

Phytochemical Content And Effectiveness Test Of Essential Oil Ointment Piper Crocatum Linn Extract In Accelerating The Healing Of Second-Degree Burns

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ABSTRACT

Burns are the second leading cause of death from non-vehicle-related accidents, with a high risk of occurring in children and the elderly, some of which most commonly occur at home, such as when cooking, heating, or using electrical appliances. This study aimed to analyze the effectiveness of red betel leaf extract (*Piper crocatum*) in treating second-degree burns in male Wistar rats. This type of research is experimental, with a pre-test and post-test control group design approach in February 2023. Research samples of Red Betel Leaf, animal samples of Wistar strain rats, with a total of 20 heads divided into four treatment groups so that each group consisted of 5 rats. The P-value of each test at each observation time <0.05 indicates a significant difference in wound contraction between each group at each observation time unit. There was a substantial difference in the epithelialization period of the standard group, 20% and 40% red betel ointment against the control group. However, there was no difference in the epithelialization period between the red betel ointment and the regular groups. This can be seen from the P-value <0.05 (P-value = 0.008). The conclusion can be drawn is that the wound healing effect of red betel leaf ointment, both 20% and 40%, and nebacetin cream as standard shows a significant difference but does not show a substantial difference in the epithelialization period parameter.

Keywords: red betel, essential oil, burns

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I. Background

Burns are the second leading cause of death from non-vehicle-related accidents, with a high risk of occurring in children and the elderly, some of which occur most frequently in the home, such as when cooking, heating, or using electrical appliances (1). Burns are defined as injury to the skin or other organic tissues caused by the transfer of energy from a heat source to the body, such as hot liquids (burns), hot solids (contact burns), or fire (flame burns) (2); (3). Other causes of radiation, radioactivity, electrical friction, or contact with chemicals are known as burns. Burns can be categorized into thermal, radiation, or chemical burns. Second-degree burns involve the epidermis and part of the dermis (4). These burns are painful, appear red, and have fluid exudation. Clinically, they can be categorized as superficial or deep partial-thickness burns (5). Deep second-degree burns damage most dermis, sweat glands, and sebaceous glands. Re-epithelialization is slow, sometimes up to months. No blistering occurs due to the thick layer of dead tissue. The wound appears white and dry (6); (7). Blood flow is impaired, making the damage susceptible to infection. These burns usually do not heal for three weeks, often resulting in scarring hypertrophy and contractures, especially in children (8).

Wound healing is divided into three stages: inflammation, proliferation, and maturation (9). Several local and systemic factors can cause wound healing to be delayed or impaired. Red betel, or in the scientific language *Piper crocatum*, is one type of betel plant, a vine with the shape of a red heart-like leaf usually living in highland areas (10); (11). Red betel plants like shady, cool places with 60-75% sunlight. Red betel plants have an exotic figure with wavy leaf surfaces accompanied by green, pink, and silver leaf colors on the upper surface of the leaves and purplish red on the lower surface of the leaves. Red betel leaves contain phytochemical compounds, namely alkaloids, saponins, tannins, and flavonoids. Flavonoids have long been recognized as an anti-inflammatory, antioxidant, hepatoprotective anti-allergen, and anticarcinogen that can inhibit bleeding if given to the skin. Flavonoids function as antibacterials by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes. Alkaloids also can be antibacterial (12). The suspected mechanism disrupts the components that make up the peptidoglycan in bacterial cells, so the cell wall layer is not formed intact and causes cell death (13). However, there is not much scientific data explaining betel leaf's role in the duration of burn wound healing. Based on this background, researchers want to know the

effectiveness of red betel leaf extract (*Piper crocatum*) on treating second-degree burns in male Wistar rats.

II. Research Methods

This type of research is experimental, with a pre-test and post-test control group design approach in February 2023. Research samples of Red Betel Leaf, animal samples of Wistar strain rats, with 20 heads divided into treatment groups, so the h group consists of 5 rats. Red betel leaves equate, lanolin, solid paraffin, cetostearyl alcohol, white vaseline, gauze, oil paper, filter paper, 1mm² sized ticked paper, oil paper, nebacetin® ointment. Maceration vessels, knives, rotary evaporators, water baths, gel containers, stirring rods, plates measuring 2 x 2 cm. A Hydro-distillation process distilled fresh red betel leaves (200 grams) for 4 hours at a temperature of 80oC; the remaining water residue in the distillation results was removed by inserting anhydrous sodium sulfate, which was then filtered to obtain the oil.

Amendments (%) = Red betel leaf Essential Oil / Sample Period Red betel leaf x100%

Evaluation of burns is carried out once every 2-4 days, with aspects evaluated from burn healing activities, including Wound contraction and epithelialization period (Handayani, Siswanto, and Pangesti, 2017). Wound contraction is measured by drooping the diameter of the wound using a ruler, and then wound contraction is calculated by the following formula (Vera Dewi Mulia, 2019):

$$\text{Wound Contraction (\%)} = \frac{(\text{Initial wound size} - \text{the size of the wound on a specific day})}{\text{Initial wound size}} \times 100\%$$

Ukuran Luka pada hari spesifik

The epithelialization period is measured by calculating the length of time that the eschar has set to release, wherein this epithelialization period is calculated in days (14). The statistical analysis used in this study was the One-Way Anova different test, followed by a post-hoc test. Before the various tests were carried out, a descriptive analysis of wound contraction and epithelialization period was carried out. If the data in this research is abnormally distributed, it will be transformed into the data so that it is usually spread.

III. Results And Discussion

Different test analyses were conducted to evaluate wound healing in each group of mice according to the study of the normality of the data from each wound healing parameter considered. The results of the data normality analysis can be seen in the table below.

Table 1. Results of Data Normality Analysis on Burn Healing Parameters.

Wound Healing Parameters	P-value
<i>Wound Contraction on To Day -3</i>	0.004
<i>Wound Contraction on To Day -6</i>	0.343
<i>Wound Contraction on To Day -9</i>	0.052
<i>Wound Contraction on To Day -12</i>	0.032
<i>Wound Contraction on To Day -14</i>	0.001
Epithelialization Period	0.002

Table 2. The results of the analysis of One Way ANOVA and Kruskal-Wallis with Wound Contraction as a Wound Healing Parameter in the Treatment Group

<i>Wound Contraction (%)</i>					
Time Observation	Control	Standard	Betel leaf ointment 20%	Betel leaf ointment 40%	P-Value
Day to -3	4.56 (8.56)	0.01 (8.75)	12.78 (25.88)	10.23 (15.56)	0.018**
Day to -6	9.46 ± 5.33	18.23 ± 10.54	34.16 ± 7.88	45.67 ± 7.66	0.013*
Day to -9	7.34± 7.22	35.11 ± 8.05	48.56 ± 6.55	51.67 ± 8.86	0.009*
Day to -12	9.89(29.12)	54.56 (20.71)	64.67 (21.75)	56.67 (8.33)	0.007**
Day to -14	27.67 (37.50)	81.71 (42.46)	76.27 (13.64)	89.29 (3.56)	0.002**

From Table 1, it can be seen that the wound contraction parameter data on day- 6 and day 9 showed a normal distribution of data. Hence, the data analysis used for the differential test was One Way ANOVA followed by the post hoc test Tukey HSD. Meanwhile, other parameters show abnormal data distribution, so the different

tests used are the Kruskal-Wallis and Mann-Whitney tests. From the data in Table 2. it can be seen that the P-value of each test at each observation time is < 0.05 ; this shows that there are significant differences in wound contraction between each group at each unit of observation time. However, in the test analysis, the difference was not clearly explained between which groups there was a significant difference.

The wound healing process is critical to prevent the occurrence of an infection as the basis of the response of the affected tissue, which is in the form of restoring tissue integrity and restoring the structure and function of the tissue, primarily through the synthesis of connective tissue matrix (15). The initial phase is the inflammatory phase. A prolonged inflammatory reaction can hinder the wound-healing process. A protracted inflammatory phase can prolong wound healing time, so some anti-inflammatory agents are needed to inhibit the unfavorable response in the tissue due to prolonged inflammatory mechanisms. Betel leaf contains several phytochemical compounds, including alkaloids, saponins, tannins, and flavonoids. The presence of flavonoids serves to limit the release of inflammatory mediators. Flavonoids also act as antioxidants that slow down the inflammatory process. The high reactivity of the hydroxyl component of flavonoids causes free radicals to become inactive so that activation of inflammatory mediators by free radicals can be inhibited. Thus, the antioxidant ability of flavonoids can optimize the wound healing process through anti-inflammatory mechanisms and inhibition of free radical activity (16). Alkaloids can be antibacterial. The suspected agent disrupts the components that make up the peptidoglycan in bacterial cells, so the cell wall layer is not formed intact and causes cell death.

Tannins have anti-inflammatory, astringent, antidiarrheal, diuretic, and antiseptic activities. As an antibacterial, the estimated mechanism is as follows: Tannin toxicity can damage the bacterial cell membrane. The antibacterial effects of tannins include reaction with the cell membrane, inactivation of enzymes, and destruction or inactivation of genetic material function (11). Astringents can cause closure of skin pores, harden the skin, and stop exudates and mild bleeding. Saponins can act as cleansers and antiseptics that kill or prevent microorganisms' growth. In addition, it spurs the formation of collagen, a structural protein that plays a role in the healing.

Epithelialization Period

The test results differed from each treatment group's epithelialization period, as seen in the table below.

Table 3. Comparative Results of Epithelialization Periods in Each Treatment Group

Treatment Groups	Epithelialization Period *	P-value
Control	22 (2) ^a	0.008
Standard	18 (2) ^b	
Red betel ointment 20%	21 (2) ^b	
Red betel ointment 40%	22 (2) ^b	

* The data is presented in a Median (Range). Lowercase letters in the same column significantly differ in the P-value < 0.05 .

From the data in Table 3, there were significant differences in the epithelialization period of the standard group, 20% and 40% red betel ointment against the control group. However, there was no difference in the period of epithelialization in the group of red betel ointments and the standard group. This can be seen from the P-value < 0.05 (P-value = 0.008). Based on the results of the above study, it can be seen that there are significant differences in the parameters of wound contraction and the epithelialization period of each treatment group. The healing activity of burns possessed by the possibility of anti-inflammatory and antibacterial activities of red betel leaves is thought to be due to the action of secondary metabolite compounds contained in red betel, namely essential oils, saponins, tannins, and flavonoids. This is evidenced through phytochemical screening, which shows that the positive red betel leaf extract contains flavonoid compounds, alkaloids, tannins, and polyphenols. In addition to flavonoids, tannins have anti-inflammatory and antioxidant activity (17).

IV. Conclusions And Suggestions

Based on the results of the study, the conclusion that can be drawn is that the wound healing effect possessed by red betel leaf ointment, both 20% and 40%, and nebacetin cream as standard shows a significant difference but does not show a substantial difference in the epithelialization period parameter.

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