The sirangak (*Cyanthillium cinereum*; Asteraceae) oil accelerates sliced-wound healing by enhancing the hematological endurance in male albino mice

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Abstract. Sirangak (*Cyanthillium cinereum*; Asteraceae) is a medicinal plant traditionally used by Minangkabaunese, West Sumatra to heal the wounds. However, the underlying mechanism of this plant in healing the wounds is scientifically unelucidated. This current research aimed to clarify that Sirangak could potently accelerate the wound healing by enhancing the hematological performances. We conducted an experiment by using adult male mice consisted of control group and Sirangak oil-topical treated group after being wounded by superficial slice cutting. Subsequently, the wound healing rate was determined and the hematological profiles were monitored periodically for a week. The results demonstrated that Sirangak oil could significantly accelerate the wound healing by 85.6% as compared to control with a 71.6% of wound recovery. The hematological analysis indicated that Sirangak oil could significantly increase the erythrocyte count, hemoglobin concentration, hematocrite, mean cell volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) particularly during the early day of treatment. However, Sirangak oil did not significantly affect the leucocyte profiles except for the granulocyte. Therefore, the Sirangak oil could potently accelerate wound healing by enhancing the physiological endurance particularly the erythrocyte and hemoglobin level. This finding underpins a scientific evidence for further use of Sirangak in medicine.

1. Introduction
Lifestyle that leads back to nature (*back to nature*) proves that something that is natural does not mean slums or obsolescence. Many researchers involved in the world of modern medicine practices are currently studying traditional wisdom in using medicinal plants. Various species of medicinal plants have been studied scientifically. The results also support the facts and evidence that medicinal plants do contain substances or compounds that are clinically proven to be beneficial to health [1,2]. Since a long time, the people of Pauh V Padang District (SUMBAR) have been used the Sirangak (*C. cinereum* (L.) H.Rob.) plant in healing various kinds of wounds. This plant grows wild around the environment. Sirangak (*C. cinereum* (L.) H.Rob.) plants can heal wounds which the criteria is the plant should be about 30 cm height because if the plants used have a large size then the work of the drug is no longer good.
Various tribes use plants as ethno-medicine ingredients with unique ingredients and ways of presentation that show the high knowledge of local ethnicity about medicinal plants \[3,4\]. For Pauh people, the way to make wound medicine from Sirangak plant is by frying the plant in a crock with sufficient cooking oil and leaving it until the plant is completely fried. Then the oil obtained is applied to the part that is not too close to where the wound occurred. The oil produced by the Sirangak plant can last a long time by heating it every time you want to use it. Based on the information on the use of Sirangak plant in traditional people, indicating that this plant has potential to be developed as a modern medicine.

Medicinal plants that contain active compounds for treatment are thought to have special secretory structures as a producer of secondary metabolites \[5\]. States that secondary metabolites found in extracts of roots, stems and leaves in *Cyanthillium cinereum* (L.) H.Rob. plant such as phenols, terpenoids, quinones and steroids \[6\]. Other studies have also been carried out by \[7\], which proved that in *Cyanthillium cinereum* fresh leaf extract contains secondary metabolites such as alkaloids, phenols, tannins, saponins, steroids, glycosides, flavonoids, carbohydrates, phlobatannins and terpenoids. Scientific information about the efficacy of plants *C. cinereum* in the form of oil is still very limited. For this reason, further research is needed on the test of the efficacy of Sirangak oil in accelerating the healing of wound. In this case with the aim that making Sirangak oil is easier than extracting and does not require much cost. In addition to the two reasons above, a very important reason is that the use of medicinal plants has no side effects as long as they are uses it as directed.

2. Material And Methods

2.1. Provision of test animals

Test animals in the form of adult male white mice strain Balb/C (2.5 months old with a weight of 25-30 grams) obtained from U.D Peternakan Wistar Yogyakarta. Mice are kept in mice cages coated with sawdust, then fed with pellet birds and *ad libitum* water. Before treatment, mice were acclimatized in the laboratory at room temperature (± 27 ° C) and normal irradiation period (12 hours dark and 12 hours bright). The procedure for providing these test animals refers to \[8\].

2.2. Provision of Sirangak oil (C. cinereum (L.) H.Rob.)

Plant samples used are fresh samples. Plants are taken from the tip of the plant bud until the roots. Plants are washed clean. Then heat the cooking oil using a can. Furthermore, the plant is fried with the heated oil until it’s getting black. After that, the oil from the Sirangak plant is ready for use. The way to use it is by applying oil is not in the place where it was injured but about 15 cm from the wound site.

2.3. Conditioning wounds in mice

Before being injured, the hair around the back is shaved with a diameter of 3 cm and cleaned with 70% alcohol. Mice are anesthetized using 10% ether with an inhalation pathway. The sling was done on the back of a mouse with a length of 2 cm and a depth of 2 mm using a sterile scapel no. 11 \[9\].

2.4. Calculation of Wound Healing Percentages

Cuts in mice were taken one by one. The photos were quantified by measuring the area of the wound area. Measurements were made with the *Macbiophotonic Image J* program \[10\]. The measurement results are in the form of a wound area in units of cm \(^2\). The area of the wound area is then converted into a form of wound healing percentage with the formula \[11\].

\[
P\% = \frac{do - dx}{do} \times 100\%
\]

\(P\%\) = Healing Percentage
\(do\) = Initial wound area
\[ dx = \text{Final wound area} \]

2.5. Hematological Measurements

Blood sampling of mice was carried out on the 1st, 4th and 7th day through the heart using a syringe and accommodated in a 1 ml tube of \textit{Ethylenediaminetetraacetic acid} (EDTA). Blood hematology examination was performed using a hematology analyzer at the Animal Hospital Laboratory, Padang City \cite{12}.

2.6. Data analysis

Data is presented in the form of mean ± SE. The Student t-test was used to test the significance of differences between treatment groups where if the P value < 0.05 then considered as significant.

3. Results And Discussion

Based on the results of the research that has been carried out, it is proven that the Sirangak oil can significantly accelerate the healing of the wound than the control. Shown in Figure 1, where the percentage of wound healing in the treatment group was higher than in the control group (85.6% vs 71.6%; \( P < 0.05 \)) after one week of treatment.

\textbf{Figure 1.} The speed of sliced-wound healing in mice treated with sirangak oil as compared with the control. Description: *) significant \((p < 0.05)\). \(n = 4\) for each treatment group. \(A1\) = picture of the initial wound condition in the control group, \(A2\) = wound in the post-treated control group, \(B1\) = the initial wound condition in the sirangak oil-treated group, \(B2\) = the post-treated wound of the sirangak oil group.

The high percentage of wound healing shows that healthy individuals have the natural ability to protect and restore themselves \cite{13}. At the end of the observation the treatment of Sirangak oil (Fig. 1B2 ) illustrates that the area around the wound has begun to grow new hair. Growth of hair in the area of the wound indicates the regeneration process and the skin condition has begun to return to normal \cite{14}. The wound process closes after the wound has undergone a scabby release process. This indicates that new cells have grown with the edges of the wound. The scab process is released where the tissue underneath is dry and the edges of the wound are drawn to the center \cite{15}.

The speed of wound healing in the treatment group is likely to be caused by substances contained in the preparation given. Plant \textit{C. Cinereum} contains secondary metabolites which can accelerate the healing process of the wound, one of which is saponin. Previous research, saponin compounds can increase collagen synthesis in skin fibroblast in wound healing process \cite{16}. Apart from saponins,
plants *C. cinereum* also has a secondary metabolite tannin type. Tannin is able to inhibit mucosal fluid hypersecretion and neutralize inflammatory proteins. Tannin has an affinity for protein so it can be concentrated in the area of the wound [17].

**Figure 2.** The average number of leukocytes, lymphocytes, monocytes and granulocytes in mice suffering from cuts in the control group and the treatment group with Sirangak oil. Description: *) significant (*p* <0.05). n = 4 for each treatment group

Furthermore, the results of hematological analysis of the components of leukocytes (total leukocytes, lymphocyte counts, monocytes and granulocytes) showed an unsignificance between the control group and the treatment group with Sirangak oil (Figure 2) except the percentage of granulocytes. Sirangak oil does not increase the quantity of leukocytes, lymphocytes and monocytes but tends to stabilize the quantity of leukocytes, lymphocytes and monocytes after injury. Leukocytes and their differentiation function as body defenses for mice. White blood or leukocytes are one of the most active blood cells moving from the body's defense system [18,19]. White blood cells function recognize and fight microorganisms on immune reactions and helping the process inflammation and healing. The irrelevance of the dynamics of leukocyte changes in the mice of the treatment group indicated that the Sirangak oil did not modulate the body's defense or immunity component in healing the wound [20].
Figure 3. The average number of erythrocytes, hemoglobin, MCHC (Mean Corpuscular Hemoglobin Concentration), Hematocrit, MCV (Mean Corpuscular Volume), RDW (Red Cell Distribution Width) in mice suffering from cuts in the control group and treatment with sirangak oil. Description: *) significant (p <0.05). n = 4 for each treatment group

The results of the analysis of the erythrocyte profile and hemoglobin indicate a significant difference between the treatment groups compared to the control group (Figure 3). From the graph of the quantity of erythrocytes it is known that erythrocytes have increased after the wound is cut which is treated with Sirangak oil. Production of the quantity of erythrocytes also increases, such as hemoglobin, hematocrit, MCHC, MVC and RDW after injury. The quantity of erythrocytes is very functional in the case of oxygen supply homeostasis for body cells. Erythrocytes are cells that are very important for living things because of the hemoglobin content in them. In physiological conditions, erythrocytes are always in the blood vessels so they can carry out their functions as oxygen carriers [21]. Sirangak oil also increases hemoglobin (Hb) levels from day 1 to day 7, to defend the durability of the mice body. This hemoglobin functions as regulating the exchange of oxygen with carbondioxide in
the body's tissues and taking oxygen from the lungs and then taking it to the whole body to be used as energy material [22].

The erythrocyte component is thought to play an important role as a mechanism for accelerating healing of wound lesions in mice treated with Sirangak oil. An increase in erythrocytes and hemoglobin is very good for defending the body condition so that the body won't lack of blood. *C. Cinereum* proves that inhibit free radical oxidative damage and related degenerative diseases involving metabolic stress, genotoxicity and cytotoxicity. This extract is also likely to inhibit oxidative damage in erythrocytes. However, further studies are needed to confirm this assumption [23].

4. Conclusion
This study proves that Sirangak oil could accelerate wound healing and defend the hematological stability, especially the components associated with erythrocytes and hemoglobin.

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