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Histopathological overview of wound healing process in white rats (*Rattus norvegicus*) using Chromolaena odorata leaf jelly extract

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ABSTRACT

Background: Various efforts have been made to accelerate the wound healing process in white rats, one of which is using *Chromolaena odorata*. The leaves contain tannins, phenols, flavonoids, saponins, and steroids, which accelerate the wound healing process. The purpose of this study was to determine the effect of *Chromolaena odorata* leaf jelly extract on the wound healing process in the inflammatory and proliferative phases in white rats microscopically (*Rattus norvegicus*).
Methods: A total of 27 healthy adult male white rats aged 3-4 months old were given open wounds on the back measuring 2x2 cm. The rats were then divided into four groups, a group administered with jelly without *Chromolaena odorata* leaf jelly extract (P0) and groups administered with *Chromolaena odorata* leaf jelly extract with concentrations of 10% (P1) and 30% (P2), respectively. *Chromolaena odorata* leaf jelly extracts were given daily by rubbing them on the wound surfaces. Skin biopsies were collected and evaluated histopathologically on days 3, 7, and 14 post-treatment. The histological assessment of wound healing was based on epithelialization and collagen density using Nagaoka criteria. The data were analyzed by analysis of variance (ANOVA) and continued with the Duncan test.
Results: The Duncan test results showed that the mean score of the degree of epithelialization and collagen density of wound tissue on days 7 and 14 was significantly higher in the P2 group compared to the P0 and P1 treatments, while the P1 group was not significantly different from P0.
Conclusions: In conclusion, the administration of *Chromolaena odorata* leaf jelly extract could accelerate the degree of epithelialization and collagen density in the wound healing process of the inflammatory and proliferative phases of white rats (*Rattus novergicus*).

Keywords: *Chromolaena odorata*, collagen density, degree of epithelialization, wound healing process.
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INTRODUCTION

Wounds are defined as a loss of tissue continuity due to various reasons. The wound occurs when there is a mechanical, chemical, or thermal trauma to the tissue resulting in the opening or damage of the skin anatomy and an imbalance of normal skin anatomy and function.[1–3] This damage occurs in the epithelial integrity of the skin. It extends to the subcutaneous tissue, followed by damage to other structures, such as tendons, muscles, blood vessels, nerves, organ parenchyma, and bones.[4] The wound healing process will begin as soon as the damage occurs. However, the mechanism and speed of recovery depend on the type of wound.[4–6] Based on the estimated incidence of injuries, chronic wounds that occur in people aged 45-65 years are 120 per 100000 people, while those over 75 years old increase to 800 per 100000 people.[5]

Previous authors have worked to accelerate the wound healing process and studied it in various ways, including through traditional medicine using plants. *Chromolaena odorata* is a type of plant which has traditional medicinal properties and has been used from generation to generation for wound treatment. *Chromolaena odorata* is also knownas *Kirinyuh* (Sunda), *Lahuna* (Bugis) or *Sikhoh-khoh* (Aceh).[7] According to phytochemical test results, this plant contains several main compounds, such as polyphenols, tannins, saponins, terpenoids, flavonoids, alkaloids, and cyanogenic glycosides.[8–10] Saputra et al. showed that the fresh leaves of *Chromolaena odorata* contain ascorbic acid, phenolic compounds, α-tocopherol, and β-carotene, which function as antioxidants. Ascorbic acid also plays an important role in the formation of collagen structure to accelerate the wound healing

process.[11] Many studies have shown that *Chromolaena odorata* leaf extract can shorten bleeding time and speed up wound healing.[12] Yenti et al. proved that 10% of *Chromolaena odorata* leaf ethanol extract formulated in a cream base had a significant effect on an open wound healing in white male rats compared to a cream containing n 10% of povidone-iodine.[13] Furthermore, several other studies have shown that *Chromolaena odorata* leaf extract can inhibit the growth of *Staphylococcus aureus* found in wounds.[14] However, to date, there has been no study proving that *Chromolaena odorata* with different bases as jelly can accelerate the healing of open wounds, especially in the early phase. This condition encourages the author to study the effect of *Chromolaena odorata* leaf jelly extract on wound healing in the inflammatory and proliferative phases of white rats (*Rattus norvegicus*).

This study used jelly base because it is suitable for topical use since the characteristics are soft, gentle, easy to apply, able to maintain skin moisture, able to prolong the contact of medicinal substances, do not irritate the skin, and do not leave an oily layer on the skin so it will be easy to wash. In addition, jelly is soothing, moisturizing, easily penetrates the skin, providing a better healing effect.[15,16] The purpose of this study was to determine the effect of *Chromolaena odorata* leaf jelly extract on the wound healing process in the inflammatory and proliferative phases in white rats microscopically (*Rattus norvegicus*). The results of this study are expected to provide scientific information regarding the effect of *Chromolaena odorata* leaf jelly extract in the wound healing process of the inflammatory and proliferative phases of white rats (*Rattus norvegicus*).

METHODS

Study Design

This study was conducted using a pure experimental method in vivo with a Randomized Post Test Only Control Group Design at the experimental animal department and the Pathology Laboratory of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh.

Animal Model

A total of 27 healthy male white rats (*Rattus norvegicus*) Wistar strain, aged 4-5 months and weighing 180-200 grams were randomly divided into three groups, namely the control group (P0), a group of rats administered with jelly without *Chromolaena odorata* leaf jelly extract and groups of rates administered with *Chromolaena odorata* leaf jelly extract with a concentration of 10% (P1), and 30% (P2) respectively. The wound was done by anesthetizing the rats using a combination of ketamine 50 mg/kgBW and xylazine 5 mg/kgBW intramuscularly. Their back fur were shaved about 3x2.5 cm, and in that area, an incision was made using a scalpel with a length and width of 2x2 cm with depth to the subcutaneous part.

Before grouping, all rats were adapted for seven days at a constant temperature (20-25°C) with a 12-hour light-dark cycle for acclimatization and fed with 20 grams of pellets and ad libitum drinking. The rats were placed in a specially designed cage with plywood walls measuring 15x30x42 cm3 covered with gauze with a husk base that was changed every two days and equipped with a container for drinking water and feed.

The Preparation of *Chromolaena odorata* Leaf Jelly Extract

The fresh leaves of *Chromolaena odorata* were plucked and separated from the stems, then washed with water until they were clean and drained. After being drained, the leaves were cut into pieces and dried using an oven temperature of 50 to 600°C. Afterward, the dried leaves were blended to become a simplicia (powder). This extract was made using maceration in which the leaves were put into a small jar, added with 96% ethanol as a solvent with a ratio of 1:3, and soaked for 6 hours and occasionally stirred to speed up the diffusion process of secondary metabolites concentration into the solvent. The next step was soaking the leaves (without stirring) for 18 hours at room temperature. The separation of macerate was carried out by filtration using a flannel cloth three times with the same solvent (remuneration) to maximize the withdrawal of metabolites that may remain in the simplicia network. All the obtained macerates were collected and concentrated with a rotary evaporator at a solvent vapor temperature of ≥ 50°C to remove the solvent, producing the pure extract without solvent or viscous extract.

Chromolaena odorata leaf jelly extract was formulated into four concentrations; 0%, 10% and 30%, and blank (jelly base) with the formula listed in **Table 1**.

Histological Preparations

Wound samples were taken from three rats on the 3rd, 7th, and 14th days. Samples were taken 1-1.5 cm from the edge of the wound with a thickness of ± 3 mm. The sample was then fixed with 10% Buffer Neutral Formalin (BNF) solution and left at room temperature for ± 24 hours. The skin tissue was then cut along 4 mm processed with an automatic tissue processor in the following order; the tissue was put in 10% formalin (BNF) (I) for 1 hour, 10% formalin (BNF) (II) for 1 hour, 85% alcohol for 1 hour, 90% alcohol (I) for 1 hour, 90% alcohol (II) for 1 hour, absolute alcohol (I) for 2 hours, and absolute alcohol (II) for 2 hours. The subsequent step was the clearing process, where the tissue was put in xylol (I) for 2 hours, xylol (II) for 2 hours. Afterward, the infiltration process was done by putting the tissue in liquid paraffin (I) for 2 hours and liquid paraffin (II) for 3 hours. The next process was embedding the tissue into a "basic mold" or a mold filled with liquid paraffin

Table 1. The formulation of *Chromolaena odorata* leaf jelly extract

Ingredient	Formula and Composition (%b/v)		
	P0	P1	P2
Extract	0	10	30
Na-CMC	5	5	5
Glycerin	10	10	10
Propylene Glycol	5	5	5
Aquadest add	100	100	100

and attached to the embedding cassette until it got cool. A tissue block was then formed and cut using a microtome with a thickness of 4 µm. After that, the sheets of tissue were floated over the water surface in a water bath and taken by object glass with spooning motion.

Hematoxylin-Eosin (HE) Staining Procedure

Tissue staining began with a paraffin removal process (deparaffinization) using xylol for three times and 2 minutes for each, continued with rehydration using an alcohol solution with a decreased concentration (absolute, 95%, 90%, 80%, and 70%) for 5 minutes and then rinsed under running water for 10 minutes. Afterward, the tissue was stained with hematoxylin for 5 minutes and rinsed again with running water for 10 minutes. After that, the tissue was stained with eosin staining for 2 minutes and continued with a graded alcohol solution, cleaned with xylol, and ended by covering the sliding tissue with a cover glass (mounting process) using an Entellan® adhesive.

Assessing the Degree of Epithelialization and Collagen Density of Wound Tissue

The degree of epithelialization and collagen density of wound tissue was measured using the Optilab® with 4x objective lens magnification and the ImageRaster® with 400x photo magnification attached to the ocular lens of the light microscope. The preparations were observed under a light microscope at 400x magnification in five fields of zigzag view. Observations were made by using blinding methods. Subsequently, the results from these five fields were averaged. The description of the degree of epithelialization and collagen density is presented in the form of a microscopic assessment score according to the Nagaoka criteria, as shown in Table 2.

Data Analysis

This research used quantitative data, in which normality test (Saphiro Wilk test) and homogeneity test (Levene test) must be tested before the statistical tests. If the data is normally distributed and homogeneous, the one-way ANOVA statistical test can be done with a 95% confidence interval ($\alpha = 0.05$) to see whether there are the significant difference among the treatment groups. Subsequently, a Post Hoc analysis using the Duncan test to determine differences between the treatment groups is conducted.[18]

RESULTS

Assessment of the Degree of Tissue Epithelialization

Epithelialization degree of skin tissue histopathological view using HE staining was observed using an Olympus EX51 series microscope with 400x magnification. Observation in each group was done on days 3, 7, and 14 as presented in Figures 1, 2, and 3. These views show that there is a difference between the control group (P0) and treatment groups (P1 and P2). Epithelialization on day 3 did not show any significant changes but continued to gradually increase until it became apparent on day 14. The increase in the degree of thickness of the epithelialization of the wound tissue in the treatment groups (P1 and P2) was significant. Meanwhile, in the control group (P0), the increase was only moderate.

The average degree of epithelialization in the control group (P0) and the treatment groups (P1 and P2) on the 3rd, 7th, and 14th day after treatment can be seen in Table 1.

The normality and homogeneity test of the degree of epithelialization indicated that the data were normally distributed ($p>0.05$) and the variants were homogeneous ($p>0.05$). The statistical test using one-way ANOVA on days 3 and 7 in the various treatment groups showed a significant difference with p value of 0.023 and 0.031, respectively. Meanwhile on day 14, the result was not significantly different from the p value of 0.272 ($p>0.05$). It can be concluded that the treatment of *Chromolaena odorata*

Table 2. The Modified Nagaoka Criteria for Assessing Wound Healing.[17]

Parameter and Description	Score
Degree of Epithelialization	
● Normal epithelialization per microscope small field of view	3
● Slight epithelialization per microscope small field of view	2
● No epithelialization per microscope small field of view	1
Degree of Collagen Density	
● Collagen density is more than normal tissue per microscope small field of view	3
● Collagen density is equal to normal tissue per microscope small field of view	2
● Collagen density is less than normal tissue per microscope small field of view	1

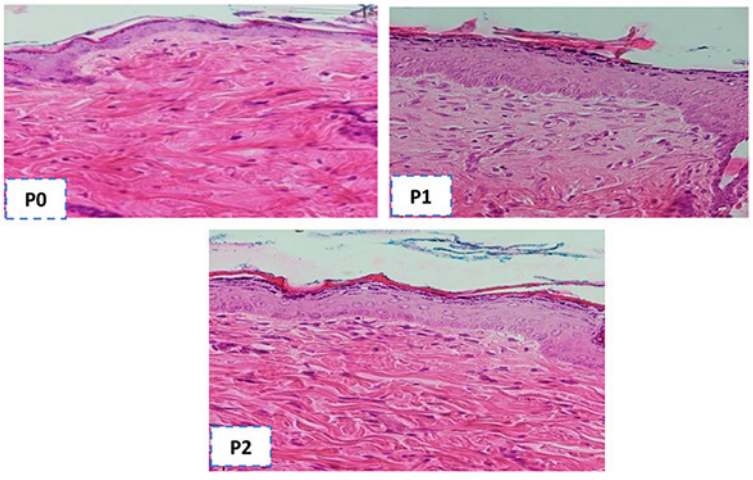

Figure 1. Histopathological view of the degree of epithelialization of open wound tissue with HE staining and with the magnification of 400x on the 3rd day after treatment on P0, P1 and P2.

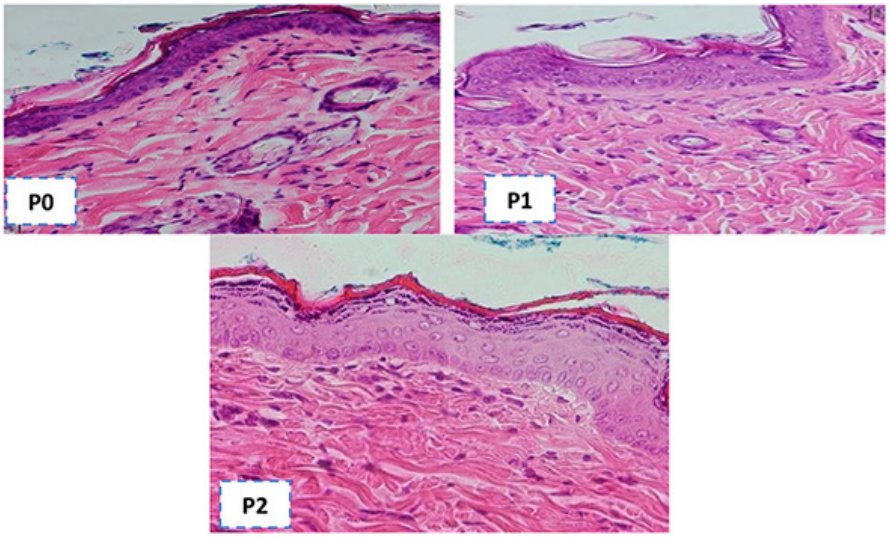

Figure 2. Histopathological view of the degree of epithelialization of open wound tissue with HE staining and with the magnification of 400x on the 7th day after treatment in groups P0, P1 and P2.

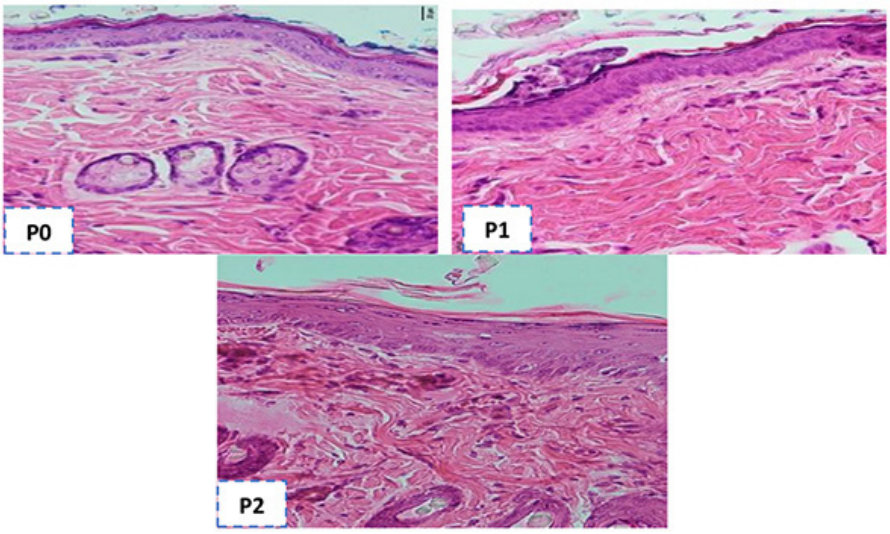

Figure 3. Histopathological picture of the degree of epithelialization of open wound tissue with HE staining and with magnification of 400x on the 14th day after treatment in groups P0, P1 and P2.

Table 3. Mean (± SD) scores of the degree of epithelialization in each group on days 3, 7, and 14

Treatment Group	Observation Time					
	Day 3	p value	Day 7	p value	Day 14	p value
P0	1.27 ± 0.12[a]		2.00 ± 0.20[a]		2.23 ± 0.20[a]	
P1	1.40 ± 0.20[a]	0.023	2.20 ± 0.20[b]	0.031	2.53 ± 0.31[b]	0.272
P2	1.80 ± 0.20[b]		2.40 ± 0.20[b]		2.60 ± 0.20[b]	

Note: different superscripts in the same column show significant differences; **P0:** rats with open wound and with no *Chromolaena odorata* jelly extract administration; **P1:** rats with open wound+10% *Chromolaena odorata* extract; **P2:** rats with open wound+30% *Chromolaena odorata* extract.

leaf jelly extract on open wounds of white rats (*Rattus novergicus*) has an effect on the degree of epithelialization density on days 3 and 7, but not on day 14. The Duncan test results showed that the mean score of the degree of epithelialization in the P2 was significantly higher than P0, but not on P1. The mean score of the epithelialization degree on P1 was not significantly different with the P0. These results prove that the administration of 30% *Chromolaena odorata* leaf jelly extract can accelerate tissue epithelialization in the healing process of open wounds of white rats on days 3 and 7 (Table 3).

Assessment of The Degree of Collagen Density

Histopathological view of the collagen density after the treatment of *Chromolaena odorata* leaf jelly extract of 0% (P0), 10% (P1) and 30% (P2) with HE staining was observed with 400x magnification using an Olympus microscope. The results on days 3, 7, and 14 observations are presented in Figures 4, 5, and 6.

The average degree of collagen density in white rats in each group on days 3, 7, and 14 can be seen in Table 4.

Normality and homogeneity test of the degree of epithelialization on days 3, 7, and 14 showed that the data were normally distributed ($p>0.05$) and the variants were homogeneous ($p>0.05$). The one-way ANOVA showed that the degree of collagen density on day 3 in each group was not significantly different with p value of 0.089 ($p>0.05$). Meanwhile, the degree of collagen density on the 7th and 14th day of observation was significantly different with a significance value of 0.011 and 0.001, respectively ($p<0.05$). These results can be concluded that *Chromolaena odorata* leaf jelly extract did not significantly affect the degree of tissue collagen density on day 3. However, it has a significant effect on days 7 and 14.

The mean of collagen density on P2 was significantly higher than P0, but the same as P1. The mean score of collagen density on P1 was the same as P0. These results prove that the administration of *Chromolaena odorata* leaf jelly extract in the concentrations of 30% could accelerate the tissue collagen density in white rats on days 7 and 14. The administration of

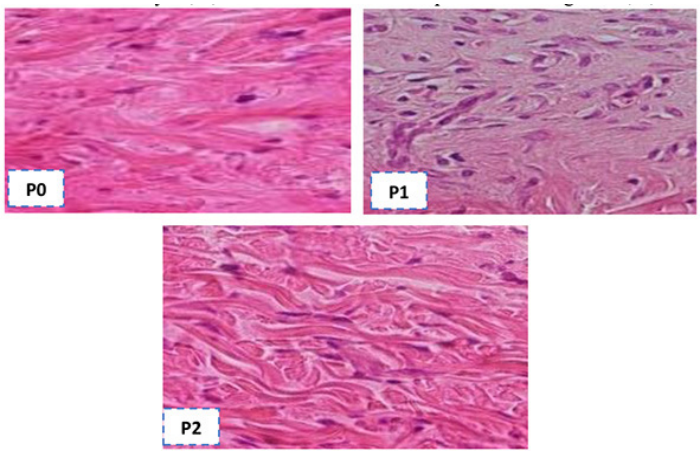

Figure 4. Histological view of the collagen density of wound skin tissue with HE staining with the magnification of 400x on the 3rd day in groups P0, P1, and P2.

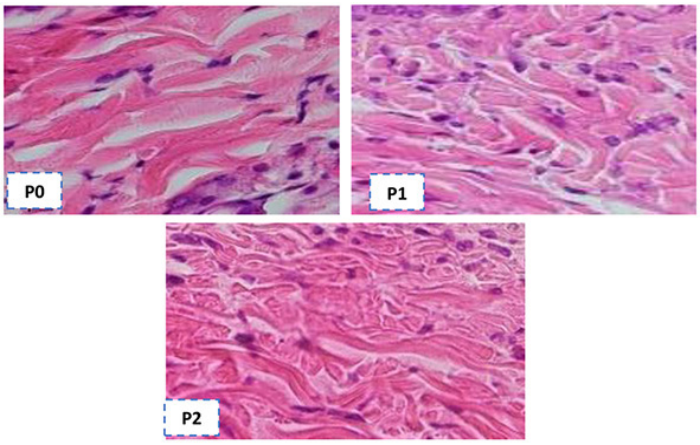

Figure 5. Histological view of the collagen density of wound skin tissue with HE staining with the magnification of 400x on the 7th day in groups P0, P1, and P2.

Chromolaena odorata leaf jelly extract in concentrations of 30% is better than 10% in accelerating the degree of tissue collagen.

DISCUSSION

Wound healing is a complex process due to the bio-cellular and biochemical activities which occur continuously. The combination of vascular response, cellular activity, and the formation of chemical compounds as mediator substances in the wound area are interrelated components in the wound healing process. When an injury occurs, the mechanism of the body will restore the damaged tissue components by forming new and functional structures.[2–4]

The wound healing process consists of three phases where the mechanism of occurrence overlaps with the subsequent phases: inflammation, proliferation, and remodeling. Each phase has a different biological process and cell role.[5-7] In the inflammatory phase, a migration process occurs, moving epithelial cells and fibroblasts in the injured area to replace the damaged or lost tissue. These cells regenerate from the edge and rapidly grow in the wound area on the part covered with blood clots and the hardening of the epithelium.[19]

The proliferation phase occurs simultaneously for 2-3 days with the migration and basal cell. The proliferation

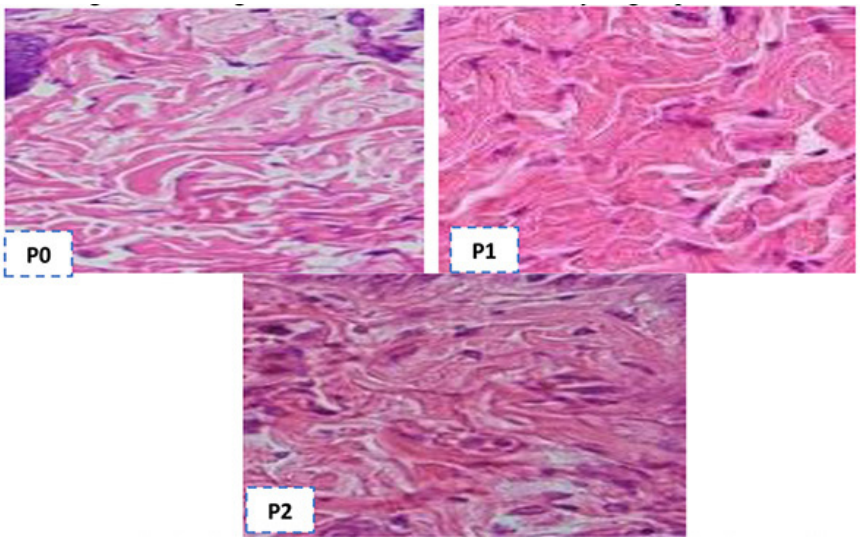

Figure 6. Histological view of the collagen density of wound skin tissue with HE staining with the magnification of 400x on the 14th day in groups P0, P1, and P2.

Table 4. Mean (±SD) scores of collagen density in each group on days 3, 7, and 14

Treatment Group	Observation Time					
	Day 3	p value	Day 7	p value	Day 14	p value
P0	1.07 ± 0.12[a]		1.73 ± 0.12[a]		2.33 ± 0.12[a]	
P1	1.27 ± 0.12[a]	0.089	2.00 ± 0.20[ab]	0.011	2.53 ± 0.12[ab]	0.001
P2	1.33 ± 0.12[a]		2.20 ± 0.20[b]		2.73 ± 0.12[b]	

Note: different superscripts in the same column show significant differences; **P0:** rats with open wound and with no *Chromolaena odorata* jelly extract administration; **P1:** rats with open wound+10% *Chromolaena odorata* extract; **P2:** rats with open wound+30% *Chromolaena odorata* extract.

phase consists of angiogenesis, formation of granulated tissue, and re-epithelialization.[20] The granulated tissue is formed by capillary and lymphatic blood vessels into the wound, and collagen is synthesized by fibroblasts and provides strength to the skin. After that, the epithelial cells harden and let the collagen repair the injured tissue. The proliferation of fibroblasts and collagen synthesis lasted for two weeks. Furthermore, the maturation stage develops with the formation of cellular connective tissue, and the strengthening of the new epithelium is determined by the size of the wound. Cellular granular tissue turns into an acellular mass within a few months to two years.[21]

The results of this study showed that on the 3rd and 7th day, the average score of the degree of epithelialization that occurred in wounds treated with 10% (P1) and 30% (P2) leaf jelly extract was higher than the control group (P0). The mean score of epithelialization on P2 was significantly higher than P0 but not significantly different with P1. Meanwhile, the mean score of the degree of epithelialization in the P1 was not significantly different with P0. In contrast to the results of observations on day 14, the mean score of the degree of epithelialization that occurred in wounds treated with *Chromolaena odorata* leaf jelly extract in the concentration of 10% (P1) and 30% (P2) was significantly higher compared to the control group (p=0.272). These results prove that the administration of 10% and 30% *Chromolaena odorata* leaf jelly extract has an effect in increasing the score of the degree of tissue epithelialization in the open wound healing process of white rats on days 3 and 7, but not on days 0 and 14.

The results of this study are in accordance with the theory of the wound healing process, where the epithelialization process begins on the 3rd or 4th day after injury and is characterized by the formation of neovascularization and accumulation of fibroblasts.[22] Twenty-four hours after injury, keratinocytes migrate laterally and regenerate the basal membrane. After the new basal membrane is formed, keratinocytes stop migrating and proliferating to peak on day 4. The epithelial layer continues to lengthen and thicken, then the newly formed epithelial tissue matures and a new corneum layer appears. Along with the regeneration of the basal membrane, the keratinocytes return to their original shape, and the hemidesmosome reattaches to the basal lamina. The migrated epithelial cells will be connected and cover the wound surface. On days 5 and 7, fibroblasts maximally secrete collagen type III, and there will be a change in the phenotype of fibroblasts to myofibroblasts.[23,24]

Epithelialization is wound healing in which keratinocyte migration and proliferation occurs, and neuroepithelium differentiate into the multi-layered epithelium. Epithelialization can be proven by measuring the thickness and width of the formed epithelial gap.[25] This is in accordance with the research of Prasetyo et al., which states that perfect epithelialization is marked by a gap in the epithelial wound that completely closes.[26] Wound healing is greatly influenced by the epithelialization process where the epithelialization speed affects the duration of wound healing. Jain's study in 2012 also states that in healing wounds, the thickness of the epithelium is closer to normal, around 0.04–1.5 mm. The results of this study are supported by previous studies which showed that administration of 20% *Chromolaena odorata* leaf methanol gel extract for 14 days increased epithelialization better than the control group in wounded rats with diabetes.[27]

This study also indicated differences in the degree of collagen density in white rats between the control group and the *Chromolaena odorata* leaf jelly extract group at each observation time. On day 3, all groups showed that the spread of collagen fibers was still thin. The mean score of the degree of collagen density in the control group did not significantly differ from the other group. These results indicate that the administration of *Chromolaena odorata* leaf jelly extract does not affect collagen density on day 3 of the wound healing process.

On the 7th day of observation, it was seen that the growth of collagen fibers was increasing, but in all the treatment groups, the composition of the fibers was not yet perfect. On the P1 and P2, the growth of collagen fibers was denser compared to the control group (P0). The mean of collagen density in the concentration of 30% was significantly higher than the control group, but it was not different with the concentration of 10%. The mean score of collagen density in a concentration of 10% was not different with the control group.

The observations on day 14 in all groups showed more mature collagen (type I) in the form of ribbons compared to day 7. The mean score of the degree of collagen density in the P0 was lower than P1 and P2. The mean of collagen density on P2 was significantly higher than P0 but was not different from the P1. The mean score of collagen density on P1 was not different with P0. These results prove that the administration of *Chromolaena odorata* leaf jelly extract in concentration of 30% is better than the concentration of 10% in accelerating collagen tissue density in the healing process on the day 14. These results are in accordance with the results of several previous studies, which stated that the administration of *Chromolaena odorata* leaf jelly extract can trigger the acceleration of wound healing. A study conducted by Besson et al. reported that *Chromolaena odorata* leaf jelly extract played a significant role in the inflammatory response, matrix remodeling, and formation of new blood vessels in wound area. In addition, the topical application of *Chromolaena odorata* leaf jelly extract was non-irritating and non-toxic to rats.[28]

In general, the results of this study signified that the administration of *Chromolaena odorata* jelly extract tended to improve wound healing indicators function (increased degree of epithelialization and collagen density). These results generally prove that topical administration of this extract can potentially affect the healing of open wounds in white rats. The administration of *Chromolaena odorata* with a concentration of 30% was better than the concentration of 10% in accelerating the healing process in white rats. The results of this study are in line with previous studies by Yenti et al., which stated that the topical application of cream was significantly different with the control groups.[13] The same study conducted by Nurhalimah reported that *Chromolaena odorata* jelly

extract played a role in the inflammatory response, matrix remodeling, and formation of new blood vessels in the wound area. In addition, the topical application of *Chromolaena odorata* leaf jelly extract was non-irritating and non-toxic to rats.[16]

The acceleration of healing after administration of *Chromolaena odorata* leaf jelly extract in the concentration of 10% and 30% was probably caused by phenolic compounds that affected the wound healing mechanism. It was reported by several previous studies that *Chromolaena odorata* contains saponins, flavonoids, tannins, and essential oils.[8,9] Saponins, flavonoids, and tannins can aggregate the wound healing process because they act as antioxidants and antimicrobials that affect wound grafting and accelerate epithelialization.[13,15] These active compounds act as an antioxidant that affects wound contraction and increases the speed of epithelialization as well as steroids (in this case sterols or alcoholic steroids) which affect wound healing, function as antioxidants and free radical scavengers, reduce lipid peroxidation, reduce necrosis cells, and increase vascularity.[11–13] This high antioxidant activity promotes wound healing because it stimulates the production of endogenous antioxidants at the wound site and provides an environment conducive to wound healing.[10,14] In the early stages of the wound healing process, there will be an inflammatory phase and the formation of reactive oxygen species (ROS) produced by neutrophils and macrophages as part of the immune system to help accelerate wound cleansing. Apart from its positive effect, ROS also has a negative impact. At low levels, hydrogen peroxide and other ROS inhibit the migration and proliferation of various cells, including skin cells (keratinocytes). On the contrary, at high levels, ROS damages tissue and even turns into neoplasms, leading the presence of ROS to inhibit wound healing. *Chromolaena odorata* leaves have a system to detoxify ROS to protect against oxidative stress. Flavonoids are powerful antioxidants that can eradicate free radicals,[9,29] protect the body against ROS, increase endogenous antioxidant function, and increase antioxidant enzymes in granulation tissue.[2,28]

The flavonoid content of *Chromolaena odorata* leaves plays an important role in enhancing the wound healing process.[13] These substances have an antimicrobial effect and are responsible for wound contraction and an increase in the rate of epithelialization.[10,11] Flavonoids have also been shown to increase epithelial cell migration and proliferation, granulation tissue formation, and myofibroblast migration and activity.[29] Administration of oral flavonoids can increase the epithelialization and formation of granulation tissue in wounds due to increased collagen production and angiogenesis.[11] This process indicates the wound healing process and suggests that flavonoids can stimulate wound healing and tissue regeneration mechanisms. A study conducted by Muralidhar et al. shows that flavonoids can significantly accelerate the wound healing process by increasing the rate of wound contraction, decreasing the epithelialization period, increasing collagen deposition, and forming granulation tissue. In addition, it was reported that flavonoids could reduce lipid peroxidase and increase the rate of epithelialization and antimicrobial.[30] The reduction of lipid peroxidase by flavonoids and phenols prevented necrosis, improved vascularity, and increased the viability of collagen fibers by increasing the strength of the collagen fiber webbing.[2,9,11]

Tannins in Chromolaena odorata play a role in stimulating epidermal growth and assisting re-epithelialization by depositing complex protein lipids and accelerating the formation of flexible scabs that cover the wound. Tannins also have cellular mechanisms, such as cleaning free radicals and reactive oxygen, improving wound healing, and increasing capillary formation and fibroblasts activation. In addition, tannin compounds are thought to play a role in regulating transcription and translation of vascular endothelial growth factor (VEGF).[31] VEGF acts as a paracrine not only on the skin vascular endothelial cells but also on keratinocytes and immune cells, promoting re-epithelialization and, at the same time, stimulating angiogenesis and restoring oxygen perfusion.[29]

In addition to the flavonoids and tannins in *Chromolaena odorata*, saponin also acts as antioxidants and antimicrobials and increases wound contraction and the speed of epithelialization.[2] Saponins can also increase the ability of the TGF-β receptor found on fibroblasts to bind TGF-β, a growth factor needed by fibroblasts in synthesizing collagen.[32–34] It has been reported that collagen plays a major role in wound healing and is an important component of connective tissue, providing a structural framework for tissue regeneration.[29,30] Collagen is the main extracellular protein present in the granulation tissue of wounds. Collagen synthesis is propagated by growth factors and cytokines, namely PDGF, FGF, TGF β and IL-1, IL-4, and IgGI produced by leukocytes and lymphocytes during collagen synthesis. In addition, the components that play a role in collagen synthesis are fibroblast cells..[6,33,34]

CONCLUSION

The administration of *Chromolaena odorata* leaf jelly extract could increase the degree of epithelialization in the inflammatory and proliferative phases on day 3 and day 7. There was a significant difference in the increase in the degree of epithelialization in the inflammatory and proliferative phases of wound healing on day 3 and day 7 between the administration of 10% and 30% *Chromolaena odorata* leaf jelly extract.

The topical administration of *Chromolaena odorata* leaf jelly extract can increase the thickness of collagen in the inflammatory and proliferative phases of the wound healing process on day 7 and day 14. There was significant difference in collagen thickness in the inflammatory and proliferative phases of the wound healing process on day 7 and day 14 between the administration of 10% and 30% *Chromolaena odorata* leaf jelly extract.

Further research is needed to evaluate the effectiveness of topical *Chromolaena odorata* leaf jelly extract at the clinical trial stage with various types of wounds.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTION

All Authors were involved in concept formulating, designing, and supervising the manuscript. All authors analyzed the data, prepare the manuscript, and agree for this final version of the manuscript to be submitted to this journal.

ETHICAL CONSIDERATION

This study has been approved by the Health Research Ethics Committee of the Faculty of Medicine, Syiah Kuala University (Permit number: 046/EA/FK-RSUZA/2020).

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