

# INVESTIGATING the EFFECTIVENESS of LEAF ETHANOL EXTRACT GEL PREPARATION RED SPINACH (*Amaranthus tricolor* L.) in HEALING of BURNS on RATS MALE WHITE (*Rattus norvegicus*)

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## ABSTRACT

Red Spinach (*Amaranthus tricolor* L.) has many health benefits, one of which is in the healing process of burns. The aim of this research was to determine the effectiveness and concentration of ethanol extract of Red Spinach leaves (*Amaranthus tricolor* L.) which is effective in the healing process of burn wounds in male white rats (*Rattus norvegicus*). The research method used is experimental. The results of the phytochemical screening of the ethanol extract of red spinach leaves contain flavonoids, alkaloids, saponins and tannins. The effectiveness test in the healing process of burn wounds used 25 experimental animals which were divided into 5 treatment groups, namely negative control group (HPMC base), positive control (Bioplacenton), and extract group with concentration variants of 5%, 10%, and 20%. Observation of the burn wound healing process was carried out by measuring the diameter of the wound on days 2, 4, 6, 8, 10, 12, 14 where the average diameter was calculated as the percentage of burn wound healing. Statistical analysis was carried out using the One Way Anova test with a significant value of p: 0.000 and continued with the LSD test. The results of the study stated that 70% ethanol extract of red spinach leaves had activity in the healing process of burn wounds in male white rats. The concentration dose that was most effective in healing burns in male white rats was 70% ethanol extract of red spinach leaves with a 20% concentration.

**Keywords :** Burns, Diameter of burns, Ethanol Extract, Male White Rat (*Rattus norvegicus*), Red Spinach Leaves (*Amaranthus tricolor* L.)

## INTRODUCTION

Plants are a very diverse and abundant biodiversity. The biodiversity of medicinal plants in Indonesia is a natural resource that has the potential to be utilized and developed by the community as raw materials for traditional medicine (Wasito, 2008). This encourages researchers to make more use of native Indonesian plants. One of the plants in Indonesia that is used as medicine is red spinach (*Amaranthus tricolor* L.).

In research by Ruswanti et al, (2014), it is stated that the flavonoid compound contains anti-inflammatory properties so it can reduce inflammation and help reduce pain if bleeding or swelling occurs. Burns (combustio) are damage and/or loss of tissue caused by contact with heat sources such as water, fire, chemicals, electricity, and radiation. The healing process of burn wounds on the skin is a complex system which is a combination of cellular and extracellular components (Dewantari et al., 2015). The speed of wound healing can be influenced by the substances contained in the drug given, this drug has the ability to stimulate faster growth of cells in the skin (Balqis et al., 2014).

Wound healing is a form of effort to repair the damage that has occurred. The main principle of treatment is to cool the burned area or reduce inflammation, prevent infection and give remaining epithelial cells the opportunity to proliferate and close the wound (Wirastuty, 2016). The wound

healing process consists of 3 phases, namely inflammation, proliferation and maturation (Nurani, 2015). The inflammatory phase is characterized by rubor (redness), calor (heat), tumor (swelling), dolor (pain), and functio laesa (loss of function). The proliferation phase is the phase of wound healing which is characterized by the process of reepithelization, fibroblast proliferation, angiogenesis and wound contraction (Wibawani et al., 2015). The maturation phase is the last and longest phase in the wound healing process. A dynamic process occurs in the form of collagen remodeling, wound contraction, and scar maturation. At the end of this healing, a mature scar is obtained which has 80% strength of normal skin (Qomariah, 2014).

Red spinach (*Amaranthus tricolor* L.) contains vitamins, proteins, carbohydrates, fats, minerals, iron, magnesium, manganese, potassium and calcium (Farisyah et al, 2014). The presence of secondary metabolite compounds in red spinach, namely alkaloids, saponins, flavonoids, tannin can be used as a source of antioxidants that can inhibit free radicals and cure burns (Septiningsih, 2008).

In Ainul's (2017) research, it was stated that 70% ethanol extract of kitolod leaves had activity in healing burns in mice, while research that 70% ethanol extract of red spinach leaves could be used as a medicine for healing burns had never been carried out, so this encouraged researchers to utilize natural ingredients as a medicinal ingredient by testing the effectiveness of an ethanol extract gel preparation of red spinach leaves (*Amaranthus tricolor* L.) on healing burns in male white Wistar rats (*Rattus norvegicus*).

The saponin compound in red spinach leaf extract is able to heal burn wounds by triggering the formation of collagen, a protein structure that plays a role in the wound healing process (Wandani, 2009). Apart from that, red spinach leaf extract also contains flavonoid compounds. According to Fitri (2015), flavonoid compounds have antibacterial activity which can be used to prevent bacterial infections in wounds.

## **METHODS**

The type of research used is experimental in the laboratory.

### **A. Tool**

The tools used in this research were analytical scales, macerated chocolate bottles, measuring cups, beakers, blenders, horn spoons, porcelain cups, water baths, test tubes, test tube racks, test tube clamps, glass funnels, stirring rods, dropper pipettes, measuring pipette, vial, micropipette, oven, desiccator, spatula, filter paper, sieve.

### **B. Material**

The materials used in this research were green spinach leaves taken from Kedungwaru District, Tulungagung and determined at UPT Materia Medika, Batu Malang City, Wistar strain white rats, 70% ethanol, distilled water, Mg powder, concentrated HCl, aquadest, FeCl<sub>3</sub> 1%, n-hexane, acetic anhydride, concentrated sulfuric acid, ether type anesthetic, bioplacenton, HPMC (gel base).

### **C. Research Variable**

Research variables include independent and dependent variables. Independent variables, also called independent variables, influence variables, stimulus variables, input, predictors or antecedents, are variables that cause the emergence or change in the dependent variable (Supardi et al., 2014). The independent variable referred to in this research is 96% ethanol extract of red spinach leaves (*Amaranthus tricolor*) with 3 different dosage concentrations, namely 5%, 10%, 20%. The dependent variable is also called the dependent variable, effect variable, response variable, output, consequent, is a variable that is a result of the existence of an independent variable (Supardi et al., 2014). The dependent variable in this study was measuring the diameter of the burn wound and calculating the percentage of burn healing.

**D. Data Collection Procedure**

**1. Taking Red Spinach Leaves and Making Red Spinach Leaf Simplicia**

Fresh red spinach leaves that have been taken are determined at UPT Materia Medika Batu Malang. Then washed with running water and dried in the air and protected from direct sunlight until dry. Next, the cherry leaves are crushed using a blender. The ground powder is stored in a glass container, tightly closed, protected from sunlight and at room temperature.

**2. Making Red Spinach Leaf Extract**

Ethanol extract of cherry leaves was carried out using the maceration method with 2,000 ml of 70% ethanol solvent. A total of 500 grams of dry cherry leaf powder was put into a vessel and then soaked in 70% ethanol solvent. The vessel was closed tightly and left for 5 days, stirring occasionally. After five days of filtering, the filter liquid is replaced with new solvent and macerated again until the simplicia is completely filtered. The filtrate that has been obtained is collected then evaporated using a rotary evaporator and concentrated using a water bath to obtain a thick extract. Next, it is weighed and the yield obtained is recorded.

**3. Preparation of Test Animals**

This research used experimental animals of the Wistar strain of white rats, weighing around 150 grams. Before treatment, the mice were adapted for 1 week in a laboratory atmosphere. The experimental animals were given enough food and drink every day and did not show any weight loss. The distribution of experimental animals was carried out randomly, namely 5 mice per treatment group.

Negative Control Group : Therapy with HPMC gel base

Positive Control Group : Therapy with Bioplacenton

Concentration Group : Therapy with red spinach leaf extract gel preparations in concentrations of 5%, 10% and 20%.

**4. Making Red Spinach Leaf Ethanol Extract Gel**

The first way to make a red spinach leaf extract gel preparation is to make an HPMC gel base by weighing 1.4g of HPMC, then developing it in distilled water with 10 times the amount of HPMC in a mortar. Leave it for 20 minutes and grind until homogeneous so that a gel mass is formed. Next, add methyl paraben to the gel mass, grind until homogeneous, then weigh 1g of red spinach leaf extract and put it in the mortar little by little while grinding until homogeneous.

**5. Creation of Burns**

The initial stage is that the hair on the rat's back is shaved to a diameter of 10 mm and disinfected using 70% alcohol. Next, they were anesthetized using a lidocaine pump spray on the rat's shaved back. After that, a burn wound was made on the skin of the rat's back using iron metal with a diameter of 10 mm and had been heated in a Bunsen fire for 1 minute and then placed for 5 seconds on the skin of the rat's back until a shallow second degree burn was formed, which was marked by the presence of color. reddish and bullae form. Rats that had

been induced with a heat source on their back skin were each given treatment based on their group. Treatment is carried out by observing the healing process of burn wounds from days 1 to 14 once a day. Burn wounds are treated openly until they heal, which is indicated by the wound closing and closing.

## RESULTS

### Test of the Activity of Ethanol Extract of Red Spinach Leaves on Healing Burns in Male White Rats

In testing the effectiveness of ethanol extract of red spinach leaves (*Amaranthus tricolor L.*) on healing burns, the aim was to determine the effect on reducing the diameter of burn wounds and the percentage of wound healing which was carried out experimentally using test animals of male white rats (*Rattus norvegicus*). The research design was to divide 25 mice into 5 treatment groups, namely a positive control given bioplacenton therapy, a negative group given HPMC gel base and three treatment groups with varying concentrations of 5%, 10% and 20% red spinach leaf extract in gel preparation. This study used Bioplacenton gel as a positive control because it contains 10% placenta extract which works to trigger the formation of new tissue and for wound healing, and contains 0.5% neomycin sulfate to prevent or treat infections of gram-negative bacteria in wounds (Rahayu, 2016).

## DISCUSSION

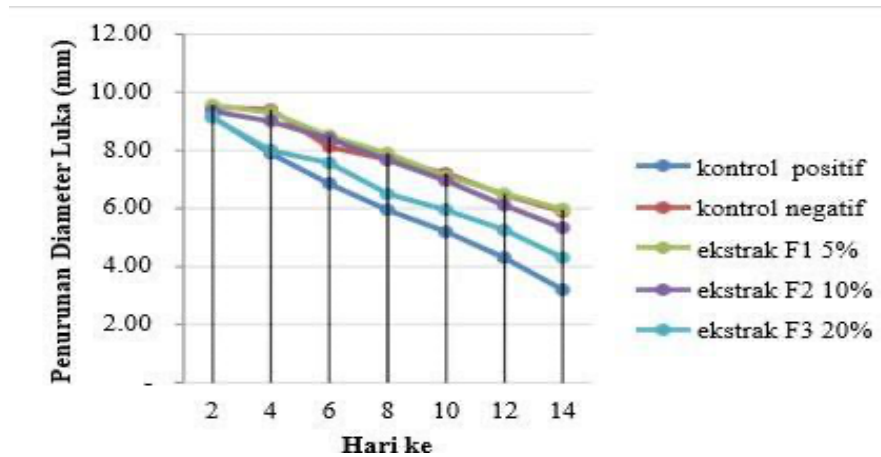
Rat anesthesia uses lidocaine in the form of a spray to temporarily relieve pain during the process of making wounds. Burns are made with 10 mm iron metal which is heated with a Bunsen flame for 1 minute then placed for 5 seconds on the back of a rat whose fur has been shaved and has been previously anesthetized. Skin affected by high- degree burns will experience blistering and damage to the epidermis tissue and parts of the dermis, reddening and peeling of parts of the skin. The burn wounds that have been created are then given therapy according to the treatment group.



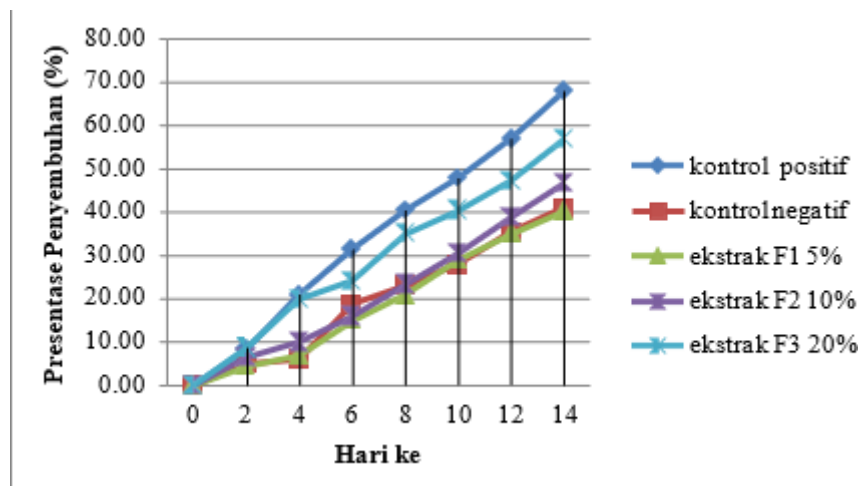
The treatment on mice was carried out once a day for 14 days. To determine the activity of healing burns and the effect of the concentration of ethanol extract of red spinach leaves, the diameter of burn wounds was measured on all groups on days 2, 4, 6, 8, 10, 12, and 14.

Within 14 days, it covers the healing phase of burn wounds, namely the inflammatory phase on day 0 or the day when the wound was made until day 4, then continued with the proliferation phase which started on day 3 and in this study on day 8 it was Scab peeling began to occur in several treatment

groups, which was then continued with the remodeling phase which actually took a long time, namely up to 20 days.



The graph above shows that there is a decrease in the diameter of the burn wound on the rat's back. This can be observed from the diameter of the burn wound which gets smaller when measurements are taken on days 2, 4, 6, 8, 10, 12 and 14. The diameter measurement data obtained is then analyzed statistically using SPSS. Statistical analysis of burn wound diameter measurement data includes the One Way Anova test and if there is a significant difference then a further test is carried out using the LSD (Least Significance Different) test. The results of data analysis of bum wound diameter measurements on the 14th day obtained from the One Way Anova test showed a significance value of  $p < 0.05$ , namely 0.000, which means that there were significant differences in each treatment group. To determine whether there were significant differences in each treatment group, the LSD (Least Significance Difference) test was continued. Based on the results of the LSD test, it can be concluded that there was one treatment group that showed no significant difference, namely the negative control group versus the 5% concentration group with a p value = 0.765.



The diameter measurement data was then converted into a percentage to see the percentage of burn wound healing produced by the 70% ethanol extract of red spinach leaves. Based on Table 4.9, the results of the average percentage of burn wound healing show that the 20% concentration has a higher percentage than the other groups. The next data analysis was carried out by the One Way Anova test with a p value <0.05, namely 0.000, which means that the percentage of healing of burn wounds on the 14th day was significantly different. Statistical analysis was then continued with the LSD (Least Significance Different) test to determine whether there were significant differences between one group and another. Based on the results of the LSD test, it shows that there is no significant difference between the negative control group and the 5% concentration group with a p value = 0.772. The LSD test results showed that there was a significant difference in the 20% concentration group compared to the positive control group, negative control, 5% concentration group and 10% concentration group. This shows that the 20% concentration group has a significant difference to the other groups with the highest percentage value among the other concentration groups.

Based on the results of observations on day 0, a second degree burn with a diameter of 10.00 mm was formed and the percentage of wound healing was still 0% with damage to the skin after the wound was applied including the epidermis and part of the dermis in the form of an inflammatory reaction accompanied by an exudation process which made the wound red and humid (Anggowarsito, 2014). Descriptive results from observing burn wounds based on color, time to dry the wound, scabs, and new hair growth can be seen in Appendix 8, in the inflammatory phase starting from day 0 to day 4. On day 4, the diameter of the burn wounds on the mice began to decrease, where the positive control group and the 20% concentration group had faster diameter reduction.

## CONCLUSION

The conclusion of this research is that the ethanol extract gel preparation of red spinach leaves (*Amaranthus tricolor* L.) is effective in healing burns in male white rats. The ethanol extract gel preparation of red spinach leaves (*Amaranthus tricolor* L.) was the most effective in healing burns in male white rats in the 20% concentration group.

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