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Efficacy test of red betel leaf ethanol extract ointment on the healing of second-degree burns in wistar rats

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Abstract
Burn injuries can occur through direct contact or exposure to thermal, electric, chemical, or radiation sources. The management of burn victims often involves the application of topical preparations to treat wounds. Red betel leaves (Piper scrotum Ruiz & Pav) are considered a potential remedy for burns due to the antibacterial properties of their essential oil, which accelerates the healing process. This experimental research, utilizing a pre-test and post-test control group design, was conducted in January 2024 to investigate the phytochemical content and effectiveness of red betel leaf essential oil in treating degree II burns in Wistar rats (Rattus norvegicus). With Wistar strain mice as samples, totaling 20, data analysis included One-Way Anova differential tests followed by a post-hoc test. Phytochemical screening of fresh red betel leaves revealed the presence of alkaloids, flavonoids, and tannins. The epithelialization period exhibited no significant differences between the red betel ointment groups (25% and 50%) and the standard group. The wound-healing effect of red betel leaf ointment (25% and 50%) and nebacetin ointment showed significant differences, with the red betel leaf ointment at 50% demonstrating a superior wound contraction rate compared to nebacetin lotion. However, neither red betel leaf ointments nor nebacetin ointments differed significantly regarding the epithelialization period parameter.

Keywords: Red betel, essential oil, burns

Introduction
Information from the Ministry of Health's Basic Health Research in 2013 indicates that burns are the sixth most common cause of unintentional injuries, particularly affecting children aged 1-4 years, with a prevalence rate reaching up to 1.5 percent (Adi, Saputra, and Yanti, 2021) [1]. This study concentrates on burns, defined as injuries resulting from direct contact or exposure to heat (thermal), electricity (electric), chemical substances (chemical), or radiation (radiation) (Rusmini *et al*., 2019) [11], specifically focusing on wounds exposed to hot objects with a degree II depth.

One approach to managing burn victims involves treating wounds with topical preparations (Marlin MR, Yamlean, and Hosea Jaya, 2020) [8]. The red betel plant (Piper scrotum Ruiz & Pav) is a medicinal plant with leaves known for their therapeutic properties in curing various diseases (Fadlilah, 2015; Syahrinastiti, Djamal, and Irawati, 2015) [5, 13]. Betel leaves contain 4.2% essential oil, primarily composed of betephenolan isomer of Eugenol allypyrocatechine Cineol Methil eugenol, Caryophyllene (cysticularpen), kavikol, kavibekol, estragole, and terpinene (Marlin MR, Yamlean, and Hosea Jaya, 2020) [8]. Essential oils act as antibacterials (Rollando and Sitepu, 2018; Effendi, P. Roswiem, and Stefani, 2014) [10, 4], disrupting the process of forming cell membranes or walls. This study aims to explore the phytochemical content and effectiveness of red betel leaf essential oil (Piper scrotum) in treating degree II burns in Wistar rats (Rattus norvegicus).

Literature Review
Red betel is scientifically known as Piper scrotum, which belongs to the Piperaceae family (Windono, 2016) [17]. The chemical content of red betel leaves of phytochemical compounds is essential oil, alkaloids, saponins, tannins, and flavonoids (Sholikhah, 2006). The chromatogram results show that red betel leaves contain phytochemical compounds, namely essential oils, tannins, polevenolad compounds, and flavonoids (Sudewo, 2010). Ulviani's research (2016) results show that active compounds in red betel leaves are suspected to help heal burns tested on experimental rabbit animals (Ulviani, Yusriadi, and Khaerati, 2016) [14].

Research Methods
This type of research is experimental, with a pre-test approach and a post-test control group design. The study was conducted in January 2024. The Red Betel Leaf research sample was obtained from a traditional Medan, North Sumatra market. Examples of Wistar strain rat experimental animals are 20 heads divided into four treatment groups so that each group consisted of 5 rats (Bachmid, 2015) [2].

Materials and Tools
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.

Making Essential Oil of Red Betel Leaf
A Hydro-distillation process distilled fresh red betel leaves (200 grams) for 4 hours at a temperature of 80; the remaining water residue in the distillation results was removed by inserting anhydrous sodium sulfate, which was

then filtered to obtain the oil (Widayani and Cahyono, 2018) [16].

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

Phytochemical Test of Red Betel Leaf

The essential oil of red betel leaves identified several groups of compounds, such as flavonoids, tannins, alkaloids, phenols, steroids/triterpenoids, terpenoids, and saponins. The phytochemical test uses the modified Farnsworth method (Serlahwaty, Sugiastuti, and Ningrum, 2011) [12]: (Fitriyani *et al*., 2011) [6]; (Diniatik, 2011) [3].

Manufacture of Ointment Preparations from Essential Oil of Red Betel Leaves

Table 1: Formulation of Topical Preparations of Each Ointment

Ingredient Name	Ointment base	Red betel leaf ointment 25%	Red betel leaf ointment 50%
Essential oil: Red betel leaves	-	1 ml	1.5 ml
Lanolin	2.5g	2.5g	2.5g
Solid paraffin	2.5g	2.5g	2.5g
Cetostearyl alcochol	2.5g	2.5g	2.5g
White vaseline	42.5g	42.5g	42.5g

Evaluation of Burn Healing Activity

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) [7].

a. Wound contraction

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) [15]:

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

b. Period of Epithelialization

The epithelialization period is measured by calculating the length of time that the eschar has set to release, wherein this epithelialization period is estimated in days (Puspita Dewi, Damriyasa, and Anom Dada, 2013) [9].

Data Analysis

The statistical analysis used in this study was the One-Way Anova different test, followed by a post-hoc test. Before the other 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 distributed.

Results and Discussion

Table 2: Phytochemical Screening Results of Red Betel Leaves

Phytochemicals	Test Methods	Result
Alkaloids	Dragendorff	+
Steroids	Maeyer Salkowsky	+ -
Saponins	Aquadest Aquadest + Alkohol 96%	- -
Flavonoids	FeCl3 5% NaOH 25%	+ -

From the results of phytochemical screening in fresh samples of red betel leaves, it was found that the phytochemical content was in the form of alkaloids, flavonoids, and tannins. The results of this study are not much different from several studies related to the content of red betels, such as Beon and Leki (2017, which reported that the fruit sample had phytochemical content in the form of phenolics, saponins, flavonoids, tannins, triterpenoids, and alkaloids. Differences in the results of this study could be due to differences in the screening methods used (Beon and Leki 2017); (Abdullah Yeni 2015); (Ulviani, Yusriadi, and Khaerati 2016) [14]; (Kusumawardhani, Aliefia Ditha. Kalsum, Umi. Rini 2015).

Wound Healing Activities.

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

Table 3: Results of Data Normality Analysis on Burn Healing Parameters

Wound Healing Parameters	P-value
Wound Contraction on To Day -3	0.012
Wound Contraction on To Day -6	0.445
Wound Contraction on To Day -9	0.089
Wound Contraction on To Day -12	0.032
Wound Contraction on To Day -14	0.012
Epithelialization Period	0.011

The table data above shows that the wound contraction parameter data on days 6 and 9 showed a normal distribution of data. Hence, the data analysis used for the differential test was One-way ANOVA followed by 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.

Table 4: The results of the analysis of OneWay ANOVA and Kruskal-Wallis with Wound Contraction as a Wound Healing Parameter in the Treatment Group

***Wound Contraction* (%)**					
Waktu Observasi	**Kontrol**	**Standar**	**Salep Daun sirih merah**	**Salep Daun sirih merah**	**Nilai**
Hari Ke-2	5.71 (8.22)	0.01 (8.70)	12.55 (25.91)	10.61 (15.91)	0.002**
Hari Ke-6	9.56 ± 5.21	18.22 ± 10.55	20.16 ± 7.88	55.67 ± 7.58	0.001*
Hari Ke-9	7.61 ± 7.92	25.11 ± 8.05	55.55 ± 6.55	51.52 ± 8.92	0.001*
Hari Ke-12	9.55 (29.17)	55.56 (20.71)	65.65 (21.75)	56.67 (8.96)	0.005**
Hari Ke-15	27.22 (27.50)	81.71 (52.56)	76.27 (12.65)	89.29 (2.65)	0.002**

From the data in Table 4. 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. Therefore, the investigation continued with the Post Hoc Test Tukey HSD and Mann-Whitney to compare two groups at each unit of observation time to determine

which groups had significant differences in wound contraction. The results of the analysis can be seen in the following table:

Epithelialization Period

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

Table 5: Comparative Results of Epithelialization Periods in Each Treatment Group

Treatment Groups	Periode Epitelialisasi*	Nilai P
Control	20 (2)a	0.014
Standard Red betel ointment 25%	17 (2)b 21 (2)b	
Red betel ointment 50%	21 (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 5. It can be seen that there were significant differences in the epithelialization period of the standard group, 25% and 50% red betel ointment, compared to 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 = 0.014). 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 also have anti-inflammatory and antioxidant properties.

Ulviani's (2016) research supported this study's results, which tested the effectiveness of red betel leaf gel on burns with rabbit test animals. The results showed that variations in extract concentrations influence the speed of wound healing. The gel with a concentration of red betel leaf extract of 3% has the most significant healing effect, with a healing percentage of 85.81%, compared to the extracted gel of 1% and 2%, with a rate of 65.32% and 76.58%. Based on these results, it is necessary to test the isolation of the group of compounds in red betel that has effectiveness as an anti-inflammatory; it is also required to formulate other topical preparations with the addition of extract concentrations because the more significant the concentration of red betel leaf extract, the greater the healing of burns (Ulviani, Yusriadi, and Khaerati, 2016) [14].

Conclusions and Suggestions

Based on the results of the study, the conclusion that can be taken is that from the effects of phytochemical screening on red betel leaves, it was found that the phytochemical content was in the form of flavonoids, alkaloids, and tannins. The wound-healing effect of red betel leaf ointment of 25% and 50% and nebacetin ointment as standard shows significant differences. The wound contraction rate of red betel leaf ointment is 50% better than that of nebacetin cream as a standard. However, in the red betel leaf ointment, 25% and 50%, and the nebacetin ointment, the bar did not show significant differences in the parameters of the epithelialization period.

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