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Potential Activity of Serunai Leaf Ethanol Extract (*Chromolaena Odorata L.*) Against the Healing of Burns

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Abstract

Burns is tissue damage caused by heat, excessive exposure to sunlight or other radiation, or chemical or electrical contact. Wounds are a problem that everyone often experiences, especially burns, so they need to be addressed. Empirically, the plant trusted by the community as a wound medicine is the serunai leaf. In South Sulawesi, serunai leaves are believed to heal wounds. This study aimed to determine whether there was an effect of healing burns on the backs of mice by administering ethanol extract of Serunai leaves (*Chromolaena odorata L.*). The research conducted is experimental. The object under study is the process of healing burns on the back of male white mice. The study was conducted on 25 male white mice aged 2-3 months with an average body weight of 20-40 grams, divided into five groups of test animals, each group consisting of 5 male white mice based on the Federer formula. The first group was given positive control (+) branded ointment, and the second group was given negative control (-) vaseline flavum, the third group was given ethanol extract of Serunai leaves with a concentration of 5%, the fourth group was given ethanol extract of Serunai leaves with a concentration of 10%. The fifth group was given ethanol extract of serunai leaves (the thick extract). Serunai leaves with a concentration of 20%, and each surface on the back of the mice was covered and calculated using AUC and analyzed by the One Way Anova test. The results showed that the ethanol extract of serunai leaves had the potential for healing burns. Each concentration has a burn healing power of 5% (48.76%), 10% (60.34%), and 20% (67.54%).

Keywords: Ethanol extract of serunai leaves (*Chromolaena odorata L.*), Animal test.

Introduction

Five compounds play a role in wound healing, namely germacrene D compounds and trans (beta)-caryophyllene compounds which belong to the sesquiterpene terpenoid group, which functions as antioxidants that can bind unstable free radicals that can damage cell membranes, cadinene compounds have antimicrobial and antifungal effects and antibiotics , hexadecanoic acid (CAS) palmitic acid is a derivative of saponin compounds which have anti-inflammatory and antifungal effects by destroying the cell wall structure of fungal membranes and octadecatrienoic acid methyl ester compounds are fatty acids and function as anti-inflammatory agents [(1)]. Several studies related to serunai leaves, including Dewi [(2)], stated that the cream with ethyl acetate fraction of the ethanolic extract of the leaves of *Chromolaena odorata L.* gave inhibitory activity to S. aureus bacteria, respectively 12.52mm, 13.90mm, and 12.42mm. In bacteria, E. aeruginosa showed the killing activity of 13.21 mm, 13.70 mm, and 12.49 mm, respectively. In E. Coli bacteria, the killing power was 13.46 mm, 13.30 mm, and 13.18 mm, respectively. Yutika [(3)], in his research results, showed inhibitory activity of the leaf extract of *Chromolaena odorata L.* against gangrene wounds. The category of the inhibitory power of the extract is in the medium category (5-10mm). Vijayaraghavan [(4)], in his research results, stated that the concentration of the *Chromolaena odorata L.* extract for excision wound healing was 5.0% (w/w). Chromolaena odorata -treated groups exhibited a faster reduction in wound area than control and Betadine-treated groups. Oso's research [(5)] *Chromolaena odorata L.* has potential therapeutic value in terms of antioxidant potential, commonly used in Nigeria for first aid and wound healing. The antioxidant potential was evaluated by measuring its ability to scavenge radicals, reduce oxidized iron and quench lipid peroxide formation. The results of Ernawaty's study [(6)] showed that the leaf juice of *Chromolaena odorata L.* had antimicrobial activity against the growth of Candida albicans and Pseudomonas aeruginosa with a strong category.

Helmidanora's research [(7)] stated that the ethanol extract of serunai leaves has potential effectiveness as an analgesic against white male mice (p 0.043 > 0.05) with the percentage of analgesic power at a dose of 50 mg/kg body weight (28.5%), a dose of 100 mg/kg. kg body weight (58.8%), dose 200 mg/kg body weight (67.3%). Komala's research [(8)] explains that the best antibacterial activity in the ethyl acetate fraction and ethanol 96% extract at a concentration of 20% with the width of the zone of inhibition values of 4.375 mm and 4 mm. This is reinforced by Fadia's [(9)] research that the ethanol extract of *Chromolaena odorata L.* leaves has an inhibitory effect on the growth of Salmonella typhi and Staphylococcus aureus. *Chromolaena odorata L.* leaves have the potential as herbal medicine against bacterial infections. Rasyid [(10)] *Chromolaena*. *odorata L.* leaf at a concentration of 100% were produced inhibition zones of 0.6 cm and 0.8 cm, respectively, against S. aureus. Yenti [(11)]

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found that the evaluation formulation results showed physically stable and provided a healing effect on the wound. The results showed that the formula cream with a concentration of 10% on the 12th day, with a 100% percentage of wound healing. Pandith [12] described *Chromolaena odorata L.* ethanol extract (70%) from the dried leaves proved to be the extract producing the highest hemostatic activity in vivo with the bleeding time of 1.85 min. The results of Sangnim's research [13] showed the 20 mg/mL herbal extract-loaded Liquid Plaster provided an antibacterial effect with admissible water vapor transmission and low skin irritation. As a result, the study demonstrates the possibility of introducing the *Chromolaena odorata L.* extract-loaded Liquid Plaster to increase the effectiveness of wound healing and the antibacterial effect on the skin. Sirinthipaporn [14] in Wound Healing Property Review of Siam Weed, *Chromolaena odorata L.* explains that *Chromolaena odorata L.* is a traditional medicinal plant widely used for its wound healing property. In particular, several parts of this herb have been used to treat wounds, burns, and skin infections.

From the research that has been done regarding Chromolaena odorata L. no one has conducted a research related to burns, so this research was carried out as a support for medicinal plants used as burns. The purpose of this study was to determine the activity of leaf extract of serunai (Chromolaena odorata L.) on the healing of burns on the backs of male white mice.

Materials and Methods

Chemicals and Drugs

70% ethanol solvent obtained from warehouse chemicals of College of Sekolah Tinggi Ilmu Kesehatan Samarinda, Vaseline Flavum, and branded ointments obtained from drugstore.

Plant materials

The plant was collected from the embun suryana street, lestari indah permain housing, sambutan village, North Samarinda district, and East Kalimantan. Samples were taken directly from plants located not far from residential areas. The plants were then identified at the Plant Physiology Laboratory, Faculty of Mathematics and Natural Sciences (F-MIPA), Mulawarman University Samarinda. Determination of plants is done to ensure the type and correctness of plants.

Extraction of the plant

The selection of extraction aims to obtain extracts from medicinal plants. Maceration is a procedure for extracting medicinal plants in this case in the form of fine powder from serunai leaves. Maceration using 70% ethanol solvent as much as 2 liters. The serunai leaf powder was weighed as much as 200 grams, put into a glass container then soaked in 70% ethanol until all ingredients were covered (1 liter) for 72 hours (3 days) and stirred using a stirrer or shaken from time to time until the extraction was complete. At the end of the extraction, the macerate was separated by filtering it using a Buchner funnel. Then re-macerated, the dregs were immersed in 1 liter of 70% ethanol in the same way as the initial maceration. The macerate is separated from the suitable solvent by evaporation over a water bath suitable for thermolabile plant material. The thick extract was then calculated the rendement and put into a storage bottle (ethanol extract of Serunai leaves).

Experimental animals

In this study, the test animals used are healthy male white mice aged 2-3 months with an average weight of 20-40 grams which will first be conditioned for 1 week with special control and treatment such as cage cleanliness and feeding and drinking. Regularly. The preparation of the test animals was carried out so that the mice could adapt to the new environment. Prepared a modified hot solder with a stainless plate measuring 0.5 x 0.5 cm^2. The hairs on the back of the mice were shaved according to the area to be wound, then induced by hot soldering onto the back of the mice for 2 seconds until the dermis and the tissue were bound so that they became blisters and peeled off the skin. All procedures conducted in this study have been approved by the Health Research Ethics Committee, Faculty of Medicine, University Mulawarman (ethical clearance number: 135/KEPK-FK/XII/2021).

Burn healing test

Prepared 25 male white mice, which were divided into 5 groups, with each group containing 5 male white mice:

Group I: (Positive Control) giving branded ointment Group II: (Negative Control) giving Vaseline flavum Group III: Giving ethanol extract of serunai leaves with a concentration of 5% Group IV: Giving 10% concentration of ethanol extract of serunai leaves Group V: Giving ethanol extract of serunai leaves with a concentration of 20%

Burn Treatment

Mice that had burns on the skin on their backs were given treatment based on their group, namely by routinely applying different preparations based on their group once a day. In addition, the cage's cleanliness and the food and drink consumed by the mice must always be controlled regularly. The burn healing process was observed from the 1st to the 14th day until the burns on the backs of the mice were covered. Burn changes were observed using millimeter block paper and measured using a caliper.

Results and Discussion

From 2500 grams of fresh Serunai leaves taken after drying, the dry leaf weight was 650 grams, and the thick extract was 40.74 grams with a rendemen of 20.37% (table 1). The size of the yield

value indicates the effectiveness of the extraction process. Important water content is determined to maintain the quality of the extract and prevent microbial growth. The smaller the water content in the extract can reduce the risk of the growth of microbes and fungi [15].

The effectiveness of the extraction process is influenced by the type of solvent, which is used as a filter for the simplicia particle size, the method, and the duration of extraction [16,17]. The temperature also plays a significant role in the extraction process. The higher the drying temperature, the lower the rendemen [18].

Table 1. Rendemen calculation

Simplisia	Thick Extract	Rendemen
200 Grams	40,74 Grams	20,37%

Testing the activity of the Ethanol Extract of Serunai Leaves against burns on the skin of the back of mice was carried out by mixing the ethanol extract obtained from the extraction with the base of Vaseline flavum ointment. The use of Vaseline flavum ointment base is widely used in the use of ointment bases in general, which are hydrocarbon (fatty ointment bases) so that they are not easily lost when exposed to water, will prolong the contact between the drug substance and the skin [19]. Hydrocarbon ointment base also acts as a bandage or cover that will inhibit water evaporation on the skin layer and soften the skin layer [20].

Adaptation of mice was carried out for 2 weeks, and this was intended to stabilize the situation, adapt to the new environment and control their health. Adaptation was carried out by feeding, drinking, and weighing the mice for 3 days before treatment using digital scales, and this aims to determine the health of the mice [21]. Tests were carried out simultaneously between a positive control group, a negative control group, and 3 variations of the serunai leaf ethanol extract concentration with Vaseline. In this study, 3 concentrations of serunai leaf ethanol extract were used, consisting of 5%, 10%, and 20%, all of which were made in 1 g of ointment stock for 2 days' supply. Each mouse was given a dose of 0.1 g one time rub. It is intended that the dose of ointment given from one mouse to another can be controlled so that the administration remains consistent.

The test animals were divided into 5 groups, where each group consisted of 5 mice based on the federer's formula {(t-1)(n-1) ≥15, description: t is number of test groups and n is sample size for each group}. The positive control group was smeared with branded ointment. The negative control group was smeared with vaseline flavum. The other three concentration variations were smeared with ethanol extract of serunai leaves with a mixture of vaseline flavum.The shaving of the mice was carried out before treating each test animal by using a hair clipper around the back of the mice and making an ointment to be applied to burns made by mixing the ethanol extract of serunai leaves and vaseline flavum with a predetermined dose. Wounds were made by attaching hot solder with a stainless plate measuring 0.5×0.5 cm2 on the back of the mice for 2 seconds previously smeared with antiseptic (alcohol swabs). The purpose of giving antiseptic was to inhibit the growth of microorganisms [22]. The ointment is given in the morning, and this is because male white mice are nocturnal animals (active at night), so to make it easier to administer in the morning, the ointment is applied to all treatments given once a day [23].

Table 2. Results of the Average Area of Burns

Day Number	Group I (cm^2)	Group II (cm^2)	Group III (cm^2)	Group IV (cm^2)	Group V (cm^2)
1	0,5	0,5	0,5	0,5	0,5
2	0,5	0,5	0,5	0,5	0,5
3	0,47	0,5	0,5	0,48	0,47
4	0,44	0,5	0,49	0,44	0,44
5	0,36	0,48	0,48	0,39	0,38
6	0,30	0,46	0,46	0,34	0,33
7	0,20	0,44	0,41	0,30	0,27
8	0,13	0,42	0,34	0,25	0,21
9	0,03	0,39	0,27	0,13	0,14
10	0,00	0,36	0,19	0,05	0,05
11	0,00	0,32	0,10	0,00	0,00
12	0,00	0,23	0,02	0,00	0,00
13	0,00	0,15	0,00	0,00	0,00
14	0,00	0,08	0,00	0,00	0,00

Information:Group I: Positive Control (n=5) , Group II: Negative Control (n=5) , Group III: Concentration of 5% Serunai Leaf Extract (n=5) , Group IV: 10% Serunai Leaf Extract Concentration (n=5) , Group V: Concentration of 20% Serunai Leaf (n=5)

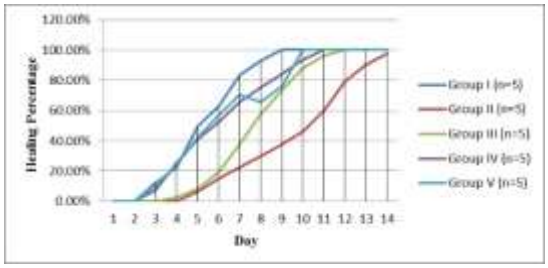

Figure 1. Graph of burn healing percentage

The study results found that the healing of burns with the highest percentage of healing was positive control (branded ointment), where the rate of healing reached 100% on the ninth day, followed by groups V, IV, and II. Bioplacenton ointment contains the main active ingredients, namely placenta extract 10% and neomycin sulfate 0.5%, where this placenta extract works to trigger tissue formation and to accelerate burn healing, while neomycin sulfate is an aminoglycoside antibiotic whose mechanism of action is to inhibit the growth and proliferation of bacteria. Groups V, IV, and III were treated with Vaseline flavum ointment base mixed with serunai leaf extract with a concentration of 20% and 10% closed wounds on the 11th (eleventh) day and 5% concentration on the 13th (thirteenth) day. Vaseline flavum is an ointment base that can inhibit the loss of water content from skin cells by forming a waterproof film so that it can retain skin moisture. Wound healing in the group with serunai leaf extract was faster than receiving only vaseline flavum, which until the 14th (fourteenth) day the wound was not completely closed. From the results of observations, serunai leaf extract can accelerate the healing of burns. This result is in line with other studies that state that serunai leaves have activity in curing wounds in rats [(24)].

From the analysis results with SPSS version 20, the Kolmogorov-Smirnov test, p-value > 0.05, which is 0.139, means that the data is normally distributed. These results continued with the One Way-Anova test and obtained a p-value < 0.005, namely 0.000, which means that the data had a significant difference in each treatment group and continued with the LSD test with a 95% confidence level. Based on the results of the LSD test, it can be concluded that group I (positive control) had better activity in healing burns compared to groups III, IV, and V (treatment group) and group II (negative control). The treatment group, namely group III (5%), group IV (10%), and group V (20%), had the same effect in healing burns where each group did not show a significant difference. So it can be said that serunai leaf extract with concentrations ranging from 5% has activity in healing burns.

Conclusion

Based on the results of the research above, it can be concluded that the serunai leaf (*Chromolaena Odorata L.*) has the potential to be used as a healing of burns.

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Ethics Statements

All procedures conducted in this study have been approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Mulawarman (ethical clearance number: 135/KEPK-FK/XII/2021).

Author Contribution

Rusdiati Helmidanora controlled experiments and data visualization and wrote the manuscript. Heri Wijaya assisted in designing experiments and proofed the data. Musakir assisted in conducting the experiments. All authors have read and approved the final manuscript.

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