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# ANALYSIS OF THE EFFECTIVENESS OF ANDALIMAN FRUIT EXTRACT (ZANTHOXYLUM PIPERITUM) IN WOUND HEALING AFTER TOOTH EXTRACTION IN WISTAR RATS

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**ABSTRACT**
*Andaliman contains phenol compounds, monoterpenes, sesquiterpenes, nones, and essential oils, a class of terpenoid compounds. Based on its chemical content and physiological activity, andaliman can be used as a spice, preservative, medicinal material and supplement, and vegetable pesticide. The study aimed to analyze the effectiveness of andaliman (Zanthoxylum piperitum) fruit extract 40% and 80% in wound healing after tooth extraction in rats. This laboratory experimental study used a complete randomized design with a post-test-only control group design pattern. The experimental animals used in this study were 32 male Wistar rats, physically healthy, 2-3 months old, with body weight between 200-250 grams. The rats will be divided into two groups, namely 16 rats treated with 40% Andaliman fruit (Zanthoxylum piperitum) and 16 rats treated with 80% Andaliman fruit (Zanthoxylum piperitum) to see the comparison of accelerated wound healing after tooth extraction. The study used a pure experiment with a non-parametric Chi-Square Test after the test showed that (p<0.05) means a significant difference between groups. The results showed that there was a meaningful relationship between the number of fibroblast tissue per visual field in Wistar rats after tooth extraction by giving Andaliman Fruit Extract (Zanthoxylum piperitum) at 40% concentration and Andaliman Fruit Extract (Zanthoxylum piperitum) 80% concentration, p=0.010 (p<0.05). Based on the results and discussion that has been done in this study, it can be concluded that Andaliman Fruit Extract (Zanthoxylum piperitum) 40% and 80% effectively accelerate wound healing time after tooth extraction of Wistar rats*

**KEYWORDS:** *Andaliman Fruit, Wound Healing, Extraction*

## I. INTRODUCTION

Tooth extraction will cause a wound in the form of exposed alveolar bone in the oral cavity (1). An injury is anatomical damage or partial destruction of tissue due to trauma. The severity of the wound depends on the amount of trauma received by the tissue. Physiologically, the body can repair damage to its skin tissue (injury), known as wound healing (2). The wound healing process can be divided into three main phases, namely, the inflammatory phase, the proliferation phase, and the remodeling phase. These phases continue from the onset of the wound until wound closure. The inflammatory phase is the body's reaction to the damage that starts after a few minutes and lasts about three days after the injury (3). The proliferation phase is characterized by the appearance of new blood vessels resulting from reconstruction and occurs within 3-24 days. The maturation phase is the final stage of the wound-healing process (4).

Andaliman is closely related to the people of the Batak tribe because andaliman fruit is often used as a spice for traditional Batak cuisine in North Sumatra. Not only utilized as a seasoning, andaliman also has benefits as a producer of terpenoid substances with antioxidant activity and antimicrobial and immunostimulant effects. Silalahi (2021) states that andaliman contains phenol compounds, monoterpenes, sesquiterpenes, nones, and essential oils, which are terpenoid compounds. Based on its chemical content and physiological activity, the utilization of andaliman can be increased; it is not just a seasoning but also a preservative, medicinal material and supplement, and vegetable pesticide (5). Several studies have reported the potential of andaliman as an antimicrobial, antioxidant, anti-inflammatory, xanthine oxidase inhibitor, and cytotoxic. Other studies have also reported the antibacterial activity of andaliman extract against food-pathogenic bacteria such as Bacillus cereus, Escherichia coli, Staphylococcus aureus, and Salmonella typhimurium (6); (7). Because of the above, the author is interested in examining the effectiveness of 40% and 80% Andaliman Fruit (Zanthoxylum piperitum) extract in accelerating wound healing time after tooth extraction in Wistar rats.

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## II. RESEARCH METHODS

This experimental laboratory study uses a randomized controlled design with a post-test-only control group design pattern. The experimental animals used in this study are Wistar rats, 32 males, physically healthy, 2-3 months old, with a body weight between 200-250 grams. The rats will be divided into two groups, namely, 16 treated with 40% Fruit Andaliman (Zanthoxylum piperitum) and 16 treated with 80% Fruit Andaliman (Zanthoxylum piperitum) to see the comparison of accelerated wound healing after tooth extraction. The Federer formula determined the sample size, namely: (t - 1) (r - 1) ≥ 15. Where t = several treatments; (2 treatments) r = several replications. Thus, the minimum sample size for each treatment was 16 rats.

$= (t-1)\ (r-1) \geq 15$
$= (2-1)\ (r-1) \geq 15$
$= (r-1) \geq 15$
$= (r-1) \geq 15$
$= r \geq 15 + 1$
$= r \geq 16$

***Tools***

Tools used in research :

1. Number-coded experimental animal cages.
2. Diagnostic set (mouth glass, sonde, tweezers).
3. Nierbeken.
4. Dental extraction forceps (a needle holder is used) under sterile conditions.
5. Syringe.
6. Gloves.
7. Mask.
8. Petri dish of jaw preparation.
9. A set of tools for making histology preparations.
10. Microscope.

***Material***

Materials used in the study:

1. Fruit Andaliman (Zanthoxylum piperitum) Extract 40%
2. Fruit Andaliman (Zanthoxylum piperitum) Extract 80%
3. Ketamine.
4. Formalin 10%.
5. Histology preparation material with Hematoxylin Eosin (HE) staining.
6. 70% alcohol as sterilization material.
7. Cotton pellet.

The type of data collected in this study is primary data obtained from the results of measurements (scoring) on the histological picture of the process of accelerating wound healing after tooth extraction by administering Fruit Andaliman (Zanthoxylum piperitum) 40% and Fruit Andaliman (Zanthoxylum piperitum) 80%. Collecting 3 kg of Fruit Andaliman (Zanthoxylum piperitum), the Fruit Andaliman (Zanthoxylum piperitum) was washed and divided into two parts to take the inner meat to obtain the gel. After washing, the flesh of the Fruit Andaliman (Zanthoxylum piperitum) was dried in an incubator at 500 ℃ for 72 hours. The dried flesh of the Fruit Andaliman (Zanthoxylum piperitum) was then pulverized using a blender until it became powder. Fruit Andaliman (Zanthoxylum piperitum) meat that had become powder was then extracted by maceration while stirring. The extraction process uses a water solvent. The powder was put into a maceration vessel or container with a watertight lid and then filtered using filter paper; the pulp was macerated up to 2 times. The obtained maceration results were collected and evaporated using a rotary vacuum evaporator at a temperature of 500C until there was no more solvent condensation on the condenser. After the solvent was evaporated using a rotary vacuum evaporator, the evaporation was continued using a 70°C water bath to obtain a pure extract. The Fruit Andaliman (Zanthoxylum piperitum) extract was then diluted with water to get 40% and 80% extract concentrations.

Before treatment, 32 rats were divided into 40% Zanthoxylum piperitum extract and 80% Zanthoxylum piperitum extract. After that, all rats were adapted for one week. Then, animals were put into cages, with five rats in each cell in the same environmental conditions, given the same food, and monitored for health. Rat tooth extraction will be performed using a modified needle holder

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under the anesthetic effect of ketamine 1000 mg/10 ml at a dose of 20 mg/kg bw intraperitoneally. One incisor tooth will be extracted from every five rats daily. After tooth extraction, observe the extraction wound and apply a tampon (cotton pellet) to stop bleeding in the wound for 5 minutes. Dropped Fruit Andaliman (Zanthoxylum piperitum) 40% in treatment group I and dropped Fruit Andaliman (Zanthoxylum piperitum) 80% in treatment group II shortly after tooth extraction as much as 0.05 ml every day. After extraction and treatment, the test animals (rats) were fed fine porridge with attention to the health of the test animals.

On the 5th day after tooth extraction, rats from each group were physically sacrificed by neck dislocation. The rat's tail was held and then placed on a surface it could reach. The rat will stretch its body; when the rat's body extends, a holder held by the left hand is placed on the nape of the neck. The right-hand pulls the tail hard so the rat's neck will be dislocated. Then, the jaw of the rat is taken out. Then, the tissue was fixed with 10% formalin for 24 hours at room temperature, and the decalcification process was carried out using Ethylene Diamine Tetra Acetic Acid (EDTA 10%) solution at room temperature. Tissue dehydration was then performed using alcohol.

First, the specimen was put into a toluol alcohol solution (1:1) using pure toluol and then into a paraffin-saturated toluol solution. The following process is infiltration in the oven by inserting the specimen into liquid paraffin. The embedding process is carried out (inserting the tissue into paraffin) and then labeled/coded. After the embedding stage, the tissue is sliced in series with a thickness of approximately 6 microns using a microtome. It was evaluating fibroblast cell response using Hematoxylin Eosin (HE) staining. The procedure that must be done is deparaffinization using xylol and alcohol solution, then continued with the rehydration process with alcohol. After that, it is washed with running water, rinsed with distilled water, and then wiped. The glass slide was then placed in Meyer's hematoxylin solution, washed with running water, and then rinsed with distilled water, after which the staining was assessed under a light microscope. If the staining has been considered good, proceed to the next step, namely the dehydration process with alcohol in stages, and then wipe. The next step was to put it into xylol solution, and the object glass was covered with deck glass and observed using a light microscope. Fibroblast density was assessed by counting the fibroblasts in 5 fields of view. Histopathology scoring parameters to determine the distribution of fibroblast tissue is done based on the field of view:

1. (-) = No fibroblast tissue found
2. (+) = small number of fibroblasts (less than 10% per field of view)
3. (++) = moderate amount of fibroblast tissue (10%-40% per field of view)
4. (+++) = large amount of fibroblast tissue (40%-80% per field of view) 4.

Data analysis using the SPSS 16 program. Research using a pure experiment with a non-parametric Chi-Square Test, after testing, showed that ($p < 0.05$) means there is a significant difference between groups.

## III. RESULTS AND DISCUSSION

Data distribution and frequency of the number of fibroblast tissue per field of view in Wistar rats after tooth extraction in groups given 40% and 80% Andaliman Fruit extract (Zanthoxylum piperitum) can be seen as follows:

**Table 1. Distribution and Frequency Data of Fibroblast Tissue Counts Per Field of View After Tooth Extraction**

| No | Number of Fibroblasts | Fruit Andaliman (Zanthoxylum piperitum) | | | |
|---|---|---|---|---|---|
| | | Concentration 40% | | Concentration 80% | |
| | | n | % | n | % |
| 1 | No fibroblast tissue was found | 0 | 0 | 0 | 0 |
| 2 | A small number of fibroblasts (less than 10% per field of view) | 8 | 25 | 3 | 9 |
| 3 | Moderate amount of fibroblast tissue (10%-40% per field of view) | 4 | 13 | 6 | 19 |
| 4 | A large amount of fibroblast tissue (40%-80% per field of view). | 4 | 13 | 7 | 22 |

Table 1 shows all samples found fibroblast tissue in the administration of Andaliman Fruit extract (Zanthoxylum piperitum) 40% and 80% after tooth extraction of Wistar Rats. The number of fibroblasts found in the small category (less than 10% per field of view) on the administration of Andaliman fruit extract (Zanthoxylum piperitum) 40% after tooth extraction of Wistar rats as many as 8 (25%) heads and on the administration of Andaliman fruit extract (Zanthoxylum piperitum) 80% as many as 3 (9%) heads. The number of fibroblasts found in the medium category (10%-40% per field of view) on the administration of Andaliman fruit extract

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(Zanthoxylum piperitum) 40% after tooth extraction of Wistar rats as many as 4 (13%) heads and on the administration of Andaliman fruit extract (Zanthoxylum piperitum) 80% as many as 6 (19%) heads. The number of fibroblasts found in the many categories (40% - 80% per field of view) on the administration of Andaliman Fruit extract (Zanthoxylum piperitum) 40% after tooth extraction of Wistar Rats as many as 4 (13%) heads and on the administration of Andaliman Fruit extract (Zanthoxylum piperitum) 80% as many as 7 (22%) heads.

**Table 2.Relationship between the number of tissue fibroblasts per field of view in Wistar rats after tooth extraction with the administration of Andaliman Fruit Extract (Zanthoxylum piperitum) at a concentration of 40% and 80%.**

| Number of Fibroblasts | Fruit Andaliman (Zanthoxylum piperitum) | | |
|---|---|---|---|
| | Concentration 40% | Concentration 80% | p |
| No fibroblast tissue was found | 0 | 0 | 0,010* |
| Small number of fibroblasts (less than 10% per field of view) 3. | 8 | 3 | |
| Moderate amount of fibroblast tissue (10%-40% per field of view) | 4 | 6 | |
| A large amount of fibroblast tissue (40%-80% per field of view). | 4 | 7 | |

*Significant p<0.05. Chi-Square Test*

From Table 2. it can be seen that there is a significant relationship between the number of fibroblast tissue per field of view in Wistar rats after tooth extraction by giving Andaliman Fruit Extract (Zanthoxylum piperitum) with a concentration of 40% and Andaliman Fruit Extract (Zanthoxylum piperitum) with a concentration of 80%, p = 0.010 (p <0.05).

Tooth extraction is the process of removing teeth, both whole and the remaining roots, from the alveolar because it cannot be treated anymore. Tooth extraction will cause injury by exposing the alveolar bone in the oral cavity. The wound is anatomical damage or destruction of part of the tissue due to trauma (1). The body will repair tissue damage (harm), known as the wound healing process, and begins from injury until wound closure. The primary cells involved in the wound-healing process are fibroblasts. The proliferation of fibroblasts determines the outcome of wound healing. This is because fibroblasts will produce collagen that will link the wound and affect the revitalization process that will close the wound.

Rat tooth extraction will be performed under the anesthetic effect of ketamine 1000 mg/10 ml dose of 20 mg/kg bw intraperitoneally. After extraction, the post-extraction wound will be observed, and a tampon (cotton pellet) will be applied to stop bleeding in the damage for 5 minutes. Andaliman Fruit Extract (Zanthoxylum piperitum) 40% was given to treatment group I. Andaliman Fruit Extract (Zanthoxylum piperitum) 80% to treatment group II shortly after tooth extraction as much as 0.05 ml daily by dropping. On the 5th day, the rat jaw was taken and fixed with 10% formalin for 24 hours at room temperature. The decalcification process used the Ethylene Diamine Tetra Acetic Acid (EDTA 10%) solution at room temperature. The tissue was then dehydrated in a toluol alcohol solution (1:1) using the pure tool.

The fibroblast cell response was evaluated using Hematoxylin Eosin (HE) staining. Fibroblast density was assessed by counting the number of fibroblasts in 3 fields of view. The sample test was carried out on the fifth day because fibroblasts are known to start growing during the third to the seventh day of the wound healing process, so researchers took the average day, namely on the fifth day (Stojanovic et al., 2011). Based on Chi-Square data analysis, there is a significant relationship between the number of fibroblast tissue per field of view in Wistar rats after tooth extraction by giving 40% Andaliman Fruit Extract (Zanthoxylum piperitum) and 80% Andaliman Fruit Extract (Zanthoxylum piperitum), p = 0.010 (p <0.05). This is seen in the distribution of data on the number of fibroblasts (40%-80% per field of view) in Fruit Andaliman (Zanthoxylum piperitum) 80% as many as eight samples and in Fruit Andaliman (Zanthoxylum piperitum) 40% only four pieces. The results of this study are supported by Shasti (2017), which states that Andaliman Fruit Extract, with a concentration of 8%, has the highest clear zone against the growth of S.aureus bacteria. In addition, the antibiotic effect of Andaliman Fruit extract at all concentrations was not significantly different, while cefotaxime with Andaliman Fruit extract at all concentrations had significant inhibition (8). Andaliman fruit has the potential to be an antioxidant and glucosidase inhibitor. Andaliman is a spice widely used by the Batak community as a seasoning. Fruit andaliman has the potential to be an antioxidant and glucosidase inhibitor. The extract of andaliman fruit has the best antioxidant activity, with IC50 reaching 30.04 ppm. Fraction C (IC50 16 ppm) has acted as the most active glucosidase inhibitor and also contains flavonoid compounds of the around and flavanone group, which are the most active compounds as glucosidase inhibitors (9).

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Anggraeni's research (2020) stated that andaliman simplistic contains 7.32% moisture content, 13.62% water-soluble juice content, 29.54% ethanol-soluble juice content, 4.80% total ash content, 0.26% acid-insoluble ash content. In addition, phytochemical screening results show that andaliman simplistic contains alkaloids, flavonoids, glycosides, saponins, tannins, and steroid/triterpenoid compounds (7). Saragih's research (2019) states that the observations made show that the seeds of andaliman contain active chemical compounds that can function as ingredients for treatment. The active chemical compounds in andaliman seeds include phenolics, saponins, flavonoids, tannins, triterpenoids, and alkaloids. These secondary metabolite compounds have antibacterial, antimicrobial, antiviral, and protein denaturing and prevent bacterial growth in digestion. Therefore, knowledge of the content of these active chemical compounds can be used as a basis for further utilization of andaliman seeds as a remedy for other diseases (10). From the results of this study, it can be seen that 80% Andaliman Fruit (Zanthoxylum piperitum) extract is more effective in the wound healing process than 40% Andaliman Fruit (Zanthoxylum piperitum) extract because the higher the concentration of the section, the content in the Andaliman Fruit (Zanthoxylum piperitum) extract is also higher so that the wound healing process is faster.

## IV. CONCLUSION

Based on the results and discussions carried out in this study, it can be concluded that andaliman Fruit Extract (Zanthoxylum piperitum) 40% and 80% effectively accelerate wound healing time after tooth extraction of Wistar rats.

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