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EFFECTIVENESS THE OINTMENT OF FOREST BETEL LEAVES EXTRACT *(Piper aduncum* L. *)* AGAINST EXCISION WOUNDS

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D e t a i l A r t i k e l

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K a t a K u n c i

Piper aduncum L.
wound excision
Ointment

P e n u l i s K o r e s p o n d e n s i

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A B S T R A C T

Excision wounds are included in open wounds and occupy the top three types of injuries experienced by the public. Forest betel (Piper aduncum L) is one of the plants that can be used in healing excision wounds because it contains several chemical ingredients such as flavonoids, steroids and saponins which can help speed up wound healing. This study aims to determine the effectiveness of ointment extracts of forest betel leaves (Piper aduncum L.) on the healing of excision wounds in male albino rats. Animals are divided into 5 groups there are control group (base used of vaselinflavum ointment), group of forest betel leaf extract concentration 5%, 10%, 15% and the comparison group (Ointment T®). Basting of preparation was done twice a day for 21 days. The parameters observed were the percentage of wound healing, epithelialization time and histopathology. The results showed on the average percentage of excision wound healing on the 7th, 14th and 21st days were control group (43.046% , 84.118%, 92.866%), concentration 5% (45.49%, 87 608%, 94.704%), concentration of 10% (46.196%, 88.24%, 96.692%), concentration of 15% (46.572%, 91.058%, 97.886%) and the comparison group (46.572%, 90.01%, 97.372%). the control group (8 days), 5% concentration (7 days), 10% concentration (6.8 days), 15% concentration (6.4 days) and the comparison group (6.6 days).For the histopathological test, the results of epithelialization, collagen fiber, and fibroblast scores were. respectively the control group (2,2,1), 5% concentration (2,2,2), 10% concentration (2,2,2), 15% concentration (3,3,3) and the comparison group (3 ,3,3). The most effective group for excision wound healing was the 15% concentration group.

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INTRODUCTION

The skin is the largest organ that covers all parts of the body and is very susceptible to injury. A wound is a condition where the continuity of normal skin tissue, structure and anatomical function is damaged due to pathological processes originating from the internal or external environment and affecting certain organs (Soniya, 2020) . A wound is an injury to a part of the body where the skin and underlying tissue lose tissue continuity (Djuddawi et al., 2019) . One type of wound is an excision wound. An excision wound is a wound caused by tissue being cut by a sharp object (Irwandi et al., 2022) . One of the type wounds is wound excision . Excision wound including open wound occupy three big types of injury suffered public that is of 22.0%, with the prevalence of injuries in West Sumatra increasing that is as much as 5.8% in Riskesdas 2013 and 8.7% in Riskesdas 2018 (Kemenkes RI , 2018).

Wound healing is a physiological process in the body, namely living tissue cells that will regenerate back to their previous structure (Primadani & Nurrahmantika, 2021) . Factors that influence wound healing consist of wound care practices, *personal hygiene* , infection, hypovolemia and the presence of foreign objects (Sinaga et al., 2022) . There are several things you can do to speed up wound healing, namely staying away from smoking, reducing stress, improving nutrition by adding supplements (protein, vitamins and minerals), controlling chronic diseases, stopping alcohol consumption, preventing infections (*wound dressings* and *antibiotics*), and avoiding drugs that can slow wound healing (Asyifa et al., 2023) .

A plant that can be used for wound healing is forest betel (*Piper aduncum* L.). Forest betel is a herb that is easily found as a wild plant in forests or on plantations. This plant does not climb like the betel plant, but its leaves and flowers resemble betel so it is called betel (Evizal, 2013) . The use of forest betel leaves as a traditional medicine is well known to the Indonesian people, which can be used to eliminate body odor, stop bleeding, help heal wounds and digestive tract disorders. Several sources state that this plant has sap in the stem which is useful as a medicine for boils and new wounds (Orjala *et al* . 2004) .

The methanol extract of forest betel leaves, it shows the presence of secondary metabolite compounds such as alkaloids, flavonoids, saponins, polyphenols, tannins, steroids and terpenoids (Nova, 2016) . Saponins, flavonoids and tannins can help the wound healing process because they function as antioxidants and antimicrobials which influence wound healing and also accelerate epithelialization (Senthil *et al.* , 2011) (Saroja *et al.* 2012) . Terpenoid compounds also have anti-inflammatory activity, because they can inhibit COX-2 expression so that prostaglandins formed during the inflammation process can be reduced (Bellik et al., 2013) . The antioxidants contained in tannins are thought to be able to reduce the presence of free radicals which can damage cell membranes and reduce the release of inflammatory cell mediators, which means they can accelerate tissue repair in the wound healing process (Nisa *et al* . 2013) .

Based on the above, researchers are interested in researching on the healing of excision wounds in male white rats using forest betel leaf extract ointment with concentrations of 5%, 10% and 15% on the wound healing process, epithelization time and collagen fiber formation.

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RESEARCH METHODS

This research was carried out for ± 3 months at the Pharmaceutical Research Laboratory and Pharmacology Laboratory, Faculty of Pharmacy, Universitas Perintis Indonesia (UPERTIS) and the Anatomical Pathology Laboratory, Faculty of Medicine, Andalas University.

The tools used in this research were cotton wool, mouse hair clippers, test tubes and racks, dropper pipettes, rulers, rotary evaporators, digital scales, tweezers, erlemeyers, measuring cups, porcelain crucibles, measuring flasks, evaporating cups, spray bottles. , pycnometer stirring rod , watch glass, funnel, refrigerator, meser bottle, spatula, mechanic and stamper, spatula, porcelain crucible, filter paper, glass beaker, glass object, spirit lamp, gegep, and tissue.

The ingredients used are forest betel leaf extract, ethanol 96%, distilled water (H_2O) , chloroform 5.0 (CHCl) , Mg powder and HCL (p) , norite, iron III chloride ($FeCl_3$) , Liebermann's reagent , 2N sulfuric acid (H_2SO_4) , 0.05 N ammonia chloroform, Mayer's reagent, 70% alcohol, 80% alcohol, 90% alcohol, 100% alcohol, 10% formalin, xylol solution ($C_6H_4(CH_3)_2$) , liquid paraffin ($C_{12}H_{26}$) , haematoxylin-eosin (HE) dye , and Tekasol ®

1. Sample Preparation

The samples used were forest betel leaves (*Piper aduncum* L.) taken in the Jorong Padang Japang area, Kenagarian VII Koto Talago, Guguak District, Limapuluh Kota Regency . Next, 1 kg of forest sirh leaves is made into an extract using ethanol solvent , then evaporated using *a rotary evaporator* until a thick extract is obtained.

2. Wound Creation

The day before the wound was made, the hair of the experimental animal was shaved on the part of the back where the incision would be made, then cleaned using cotton wool treated with 70% alcohol, and the mice were anesthetized using sufficient ether. Next, a circular wound was made with a diameter of ± 2 cm and a depth of ± 1 mm by lifting the rat's skin on the back with tweezers and then cutting it with surgical scissors (Cahaya et al., 2017).

a. Grouping Experimental Animals

In this study, mice were divided into 5 groups, each group consisting of 5 mice, where each mouse was given treatment according to its group. The group divisions are:

- Group I (control) is a group of mice that will be given wounds without any treatment and only apply an ointment base.
- Group II (treatment) was a group of mice that had forest betel leaf extract ointment applied at a concentration of 5% to the wounds.
- Group III (treatment) was a group of mice that had forest betel leaf extract ointment applied at a concentration of 10% to the wounds.
- Group IV (treatment) was a group of mice that had forest betel leaf extract ointment applied with a concentration of 15% to the wounds.
- Group V (comparison) was a group of mice that were applied with a circulating preparation, namely Tekasol® ointment.

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Forest sirh extract ointment was applied to the backs of rats that had been injured twice a day for 21 days. Preparations were given to each group according to their grouping (Cahaya et al., 2017).

3. Parameters Measured in Wound Healing

a. Wound Healing Percentage

The percentage of wound healing on days 7, 14 and 21 was calculated with the formula:

$$\%\ Luas\ Penyembuhan\ Luka = \frac{\text{Luas luka awal} - \text{Luas luka akhir}}{\text{Luas luka awal}} \text{x } 100\%$$

b. Epithelialization Time

The time required for complete formation of new epithelium in the wound area. In this case, it is recorded on what day the scab tissue peels from the wound without leaving any wound residue.

c. Histopathology

Observations were made on the excision wound tissue. Three mice were taken from each group to be decapitated on the 21st day. Samples of wound tissue are taken 0.3 cm from the edge of the wound, fixation of the wound tissue is soaked in 10% formalin solution for 1-4 days (wet tissue).

– **Microscopic Examination of Histological Preparations of Wound Tissue**

The preparations that have been covered with a cover glass are then observed under a microscope and a score is made using the criteria for scoring epithelialization, collagen fibers and fibroblasts as follows :

- Collagen (Burkitt criteria)

0: no collagen
1 : spread thinly a little
2 : spreading while there is visible union
3 : compacted and perfectly bound

- Fibroblasts

0 : 0 cells
1 : 5-10 cells
2 : 10-50 cells
3 : > 50 cells

- Epithelialization

0 : absent
1 : starting

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2 : incomplete
3 : complete

4. Data analysis

The value obtained from each parameter was calculated as the mean ± standard deviation (SD). The significance of the difference in average values due to this treatment in the control group was analyzed using *One Way Anova* and *Two Way Anova* with the SPSS 23 program and continued with the Duncan distance test to see the significance of the average difference caused by the difference in treatment.

RESULTS AND DISCUSSION

The percentage of wound healing, namely measuring the initial wound area compared to the wound area on days 7, 14, 21, measuring the area of the healed wound area, is the first parameter used to assess the effect of wound healing, where a high percentage indicates effective wound healing as the size decreases. wound.

The diagram of the average percentage of wound healing area on day 7 shows that the comparison group had a higher percentage of wound healing area than the other groups, followed by the 15% concentration which was higher than the 5% and 10% concentrations. This is because at a concentration of 15% more compounds can help speed up wound healing. So on day 7, the comparison group and a concentration of 15% indicated effective wound healing with a higher percentage of wound healing. It can be seen in figure 1

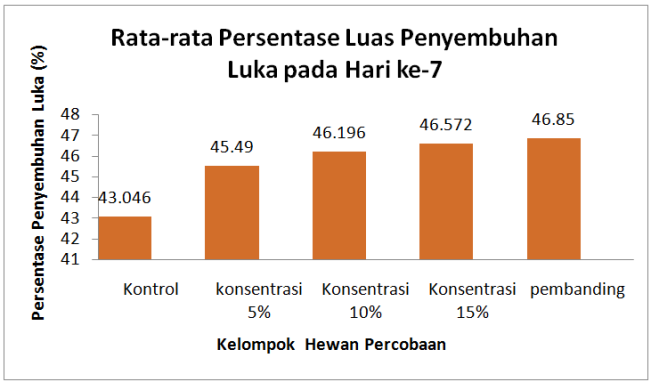

Figure 1. Diagram of the percentage of wound healing on the 7th day

In the diagram of the average percentage of wound healing area on the 14th day, it shows that the group with a concentration of 15% had a higher percentage of wound healing area than the other groups, this is because compounds that can help wound healing are more abundant at a concentration of 15%. Compared with day 7, the average percentage on day 14 rose higher, indicating the size of the wound was decreasing. This happens because collagen

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production occurs rapidly from day 7 to day 14. So on day 14 the 15% concentration group indicates effective wound healing. This can be seen in Figure 2.

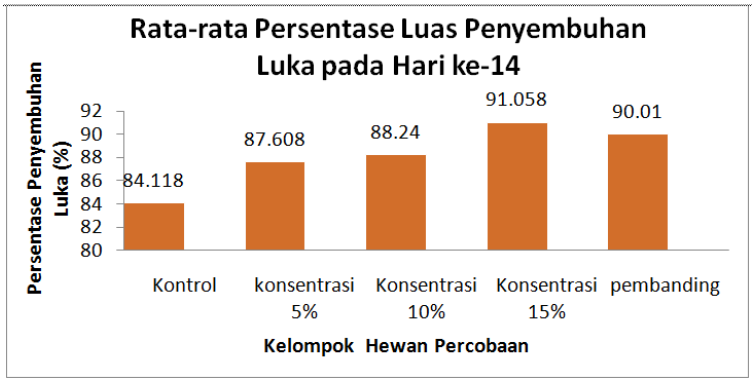

Figure 2. Diagram of the percentage of wound healing on the 14th day

The diagram of the average percentage of wound healing area on day 21 shows that the group with a concentration of 15% had a higher percentage of wound healing area compared to all groups, followed by the comparison group which was applied with T ® ointment . Meanwhile, the control group that was applied with vaseline flavum gave the smallest average percentage of wound healing among all groups. This is because vaseline flavum does not contain active ingredients/substances that can speed up wound healing and is only able to provide a resistance reaction to simply cover the wound, whereas at a concentration of 15% there are more compounds that can help speed up wound healing. So on the 21st day the 15% concentration group indicates effective wound healing with a higher percentage of wound healing which indicates a decreasing size of the wound. It can be seen in figure 3.

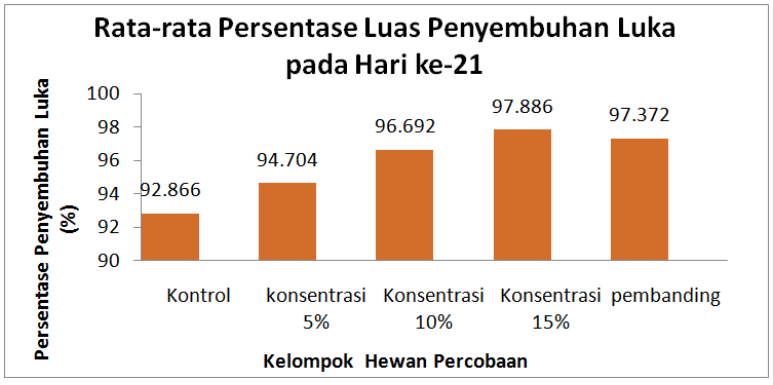

Figure 3. Diagram of the percentage of wound healing on 21st day

Based on the results of statistical analysis using the ANOVA test, a significance value of 0.000 ($p<0.05$) was obtained, meaning that it could be concluded that administration of forest betel leaf extract affected the extensive parameters of wound healing. From the results of Duncan's follow-up test, it was seen that the control had a wound healing effect with

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significantly different significance from the 5%, 10%, 15% and comparison concentration groups. A 5% concentration has the same significance as a 10% concentration. The 15% concentration has the same significance as the comparison.

Epithelialization time is the time recorded for how many days the scab peels off without leaving wound residue, where the faster the epithelialization time indicates the faster the wound will heal.

From the results of epithelialization time measurements, it can be seen that those applied with forest betel leaf extract ointment with a concentration of 15% resulted in the fastest average epithelialization time compared to all groups, followed by the comparison group applied with T® ointment. Meanwhile, the control group that was applied vaseline flavum gave the longest average epithelialization time among all groups, this shows that the administration of vaseline flavum alone did not affect the speed of wound healing. The speed of scab peeling indicates the speed of wound healing, the faster the scab is removed, the faster the healing. The wound is getting faster. It can be seen in Figure 4.

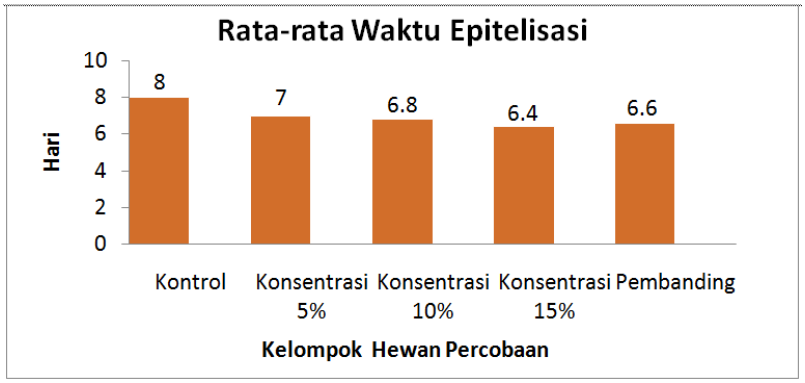

Figure 4. Epithelialization time diagram

Based on the results of statistical analysis using the one-way Anova test, the significance value was 0.004 ($p<0.05$), meaning that it could be concluded that administration of forest betel leaf extract influenced the epithelialization time parameters. From the results of Duncan's follow-up test, it was seen that the control had a wound healing effect with significantly different significance from the 5% concentration, 10% concentration, 15% concentration, and the comparison group. However, concentrations of 5%, 10%, 15% and comparison have the same significance.

The histopathological test carried out is observation of epithelialization, collagen fibers and fibroblasts from skin tissue that has grown back on the 21st day. To observe epithelialization, look at the score parameters, there are 4 scores, namely score 0 (absent) there is no epithelium , score 1 (strating) begins to show epithelium, score 2 (incomplete) thin epithelium, and score 3 (complete) thick epithelium. To observe collagen fibers, there are 4 score parameters, namely score 0, there is no collagen, score 1, the collagen is spread thinly and a little, score 2, the collagen is moderately spread and is starting to appear united, and score 3, the collagen is dense and perfectly bound. To observe fibroblasts there are also 4

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score parameters, namely score 0 there are no fibroblast cells, score 1 there are 5-10 fibroblast cells, score 2 there are 10-50 fibroblast cells, and score 3 there are more than 50 fibroblast cells.

Table 1. Histopathology score results

Group	Histological picture	Score
Control	Incomplete epitheliation	2
	Medium collagen	2
	Fibroblasts	1
Concentration 5%	Incomplete epitheliation	2
	Medium collagen	2
	Fibroblasts	2
Concentration 10%	Incomplete epitheliation	2
	Medium collagen	2
	Fibroblasts	2
Concentration 15%	Complete epitheliation	3
	Dense collagen	3
	Fibroblasts	3
Comparison	Complete epitheliation	3
	Dense collagen	3
	Fibroblasts	3

The histology in the control group, 5% concentration, and 10% concentration showed incomplete epitheliation with a score of 2 which showed crusty areas in the wound and thin epithelium (figures 5, 6, 7). Meanwhile, the 15% group and the comparison group showed complete epitheliation with a score of 3 which showed no crusting in the wound and thick epithelium (figures 8,9). The histological picture of the density of collagen fibers shows that in the control group, the 5% concentration, and the 10% concentration showed the collagen fibers were moderately spread and appeared to be united with a score of 2 (figures 5, 6, 7). Meanwhile, at a concentration of 15% and the comparison group showed collagen fibers that were condensed and perfectly bound with a score of 3 (figure 8.9). The histology in the control group showed few fibroblast cells with a score of 1 which only had 5-10 cells (figure 5). The 5% and 10% concentration groups showed a moderate fibroblast cell population with a score of 2 which only had 10-50 cells (figures 6, 7). Meanwhile, at a concentration of 15% and the comparison group showed a high population of fibroblast cells with a score of 3 where there were more than 50 cells (figures 8.9).

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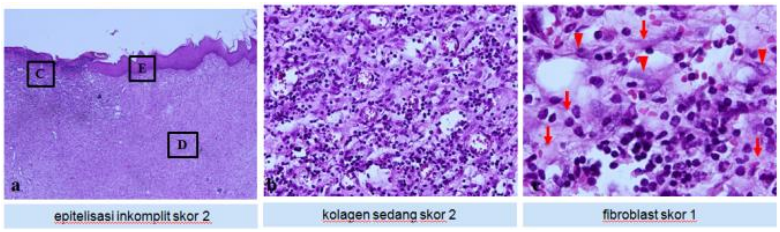

Figure 5. Control group

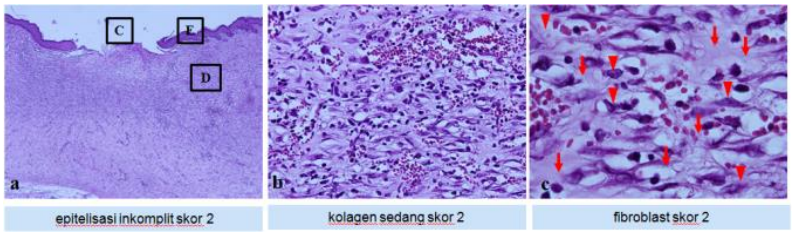

Figure 6. 5% concentration group

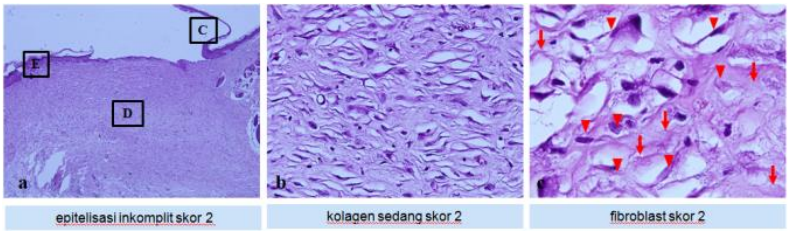

Figure 7. 10% concentration group

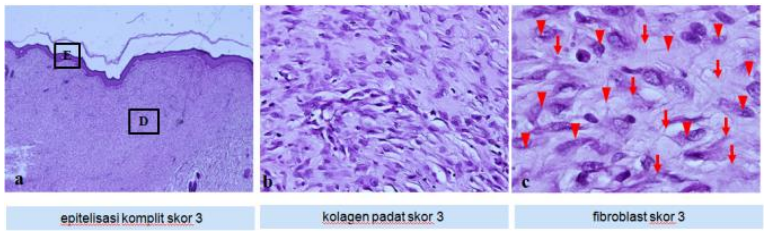

Figure 8. 15% concentration group

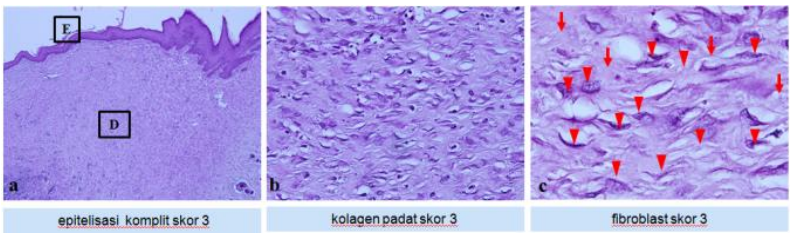

Figure 9. Comparison group

Based on these three test parameters, it can be seen that the group with a concentration of 15% showed better wound healing than the other groups, followed by the comparison group. This is because the 15% concentration group contains more active compounds which can accelerate wound healing. The controls who were only given vaseline flavum were far

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below the percentage compared to the other treatment groups. This was because vaseline flavum did not provide a significant healing effect. After all, it did not contain active ingredients/substances which were only able to provide a resistance reaction to simply cover the wound.

CONCLUSION

From the results of research testing the effectiveness of forest betel leaf extract ointment on healing excision wounds in male white rats, the following conclusions can be drawn:

1. Administration of forest betel leaf extract ointment (*Piper aduncum* L.) is effective in healing excision wounds in male white rats.
2. Variations in concentration affect the effective level of healing of excision wounds in male white rats. The treatment group with a concentration of 15% had a better wound healing effect than all groups as seen from 3 parameters, namely wound healing percentage, epithelialization time, and histopathology.

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