

Effectiveness of ethanol extract of kirinyuh leaf (*Chromolaena odorata* L) on the increase the fibroblasts and angiogenesis in nose mucosa of rabbit (*lepus curpaneums*)

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ABSTRACT. Wounds are the loss or damage to a part of the body's tissue that disrupts the tissue's continuity. Kirinyuh plants have been used as a traditional wound treatment in various cultures worldwide. Purpose: To prove the effectiveness of topical ethanol extract of kirinyuh leaf ointment on increasing angiogenesis and fibroblasts in healing rabbit nasal mucosal incisions. Methods: This research is an experimental study with a posttest-only control group design. The research subjects consisted of 25 rabbits divided into five groups; one positive control group, one negative control group, and three treatment groups. The positive control group was given betadine ointment, the negative control group was made by incision of the nasal mucosa, and the three treatment groups were given 5%, 10%, and 15% concentrations of kirinyuh leaf ethanol extract ointment. Results: The results showed that administering kirinyuh leaf ethanol extract ointment with concentrations of 5%, 10%, and 15% could improve the healing of rabbit nasal mucosal incisions (*Lepus Curpaneums*), which were characterized by an increase in the number of fibroblasts and angiogenesis. In general, the increase in fibroblast cells between the concentrations of the ethanol extract of kirinyuh leaf ointment was significantly different ($p < 0.05$) with a negative correlation ($r = -0.538$). Meanwhile, there was no significant difference in the amount of angiogenesis between concentrations ($p > 0.05$) with a positive relationship ($r = 0.099$). Conclusion: the ethanol extract of kirinyuh leaf ointment applied topically improves the healing of rabbit nasal mucosal incisions

KEYWORDS: ethanol extract of kirinyuh leaf ointment, wound healing, fibroblast, angiogenesis

INTRODUCTION

Wounds are loss or damage to somebody's tissues caused by sharp or blunt trauma, changes in temperature, chemicals, explosions, electric shocks, or animal bites. Injuries to the nasal mucosa can be caused by trauma, radiotherapy, chronic infections such as sinusitis, and rhinoplasty.^{1,2,3}

The wound healing process consists of various complex processes to restore tissue integrity. Blood clots, acute and chronic inflammatory responses, neovascularization, cell proliferation, and apoptosis occur during this process. Wound healing consists of several stages and is influenced by many factors, both internal and external, starting with inflammation and followed by the process of

fibroplasia, then tissue remodeling and scar tissue formation^{4,5}

Kirinyuh or siam weed (*Chromolaena odorata* (L)) is a medicinal plant used by the community as a medicine for wounds, treating infections, headaches, and diarrhea, as an astringent, antispasmodic, antihypertensive, anti-inflammatory, and diuretic.⁶

Ayyanar et al., 2009, determined that extracts from *Chromolaena odorata* (L) help treat wounds. In traditional use, the leaves are ground into a paste and applied to the affected area to heal the wound. Thang et al. (1998 and 2001) reported that *Chromolaena odorata* (L) extract stimulated the proliferation of keratinocytes in human epidermal keratinocytes at low concentrations (0.1–5 µg/mL).⁸

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This study was conducted to evaluate the role of *Chromolaena odorata* (L)) ethanol extract ointment in increasing the acceleration of wound healing in

the nasal mucosa of rabbits in increasing fibroblast cells and angiogenesis so that it can be used as a wound medicine candidate.

MATERIALS AND METHODS

This research was an experimental study with a posttest-only control group design, and the research subjects were male rabbits (*Lepus curpaeums*), body weights 2000-2500 grams, and the ethanol extract of kirinyuh leaf (*Chromolaena odorata* (L)) obtained from the Darussalam area.

The experimental group was divided into five different groups:

1. Treatment group P1, the experimental animal group, by gave 5% topical ethanol extract of kirinyuh leaves ointment
2. Treatment group P2, the experimental animal group, offers 10% topical kirinyuh leaf ethanol extract ointment
3. P3 treatment group, experimental animal group by giving 15% topical kirinyuh leaf ethanol extract ointment
4. KN Negative Control Group, an experimental group of animals with nasal mucosal incisions.
5. KP Positive Control Group, an experimental group of animals by administering betadine ointment to incisions of the nasal mucosa

RESULTS

Figure 1 shows that the quantity of fibroblast cells increased in all treatment groups. These results indicate that the concentration does not affect the development of fibroblast cells. It means that the percentage distribution of fibroblast cells in the treatment group did not follow the concentration of

the ethanol extract of kirinyuh leaf ointment as a test material for wound healing of nasal mucosal incisions. In this study, it was also shown that the ability to induce the development of fibroblast cells in the ethanol extract of kirinyuh leaf ointment was better than positive and negative controls.

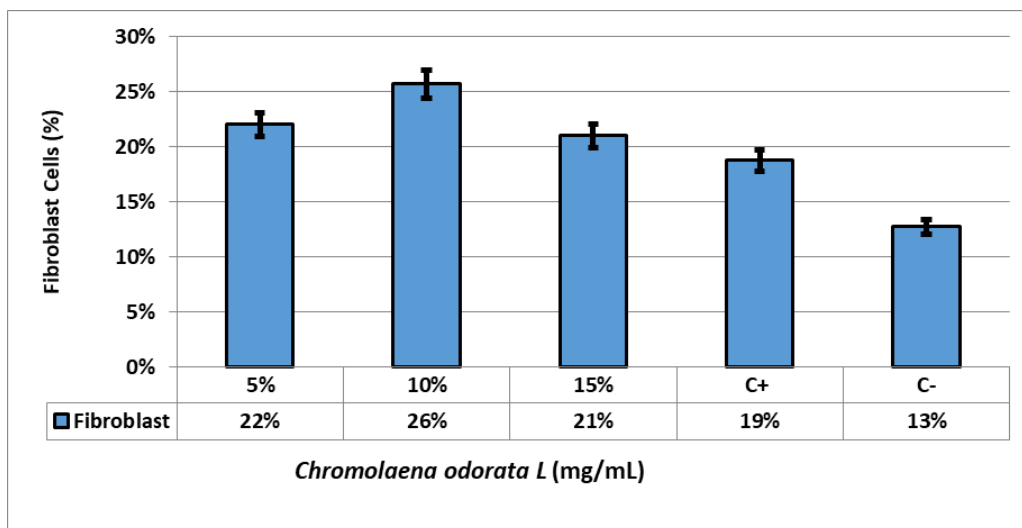


Figure 1. Increase in the number of Fibroblast cells

Figure 2 shows that the highest angiogenesis occurred in the positive control group, while the

treatment group had the same amount of angiogenesis as the negative control group.

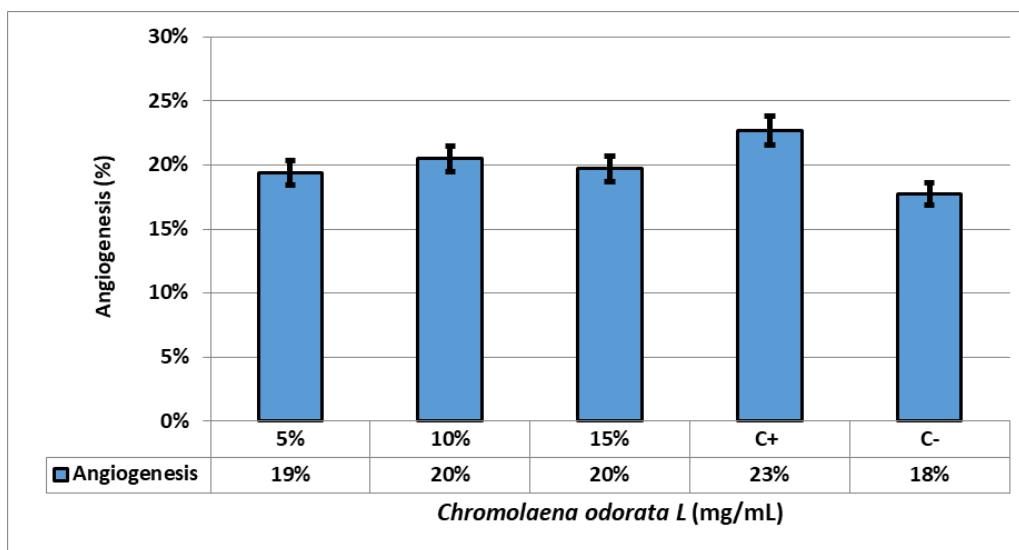


Figure 2. Increased Number of Angiogenesis

Figure 3 shows a graph of the relationship between fibroblast cell variables and angiogenesis. The 15% kirinyuh leaf ointment concentration had a better relationship than the other treatments. It means that in wound healing, this concentration has an

excellent response to the host immune system involved in wound healing. The presence of fibroblast cells simultaneously increases angiogenesis in the wound area.

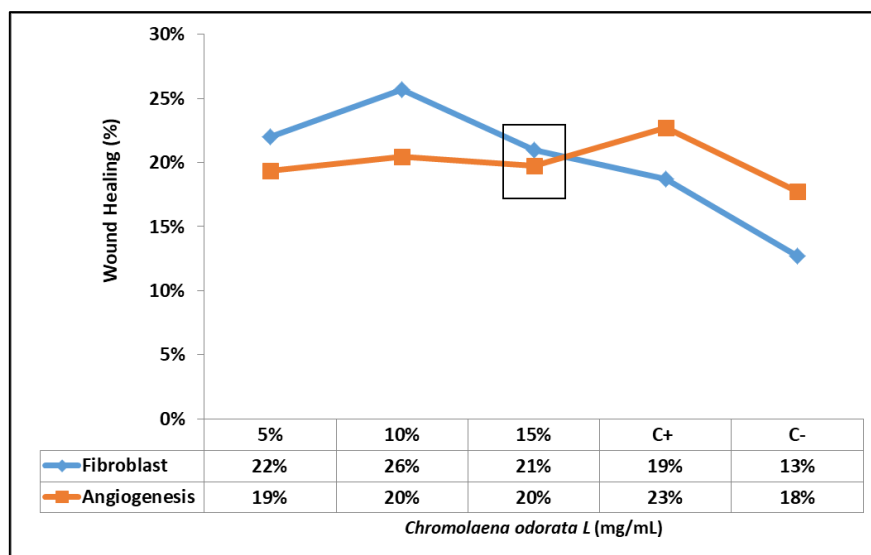


Figure 3. Relationship of Increased Angiogenesis with Fibroblast Cells

Table 1 shows the distribution and frequency of involvement of fibroblast cells and angiogenesis in wound healing. In general, of all concentrations, the increase in fibroblasts reached 69% while the

increase in angiogenesis reached 60%. It means that the activity of fibroblast cells determines the development of angiogenesis.

Table 1. Distribution and frequency of development of fibroblast cells and angiogenesis in rabbit nasal mucosal incisions after being given kirinyuh leaf ethanol extract ointment Kirinyuh

Concentration	N	Fibroblast				P	Angiogenesis				P
		Cell	SDV	Freq (%)	Scale		Blood Vessel	SDV	Freq (%)	Scale	
5%	5	8.6	1.19	22%	Moderate	p<0.05 (0,04)	2.8	0.40	19%	Low	p >0.05 (0.685)
10%	5	10.0	1.23	26%	High		3.0	0.62	20%	Moderate	
15%	5	8.2	0.74	21%	Moderate		2.9	0.49	20%	Moderate	
C+	5	7.3	0.50	19%	Moderate		3.3	0.33	23%	High	
C-	5	5.0	0.81	13%	Low		2.6	0.25	18%	Low	

DISCUSSION

This study evaluates the role of kirinyuh leaf ethanol extract ointment in improving the healing of incisions on the nasal mucosa of rabbits. Specifically, it estimates the increase in fibroblast cells and angiogenesis as a reference for wound healing.

Gonzales (2016) reported that fibroblasts are the primary cells in wound healing. These cells are cellular elements commonly found in connective tissue that proliferate and actively synthesize matrix components in wound healing and repair of damaged tissue.⁹ Fibroblasts are the primary material for forming scar tissue and collagen, providing tensile strength in wound healing of soft tissue. When the tissue is inflamed, the primary cells that migrate toward the wound are fibroblasts.¹⁰ In addition, the assessment of angiogenesis in this study relates to forming new blood vessels. It is also involved in healing and developing new tissue after damage. Fibroblasts naturally play an important role in wound healing and reproduction

Figure 1 shows that the ethanol extract of kirinyuh leaf ointment can increase the number of fibroblast cells, which means it can accelerate the wound healing process. This phenomenon can be justified because several chemical compounds in the *Chromolaena odorata* (L) extract act as antiplatelet, anti-inflammatory, and antioxidants.¹⁰ Sanchez (2018) reported that the Squalene compound is an antioxidant that can help increase the cell's defense response against infectious agents. While increasing the intensity of fibroblast cell production. In addition, an increase in fibroblast cells is also possible due to a decrease in infection or inflammation due to the influence of these chemical compounds.¹² Phenol compounds, (3S)-7-O-Methoxymethylvestitol and 9,12,15-Octadecatrienoic acid, methyl esters contained in *Chromolaena odorata* (L) can work as an anti-inflammatory. Decreased

inflammation can help increase the migration of fibroblast cells to the wound area.

The wound-healing phase consists of hemostasis, inflammation, proliferation, and remodeling. Several factors can influence this physiological process, and abnormalities in these phases can lead to impaired wound healing.¹⁴ The results of this study have illustrated the physiological role of *Chromolaena odorata* (L) in accelerating the formation of tissue or matrix synthesized by fibroblast cells. In addition, *Chromolaena odorata* (L) can act as an antithrombotic (antiplatelet), which works to prevent the accumulation of metabolic waste cells (coagulation) in the blood vessels.¹⁵ so that the blood can remain thin. In general, based on the results of this study, *Chromolaena odorata* (L) can be involved in the process of wound healing, starting from stopping bleeding (hemostasis) and inflammation (inflammation), forming new tissue, and strengthening the tissue.

Figure 2 shows the wound area where angiogenesis is formed, indicating that *Chromolaena odorata* (L) has a good effect in increasing Nitric Oxide production, increasing VEGF release, and activating the actin signaling pathway. The research findings from Figure 2 also show that *Chromolaena odorata* (L) can increase the mobilization of endothelial progenitor cells (fibroblast cells) and accelerate reendothelialization after vascular injury (Figure.3). In addition, *Chromolaena odorata* (L) may be angiogenic by initiating the release of protease enzymes from activated endothelial cells.¹⁶ The formation of vascular blood vessels is characterized by degradation of the extracellular matrix, migration, and proliferation of endothelial cells, and the production of new extracellular matrix, followed by controlled and modulated blood vessel maturation/stabilization to meet tissue needs.¹⁷ This

phenomenon could be possible that *Chromolaena odorata* (L) can reduce wound healing time while simultaneously increasing collagen deposition, fibroblast number, and blood vessel density (Figure. 3). In addition, it has been reported that chemical substances/compounds from *Chromolaena odorata* (L) function as pro-angiogenic agents during wound healing and wound repair by regulating TGF- β .¹⁸

Theoretically, excessive angiogenesis occurs when abnormal cells produce high amounts of angiogenic factors that inhibit the effect of angiogenesis inhibitors. Conversely, if the production of angiogenic factors is low, it can inhibit the regeneration of blood vessel cells, resulting in atherosclerosis, stroke, infertility, ulcers, and delayed wound healing. Abnormalities can result in tissue death.

Angiogenesis is also reported as forming new blood vessels from pre-existing blood vessels through developing or remodeling existing blood vessels.²⁰ The mechanism of blood vessel formation by angiogenesis begins with cell division facilitated by angiogenic substances and then travels to nearby blood vessels and activates its endothelial cell receptors

Figure 3 shows a graph of the relationship between fibroblast cell variables and angiogenesis. Based on the chart, it is explained that fibroblast cells can play a role in increasing angiogenesis in the healing of cuts in the nasal mucosa of rabbits. These results show fibroblasts secrete factors other than VEGF that induce an angiogenic phenotype in endothelial cells. Fibroblasts and fibroblasts-CM support endothelial cell growth and lumen formation.²¹ Meanwhile, VEGF (Vascular Endothelial Growth Factor) promotes angiogenesis through several mechanisms, including increased proliferation and survival of endothelial cells; increased migration and invasion of endothelial cells; increased permeability of existing blood vessels, forming a lattice network for endothelial cell migration; and increase chemotaxis and homing.

This study's findings also reported that fibroblast cells increased by 69% in general while angiogenesis reached 60%. It means that the activity of fibroblast cells determines the development of angiogenesis. Tracy (2016) reported that fibroblasts' specific contribution to angiogenesis is still largely unknown. Still, derivatives of fibroblasts may act as angiogenic in endothelial cells to increase matrix rigidity.²³

The role of fibroblasts in wound healing was reported by Tonnesen (2000), where angiogenic capillary buds invaded the wound clot, which is rich in fibrin/fibronectin and, within a few days, formed

a microvascular network throughout the granulation tissue. When collagen accumulates in the granulation tissue of a scar, it reduces the density of blood vessels.²⁴ Angiogenesis plays a significant role in wound healing. The angiogenic response is required to deliver immune cells, remove debris, and provide nutrients for tissue regeneration. Defects in the regulation of vascular growth can lead to dehiscence and ulceration.

Angiogenesis plays an essential role in the development of many diseases. Disruption of the balance between pro-angiogenic and anti-angiogenic factors causes excessive or inadequate levels of angiogenesis.²⁶ *Chromolaena odorata* (L) extract used in this study tends to act pro-angiogenic because it is required for wound healing. It is believed that several active compounds contained in it have worked based on pharmacodynamic and pharmacokinetic properties, where their role, apart from increasing bioavailability, also increases their solubility and rate of absorption without changing their biological effects while providing a healing response.²⁷ In general, several natural compounds in plant or herbal extracts have angiogenesis-modulating effects.²⁸ These plant-derived compounds are mostly phytochemicals that have significant physiological effects on the body. This molecule acts as an antioxidant, stimulates enzyme activity, mimics hormones, interferes with DNA replication, or binds to cell wall proteins.²⁹ Several research reports also describe the synergistic effect of plant-based compounds as anti-angiogenic or pro-angiogenic agents.²⁸

The polyphenols in *Chromolaena odorata* (L) have a proliferative effect to enhance wound healing. In addition, flavonoids, including flavones, flavonols, flavanones, anthocyanins, and isoflavones, belonging to another class of polyphenols, also play a pro-angiogenic role as well as play a role in wound healing.¹⁶ A number of compound groups in *Chromolaena odorata* (L) plants increase angiogenic capillary bud to invade wound clots rich in fibrin/fibronectin to form granulation tissue.²⁷ The Phyto-response properties of *Chromolaena odorata* (L) can increase collagen accumulation in granulation tissue in scars and reduce blood vessel density.²⁴

Molecularly Tonnesen (2000) reported that this healing process occurs due to interactions between endothelial cells and angiogenic cytokines, such as FGF, VEGF, TGF-beta, angiopoietin, mast cell tryptase, and the extracellular matrix environment.^{11,24} These references are strengthened by Feng (2013) explained that endothelial cell-extracellular matrix receptors are crucial for morphogenetic changes in blood vessels during

wound repair. In particular, $\alpha(v)\beta3$, the integrin receptor for fibrin and fibronectin, is involved in wound angiogenesis. $\alpha(v)\beta3$ is expressed at the bud tips of angiogenic capillaries that invade the wound clot and inhibit the formation of granulation tissue.^{11,24,29} Recent findings have been reported that the extracellular matrix in the wound area can regulate angiogenesis by modulating the expression of integrin receptors.³⁰

Levels of mRNA $\alpha(v)\beta3$ in human skin microvascular endothelial cells either coated with fibronectin or covered with fibrin gel were higher than in cells coated with collagen or coated with collagen gel.³¹ Wound angiogenesis is also regulated by the interaction of endothelial cells with the extracellular matrix environment in the wound area.³¹

CONCLUSION

Based on the research objectives, it can be concluded that the ethanol extract of kirinyuh leaves (*Chromolaena odorata* (L)) ointment applied

topically improves the healing of cuts on the nasal mucosa of rabbits (*Lepus curpaneums*).

CONFLICT OF INTEREST

There is no conflict of interest in this research

ETHICAL STATEMENTS

Number of ethical contributions KEPPKN Registration Number: 1171012P Description of ethical exempted "ethical exempted" Number: 130/EA/FK-RSUDZA/2020

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