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ANGIOGENESIS ACTIVITY OF *Chromolaena odorata* L. LEAF EXTRACT GEL IN OPEN WOUNDS INCISION PHASE II OF RATS

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ABSTRACT

This study aims to determine the effect of administering *Chromolaena odorata* L. leaf extract gel on increasing angiogenesis in the healing of open incisional wounds in rats (*Rattus norvegicus*). In this study, 27 healthy male rats, aged 3-4 months with a body weight of 150-200 g were used. The rats were anesthetized and incised in the back area with a size of 2x2 cm (WxL) and a depth reaching subcutaneous. The rats were divided into three groups (n= 9), the group only given gel (P0), the group of rats that were given 10% *Chromolaena odorata* leaf extract gel (P1), and the group of rats that were given 20% *Chromolaena odorata* leaf extract gel (P2). Collection of wound skin tissue samples for histopathological preparations was carried out on days 3, 7, and 14 after treatment, 3 individuals per group for each collection. The results showed that application of *Chromolaena odorata* leaf extract gel on P1 and P2 could increase angiogenesis significantly (P<0.05) compared to P0 on days 3 and 7, but was not significantly different (P>0.05) on day 14. The average angiogenesis in P1 and P2 on day 3 was similar, while on day 7 the average angiogenesis on P1 was significantly different compared to P2. It was concluded that administration of *Chromolaena odorata* leaf extract gel could increase angiogenesis in rats during phase II of the open incision wound healing process.

Key words: *Chromolaena odorata* L., new blood vessels, white rats, wound healing

ABSTRAK

Penelitian ini bertujuan mengetahui pengaruh pemberian gel ekstrak daun Chormolaena odorata L. terhadap peningkatan angiogenesis pada penyembuhan luka insisi terbuka tikus (Rattus norvegicus). Dalam penelitian ini digunakan 27 tikus jantan, sehat, berumur 3-4 bulan dengan bobot badan 150-200 g. Tikus dianestesi untuk pembuatan luka insisi pada area punggung dengan panjang dan lebar luka 2x2 cm dengan kedalaman mencapai subkutan. Tikus dibagi tiga kelompok (masing-masing n= 9) yakni kelompok tikus yang hanya diberi gel (P0), kelompok tikus yang diberikan gel ekstrak daun Chromolaena odorata 10% (P1), dan kelompok tikus yang diberikan gel ekstrak daun Chromolaena odorata 20% (P2). Koleksi sampel jaringan kulit luka untuk pembuatan preparat histopatologi dilakukan pada hari ke 3, 7, dan 14 setelah perlakuan, masing-masing 3 ekor per kelompok untuk tiap koleksi setelah tikus dieutanasi terlebih dahulu. Hasil penelitian menunjukkan bahwa pemberian gel ekstrak daun Chromolaena odorata L. pada P1 dan P2 dapat meningkatkan angiogenesis secara signifikan (P<0,05) dibandingkan dengan P0 pada hari ke-3 dan ke-7, namun tidak berbeda secara signifikan (P>0,05) pada hari ke-14. Rerata angiogenesis hari ke-3 pada P1 tidak berbeda dengan P2, sedangkan pada hari ke-7 rerata angiogenesis pada P1 berbeda secara signifikan dibandingkan P2. Disimpulkan bahwa pemberian gel ekstrak daun Chromolaena odorata dapat meningkatkan angiogenesis pada tikus pada proses penyembuhan luka insisi terbuka fase II.

Kata kunci: Chromolaena odorata L., pembuluh darah baru, tikus putih, penyembuhan luka

INTRODUCTION

A wound is a condition where tissue damage occurs due to disruption of tissue continuity due to various causes, such as mechanical, chemical or temperature trauma which results in damage to the skin and an imbalance in the function and anatomy of the skin (Velnar *et al*. 2009; Tsala *et al*. 2013). This damage can occur in the integrity of the skin epithelium, and can even extend to subcutaneous tissue, followed by damage to other structures such as tendons, muscles, blood vessels, nerves, parenchymal organs and bones (Perdanakusuma 2007).

The wound healing process is commenced immediately after damage occurs, however the mechanism and speed of repairing damaged tissue depends on the type of wound (Perdanakusuma 2007; Rodrigues *et al*. 2019). The wound healing process is basically a complex cellular process and focuses on restoring the integrity of damaged tissue (Tsala *et al*. 2013). In general, the wound healing process consists of four phases; hematoma, inflammation, proliferation and remodeling phases (Velnar *et al*. 2009; Sorg *et al*. 2017; Tottoli *et al*. 2020).

Angiogenesis is the process of forming new blood vessels from pre-existing blood vessels. Angiogenesis is one of the main components in the proliferation phase because it can maintain the function of various tissues by providing the supply of nutrients and oxygen needed for metabolic processes during the wound healing process (Frisca and Sandra 2009). There are several stimulators that play a role in the angiogenesis process during wound healing, including high levels of lactic acid, acidic pH, Reactive Oxygen Species (ROS), and a decrease in oxygen tension in tissues as well as cytokines and growth factors. Cytokines and growth factors involved in the angiogenesis process include basic Fibroblast Growth Factor (bFGF), Transforming Growth Factor (TGFα, TGFβ), Vascular Endothelial Growth Factor (VEGF) and prostaglandins. The surface

of endothelial cells has growth factor receptors which play an active role in dissolving the extracellular matrix to facilitate the process of subsequent migration and proliferation of endothelial cells (Velnar *et al*. 2009; Sorg *et al*. 2017; Rodrigues *et al*. 2019). Several studies has been performed to evaluate the use of medicinal to accelerate the angiogenesis process in the wound healing process, including through the use of traditional medicinal plants (Nagori and Solanki 2011; Thakur *et al*. 2011).

The *Chromolaena odorata* plant is a weed that disrupts the growth of other plants and reduces soil fertility (Andika *et al*. 2020). Traditionally, *Chromolaena odorata* leaf have been used to treat various diseases in tropical countries such as to stop bleeding, wounds healing, cough, malaria, antimicrobial, headache, antidiarrheal, astringent, antispasmodic, antihypertensive, antiinflammatory, and diuretic (Prawiradiputra 2007; Ngozi *et al*. 2009; Soni and Singhai 2012). The results of phytochemical tests on the leaf of the *Chromolaena odorata* plant contain several main compounds such as flavonoids, saponins, tannins, terpenoids, alkaloids and steroids, which function as antioxidants, antibacterials and anti-inflammatory which are important in accelerating wound healing (Akinmoladun *et al*. 2007; Vital and Rivera 2009; Saputra *et al*. 2017). The flavonoid content in *Chromolaena odorata* leaf also has an effect in increasing wound healing by accelerating the rate of epithelialization through inducing the production of TGFβ (Yuslianti *et al*. 2016; Zulkefli 2023). Saponins are known to stimulate VEGF, accelerate angiogenesis and stimulate the formation of collagen which is a structural protein that plays a role in the wound healing process (Kim *et al*. 2011). Tannins have the function of stimulating the growth of epidermal tissue, helping the re-epithelialization process by precipitating complex protein lipids and accelerating the formation of elastic scabs that cover wounds. Steroids are useful as anti-inflammatories which can prevent stiffness and pain in the wound (Thakur *et al*. 2011; Soni and Singhai. 2012; Vijayaraghavan *et al.* 2017).

The results of several previous studies reported that *Chromolaena odorata* leaf extract has activity in increasing fibroblast proliferation, spurring keratinocyte proliferation in human epidermal keratinocytes (Prabhudutta and Arpita 2010). The research conducted by Yenti *et al*. (2011) in male rats proved that 10% ethanol extract of *Chromolaena odorata* leaf formulated in a cream preparation, had a good effect on healing open wounds compared to a cream base containing 10% povidone iodine. Yudika *et al*. (2021) reported that administering 10-30% ethanol extract gel from *Chromolaena odorata* leaf could accelerate the healing of open wounds in white rats. Husni *et al.* (2023), compared gel concentrations of 5%, 10%, and 15% of *Chromolaena odorata* leaf extract on angiogenesis in white rat nasal mucosal wounds and found that the 15% cell concentration had a better result compared to other concentrations. However, until now research examining the potential of *Chromolaena odorata* leaf extract on angiogenesis in the proliferation phase in wound healing is still limited. Therefore, this study is conducted to examine the effectiveness of administering *Chromolaena odorata* leaf extract gel to increase angiogenesis in the phase II open wound healing process in white rats as an animal model.

MATERIALS AND METHODS

This research was a laboratory experimental study using a Completely Randomized Design (CRD). In this study, 27 male rats, healthy, aged 3-4 months with a body weight of 150-200 g were used. The rats were anesthetized to make an incision wound in the back area with a wound length and width of 2x2 cm, and a depth reaching subcutaneous. The rats were divided into three groups (n= 9), namely the group of rats that were only given gel (P0), the group of rats that were given 10% *Chromolaena odorata* L. leaf extract gel (P1), and the group of rats that were given 20% *Chromolaena odorata* L. leaf extract gel (P2). Collection of wound skin tissue samples for histopathological preparations was carried out on days 3, 7, and 14 after treatment, 3 individuals per group for each collection after the rats were euthanized. This research had been approved by the Research Ethics Commission of the Faculty of Veterinary Medicine, Syiah Kuala University (No. 230/KEPH/VII/2023).

Preparation of *Chromolaena odorata* L. Leaf Extract

A total of 4000 g of fresh local *Chromolaena odorata* leaf, which were dark green in color, were taken and washed with running water until clean. The *Chromolaena odorata* leaf were dried at a temperature of ± 30° C in a drying cabinet for 1 day. Next, the dried *Chromolaena odorata* leaf were weighed 1000 g each, ground using an electric grinder (blender) and sieved using 40 mesh.

The resulting leaf were sifted, and then 500 g was taken and divided into 10 parts, 50 g each. Each part of the dry simplicia powder was put into 10 dark macerator bottles, added with 10 parts of 96% ethanol solvent (± 500 mL) and soaked for the first 6 hours while stirring occasionally to speed up the diffusion process of secondary metabolite concentrations into the solvent. Next, the simplicia were soaked for 18 hours at room temperature. The macerate was separated by filtration using flannel cloth, repeated three times with the same solvent to maximize the withdrawal of metabolites that might remain in the simplicia. All the macerate obtained was then concentrated using a rotary evaporator at a temperature of 50° C, to remove the solvent so that a thick extract was obtained.

Gel Formulation

The *Chromolaena odorata* leaf extract gel formulation was made in a concentration of 10%. The formulation of gel base and extract gel is presented in Table 1.

Table 1. Formulation of *Chromolaena odorata* leaf extract gel

Material	Gel formulation and composition (w/v)		
	0 % *Chromolaena odorata leaf* extract	10 % *Chromolaena odorata* leaf extract	20 % *Chromolaena odorata* leaf extract
Chromolaena odorata leaf extract	0	10	20
Na-CMC	5	5	5
Glycerin	10	10	10
Propylene glycol	5	5	5
Aquadest	100	100	100

Adaptation of Experimental Animals

Rats were adapted for two weeks and kept in cages. Rats were weighed and rectal temperatures were measured. The temperature in the cage was set at room temperature (25° C). Every day the rats were fed 10 g of BR I pellets and drinking water was provided *ad libitum*. At the end of the adaptation period, the health condition of the experimental ratswere observed and their body weight was measured. Next, the experimental rats were randomly divided according to treatment groups.

Wound Incision

The wound incision was performed at the back area close to the tail so that the rat cannot scratch or lick it. The experimental rats were anesthetized using a combination of ketamine at a dose of 10 mg/kg BW and xylazine at a dose of 2 mg/kg BW intramuscularly. After the rats were anesthetized, the dorsal surface was shaved with a sterile razor, then the skin was cleaned using 70% alcohol. The excision wound was made by first drawing it using a marker on the rat's back in a cube shape measuring 2x2 cm with a depth of 2 mm. Then, with the help of chirurgis tweezers, a scalpel and scissors, a wound was made on the part that has been drawn. The skin and subcutaneous tissue are removed using tweezers, then cut according to a predetermined circle shape.

Wound Care and Treatment

A total of 27 rats that had had open incision wounds were given wound care according to the treatment group. Gel was applied to open incision wounds twice a day using a cotton bud evenly to cover the entire wound area, and the wound was left open. At the end of this study, the wound was first measured to assess wound healing microscopically, then skin samples were taken to assess wound healing microscopically.

Skin Tissue Sampling

Wound skin tissue samples were taken on days 3, 7, and 14, consisting of 3 rats per treatment group. Skin samples were collected by first anesthetizing the rats. After the rat was anesthetized, cervical dislocation was performed to terminate the rat. The skin sample was taken by cutting the skin 1-1.5 cm long with a thickness of ± 2-3 mm, until it reached the entire thickness of the skin (subcutis). The skin tissue that has been collected was fixed in 10% Buffered Neutral Formalin (BNF) solution and left at room temperature for ± 24 hours.

Histopathology Preparations

Histopathological preparations were made in the histology laboratory, Faculty of Veterinary Medicine, Universitas Syiah Kuala-Banda Aceh using the Kiernan method (1999). Skin tissue samples that had been fixed in 10% BNF were cut into 4 µm. The tissue was then processed further with an automatic tissue processor in the following order; the tissue was placed 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, absolute alcohol (II) for 2 hours. For clearing process, the tissue was immersed in xylol (I) for 2 hours and xylol (II) for 2 hours. The tissue was tehn placed in liquid paraffin (I) for 2 hours and liquid paraffin (II) for 3 hours (infiltration process). The embedding process was carried out by embedding the tissue into a "basc mold" or mold filled with liquid paraffin and attached to the embedding cassette until it cooled and a block of tissue was formed. The tissue was cut using a microtome with a thickness of 4 µm. The tissue sheet was then floated on the surface of the water in a water bath and taken using a glass slide with a scooping motion. Tissue staining initiated with a deparaffinization process (removing alcohol from skin tissue) by soaking with xylol three times for 5 minutes each, followed by a rehydration process using absolute alcohol, 95% alcohol, 90% alcohol, 80% alcohol, and 70% alcohol each for 5 minutes, then rinsed with running water for 10 minutes. The tissue was stained with hematoxylin for 5 minutes and rinsed with running water for 10 minutes. The tissue was stained with eosin staining for 2 minutes and followed by using a graded alcohol solution, a clearing process with xylol, and a mounting process using Entellan® adhesive. Next, observations were made using a light microscope with 400x magnification to observe angiogenesis and epithelial thickness of the epidermis.

Angiogenesis Assessment

Assessment of angiogenesis in the wound tissue was carried out on days 3, 7, and 14. The preparations were observed under a light microscope at 400x magnification in 5 fields of view shifted in a zig zag manner. The counting started at the left end and then to the right end. Next, the number of angiogenesis (new blood vessels) wascalculated and scored based on the scoring system by Karayannopoulou *et al.* (2011) in which no new blood vessels found was considered 0, 1-10 new blood vessels found is 1,11-30 new blood vessels found is 2, and more than 31 new blood vessels found is 3.

Data Analysis

Data from observations of angiogenesis scores (formation of new blood vessels) in skin tissue were analyzed using analysis of variance (ANOVA) and followed by the Duncan test to see differences among treatment groups.

RESULTS AND DISCUSSION

The observations of histopathological images of angiogenesis (formation of new blood vessels) on days 3, 7, and 14 are presented in Figure 1, Figure 2, and Figure 3. Based on Figure 1, Figure 2, and Figure 3 and after scoring, the angiogenesis scores (Table 2) was different between the P0 group and the P1 and P2 groups. On day 3, the angiogenesis score was still low and the valuein the P0 group was less than in the P1 and P2 groups. On the 7th day, the angiogenesis score was higher, where in the P1 group the angiogenesis score was seen to be the highest compared to the P0 and P2 groups. On the 14th day of observation, the angiogenesis scores in groups P1 and P2 seemed to start to slow down and even tended to decrease, while in the P0 group the angiogenesis scores continued to increase.

The results of statistical tests showed that the skin tissue angiogenesis scores on days 3 and 7 after treatment were significantly different (P0<0.05), while on day 14 they were not significantly different (P>0.05).

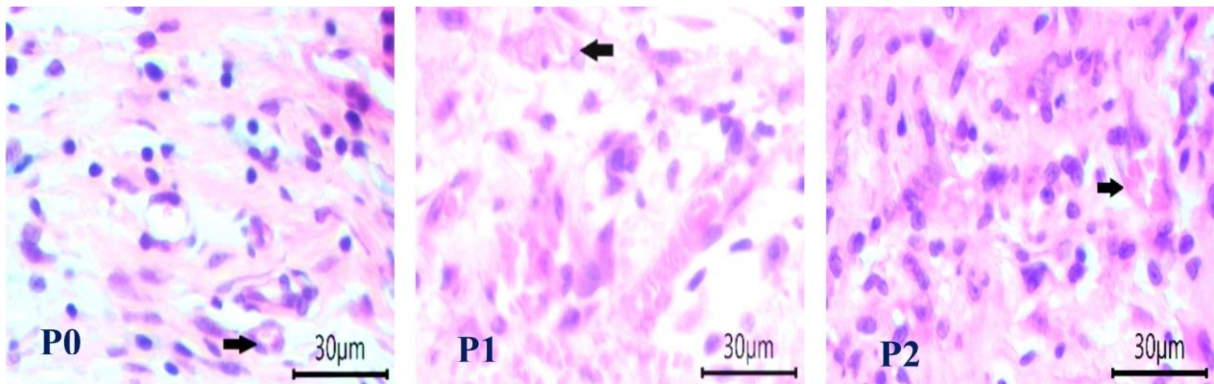

Figure 1. Photomicrograph of angiogenesis (arrow) of rats skin on day 3. P0= The control group was only given gel without *Chromolaena odorata* extract, P1= Groups of rats given 10% *Chromolaena odorata* leaf extract gel and P2= Groups of rats were given 20% *Chromolaena odorata* leaf extract gel, HE 400x

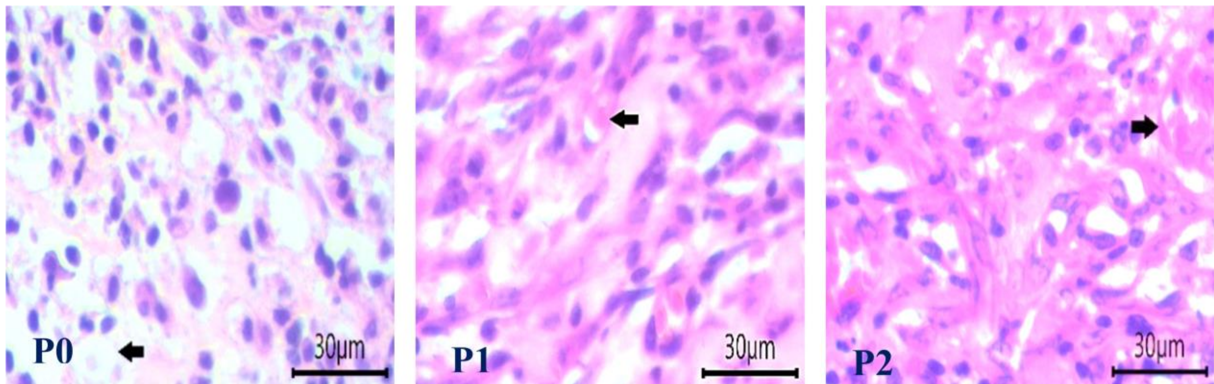

Figure 2. Photomicrograph of angiogenesis (arrow) of rats skin) on day 7. P0= The control group was only given gel without *Chromolaena odorata* extract, P1= Groups of rats given 10% *Chromolaena odorata* leaf extract gel and P2= Groups of rats were given 20% *Chromolaena odorata* leaf extract gel, HE 400x

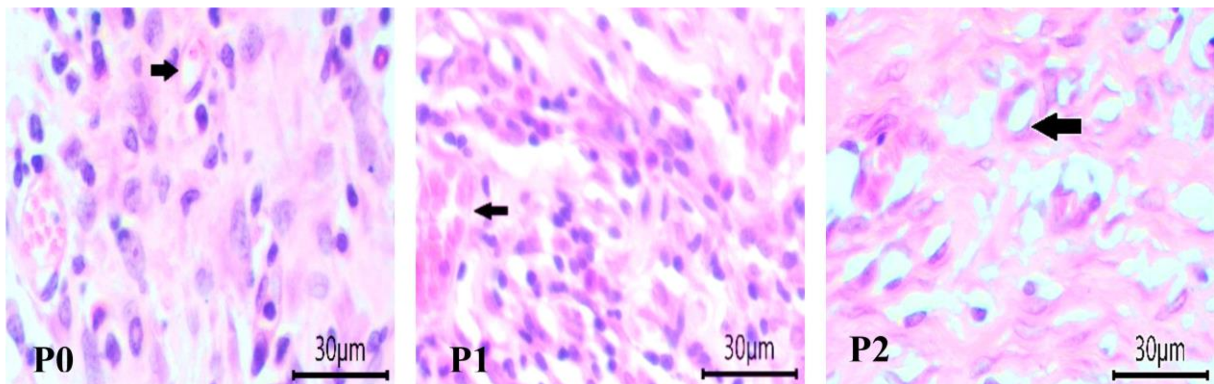

Figure 3. Photomicrograph of angiogenesis (arrow) of white mouse skin on day 14. P0= The control group was only given gel without *Chromolaena odorata* extract, P1= Groups of rats given 10% *Chromolaena odorata* leaf extract gel and P2= Groups of rats were given 20% *Chromolaena odorata* leaf extract gel, HE 400x

Table 2. Mean (±SD) angiogenesis scores in skin tissue of rats in various observed group on day 3, 7, and 14

Observation time	n	Treatment group		
		P0	P1	P2
Day 3	3	0.80±0.20[a]	1.40±0.20[b]	1.33±0.20[b]
Day 7	3	1.47±0.12[a]	2.20±1.40[c]	2.00±0.20[b]
Day 14	3	1.80±0.20[a]	2.00±0.20[a]	1.93±0.3[a]

[a,b,c] Different superscripts within the same row indicate significant difference (P<0.05). P0= The control group was only given gel without *Chromolaena odorata* extract, P1= Groups of rats given 10% *Chromolaena odorata* leaf extract gel and P2= Groups of rats were given 20% *Chromolaena odorata* leaf extract gel

These results prove that the treatment with 10% and 20% *Chromolaena odorata* leaf extract gel had a significant effect (P<0.05) on increasing skin tissue angiogenesis scores on days 3 and 7, but did not have a significant effect (P>0.05) on day 14 of wound healing. On day 3 after treatment the mean angiogenesis score at P1 and P2 was significantly higher compared to P0, but there was no significant difference between P1 and P2. On the 7th day after treatment, the mean skin tissue angiogenesis score at P1 was significantly higher compared to P2 and P0. The mean skin tissue angiogenesis score at P2 was significantly higher compared to P0. These results prove that on day 3, the skin tissue angiogenesis score on P1 was not significantly different (P>0.05) compared to P2, whereas on day 7 observations the skin tissue angiogenesis score on P1 was significantly higher (P<0.05) compared to P2. The results of this study are in line with several previous studies that *Chromolaena odorata* leaf extract at various concentrations can influence the healing process of incision wounds of white rat. Applying 5% and 10% *Chromolaena odorata* leaf extract cream can suppress inflammation topically, however at 20% the effect can be lower than at 10% (Yenti *et al.* 2011). Similarly, Yudika *et al.* (2021) found that administration of 10% *Chromolaena odorata* leaf extract gel had a faster healing effect compared to 30% *Chromolaena odorata* leaf extract gel.

The increase in angiogenesis (formation of new blood vessels) of skin tissue after administration of 10% and 20% *Chromolaena odorata* leaf extract gel in this study is likely due to the positive effect of the active ingredients contained in *Chromolaena odorata* leaf extract gel which is able to induce endothelial cell activity and encourage endothelial migration into the collagen matrix to form capillary like tubules (Veith *et al.* 2019). Regarding the wound healing process, angiogenesis plays an important role in the process of removing debris, providing nutrients and oxygen for metabolic processes during the tissue repair process in the wound area. The angiogenesis process takes place in the proliferative phase, namely between day 3 and reaches its peak on days 7 and 14. The massive growth of blood vessels will immediately initiate the vascular remodeling process. The process of forming new blood vessels are stimulated by angiogenic growth factors such as TGF-β and VEGF. These growth factors associate with receptors on the surface of the endothelium. The activated endothelial cells then proliferate and grow out through the basement membrane to form capillary shoots which become new blood vessels. Suboptimal angiogenesis causes ischemia which impacts the duration of the wound healing process (Sorg *et al*. 2017; Veith *et al*. 2019).

Several previous researchers reported that *Chromolaena odorata* leaf contain many bioactive compounds such as flavonoids, tannins, saponins and steroids which function as antioxidants, antibacterials and anti-inflammatories which are important in accelerating wound healing (Ngozi *et al*. 2009; Rao *et al*. 2010; Saputra *et al*. 2017; Maulida *et al*. 2019). High antioxidant activity can accelerate wound healing because it can stimulate the production of endogenous antioxidants at the wound site, function as a free radical scavenger, reduce lipid peroxidation, reduce cell necrosis, and increase vascularization, as well as providing a conducive environment for wound healing to occur (Rao *et al*. 2010; Saputra *et al*. 2017; Maulida *et al*. 2019). The flavonoid content of *Chromolaena odorata* leaf is a strong antioxidant, could eradicate free radicals, protect the body against ROS, increase endogenous antioxidant function, which was important in improving the wound healing process (Kantha *et al*. 2023). Flavonoids are also responsible for increasing the migration and proliferation of epithelial cells, the formation of granulation tissue, and increasing the migration and activity of myofibroblasts (Zulkefli *et al*. 2023). This had been proven by administering oral flavonoids to increase epithelialization and formation of granulation tissue in wounds by increasing collagen production and angiogenesis in wounds. Studies conducted (Muralidhar *et al*. 2013), showed that flavonoids could significantly accelerate the wound healing process by increasing the rate of wound contraction, decreasing the epithelialization period, increasing collagen deposition and the formation of granulation tissue. In addition, it was reported that flavonoids can reduce lipid peroxidase, increasing the speed of epithelialization. Reducing lipid peroxidase by flavonoids will prevent necrosis, improve vascularization and increase the viability of collagen fibers by increasing the strength of the collagen fiber weave (Zulkefli *et al*. 2013). The angiogenesis process, which is characterized by the formation of new blood vessels in the proliferation phase, occurs more rapidly in large quantities, thus accelerating the synthesis and maturation of collagen in the wound healing process.

The saponin content in *Chromolaena odorata* leaf is important for stimulating VEGF, accelerating angiogenesis and stimulating the formation of collagen which is a structural protein that plays a role in the wound healing process (Kim *et al*. 2011). The tannin content in *Chromolaena odorata* leaf is important for stimulating epidermal growth and helping re-

epithelialization by precipitating complex protein lipids and accelerating the formation of flexible scabs that cover wounds. Tannins play a role in the regulation of transcription and translation of VEGF. VEGF which acts in a paracrine manner not only acts on skin vascular endothelial cells but also on keratinocytes and immune cells promoting re-epithelialization (Li *et al*. 2011). This was seen in the treatment group given 10% (P1) and 20% (P2) *Chromolaena odorata* leaf extract gel, where the angiogenesis score was higher compared to the control group (P0). Angiogenesis scores continued to decrease in line with wound healing and maturation of granulation tissue based on the gel concentration of *Chromolaena odorata* leaf extract as seen in group P2. The results of this study also showed that the angiogenesis score in the 20% *Chromolaena odorata* leaf extract gel treatment was lower than 10%. This was assumed that the absorption of the *Chromolaena odorata* leaf extract gel with a higher concentration was more difficult, so the onset of the active ingredient in the *Chromolaena odorata* leaf extract was slower. Apart from that, it was also due to an increase in the content of active ingredients in *Chromolaena odorata* leaf extract in the body, such as flavonoids, saponins and tannins. These results were in accordance with those reported by Kantha *et al*. (2023), that the administration of flavonoid, saponin and tannin compounds at high concentrations could cause antioxidant activity to change to pro-oxidants which could damage cells. When oxidant levels were high but pro-oxidants were low, the body will form pro-oxidant compounds to balance the levels with antioxidants. This will continue to increase the production of free radical cells. Pro-oxidant activity could reduce levels of antioxidant enzymes in the body such as glutathione peroxidase and catalase. An imbalance between antioxidant and pro-oxidant enzymes in the body will damage endothelial cells, causing the formation of new blood vessels to be disrupted (Arief and Widodo 2018; Kantha *et al*. 2023).

CONCLUSION

The administration of *Chromolaena odorata* leaf extract gel can increase angiogenesis (formation of new blood vessels) in the wound healing process on days 3, 7, and 14. Application of 10% *Chromolaena odorata* leaf extract gel is better than concentration of 20% in increasing angiogenesis in the phase II wound healing process.

REFERENCES

Akinmoladun A, Ibukun E, Dan-Ologe I. 2007. Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata*. *Scientific Research and Essays,* 2(6):191-194.

Andika B, Halimatussakdiah H, Amna U. 2020. Analisis kualitatif senyawa metabolit sekunder ekstrak daun gulma siam (*Chromolaena odorata* L.) di Kota Langsa, Aceh. *QUIMICA: Jurnal Kimia Sains dan Terapan,* 2(2):1-6.

Arief H, Widodo MA. 2018. Peranan stres oksidatif pada proses penyembuhan luka. *Jurnal Ilmiah Kedokteran Wijaya Kusuma.* 5(2):22-28.

Frisca SC, Sandra F. 2009. Angiogenesis: Patofisiologi dan aplikasi klinis. *Maranatha Journal of Medicine and Health,* 8(2):174-187.

Husni TT, Darmawi D, Azwar A, Jamil KF. 2023. Bioactive phytoconstituents of ethanolic extract from *Chromolaena odorata* leaves interact with vascular endothelial growth factor and cyclooxygenase-2: A molecular docking study. *Journal of Advanced Pharmaceutical Technology & Research,* 14(1):29-33.

Kantha P, Phiw-on S, Pinyakong O, Teepoo S, Bootchanont A, Barnthip N. 2023. Preparation of *Chromolaena odorata* (Siam weed) loaded hydrogels and the effect of electrical potentials on the total flavonoids releasing behavior for wound healing application. *Soft Materials*, 21(1):1-10.

Karayannopoulou M, Tsioli V, Loukopoulos P, Anagnostou T, Giannakas I, Savvas N, Papazoglou L, Kaldrymidou E. 2011. Evaluation of the effectiveness of an ointment based on alkannins/shikonins on second intention wound healing in the dog. *The Canadian Journal of Veteriner Respiration*, 75(1):42-48.

Kim HO, Kim HS, Youn JC, Shin EC, Park S. 2011. Serum cytokine profiles in healthy young and elderly population assessed using multiplexed bead-based immunoassays. *Journal of Translational Medicine,* 9(113):1-7.

Kiernan JA. 1990. *Histological and Histochemical Methods: Theory and Practice.2th ed.*. Pergamon, England.

Li K, Diao Y, Zhang H, Wang S, Zhang Z, Yu B, Huang S, Yang H. 2011. Tannin extracts from immature fruits of *Terminalia chebula* Fructus Retz. promote cutaneous wound healing in rats. *BMC Complementary and Alternative Medicine,* 11(86):2-9.

Maulida PA, Putri DA, Fatmawati S. 2019. Free radical scavenging activity of *Chromolaena odorata* L. leaves. *IPTEK,* 30(3):73-75.

Muralidhar A, Babu KS, Sankar TR, Reddanna P, Latha J. 2013. Wound healing activity of flavonod fraction isolated from the stem bark of *Butea monosperma* (Lam) in albino wistar rats. *European Journal of Experimental Biology,* 3(6):1-6.

Nagori BP, Solanki R. 2011. Role of medicinal plants in wound healing. *Research Journal of Medicinal Plant,* 5(4):392-405.

Ngozi IM, Jude IC, Catherine IC. 2009. Chemical profile of *Chromolaena odorata* L. (King and Robinson) leaves. *Pakistan Journal of Nutrition,* 8(5):521-524.

Perdanakusuma D. 2007. Anatomi Fisiologi Kulit Dan Penyembuhan Luka, Plastic Surgery Departement, Airlangga University School of Medicine-Dr. *Soetomo General Hospital, Surabaya.* Perdanakusuma D. (Ed). 1th ed Surabaya.

Prabhudutta P, Arpita G. 2010. Formulation and evaluation of topical dosage form of *Eupatorium odoratum* Linn. and their wound healing activity. *International Journal of Pharma and Bio Sciences,* 1(2):532-41.

Prawiradiputra BR. 2007. Kir inyuh (*Chromolaena odorata* (L) RM King dan H Robinson): Gulma padang rumput yang merugikan. *Wartazoa,* 17(1):46-52.

Rao KS, Chaudhury PK Pradhan A. 2010. Evaluation of anti-oxidant activities and total phenolic content of *Chromolaena odorata*. *Food and Chemical Toxicology,* 48(2):729-732.

Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. 2019. Wound healing: A cellular perspective. *Physiological reviews* 99(1):665-706.

Saputra A, Gani A, Erlidawati E. 2017. Uji aktivitas antioksidan daun gulma siam (*Chromoleana odorata* L.) dengan metode 1, 1-difenil-2-pikrilhidrazil. *Jurnal IPA & Pembelajaran IPA,* 1(2):131-142.

Soni H, Singhai AK. 2012. A recent update of botanicals for wound healing activity. *International Research Journal of Pharmacy,* 3(7):1-7.

Sorg H, Tilkorn DJ, Hager S, Hauser J, Mirastschijski U. 2017. Skin wound healing: an update on the current knowledge and concepts. *European Surgical Research,* 58(1-2):81-94.

Thakur R, Jain N, Pathak R Sandhu SS. 2011. Practices in wound healing studies of plants. *Evidence-based Complementary and Alternative Medicine,* 438056. Doi:10.1155/2011/438056.

Tottoli EM, Rossella D, Ida G, Enrica C, Silvia P, Bice C. 2020. Skin wound healing process and new emerging technologies for skin wound care and regeneration. *Pharmaceutics,* 12(8):735-765.

Tsala D, Amadou D, Habtemariam S. 2013. Natural wound healing and bioactive natural products. *Phytopharmacology,* 4(3):532-560.

Yenti R, Afrianti R, Afriani L. 2011. Formulasi krim ekstrak etanol daun kirinyuh (*Euphatorium odoratum* L) untuk penyembuhan luka. *Majalah Kesehatan PharmaMedika,* 3(1):227-230.

Yuslianti ER, Bachtiar BM, Sastradipura DFS, Sutjiatmo AB, Mozef T. 2016. Effect of rambutan-honey and its flavonoid on TGF-β1 induce fibroplasia oral wound healing. *Research Journal of Medicinal Plant,* 10(8):435-442.

Veith AP, Henderson K, Spencer A, Sligar AD, Baker AB. 2019. Therapeutic strategies for enhancing angiogenesis in wound healing. *Advanced drug Delivery Reviews,* 146(2):97-125.

Velnar T, Bailey T, Smrkolj V. 2009. The wound healing process: An overview of the cellular and molecular mechanisms. *Journal of International Medical Research,* 37(5):1528-1542.

Vijayaraghavan K, Rajkumar J, Seyed M. 2017. Efficacy of *Chromolaena odorata* leaf extracts for the healing of rat excision wounds. *Veterinární Medicína,* 62(10):565-578.

Vital PG, Rivera WL. 2009. Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L. f.) King and Robinson and Uncaria perrottetii (A. Rich) Merr. extracts. *Journal of Medicinal Plants Research,* 3(7):511-518.

Zulkefli N, Che, NM, Che Z, Nor HS, Ammar AK, Norazalina S, Hamizah SH, Hamidun B, Syarul NB, Ahmed M, Qamar UA, Ahmad FHI, Murni NS. 2023. Flavonoids as potential wound-healing molecules: Emphasis on pathways perspective. *International Journal of Molecular Sciences,* 24(5):4607-4636.