

THE EFFECT OF 15% ETHANOL EXTRACT OINTMENT OF MENIRAN LEAVES (*PHYLLANTUS NIRURI L.*) ON HYDROXYPROLINE LEVELS HEALING OF BURN WOUNDS IN WHITE MALE RATS

Sanubari Rela Tobat^{*1}, Diana Agustin¹, Aulia Sari¹

¹Faculty of Pharmacy, Perintis University Indonesia, Padang, West Sumatra, 25586, Indonesia.

Article Received: 7 January 2026 | Article Revised: 28 January 2026 | Article Accepted: 17 February 2026

*Corresponding Author: Sanubari Rela Tobat

Doctoral Program, Department of Biomedical Sciences, Faculty of Medicine, Andalas University, Padang, West Sumatra, 25163, Indonesia.

DOI: <https://doi.org/10.5281/zenodo.18798544>

How to cite this Article: Sanubari Rela Tobat, Diana Agustin, Aulia Sari (2026) THE EFFECT OF 15% ETHANOL EXTRACT OINTMENT OF MENIRAN LEAVES (*PHYLLANTUS NIRURI L.*) ON HYDROXYPROLINE LEVELS HEALING OF BURN WOUNDS IN WHITE MALE RATS. World Journal of Pharmaceutical Science and Research, 5(3), 01-15. <https://doi.org/10.5281/zenodo.18798544>



Copyright © 2026 Sanubari Rela Tobat | World Journal of Pharmaceutical Science and Research.

This work is licensed under creative Commons Attribution-NonCommercial 4.0 International license (CC BY-NC 4.0).

ABSTRACT

Meniran (*Phyllanthus niruri L.*) is a plant that contains phytochemical constituents such as alkaloids, phenolics, flavonoids, terpenoids, steroids, and saponins which can heal burn wounds. This study aims to determine the effectiveness of 15% *Phyllanthus niruri L.* leaf ethanol extract ointment on wound healing. This study was conducted using an experimental method using white male rats with 3 parameters, namely the percentage of wound healing area, epithelialization time, and hydroxyproline levels. Group I was applied with ointment base, group II was applied with T[®], and group III was applied with 15% *Phyllanthus niruri L.* leaf ethanol extract ointment. The results showed that the results of the percentage of wound healing area on day 3 of groups I, II, and III were on average 1.17%, 6%, and 3.61%. The results of the percentage of wound healing area on day 7 of groups I, II, and III were on average 13.83%, 34.19%, and 29.54%. The results of the percentage of wound healing area on day 14 of groups I, II, and III were on average 39.41%, 83.93%, and 71.36%. The average epithelialization time of groups I, II, and III was on day 10, day 7, and day 7. The results of the percentage of hydroxyproline levels on day 3 of groups I, II, and III were on average 10.48%, 19.92%, and 17.59%. The results of the percentage of hydroxyproline levels on the 7th day of groups I, II, and III were on average 11.42%, 28.02%, and 20.77%. The results of the percentage of hydroxyproline levels on the 14th day of groups I, II, and III were on average 12.88%, 42.62%, and 20.95%. The results of statistical analysis using one way and two way ANOVA followed by Duncan test (SPSS 25.0) showed a significant difference in groups I, II and III on the percentage of wound healing area and hydroxyproline levels. Epithelialization time ($p > 0.05$), it can be concluded that groups I, II and III were not significantly different. The 15% of ethanol extract of Meniran leaf ointment is quite effective in the percentage of wound healing area, epithelialization time, and hydroxyproline levels in male white rats.

KEYWORDS: Phyllanthus niruri L., ethanol extract, burn wounds, wound healing, hydroxyproline levels.

INTRODUCTION

A wound is a condition in which body tissue is damaged or lost due to a factor that disrupts the body's defense system. These factors include trauma, temperature changes, chemicals, explosions, and electric shocks, animal's bite.^[1] There are several types of wounds, one of which is burn wounds. Wound burns are a form of tissue damage caused by several source such as very high temperature of water, fire, chemical substance, electricity, and radiation. When there is contact with a thermal source or other cause, resulting numerous of chemical reaction from the body that drain energy from the tissue so that cells are reduced and damage.^[2] The most common cause of wound burns is direct fire which can be triggered or worsened by the presence of flammable liquids such as gasoline, stove gas, and fluid from lighter and sun burns.^[3]

The primary goal of burn treatment is to restore normal skin tissue function and form with minimal local complications. When a burn occurs, the tissue undergoes a healing process, which is a complex phenomenon involving several processes. Wound healing is a complex process consisting of three phases: the inflammatory phase, the proliferation phase, and the maturation phase. The healing process of burns can be accelerated by doing treatment of burns.^[4] One of alternative treatment of burn wounds is the application of medicinal plant such as *Phyllanthus niruri* L that has long been used for generations as an Indonesian medicinal plant to treat burns.^[5] Meniran is a plant that contains several phytochemical constituents such as high levels of alkaloids and phenols, flavonoids, terpenoids, steroids, *cardiac glycosides*, saponins, tannins, glycosides and cyanogenic.^[6] The benefits of meniran include increasing urine flow (diuretic), increasing body resistance, reducing fever, treating stomach ulcers, destroying kidney stones, destroying gallstones, treating malaria, relieving menstrual pain, reducing weight, eliminating acne, curing toothache, treating coughs, healing burns, and treating epilepsy.^[7]

Phyllanthus niruri L. leaves can be used as a medicine to improve the immune system which very beneficial in wound treatment. They have a bitter taste, an aromatic smell, and a cooling effect. All parts of the plant can be used medicinally. *Phyllanthus niruri* L leaves contain numerous substances, some of which have the ability to kill bacteria.^[8] In cases of open wounds, infection often occurs due to the entry of germs or bacteria into the wound, which will slow the skin's healing process. The condition will worsen if antiseptic treatment is not administered promptly.^[9]

According to study that has been done by Alfinda (2024),^[10] the administrations of subfraction ethyl 10% and 15% acetate can heal excision wounds, as seen from the good wound healing percentage parameters found in the ethyl acetate subfraction group. 15% and not far different from group ointment subfraction ethyl acetate 10% and control group. In addition, Shanbhag *et al* (2010),^[11] proved that administering meniran leaf extract, either orally or topically, can speed up the healing process of burn wounds which marked by faster epithelialization period.

Based on description in on, so researchers have conducted research on the effectiveness of 15% ethanol extract of *Phyllanthus niruri* L. leaves on hydroxyproline levels. on the day the 3, 7, And 14 in burn wounds. The parameters observed in male white mice were the percentage of burn wounds area, time epithelialization and hydroxyproline levels in male white rats.

Research Objectives: First to determine the effect of administering ethanol extract ointment of meniran leaves (*Phyllanthus niruri* L) with a concentration of 15% on the percentage of burn wounds healing, epithelialization time, and expression of hydroxyproline levels in male white mice. Second, to determine the effect of the duration of administration of meniran leaf ethyl ethanol extract ointment (*Phyllanthus niruri* L) with a concentration of 15% on days 3, 7, 14 on the percentage of wound healing, epithelialization time, and hydroxyproline levels in male white mice.

RESEARCH METHODOLOGY

The research was carried out from June to December 2024 at the Pharmacology Research Laboratory, Faculty of Pharmacy, Perintis Indonesia University and the Anatomical Pathology Laboratory, Faculty of Medicine, Andalas University.

Tools

Mouse cage, cotton wool, hair clipper, 2 cm metal cylinder, scissors, droppers, rulers, razor blade (tiger®), digital weighing scale, animal scale, gloves, mask, oven, stir bar, knives, tweezers, measuring cup, volume pipette, erlenmeyer, filter paper, *beaker glass*, tongs, spatula and universal pH stick, rotary microtome, heat induced epitope, microwave, deck glass, pot ointment, syringe, plate drops, glass object microscope and UV- Vis spectrophotometer.

Material

The materials used are 250 grams of meniran leaves dry, ethanol 70%, ethanol 96%, carbo adsorbent, chloroform ammonia 10%, H₂SO₄ concentrated, H₂SO₄ 2N, Mayer's reagent, Mg powder, concentrated HCl, T[®] ointment, vaseline flavum, alcohol swab, CuSO₄, NaOH, anhydrous acetic acid, H₂O₂, HCl 6N, H₂SO₄ 3M, 4-dimethylaminobenzaldehyde, n-hexane, ethyl acetate and lidocaine cream, rat food and drink, hair removal cream, distilled water, aquabidest, ether, FeCl₃, hydroxyproline, NH₄Cl, NH₄OH, C₄H₆O₃, NH₃, HgCl₂, KI.

Sampling

The samples used were fresh meniran leaves (*Phyllanthus niruri* L.) were taken from Lubuk Buaya, Koto Tangah, Padang, West Sumatra, Indonesia.

Sample Identification

Sample identification was carried out at the Andalas University Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang.

Preparation of Ethyl Acetate Subfraction of Meniran Leaves

Meniran leaf taken and collected later cleaned from impurities with water flow, drained in on winnowing basket which covered with a paper. Next, the wet weight was measured at 3 kg, then dried by airing it in a room away from direct sunlight for 11 days. The dried samples were separated into leaves and stems of *Phyllanthus niruri* L and grind with blender until become coarse powder and obtained heavy sample as 250 grams, then the sample is macerated in the following way, as much as 250gram sample is put into a 4 liter dark colored bottle and soaked using 2.5 liters of 70% ethanol solvent (1:10) for 3x24 hours while stirring occasionally. After 3x24 hours of soaking, filter with filter paper to get the macerate and repeat 3 times until the macerate is clear, then the macerate evaporated with *rotary evaporator* on temperature 60⁰ C until ethanol extract is obtained.

Evaluation of Ethanol leaf extract.^[12]**1. Organoleptic Examination**

The inspection was carried out visually, namely, by observing the shape, colour, smell and taste.

2. Determination of pH Ethanol Extract

This test was performed using a pH meter. One gram of *Phyllanthus niruri* L leaf ethanol extract was weighed and diluted with 10 mL of distilled water. The electrode was then rinsed with distilled water and dried with a tissue. The electrode was then dipped in the solution until a constant reading was obtained. Three measurements were taken, and the average value was taken.

3. Determination of

The ethanol extract yield is calculated using the equation:

$$\% \text{ Yield} = \frac{\text{Weight of ethanol extract (g)}}{\text{Weight of dry simple substance (g)}} \times 100 \%$$

4. Drying Shrinkage Check

Dry porcelain crucible and lid in the oven at 105⁰ C for 30 minutes, then let it cool and then weigh it. Put the ethanol extract into the crucible until the weight is 1-2 grams beyond the weight of the previously known crucible with lid. Shake the crucible gently so that the extract is evenly distributed and put it back in the oven, open the lid, and leave the lid in the oven. The crucible containing the ethanol extract was heated in an oven at 105⁰ C until constant weight. After that, the crucible is removed and cooled in a desiccator, then weighed. Repeat as above until a constant weight is obtained.

Calculate the drying loss using the formula:

$$\% \text{ Drying shrinkage} = \frac{(B-A)-(C-A)}{(B-A)} \times 100 \%$$

Explanation:

A = Weight of empty crucible

B = Crucible weight + ethanol extract before drying

C = Crucible weight + ethanol extract drying

5. Ash Content

Weigh 2-3 grams of *Phyllanthus niruri* leaf ethanol extract, place it in a crucible that has been heated and tared, then leveled. Gently heat it until the charcoal is gone, cool it in a desiccator, and weigh it. Then, the crucible is placed in a furnace for 6 hours at 600°C, until ash forms, cool it in a desiccator, and weigh the resulting ash. Ash content is calculated using the formula:

$$\% \text{ Ash content} = \frac{(C-A)}{(B-A)} \times 100 \%$$

Description:

A = Weight of empty porcelain crucible, expressed in g

B = Weight of crucible + sample before heating, expressed in g

C = Weight of crucible + sample after heating,

Preliminary Examination of Chemical Content.^[12]

The ethanol extract (0,5g) from meniran leaves (*Phyllanthus niruri* L.) was put into a test tube, added with 5 ml of distilled water and 5 ml of chloroform acetate, left until two layers were formed

a. Flavonoid Test (Cyanidine Test Method)

Take a layer of 1-2 drops of water, drop it on the drip plate, and then add Mg powder and HCl (p), the formation of a red color indicates the presence of flavanoids.

b. Saponin Test

Take a layer of water, then shake vigorously in a test tube, then permanent foam forms (\pm 15 minutes) indicating the presence of saponins.

c. Terpenoid and Steroid Test ("Simes" method)

Take a small layer of chloroform using a dropper containing cotton wool and norit. drop the filtrate on the drip plate. Let it dry. The residue is added to 1 drop of anhydrous acid and 2 drops of H₂SO₄ (p), the formation of a purple blue color indicates the presence of steroids, if a red color is formed, it indicates the presence of terpenoids.

d. Alkaloid Test ("Culvenore - Fristgerald" Method)

Take a small layer of chloroform and add 10 ml of 0.05 N chloroform ammonia, stir slowly, then add 2-3 drops of H₂SO₄ 2N then shake gently, and let stand until separated. Acid layers adding 2 drops of Mayer's reagent, a positive alkaloid reaction is indicated with white mist to white lumps.

e. Phenolic Test

Take a layer of 1-2 drops, drop it on the drop plate, and then add the FeCl₃ 1-2 drops. The formation of a blue color indicates the presence of phenolic content.

Preparation of ethanol extract ointment

The ointment preparation that will be made in this research has a concentration of 15% of ethanol extract of Meniran leaf and the preparation that will be made is 30 g. Add 4,5 grams of meniran leaf ethanol extract of (*Phyllanthus niruri* L) into a mortar, then weigh 25,5 g of the ointment base (vaselin flavum), put it in a mortar and then grind until homogeneous. Remove from the mortar; put it into the prepared container.

Evaluation of Meniran Leaf Ethanol Extract Ointment^[13]**1. Organoleptic Examination**

The inspection is carried out visually, namely, by observing the shape, colour and smell.

2. Homogeneity Check

The examination was carried out by smearing 0.1 gram of the preparation mass on a glass object, then leveling it with another glass object at a slope of 45⁰, pulling quickly with the same pressure. The arrangement was observed under a microscope and no coarse grains were visible.

3. Ointment pH Check

This test was performed using a pH meter. One gram of *Phyllanthus niruri* L. leaf ethanol extract ointment was weighed and diluted with 10 mL of distilled water. The electrode was then rinsed with distilled water and dried with a tissue. The electrode was then dipped in the solution until a constant reading was obtained. Three measurements were taken and the average value was taken.^[14] A good pH value for the ointment is 4.5-6.5, which corresponds to the pH of human skin.^[13]

Experimental Animal Preparation

Testing the effect of administering the ethanol extract of meniran leaves on wound healing will be carried out using experimental animals of male white rats weighing ± 200 grams. A total of 27 mice were divided into 3 main groups, and then each group was examined on days 3, 7, and 14 after the mice were given excision wounds. On each day of examination, the animals were sacrificed using isoflurane and then the skin tissue of the experimental animals was taken for examination of hydroxyproline levels. Before use, mice were first acclimatized for 7 days. Animals are declared healthy if during acclimatization they do not show deviations in body weight of more than 10% and visually there are no symptoms of disease.

The day before the wound was made, the fur of the experimental animal was shaved on the back where the incision would be made, and then cleaned using cotton wool treated with 70% alcohol, and then anesthetized the mouse using isoflurane. 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. In the research, each group of mice was given the following treatment:

- Group I (control) is a group of mice that will be given burn wounds without being given treatment and only smear vaseline flavum ointment based on the wound and check the percentage of wound healing area, epithelialization time, and hydroxyproline levels in white male mouse on the 3th, 7th, and 14th days.
- Group II (comparison) is a group of mice that will be given burn wounds and being given treatment of comparison preparation on the market (Tekasol[®]) twice daily.
- Group III (treatment) was a group of mice that applied ethanol extract ointment with a concentration of 15% to the wound and examined the percentage of wound healing area, epithelialization time, and expression of hydroxyproline levels in white male mouse on days 3, 7, and 14.

Making Wound Burn

The mice were prepared and acclimatized for 7 days. Then, 3 cm area of fur was shaved on the back using a hair removal cream. Afterward, the fur was cleaned with an alcohol swab, part back which shaved. Prior to treatment, the mice were anesthetized with ether, and then lidocaine cream was applied to their backs. Wounds were made using a 2-cm-diameter metal rod soaked in hot water for 5 minutes. The heated metal rod was placed on the mice's backs for 30 seconds, resulting in a burn wound.

Wound Healing Activity Assay

- The ointment was applied to the back of the rat 2 times/day.
- Rats were also given an anesthetic/analgesic cream, namely, 5% lidocaine prilocaine cream to treat pain 2 times/day.
- The ointment preparation was given to groups of mice that had been grouped.

- Next, the wound healing parameters were observed.

Wound Healing Parameters

The percentage of wound healing area by calculating the wound area on the first day after being injured, and then the wound healing area on days 3, 7, and 14 in each group. Then look for the percentage of wound healing which is calculated using the formula:

$$\% \text{ wound healing} = \frac{(\text{Initial wound area}) - (\text{final wound area})}{(\text{initial wound are})} \times 100 \%$$

The time required for the formation of new epithelium to completely cover the wound area. In this case, the day the scar tissue peels from the wound is recorded without leaving a scar residue in the excision area.

Epithelialization Time

The day after the scab peels off the wound without leaving a scar is recorded.

Determination of Hydroxyproline Levels

Hydroxyproline Levels Reagent Preparation:

a. Making reagent HCl 6 N

Pipette 50 mL of 6 N HCl solution into a 100 mL volumetric flask, add distilled water up to the mark and shake until homogeneous.

b. Making reagent 0.01 M CuSO₄

Powder reagent of CuSO₄ 0.01 N weighed as much as 0.25 grams was then placed into a 100 mL volumetric flask, add distilled water up to the mark and shake until homogeneous.

c. Making 2.5 N NaOH reagent

Weighed as much as 10 grams of NaOH, then entered to volumetric flask 100 mL, added distilled water until sign limit and shake until homogeneous.

d. Making Reagent H₂O₂ 6%

Pipette as much as 20 mL H₂O₂ solution of the solution into a 100 mL volumetric flask, add distilled water up to the mark and shake until homogeneous.

e. Making Reagent H₂SO₄ 3N

Pipette 16.6 mL H₂SO₄ solution into a 200 mL volumetric flask, add distilled water up to the mark and shake until homogeneous.

f. Preparation of reagent 4-dimethylaminobenzaldehyde 5%

Weighed 5 grams of 4-dimethyl amino benzaldehyde (PDAB) powder into a 100 mL volumetric flask, add 96% ethanol up to the mark and shake until homogeneous.

g. Preparation of Buffer Reagents pH 7

Solution of NH₄Cl 0.2 M: weighed as much as 1.07 grams NH₄Cl, entered into a 100 mL volumetric flask, add distilled water up to the mark and shake until homogeneous.

Solution of NH_4OH 0.2 M: pipette 15 drops of NH_4OH solution then put into beaker glass, added distilled water to 25 mL.

The two solutions are mixed in beaker glass by adding 90 mL of 0.2 M NH_4Cl solution. The pH of the solution is measured using a pH meter, and 0.2 M NH_4OH solution is added little by little while stirred until obtained pH 7.

Hydroxyproline Standard Solution 500 ppm

The solution was made by weighing 50 mg of hydroxyproline powder, then put into a 100 mL volumetric flask then dissolved with distilled water until sign limit and obtained a standard solution of 500 ppm hydroxyproline.

Determination Long Wave Maximum Hydroxyproline Uptake

As much as 0.6 mL of 500 ppm stock solution was pipetted into a 10 mL measuring flask and added with aquabidest up to 1 mL, then 1 mL of 0.01 M CuSO_4 was added, and then 1 mL NaOH 2.5 N and 1 mL H_2O_2 6% were also added. The solution was then stirred and incubated at 80°C for 5 minutes. After the incubation process was complete, the solution was cooled. And added 4 mL H_2SO_4 3 N and 2 mL of 5% 4-dimethylaminobenzaldehyde. The solution was re-incubated at 70 °C for 16 minutes, cooled to 20° C and the absorbance was measured using a UV -Vis spectrophotometer at a wavelength of 400-800 and the maximum wavelength was determined.

Determination of Hydroxyproline Levels

From the 500ppm stock solution, five different concentration variations were prepared in five 10 mL volumetric flasks, as follows:

- Pipette 0.2 mL to obtain 10ppm hydroxyproline
- Pipette 0.4 mL to obtain 20ppm hydroxyproline
- Pipette 0.6 mL to obtain 30ppm hydroxyproline
- Pipette 0.8 mL to obtain 40ppm hydroxyproline
- Pipette 1 mL to obtain 50ppm hydroxyproline

Pipette 500 ppm stock solution of 0.2 mL; 0.4 mL; 0.6 mL; 0.8 mL; and 1 mL were put into a 10 mL measuring flask and added with aquabidest up to 1 mL, then added 1 mL of 0.01 M CuSO_4 , 1 mL of 2.5 N NaOH, and 1 mL of 6% H_2O_2 . The solution was then stirred and incubated at 80oC for 5 minutes. After the incubation process was complete, the solution was cooled and 4 mL of 3N H_2SO_4 and 2 mL of 5% 4-dimethylaminobenzaldehyde were added. The solution was incubated again at 70oC for 16 minutes, cooled at 20oC. Then the absorbance was measured at the maximum wavelength and then a calibration curve was made to obtain the regression equation $y = a + bx$. This equation is used to determine the levels of hydroxyproline in skin tissue.

Determination of Hydroxyproline Levels in Skin Tissue

Biopsy was performed on the skin of the scarred rats on days 3, 7, and 14. The skin tissue was then dried at 60oC for 12 hours and hydrolyzed with 6 N HCl for 24 hours at 110oC. Next, it was neutralized by adding 2 mL of NaOH, 1 mL of pH 7 buffer, and 1 mL of distilled water with a total neutralization volume of 4000 μl . Then, 30 μl was taken and added with distilled water to 1000 μl , added with 1 mL of CuSO_4 , 0.01 M, 1 mL of 2.5 N NaOH, and 1 mL of 6% H_2O_2 . Then, it was stirred and incubated at 80oC for 5 minutes. After the incubation process was complete, the solution was

cooled and 4 mL of 3N H₂SO₄ and 2 mL of 5% 4-dimethylaminobenzaldehyde were added. Then, the sample was incubated again at 70°C for 16 minutes, cooled to 20°C, and its absorbance was measured at maximum wavelength using a UV-Vis spectrophotometer. The amount of hydroxyproline in the sample was calculated against a standard curve of hydroxyproline.

3.5.4 Data Analysis

The data analysis used in this research is analysis of variance (ANOVA). This ANOVA is used because the data obtained is objective, categorical, and numerical. In this study, data from wound healing parameters, namely, the results of the percentage of wound healing area, epithelialization time, and hydroxyproline levels were used for statistical analysis using one-way ANOVA. If the results obtained are significant ($p < 0.05$). Data analysis was continued with the Duncan test which aims to determine the significance of the differences in results between each treatment group.

RESULTS AND DISCUSSION

After conducting research on the effect of administering ethanol extract ointment of meniran leaves (*Phyllanthus niruri* L.) percentage of wound healing area, epithelialization time, and hydroxyproline levels in white male mice, the following results were obtained:

1. Based on the results of sample identification, it shows that the sample used in this research is a meniran leaf plant (*Phyllanthus niruri* L.) from the family *Phyllanthaceae* with sample identification code number: 560/K-ID/ANDA/VII/2024
2. Based on the information that passed the ethical review from KEPK UPERTIS with number 923/Kepk.F2/ETIK/2024 we have approved the protocol for this study.
3. The results of organoleptic observations of the meniran leaf ethyl acetate subfraction ointment showed that it was a semisolid preparation, dark green in color and had a distinctive smell.
4. The results of examining the pH of the meniran leaf ethyl acetate subfraction ointment showed a pH of 4.42 in the ointment preparation with a concentration of 15%.
5. From 250 grams of dry simplex, 56.068 grams of thick meniran leaf extract was obtained with an extract yield percentage of 22.427%.
6. Results of drying shrinkage examination of ethanol extract of *Phyllanthus niruri* leaves is 6,845.

Table 1: Results of drying shrinkage examination of ethanol extract of *Phyllanthus niruri* leaves.

Weight of empty crucible A (g)	Crucible weight + ethanol extract before drying B (g)	Crucible weight + ethanol extract after drying C (g)	% drying shrinkage	Average ± SD
36,1164	37,1668	37,0949	6,845	6,894
39,3860	40,4127	40,3514	5,971	±
35,4538	36,5685	36,4808	7,868	0,949

$$\begin{aligned}
 \% \text{ Drying shrinkage} &= \frac{(B-A)-(C-A)}{(B-A)} \times 100 \% \\
 &= \frac{(37,1668-36,1164)-(37,0949-36,1164)}{(37,1668-36,1164)} \times 100 \% \\
 &= \frac{(1,0504)-(0,9785)}{(1,0504)} \times 100 \% \\
 &= 6,845 \%
 \end{aligned}$$

7. Results of ash content examination of ethanol extract of *Phyllanthus niruri* leaves is 5,950 %

Table 2: Results of ash content examination of ethanol extract of *Phyllanthus niruri* leaves.

Weight of empty crucible A (g)	Crucible weight + ethanol extract before drying B (g)	Crucible weight + ethanol extract after drying C (g)	% Ash content	Average ± SD
36,1176	38,6874	36,2705	5,950	6,404 ± 0,449
41,6709	44,2102	41,8453	6,8483	
41,6899	44,1923	41,8504	6,4138	

$$\begin{aligned} \text{\% Ash content} &= \frac{(C-A)}{(B-A)} \times 100 \% \\ &= \frac{(36,2705 - 36,1176)}{(38,674 - 36,1176)} \\ &= \frac{(0,1529)}{(2,5698)} \times 100 \% \\ &= 5,950 \% \end{aligned}$$

8. Results preliminary examination of chemical content:

Positive: flavonoid and fenolic.

Negative: saponin, terpenoid, steroid, alkaloid.

9. Results of Organoleptic observation results of ethanol extract ointment of meniran leaves.

Table 3: Results of Organoleptic observation results of ethanol extract ointment of meniran leaves.

Organoleptic	Observation result	
	F0	F1
Shape	Semisolid	Semisolid
Colour	yellow	Blackish Green
Odour	typical vaseline	distinctive smell

10. Results of pH of ethanol extract ointment of meniran leaves.

Table 3: Results of pH observation results of ethanol extract ointment of meniran leaves.

pH of Ointment	Mean ± SD
4,60	4,63 ± 0,026
4,64	
4,65	

In research regarding the effect of healing burn wounds, the sample used was an ethanol extract ointment of meniran leaves (*Phyllanthus niruri* L.) as a test material. Meniran plants were taken from from Lubuk Buaya, Koto Tengah, Padang, West Sumatra, Indonesia. Before the research was carried out, the samples were first identified in the ANDA herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University. Identification is the first step so that the identity of the sample can be known so that there are no errors regarding the plants used in the research.

Evaluation of ethanol extract of meniran leaf ointment was an organoleptic test with a semisolid dosage form, blackish green in color and containing white crystals and a distinctive smell, a homogeneous preparation characterized by the absence of lumps on the smear. Then a pH test of the ointment was carried out using a pH meter where the ointment preparation had a concentration of 10%, pH 4.63. This ointment has a good pH because it corresponds to the pH of human skin, namely, 4.5-6.0.^[12]

The experimental animals used in this research were male white rats which had previously been acclimatized for 7 days. The experimental animals used were divided into 3 groups, namely, group 1 (control), rats which were injured and smeared with an ointment base (vaseline flavum), group 2 rats which were injured and smeared with T® ointments as comparison group and group 3 (treatment) rats which were injured and smeared with 15% concentration of meniran leaf ethanol extract ointment.

Topical ointment was given to each group twice a day in the morning and evening, given for 3, 7 and 14 days at a rate of ± 80 mg with the aim of observing wound healing in the inflammatory and proliferation phase. Specific in proliferation phase, the role of fibroblasts is very dominant in the repair process, which plays a role in preparing to produce protein products that will be used during the tissue reconstruction process. This proliferation phase starts from the third day and ends in two weeks marked by the formation of granulation tissue in the wound.^[15,16]

Testing the effect of administering the ethanol extract of meniran leaves on wound healing will be carried out using experimental animals of male white rats weighing ± 200 grams. A total of 27 mouse were divided into 3 main groups, and then each group was examined on days 3, 7, and 14 after the mouse were given excision wounds. On each day of examination, the animals were sacrificed using isoflurane and then the skin tissue of the experimental animals was taken for examination of hydroxyproline levels. Before use, mouse were first acclimatized for 7 days. Animals are declared healthy if during acclimatization they do not show deviations in body weight of more than 10% and visually there are no symptoms of disease.

Measuring the percentage of wound area that heals is the first parameter used to assess the effect of effective wound healing as the size of the wound decreases from day to day. The purpose of choosing to examine the effect of wound healing on day 3, day 7, and day 14 was to see the effect of healing burns wound in the proliferation phase. In this phase, fibroblast formation occurs. Fibroblasts are mesenchymal cells in the form of collagen fibers which play a role in wound healing, where collagen is a parameter for tissue formation or skin regeneration. Collagen is found in the dermis layer of the skin. The formed fibroblasts will move towards the wound area and will produce large amounts of collagen matrix so that the wound fills and the wound closes.

From the results of measuring the percentage of wound healing on the 3th day, the 7th day, and the 14th day, the group of animals given 15% meniran leaf ethanol extract ointment produced a higher average percentage of wound healing than the control group. Wound diameter measurements were carried out on day 3, day 7, and day 14 to calculate the percentage of wound healing. The percentage of wound healing observed was the initial area measurement with final area measurements on the 3th day, 7th day, and 14th day. A high percentage was indicated by the smaller the size of the wound, the better the wound healing.

Table 4: Results of measuring the percentage of wound healing area on the 3th day.

Group	Animal	Initial Diameter 1 st Day	Final Diameter 3 th Day	Initial Wound Area 1 st Day	Final Wound Area 3 th Day	% wound healing	Average \pm SD
I	1	2.17	2.16	3.711	3.677	58.19	1.17 \pm 0.3963
	2	2.03	2.02	3.249	3.196	45.11	
	3	2.05	2.04	3.313	3.281	54.63	
II	1	2,06	1.99	3.345	3.101		6 \pm 1.1360
	2	2,03	1.97	3.238	3.060		
	3	2,03	1.98	3.238	3.07		

III	1	2.06	2.02	3.324	3.196	55.71	3.61 ± 0.4013
	2	2.1	2.06	3.454	3.345	52.00	
	3	2.06	2.02	3.345	3,217	53.00	

Table 4: Results of measuring the percentage of wound healing area on the 7th day.

Group	Animal	Initial Diameter 1 st Day	Final Diameter 3 th Day	Initial Wound Area 1 st Day	Final Wound Area 3 th Day	% wound healing	Average ± SD
I	1	2.07	1.92	3.367	2.897	13.97	13.8 ± 0.18
	2	2.08	1.93	3.399	2.928	13.9	
	3	2.41	2.24	4.551	3.931	13.63	
II	1	2,09	1.71	3.421	2.289	33.11	34.19 ± 0.967
	2	2,05	1.66	3.291	2.156	34.48	
	3	2,08	1.68	3.410	2.218	34.97	
III	1	2.08	1.72	3.399	2.334	31.35	29.5 ± 1.821
	2	2.27	1.93	3.049	2.927	27.71	
	3	2.05	1.72	3.313	2.334	29.56	

Table 5: Results of measuring the percentage of wound healing area per day 14th.

Group	Animal	Initial Diameter 1 st Day	Final Diameter 3 th Day	Initial Wound Area 1 st Day	Final Wound Area 3 th Day	% wound healing	Average ± SD
I	1	2.08	1.69	3.399	2.235	34.24	39.41 ± 4.582
	2	2.10	1.58	3.454	1.97	42.97	
	3	2.08	1.6	3.410	2.011	41.02	
II	1	2,07	1.99	3.367	0.478	85.80	83.93 ± 2.806
	2	2,08	1.97	3.388	0.499	85.28	
	3	2,09	1.98	3.421	0.661	80.70	
III	1	2.09	1.15	3.421	1.033	69.80	71.36 ± 1.57
	2	2.06	1.07	3.345	0.905	72,94	
	3	2.08	1.11	3.399	0.974	71.35	

The second parameter is epithelialization time. Epithelialization time is the time recorded from the first day of spontaneous scab peeling without leaving residual wounds. From the results of observations carried out for 14 days in experimental animals in the treatment group with 15% ethanol extract, the average epithelialization time was on the 7th day, and in the control group the epithelialization time was on the 10th day.

Table 6: Epithelialization Time.

Group	Animal	Epithelialization time	Average ± SD
I	1	9	9.67 ± 1.155
	2	11	
	3	9	
II	1	6	6.66 ± 0.577
	2	7	
	3	7	
III	1	9	7.33 ± 1.528
	2	7	
	3	6	

The third parameter is hydroxyproline levels is the determination of hydroxyproline levels. The first thing to do is to determine the maximum absorption wavelength of hydroxyproline; in this study, the maximum wavelength was 559 nm. Furthermore, a calibration curve was made to obtain a regression equation. The regression equation obtained from the calibration curve using a series Duncan test of wound healing parameter by day of standard solutions is $y = 0.1661 + 0.06025x$, with a correlation coefficient (r) = 0.99878. Hydroxyproline is an amino acid with no chromophore group, so it does not have absorption in the UV-Vis region. The chromophore group is an unsaturated

covalent group that provides absorption in the ultraviolet and visible regions. Therefore, derivatization is carried out to determine the hydroxyproline levels. Derivatization aims to change hydroxyproline into color and its absorption can be read on a UV-Vis spectrophotometer. The derivatization process is carried out by making a hydroxyproline solution with the desired concentration, then adding reagents to change the hydroxyproline solution into a colour solution. The derivatization process in this study was carried out by making a hydroxyproline solution with a concentration of 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm, then adding reagents to change the hydroxyproline solution into color, the reagents added to the hydroxyproline solution are buffer, CuSO_4 , NaOH , H_2O_2 , H_2SO_4 and 4-dimethylamino benzaldehyde. The addition of a buffer solution or buffer solution to the hydroxyproline solution serves to maintain the pH value (acidity) so that it does not change much during the reaction by adding a strong base and dilution with water, then the solution is made alkaline by adding NaOH then adding CuSO_4 solution and H_2O_2 solution which functions as an oxidizer. Furthermore, the solution is incubated at a temperature of 80°C for 5 minutes. Allowed to cool, and a PDAB (para-dimethylamino-benzaldehyde) solution was used as a complexing agent to change the solution into color and sulfuric acid, which functions as a catalyst. The chemical mechanism of this process can be described as follows. This process involves the oxidation of amino acids to pyrrole-2-carboxylate or pyrrole, then the formation of chromophores by adding Ehrlich's reagent (p-dimethyl amino-benzaldehyde). The resulting compound is a very colored quinoid compound (color depends on the substituent and varies from orange-purple). The hydroxyproline solution that has been added with PDAB solution will be yellow, then re-incubated the solution will turn red, the function of heating itself so that the reaction occurs faster. The higher the hydroxyproline content, the more concentrated the red color produced. Derivatized hydroxyproline has absorption in the visible area. After that, the hydroxyproline levels were examined in the mouse skin tissue. The scar skin was taken; previously, the experimental animal was sacrificed first, then the skin tissue above the scar was taken using tweezers and surgical scissors. The skin tissue is then dried for 12 hours at a temperature of 60°C , the aim being to dry the water content in the skin. Next, it is hydrolyzed with 6N HCl for 24 hours at a temperature of 110°C to destroy or break down skin tissue into smaller pieces with the help of heating. After hydrolysis, the sample is neutralized with 2 ml of NaOH , and 1 ml of aquabidest and 1 ml of buffer solution are used so that the pH remains the purpose of adding buffer to maintain the pH value because there is a mixture of strong base with strong acid and dilution with water. The sample is pipetted as much as $200\ \mu\text{l}$ ad with aquadest up to 1 ml and mixed with 1 ml of 0.01 M CuSO_4 , 1 ml of 2.5 N NaOH , and 1 ml of 6% H_2O_2 , all three as oxidants. The addition of oxidant solution functions to convert hydroxyproline into pyrrole-2-carboxylate or pyrrole. The solution is then stirred and incubated at a temperature of 80°C for 5 minutes; the incubation functions so that the oxidant solution can react optimally. After the incubation process is complete, the solution is cooled and added with 4 ml of 3 N H_2SO_4 as a catalyst or to accelerate the reaction and 2 ml of 5% 4-dimethylamino benzaldehyde functions as a color complexing agent to change the sample to yellow, the sample is re-incubated at 70°C for 16 minutes, cooled at 20°C the solution will turn red, the function of self-heating so that the reaction occurs faster. The higher the hydroxyproline content, the more concentrated the red color produced. Hydroxyproline resulting from derivatization has absorption in the visible region. The visible region is in the 380-780 nm region. Determination of hydroxyproline levels is carried out on the 5th day, the 10th day, and the 15th day after the wound because these days have entered the proliferation phase where the proliferation phase of fibroblast formation occurs. Fibroblasts will synthesize collagen, which is the main element of the extracellular matrix which is useful for forming scar tissue strength in wounds. The amount of collagen in the skin can be determined by measuring hydroxyproline levels. From the calculation results of the percentage of hydroxyproline levels, the comparison group gave the highest average percentage of hydroxyproline levels compared to

all groups, followed by the treatment group that was applied with ethyl acetate subfraction ointment with a concentration of 5%. The group that was applied with vaseline flavum gave the smallest average percentage of hydroxyproline levels among all groups. Based on the calculation results of the percentage of hydroxyproline levels in this study, it was seen from the maximum wavelength, determination of the regression equation and absorbance value in the sample solution.

Table 7: Results of hydroxyproline levels on day 3.

Group	Experimental animals	Absorbance	% hydroxyproline content	Average \pm SD
I	1	0.280	10.18	10.4778
	2	0.293	11.00	\pm
	3	0.281	10.24	0.4547
II	1	0.748	17.74	19.9163
	2	0.753	19.19	\pm
	3	0.789	22.81	2.6079
III	1	0.668	21.76	17.586
	2	0.667	13.86	\pm
	3	0.666	17.12	3.9743

Table 7: Results of hydroxyproline levels on day 7.

Group	Experimental animals	Absorbance	% hydroxyproline content	Average \pm SD
I	1	0.324	7.49	11.4241
	2	0.541	14.16	\pm
	3	0.324	12.61	3.4898
II	1	0.611	28.16	28.0186
	2	0.584	27.64	\pm
	3	0.639	28.23	0.3224
III	1	0.490	13.58	20.7653
	2	0.594	18.76	\pm
	3	0.599	29.94	8.3639

Table 7: Results of hydroxyproline levels on day 14.

Group	Experimental animals	Absorbance	% hydroxyproline content	Average \pm SD
I	1	0.549	12.95	12.8776
	2	0.546	12.86	\pm
	3	0.544	12.80	0.0756
II	1	0.615	42.13	42.6178
	2	0.626	43.07	\pm
	3	0.621	42.64	0.4669
III	1	0.510	16.18	20.9522
	2	0.524	23.21	\pm
	3	0.515	23.45	4.1289

CONCLUSION

Based on the results of research on the healing effect of excision wounds from meniran leaves (*Phyllanthus niruri* L) with a concentration of 10% on male white rats, it was concluded that:

1. Applying meniran leaf ethyl acetate subfraction ointment can provide a better effect in the wound healing process.
2. The duration of administration of meniran leaf ethyl acetate subfraction ointment can provide a good healing effect. Based on the results obtained in statistical analysis using the anova test, a significant value was obtained ($p < 0.05$).

BIBLIOGRAPHY

1. Wahyuni, W., Aliah, A. I., & Sembah, E., Formulasi Gel dan Uji Efektivitas Ekstrak Etanol Daun Meniran (*Phyllanthus niruri* L.) terhadap Penyembuhan Luka Sayat pada Kelinci Jantan (*Oryctolagus Cuniculus*). *Media Kesehatan Politeknik Kesehatan Makassar*, 2021; 16(1): 76. <https://doi.org/10.32382/medkes.v16i1.1798>.
2. Moenadjat Y. Luka Bakar: Masalah dan Tata Laksana. Balai Penerbit FKUI. Edisi 4, 2009: 1-38.
3. Sjamsuhidajat, R., Buku ajar ilmu bedah Sjamsuhidajat-de jong. *Sistem Organ dan Tindak Bedahnya (1)*. 4th ed. Jakarta: Penerbit Buku Kedokteran EGC, 2017; 332-339.
4. Primadina, N. *et al.* 2019. Proses Penyembuhan Luka Ditinjau dari Aspek Mekanisme Seluler dan Molekuler. *Qanun Medika - Medical Journal Faculty of Medicine Muhammadiyah Surabaya*. 3, 1 (Jan. 2019), 31–43. DOI:<https://doi.org/10.30651/jqm.v3i1.2198>.
5. Rivai, H., Nurdin, H., Suyani, H & Bakhtiar, A., *Pengaruh Cara Pengeringan terhadap Mutu Herba Meniran (Phyllanthus niruri L)*. Padang: Fakultas Farmasi Universitas Andalas, 2011.
6. Danladi, S., Idris, M., & Umar, I., Review on Pharmacological Activities and Phytochemical Constituents of *Phyllanthus niruri* (Amarus). *The Journal of Phytopharmacology*, 2018; 7(3): 341–348. <https://doi.org/10.31254/phyto.2018.7318>
7. Sulaksana, J., & Jayusman, D.I., *Meniran Budidaya dan Pemanfaatan Obat*. Jakarta: Swadaya, 2004.
8. Sudibyoy, M., *Alam Sumber Kesehatan: Manfaat dan Kegunaan Daun Meniran*. Jakarta: Balai Pustaka, 1998.
9. Nagori, B., & Solanki, R., Role of Medicinal Plants in Wound Healing. *Research Journal of Medicinal Plant*, 2011; 5: 392–405. <https://doi.org/10.3923/rjmp.2011.392.40>
10. Alfinda, Y. T. N. (2024). *Pengaruh SubfraksiI Etil Asetat 10% dan 15% Daun Meniran (Phyllanthus niruri L.) terhadap Hidroksiprolin Tikus Putih Jantan Hari Ke- 3, 7, dan 14*. Padang:Universitas Perintis Indonesia.
11. Shanbhag, T., Amuthan, A., & Shenoy, S., Effect of *Phyllanthus niruri*. Linn on burn wound in rats. *Asian Pacific Journal of Tropical Medicine*, 2010; 3(2): 105–108. [https://doi.org/10.1016/S1995-7645\(10\)60045-4](https://doi.org/10.1016/S1995-7645(10)60045-4).
12. Departemen Kesehatan Republik, Indonesia. Indonesian Pharmacopoeia IV. Republic of Indonesia Ministry of Health. Jakarta, 1995.
13. Harbone JB. Physicochemical Methods Guiding Modern Ways of Analyzing Plants. Second Issue. ITB Bandung, 1987.
14. Wasitaatmadja, S. M., *Penuntun Ilmu Kosmetik Medik*. Jakarta: UI-Press, 1997.
15. Gushiken LFS, Beserra FP, Bastos JK, Jackson CJ, Pellizzon CH. Cutaneous Wound Healing: An Update from Physiopathology to Current Therapies. *Life (Basel)*, 2021; 11(7): 665. DOI: 10.3390/life11070665. PMID: 34357037, PMCID: PMC8307436.
16. Tara S, Arul A, Smita S, Sudhakar. Effect of *Phyllanthus niruri*. Linn on burn wounds in rats. *Asian Pacific Journal of Tropical Medicine*, 2010; 105-108.