

# Analysis of Grade II Burns Healing from Andaliman Fruit Ethanol Extract Ointment (*Zanthoxylum Acanthopodium* Dc.) in Wistar Rats (*Rattus Norvegicus*)

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**Abstract:** *The prevalence of injuries has increased every year, from risked results data in 2013 reported that burns are the sixth cause of accidental injuries with a prevalence of 0.7 percent of the Indonesian population. Andaliman fruit extract with various phytochemicals has active physiological activity as a potential antioxidant and antimicrobial. The study aims to explore the wound healing effects of andaliman fruit ethanol extract. This study was an experimental study using 20 rats divided into 4 groups (Control, Standard, Essential Oil 10%, and Essential Oil 15%). Wound healing parameters are assessed through wound contraction and epithelial period. Samples of andaliman fruit used in this study were obtained from one of the traditional markets in the city of Medan. Essential oils are obtained from the hydrodistillation process. The results of the study showed significant differences in the epithelial period of the standard group, 10% and 15% in the control group. However, in the andaliman ointment group and the standard group, there was no difference in the epithelial period. This is evident from the value  $P < 0.05$  (Value  $P = 0.029$ ). The conclusion of the healing effects of burns possessed by andaliman ointment both 10% and 15% and nebacetin ointment as standard shows a significant difference. Where the wound contraction rate of your ointment is 15% better than nebacetin ointment as standard. But in both andaliman ointments, both 10% and 15% and nebacetin ointment as standard did not show significant differences in the parameters of the epithelial period.*

**Keywords:** *andaliman; essential oils; burns*

## I. Introduction

The prevalence of injuries has increased every year, from the results of research conducted by Monuteaux, Fleegler, & Lee (2017) in the United States, 1.4 million adults were treated for violent injuries between 2000 and 2010, with a prevalence of 1.6% of all adult patients in emergency units in the United States, In Libya (Monuteaux et al., 2017). The Ministry of Health's 2013 Basic Health Research data noted that burns rank sixth cause of unintentional injury after falls, motorcycles, sharp/blunt objects, other land transportation, and falls with a prevalence rate of 0.7 percent of the Indonesian population. It also emphasized that children ages 1-4 are the most vulnerable age group to burns with a prevalence rate of up to 1.5 percent (Dinas Kesehatan Republik Indonesia, 2013). Combustion is an injury (injury) as a result of direct contact or exposure to sources of heat (thermal), electricity, chemicals, or radiation (Tutik Rahayuningsih, 2012). Wound healing that must be treated and healed immediately, several healing phases such as the inflammatory phase, proliferative phase, and maturation phase. The inflammatory phase is characterized by hemostasis, chemotaxis, and increased permeability of blood vessels that limit further damage, close wounds, remove cellular debris and bacteria and encourage cellular migration (Suryadi, 2013). The proliferative phase is characterized by the formation of granulation tissues, recapitalization, and neovascularization. This phase can

last several weeks. The maturation and remodeling phase is where the wound reaches maximum strength at maturity ((Suryadi, 2013); (Suryadi, 2013).

Andaliman fruit extract is reported to have active physiological activity as a potential antioxidant and antimicrobial so the components that play a role in these unique properties need to be identified (Sitanggang et al., 2019). Some studies prove that its terpenoid content has antioxidant and antimicrobial activity repellent and kills insects (Mery Sukmawati, Sumarto, 2013). Antioxidant and immunostimulant activity of andaliman fruit that can increase the number of lymphocyte cells and can reduce free radicals to 71.90 mM which is different from control (114.81 mM) (Winarti et al., 2018). But until now, information related to the biological activity of the nano herbal andaliman, especially in increasing antioxidant and immunostimulatory activity related to prevention or accelerating the healing of burns on the skin is still not much. The purpose of this study was to find out the phytochemical content and effect of andaliman fruit ointment (*Zanthoxylum acanthopodium* DC.) in curing grade II burns in Wistar rats (*Rattus norvegicus*).

## II. Review of Literature

Andaliman (*Zanthoxylum acanthopodium* DC.) is a Rutaceae family that is widely found in North Sumatra, and the fruit is widely used as a traditional cooking spice by the Batak tribe (Asbur & Khairunnisyah, 2018). Some studies prove that the content of andaliman terpenoids has antioxidant and antimicrobial activity, also has immunostimulating effects (Asbur & Khairunnisyah, 2018). Most plants that contain bioactive compounds such as glycosides, alkaloids, terpenoids, and flavonoids have antioxidant and antidiabetic activity (Ergina et al., 2014). Burns can usually be prevented, and different treatments are applied based on the severity of the burn. Sometimes, ointments, creams, biological and nonbiological dressings, and antibiotics are recommended levels 2, 3, and 4 burns, while misuse of these drugs increases the risk of antibiotic resistance and fungal infections, even slowing wound healing and increasing the depth of burns (Agussalim Bukhar, 2018). Alternative treatments for burns other than with pharmacology are honey, aloe vera, oatmeal, eggs, mud, leaves, or cow dung, which have been used for the treatment of burns (Budiyanto, n.d.); (Hakim, 2020). The skin is part of the integument system and is considered the largest organ of the human body. There are three main layers of skin: epidermis, dermis, and hypodermis (subcutaneous fat). Skin appendages such as sweat glands, hair follicles, and sebaceous glands are in-depth reviewed elsewhere (Kalangi, 2014).

## III. Research Methods

This research is experimental with a pre-test control group design approach. The study was conducted at Riwandi Pet Shop and Animal House from February-March 2021. The study sample is andaliman plant, animal samples try Wistar strain rats as many as 20 male Wistar rats (*Rattus norvegicus*) divided into 4 treatment groups, so that each group consists of 5 rats (Lahamendu et al., 2019). Independent variables include giving several topical formulations (base ointment, nebacetin ointment®, andaliman ointment (*Zanthoxylum acanthopodium*) 10% and andaliman ointment (*Zanthoxylum acanthopodium*) 15%, as well as independent variables that include wound contraction and epithelialization period (Izzati, 2015); (Hariani, 2016).

### 3.1 Tool

Maceration vessel, knife, rotary evaporator, water handler, gel container, stirrer rod, plate measuring 2 x 2 cm.

### 3.2 Material

Andaliman fruit, aquades, lanolin, solid paraffin, Cetostearyl alcohol, white vaseline, gauze, oil paper, filter paper, 1mm<sup>2</sup>-sized paper, oil paper, ointment nebacetin®.

### 3.3 Research Procedure

- a. Making Essential Oil of Andaliman fruit. Fresh fruit andaliman as much as (200 grams) distilled by hydro-distillation process for 4 hours at a temperature of 80°C, the remaining water residue in the distillation is removed by inserting anhydrous sodium sulfate, which is then filtered to obtain the oil (Asbur & Khairunnisyah, 2018).

- b. Calculation of The Yield of Essential Oil andaliman fruit. As for the calculation of the yield of essential oils from Andaliman fruit:

$$\text{Rendeman (\%)} = \frac{\text{Andaliman Fruit Essential Oil}}{\text{Andaliman Fruit Sample Time}} \times 100\%$$

- c. Phytochemical Test (Identification of compound groups).

Andaliman fruit identified several groups of compounds such as flavonoids, tannins, alkaloids, phenols, steroids/triterpenoids, terpenoids and saponins. The phytochemical test uses Fransworth's modified method. Each work procedure as stated below (Rahmawati et al., 2016);

- Identification of Flavonoids. As much as 1 ml of test solution is each put into 3 test tubes. Tube 1 as a control, tube 2 coupled with 1 mL of 5% FeCl<sub>3</sub> solution, flavonoid positive if there is a dark green/blue discoloration. Tube 3 coupled with a few drops of NaOH 10% formed yellow color if it contains flavonoids (Ingkasupart *et al.*, 2015).
- Identification of Tannins. Tannins As much as 2 mL of test solution inserted into the test tube is added with a few drops of FeCl<sub>3</sub> 1% solution, a positive sign of tannin if the color formed is dark green / blue (Ergina et al., 2014).
- Identification of alkaloids. For alkaloid tests, as much as 2 mL of test solution is evaporated on a porcelain cup until residue is obtained. The residue is then dissolved with 5 mL HCl 2N. Once cool, the solution is filtered. The solution obtained is divided into 3 test tubes. The first tube serves as a control. The 2nd tube is added 3 drops of the dragendorff reagent and the third tube is added 3 drops of mayer reagent (through the tube wall). The formation of orange deposits in the second tube and yellow deposits in the third tube indicates the presence of alkaloids (Sakamoto et al., 2020).
- Identification of phenols. Tests are carried out in drip plates, the test solution is added FeCl<sub>3</sub> (1% dalaim water / ethanol), if there is a change in color green / red / purple / blue / black shows the presence of phenol content (Rahmawati et al., 2016).
- Identification of Terpenoids. The modified Salkowaski test. For 2 mL of water plant extract, added 2 mL of chloroform and this is followed by the addition of a few drops of concentrated sulfuric acid. The solution is well shaken. The formation of a yellow lower layer indicates the presence of terpenoids (Lu et al., 2020).

- d. Identification of Saponins. Saponins 4 mL test solution is added with 5 mL aqua dest, shake, see the presence of a stable foam. A little extract is added 5 mL of water, shake in a test tube, form a stable foam (foam as high as 1 cm and stable for 30 minutes) 4 mL of test solution is inserted into the test tube as a control (Lukitaningsih et al., 2020).

The various materials used are heated according to the melting point of each material. Formulations are made with varying concentrations as shown in the following table.

**Table 1.** Topical Preparation Formulations of Each Ointment

Material Name	Ointment base	Fruit ointment Andaliman10%	Fruit ointment Andaliman 15%
Andaliman Fruit Essential Oil	-	1 ml	1.5 ml
Lanolin	2.5g	2.5g	2.5g
Solid paraffin	2.5g	2.5g	2.5g
<i>Cetostearyl alcochol</i>	2.5g	2.5g	2.5g
White vaseline	42.5g	42.5g	42.5g

#### e. Testing on Animal Trials

All animals try in the form of wistar rats are done need by using electrical solder that has been motivated with a round-shaped tip then found on the dorsal part of the rat for 10 seconds, before doing the need for the rats dianastesi using ketamine (50 mg / kg i.m) that has previously been satisfied. Before continuing with the sampling of extract gels and controls, different tests are carried out to assess the degree and extent of the grade II burn (Febiati, 2016); (Sumiati, 2017).

#### f. Evaluation of Burns Healing Activities

Burns evaluation is carried out every 2-4 days, with aspects evaluated from the healing activities of the burn including wound contraction and epithelialization periods (Febiati, 2016).

#### g. Epithelialization Period

The epithelialization period is measured by calculating the length of time it takes for the eschar to detach, during which the epithelial period is calculated in days (Palumpun et al., 2017).

#### h. Data Analysis

The statistics analysis used in the study was Anova's One-Way test, followed by a post-hoc test. Before another test is done descriptive analysis of wound contraction and epithelial period. If the data in this study is distributed abnormally, then there will be a transformation of the data so that the data is distributed normally.

## IV. Discussion

### 4.1 Karaketristic Essential Oils

Characteristics of the essential oils evaluated in the study include the initial weight of the fruit, the weight of the resulting essential oil, the volume of the essential oil, and the yield. Of the 1500 grams of andaliman fruit time used after distillation was found as much as 2.54 ml of andaliman essential oil. So found a yield value of 0.25%.

Based on the results of rienoviar and setyaningsih (2018) study reported that extracts from andaliman fruit extracted by maceration method found the amount of yield from ethanol extract is 6.54%, 5.35% from ethyl acetate extract, and 6.09% from ethanol extract: ethyl astet (1:1) (Rienoviar & Setyaningsih, 2018). The yield value of essential oils with hydrodestilation methods is of the least value compared to the andaliman fruit extract that is accelerated, this shows that the essential oil from andaliman oil has better quality than andaliman fruit extract made by the maceration method. This is because the yield value is inversely proportional to the quality of essential oils (Rienoviar & Setyaningsih, 2018).

#### 4.2 Phytochemical Screening of Andaliman Fruit

The andaliman fruit used in this study conducted a phytochemical screening test to find out the phytochemical content of fresh samples of andaliman fruit. The results of the screening can be seen in the table below.

**Table 3.** Fruit Phytochemical Screening Results Andaliman (*Zanthoxylum acanthopodium*)

Fitokimia	Metode Uji	Result
Alkaloid	Dragendorff	+
Steroid	Maeyer Salkowsky	+
Saponin	Aquadest Aquadest + Alkohol 96%	-
Flavonoid	FeCl <sub>3</sub> 5% NaOH 10%	+
Tanin	FeCl <sub>3</sub>	+

From the results of phytochemical screening in fresh samples of andaliman fruit found phytochemical content in the form of alkaloids, flavonoids, and tannins. The results of this study are not much different from the results of research conducted by Saragih and Arsita (2019) on Andaliman fruit from the Toba Samosir and North Tapanuli regions that reported that the fruit sample contained phytochemicals in the form of phenolics, saponins, flavonoids, tannins, triterpenoids, and alkaloids (Saragih & Arsita, 2019). The difference in the results of this study could be due to differences in screening methods used. In comparison, the results of the Coal et al. (2017) study reported that in andaliman leaf extract only found phytochemicals in the form of alkaloids, steroids, and saponins. This suggests that in ethanol extracts andaliman leaves have less phytochemical content, especially phytochemical content with phenol groups such as phenolics, tannins, and flavonoids (Batubara et al., 2018).

#### 4.3 Wound Healing Activities

To evaluate wound healing in each group of mice, different test analyses were conducted in accordance with the results of the normality analysis of data from each of the wound healing parameters evaluated. The results of the analysis of the normality of the data can be seen in the table below:

**Table 4.** Results of Data Normality Analysis on Burn Healing Parameters

Wound Healing Parameters	Score P
Wound Contraction On the 3rd Day	0.009
Wound Contraction On the 6th day	0.352
Wound Contraction On the 9th day	0.056
Wound Contraction On the 9th day	0.035
Wound Contraction On the 14th day	0.001
Period Epitelialisasi	0.001

From the table data above it can be seen that the wound contraction parameter data on day 6 and day 9 shows a normal data distribution, so the analysis of the data used for other tests is One Way Anova followed by Post Hoc Test Tukey HSD. Meanwhile, other parameters show an abnormal distribution of data so that different tests used are kruskal-wallis and mann-whitney tests.

#### 4.4 Wound Contraction

As one of the parameters of wound healing the test results differ from wound contraction in each treatment group shown in the following table.

**Table 5.** Wound Contraction Analysis Results (%) Data on Burn Healing Parameters

Observation Time	Wound Contraction (%)				Score
	Control	Standard	Andaliman Ointment 10%	Andaliman Ointment 15%	
Day 3	4.71 (8.33)	0.01 (8.70)	12.54	10.61	0.003*
Day 6	9.46 ± 5.21	18.22 ±	30.16 ± 7.88	45.67 ±	0.001*
Day 9	7.61 ± 7.92	35.11 ±	45.45 ± 6.55	51.42 ±	0.001*
Day 12	9.54 (29.17)	54.56	64.64	56.67 (8.96)	0.005*
Day 14	27.23 (37.50)	81.71	76.27	89.29 (3.64)	0.002*

#### 4.5 Epithelialization Period

In addition to wound contraction, another parameter that is also evaluated in assessing burn healing is the epithelial period, the results of different tests from the epithelialization period of each treatment group can be seen in the table below.

**Table 6.** Results of Epithelial Period Comparison in Each Treatment Group

Treatment Group	Epithelialization Period*	Value P
Control	21 (2) <sup>a</sup>	0.029
Standard Ointment Andaliman 10%	17 (2) <sup>b</sup>	
Ointment Andaliman 15%	22 (2) <sup>b</sup>	

\* Data is presented in Median (Range). Different lowercase letters in the same column shows a significant difference in the value of P < 0.05

From the table data above it can be seen that there are significant differences in the epithelial period of the standard group, 10% and 15% in the control group. However, in the andaliman ointment group and the standard group there was no difference in epithelial period. This is evident from the value  $P < 0.05$  (Value  $P = 0.029$ ). Based on the results of the above study, it can be seen that there are significant differences in the wound contraction parameters and epithelialization periods of each treatment group. The healing activity of the burn owned by the andaliman fruit is related to the antioxidant, anti-inflammatory, and antimicrobial activity of this andaliman fruit, because through the antioxidant and antimicrobial activity owned by this fruit it will create a good environment (Microenvironment) for wound healing.

This is supported by the results of research conducted by Winarti *et al.* (2018) who reported that ethyl acetate extract from Andaliman fruit has high antioxidant activity with ic50 value of 66.91 BPJ, antioxidant activity in ethyl acetate extract is due to the presence of compound 2-methoxy-4-vinylphenol (Winarti *et al.*, 2018). Another study that exports anti-inflammatory activity from andaliman fruit is the result of research Sefanny, Nicki L, (2020), It is reported that andaliman fruit ethanol extracts can significantly inhibit the expression of certain types of inflammatory biomarkers at the level of protein synthesis (TNF- $\alpha$ , COX-2, and MMP-9 proteins) and genes (TNF- $\alpha$ , IL-6, iNOS, COX-2, and MMP-9) in macrophage induced with lipopolysaccharides *in vitro* (Sefanny, Nicki L, 2020).

## V. Conclusion

The conclusions that can be drawn from this study are as follows, the healing effects of burns possessed by andaliman ointments are both 10% and 15% and nebacetin ointment as standard shows significant differences. Where the wound contraction rate of your ointment is 15% better than nebacetin ointment as standard. But in both andaliman ointments, both 10% and 15% and nebacetin ointment as standard showed no significant difference in the parameters of the epithelial period.

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