positive controls (Bioplacenton®), the calculated kolmogorov value was 0.107 with N= 35. Table data with N=35 is 0.224, then 0.107 < 0.224. Asymp data. Sig. (2

tailed) shows a value of 0.746 which means the data for wound recovery using Bioplacenton® is normally distributed, this means that the overall data is normally distributed (Nuryadi, et al. 2017)**.**

Table 5. Kolmogorov-Smirnov Normality Test Results

		Wound Healing (Extract)	Wound Healing (Bioplacenton®)
N		140	35
Normal Parameters[a,b]	Mean	1.0970	1.0285
	Std. Deviation	.56998	.63318
Most Extreme Differences	Absolute	.080	.107
	Positive	.090	.098
	Negative	-.089	-.107
Kolmogorov-Smirnov Z		1.063	.644
Asymp. Sig. (2-tailed)		**.196**	**.746**
a. Test distribution is Normal.			
b. Calculated from data.			

Table 6. Test Results of the Effect of Extract Administration on the Healing of Cut Wounds

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Red Betel Extract	Between Groups	6.843	3	2.281	8.095	.002
	Within Groups	38.316	136	.282		
	Total	45.159	139			

The table above shows the calculated F value of 8,095. To find the value in the F value table for df = 3/136 with a probability (α) of 0.05, an F-table value of 2.67 is obtained. So that the value of F-count > F-table which means that overall there is a real influence on the administration of red betel extract on the healing of cut wounds. To emphasize this hypothesis test, it can be seen in the Sig value. calculate by 0.002 while the value of Sig (α) is 0.05 which means the value of Sig. count < Sig (α). This means that there is a noticeable influence of the administration of red betel extract on the healing of cut wounds in rats.

Table 7. Test Results of the Effect of Bioplacenton® Administration (positive control) on Wound Length

ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	11.927	6	1.987	32.664	.001
Within Groups	1.704	28	.061		
Total	13.631	34			

Based on the table above, it can be seen that the F-count value is 32.664 while the F-table value is 2.45 which means F-count > F-table. If you look at the sigifikansi value, the calculated signinifikan value is 0.001 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 the recovery of cut wounds (Datta et al, 2019). The wound healing process becomes important because the skin is a single organ that is exposed to the outside world. The skin has specific functions for the body, namely protective, sensory, thermogulatoric, metabolic, and sexual signal functions. When the skin loses its continuity, then those functions cannot work as they should (Mescher, 2012). Therefore, the wound healing process requires proper management and treatment so that the wound area does not become infected and eventually cause chronic wounds (Wintoko et al., 2020). Bioplacenton® is one of the gels that can be used for

wound healing. The gel contains 10% placenta extract and 0.5% neomycin sulfate. Placenta extract works to trigger the formation of new tissues and neomycin sulfate prevents infection of the wound area (Hendriati et al., 2018). Placenta extract has long been used in various countries for cosmetic and wound healing purposes (Yi Pan et al., 2017). Wounds can be classified by nature, anatomical structure, healing process, duration of healing, as well as the depth and extent of the wound. By their nature, wounds are divided into abrasion wounds, contusions, incisions, lacerations, penetrations, punctures, sepsis, and others.

Based on the healing process, wounds can be classified into three, namely (namely primary wound healing, secondary wound healing and Delayed Primary Healing. In the primary healing process, the edges of the wound can be reunited, the surface is clean, and no tissue is lost (Novitasari et al., 2017). Secondary healing occurs when the edges of the skin are separated far apart and part of the tissue is lost. The healing process takes place starting from the formation of granulation tissue at the base of the wound and its surroundings. Wound healing is a natural process of improvement against tissue injury by involving inflammatory mediators, blood cells, extracellular matrices, and cell parenchyma (Singh et al., 2017). Wound healing is a complex biological process that results in the restoration of integrity tissues. Physiologically, the wound healing process can be divided into four phases of values from hemostasis, inflammation, proliferation and tissue rermulelling. Many factors are known to slow wound healing, namely poor nutrition, hypoxia, immunosuppression, chronic diseases and postoperative states (Kosol et al., 2020). It is very important for surgical ahii to understand the physiological processes involved in wound healing in order to minimize the patient's morbidity of the delayed wound healing process (Singh et al., 2017).

4. CONCLUSIONS

Based on the results of the study, it can be concluded that red betel ethanol extract has several bioactive compounds such as alkaloids, flavonoids, saponins, and tannins that play a role in wound healing. The optimum concentration of red betel ethanol extract that can heal cut wounds in white rats is 5%. The highest percentage of recovery on k-14 days was in positive control (Bioplacenton®) which was 95% and followed by extract 5% v /v with a cure percentage of 90%. Gel preparations of red betel ethanol extract have an ability close to Bioplacenton® in healing cut wounds in rats.

5. REFERENCES

Armansyah, T., Siregar, T. N., Suhartono, & Sutriana, A. (2022). Phytochemicals, characterization and antimicrobial tests of red betel leaves on three solvent fractions as candidates for endometritis phytotherapy in Aceh cattle, Indonesia. *Biodiversitas*, *23*(4), 2111–2117. https://doi.org/10.13057/biodiv/d230446

Calsum, U., Khumaidi, A., & Khaerati, K. (2018). Aktivitas Ekstrak Etanol Kulit Batang Kayu Jawa (Lannea coromandelica) terhadap Penyembuhan Luka Sayat pada Tikus Putih (Rattus Norvegicus L.). *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, *4*(2), 113–118. https://doi.org/10.22487/j24428744.2018.v4.i2.11078

Dictara, A. A., Angraini, D. I., & Musyabiq, S. (2018). Efektivitas Pemberian Nutrisi Adekuat dalam Penyembuhan Luka Pasca Laparotomi Effectiveness of Adequate Nutrition in Wound Healing Post Laparotomy. *Majority*, *7*(71), 249–256.

Govindaraju, P., Todd, L., Shetye, S., Monslow, J., & Puré, E. (2019). CD44-dependent inflammation, fibrogenesis, and collagenolysis regulates extracellular matrix remodeling and tensile strength during cutaneous wound healing. *Matrix Biology*, *75–76*(2017), 314–330. https://doi.org/10.1016/j.matbio.2018.06.004

Hendriati, L., Hamid, I. S., Widodo, T., Wandasari, C., & Risata, P. M. (2018). Effect of Egg White Gel againts Burn Healing on White Rat (Rattus novergicus). *Jurnal Ilmu Kefarmasian Indonesia*, *16*(2), 231. https://doi.org/10.35814/jifi.v16i2.532

Hesketh, M., Sahin, K. B., West, Z. E., & Murray, R. Z. (2017). Macrophage phenotypes regulate scar formation and chronic wound healing. *International Journal of Molecular Sciences*, *18*(7), 1–10. https://doi.org/10.3390/ijms18071545

Kosol, W., Kumar, S., Marrero-BerrÍos, I., & Berthiaume, F. (2020). Medium conditioned by human mesenchymal stromal cells reverses low serum and hypoxia-induced inhibition of wound closure. *Biochemical and Biophysical Research Communications*, *522*(2), 335–341. https://doi.org/10.1016/j.bbrc.2019.11.071

Megawati, S., Nur'aini, N., & Kurniasih, D. (2020). UJI EFEKTIVITAS GEL EKSTRAK ETANOL 96% DAUN SINGKONG (Manihot esculenta Crantz.) PADA PENYEMBUHAN LUKA SAYAT KELINCI JANTAN GALUR New Zealand White. *Jurnal Farmagazine*, *7*(1), 1. https://doi.org/10.47653/farm.v7i1.159

Novitasari, A. I. M., Indraswary, R., & Pratiwi, R. (2017). Pengaruh Aplikasi Gel Ekstrak Membran Kulit Telur Bebek 10% Terhadap Kepadatan Serabut Kolagen Pada Proses Penyembuhan Luka Gingiva. *ODONTO : Dental Journal*, *4*(1), 13. https://doi.org/10.30659/odj.4.1.13-20

Singh, S., Young, A., & McNaught, C. E. (2017). The physiology of wound healing. *Surgery (United Kingdom)*, *35*(9), 473–477. https://doi.org/10.1016/j.mpsur.2017.06.004

Tandi, J., Lalu, R., Magfirah, Kenta, Y. S., & Nobertson, R. (2020). Uji Potensi Nefropati Diabetes Daun Sirih Merah (Piper croatum Ruiz & Pav) pada Tikus Putih Jantan (Rattus norvegicus). *KOVALEN: Jurnal Riset Kimia*, *6*(3), 239–251. https://doi.org/10.22487/kovalen.2020.v6.i3.15323

Wintoko, R., Dwi, A., & Yadika, N. (2020). Manajemen Terkini Perawatan Luka Update Wound Care Management. *JK Unila*, *4*, 183–189.

Yi Pan, S., K.S. Chan, M., B. F. Wong, M., Klokol, D., & Chernykh, V. (2017). Placental therapy: An insight to their biological and therapeutic properties. *Journal of Medicine and Therapeutics*, *1*(4). https://doi.org/10.15761/jmt.1000118