

The effect of ethanol extract of kirinyuh leaf (*Chromolaena odorata* L) on the allergy healing of rabbit (*lepus curpaneums*)

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ABSTRACT. Allergic rhinitis is one of the world's most common diseases and remains alive. The following diseases are often found in otorhinolaryngology and constitute a significant health problem worldwide. Symptoms of nasal hyperactivity and hyperresponsiveness are caused by the role of eosinophils, basophils, neutrophils, and other inflammatory mediators. The development of medicinal plants in other countries is overgrowing due to many adverse drug reactions. *Chromolaena odorata* (L) has been used as a traditional medicine because it has ethnopharmacology effects such as alkaloids, essential oils, phenolics, flavonoids, steroids, phenolic compounds, glycosides, phytates, saponins and tannins which are suppress allergenic. To determine the effect of ethanol extract ointment of kirinyuh leaves (*Chromolaena odorata* (L)) on the levels of eosinophils, basophils, and neutrophils of allergic that induced in a rabbit nasal mucosa. Experimental research with a pretest-posttest control group design uses rabbits as a research subject. The extract of ethanol ointment from *Chromolaena odorata* (L) leaf can reduce the nasal mucosa's allergen response. One-way ANOVA statistical analysis showed no significant difference in the number of inflammatory cells between basophils, eosinophils, and neutrophils ($p > 0.05$; 1.00). Furthermore, based on the analysis of the Kruskal Wallis Test, it showed that there were differences in the number of inflammatory cells based on the concentration of the test material, related to the allergen response ($p < 0.05$; 0.024) with a strong relationship ($r = 0.907$). Extracting ethanol ointment from *Chromolaena odorata* (L) leaf, which is applied topically, can reduce allergic reactions and inflammation.

KEYWORDS: allergic rhinitis, eosinophils, basophils, neutrophils, *Chromolaena odorata* (L)

INTRODUCTION

Allergic rhinitis is one of the most common diseases in the world and usually persists throughout life.^{1,2} The self-reported prevalence of allergic rhinitis is estimated to be around 2% to 25% in children and 1% greater than 40% in adults.¹ According to the World Allergy Report 2008, the prevalence of allergic rhinitis in low- and middle-income countries in the Asia Pacific region is estimated at around 5–45%.² Unfortunately, the prevalence rate in Indonesia is still unknown. Although rarely life-threatening, allergic rhinitis causes poor sleep and a lack of productivity in industry and education

Allergic rhinitis is an inflammatory disease with symptomatic abnormalities in the nose induced by inflammation caused by immunoglobulin E (IgE) due to exposure to foreign substances called allergens. It is characterized by one or more symptoms of nasal pruritus, sneezing, discharge, and congestion. In addition, the sense of smell and taste is also impaired.^{1,4} Most allergens are between 5 and 20 μm in diameter, allowing inhalation by the nose.⁴ Allergens that cause hypersensitivity responses in individuals with atopy are proteins or protein-bound chemical substances. Typical allergens include protein in pollen, house dust

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mites, animal dander, food, mold, and drugs such as the antibiotic penicillin. Inhalant allergens play a significant role in the occurrence of allergic rhinitis.⁴

There have been many studies that have studied to treat allergic rhinitis. There are three options for the management of allergic rhinitis: (1) avoidance and environmental control, (2) pharmacotherapy, and (3) immunotherapy.⁴ One alternative treatment that can be used is herbal medicine.^{6,7} The development of medicinal plants at home and abroad is growing due to many adverse drug reactions.⁸ The World Health Organization (WHO) recognizes traditional medicine as one of the essential elements of primary health care: *Saving plants saves lives*.^{9,10}

Chromolaena odorata (L), commonly known as the Siamese weed, belongs to the sunflower family Asteraceae. *Chromolaena odorata* (L) is an important medicinal plant that can easily be found in the tropics of Asia, West Africa, and parts of Australia.

Several literature studies reveal several important pharmacological activities such as antiallergic, anticancer, antidiabetic, antidiarrheal, anticonvulsant, anti-inflammatory hepatoprotective activity, antihelmintic, antimalarial, analgesic, anti-inflammatory, antipyretic, antispasmodic, antioxidant, antigonorrhea, antimycobacterial, insecticide, fungicide, wound healing and hemostatic, diuretic, blood clotting, antibacterial¹¹ and antitrypanosomal, as well as antihypertensive.¹² The value of medicinal plants lies in bioactive compounds such as alkaloids, flavonoids, tannins, and phenolic compounds, which produce specific physiological actions in the human body.¹³

Quercetin is an active bioflavonoid found in many plants and is very safe for treating allergic rhinitis. Flavonoids inhibit enzymes that increase histamine release from mast cells and basophils: cAMP phosphodiesterase and calcium-dependent ATPase. Large amounts of the cAMP act by blocking the intracellular histamine reservoir. Calcium-

dependent ATPases also degrade ATP to release energy to facilitate the Ca^{2+} gate across cell membranes: high intracellular Ca^{2+} levels also cause histamine release from cellular storage granules. Quercetin is a flavonoid with a strong affinity for mast cells and basophils. Quercetin stabilizes their cell membranes, thereby preventing them from shedding histamine. By inhibiting the release of histamine and leukotrienes into the bloodstream, quercetin prevents allergy symptoms such as swollen nasal passages, stuffy nose, sneezing, watery eyes, and itchy eyes and nose.

According to the results of research conducted by Oluwaseun Ruth Alara in 2019, significant variables include microwave power (400-800 W), irradiation time (1-5 minutes), and ethanol concentration (20-60%) at a constant temperature (70°C) and solvent ratio (10:1) mL/g can be further optimized to achieve maximum yield of total phenolic and flavonoid content (TFC) from *Chromolaena odorata* (L) leaves.¹⁵ Animal allergy experiments usually use ovalbumin (OVA). One of the uses of ovalbumin is stimulating allergic reactions in various experimental animals. Allergy induction with ovalbumin is given gradually, starting with sensitization and continuing with allergen provocation (allergen challenge).^{16,17} Murat Kar et al., 2019 stated 0.4% OVA solution is prepared with OVA emulsified in Tween 80 with $\text{Al}(\text{OH})_3$ in 100mL saline. Experimental animals were sensitized by intraperitoneal administration of 5mL OVA on days 0, 2, 4, and 6. Furthermore, 10 μ L of a similar solution was administered intranasally once a day for seven days.¹⁸ From the description above, the researcher wanted to know the effect of giving kirinyuh leaf (*Chromolaena odorata* (L)) ethanol extract ointment on the levels of eosinophils, basophils, and neutrophils in the nasal mucosa of rabbits.

MATERIALS AND METHODS

This research is an experimental laboratory study with a pretest-posttest control group design using rabbits as the research subject. The time plan for this research is from October 2020 to March 2021.

This research was conducted at the Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, to maintain and treat experimental models. Preparation of ointments from the ethanol extract of kirinyuh leaves (*Chromolaena odorata* (L)) at the Faculty of Pharmacy, Syiah University Kuala Banda Aceh.

The subjects of this study were male rabbits with the inclusion criteria for male rabbits, body weight between 1000-1500 grams, and rabbits in good health (ears erect and clean, eyes round and clear, dry nasal surface, strong teeth).

While the exclusion criteria were that the experimental animals looked sick during the adaptation, decreased body weight during adaptation >10%, experimental animals died during the experiment. This study had five groups: three treatment groups, one positive control group, and one negative control group. The sample of this

research is five samples for each treatment, so the required selection is 25 rabbits.

The research procedure includes: 1.) Sterilization of tools done by steam sterilization. All tools to be used are washed and cleaned. 2.) The preparation of the ethanol extract of kirinyuh leaves is air-dried for 72 hours. After drying, it is ground using a vortex to become powder. Dry kirinyuh leaf powder was extracted by maceration employing fine kirinyuh leaf powder soaked in 7 liters of 70% ethanol for 72 hours to obtain the first filtrate. Furthermore, the fine powder of kirinyuh leaves was rewashed with 7 liters of 70% ethanol for 72 hours, so the second filtrate was obtained. Then the first and second filtrates were combined, and the solvent was evaporated with a rotary evaporator to obtain a thick extract of 20 grams. 3.) Making a variable concentration of kirinyuh leaves, the extract that has been obtained is then diluted with distilled water to obtain concentrations of 5%, 10%, and 15%. 4.) Preparation of ointment Evaluation of the ethanol extract of kirinyuh leaf ointment in this experiment was carried out to find out the results of the tests parallel to the theoretical results which included organoleptic and pH tests, homogeneity, viscosity, and spreadability tests of the ointment. 5.) Preparation of Experimental Animals The research samples, before treatment, were weighed and adapted for two weeks, homogenized in the cage, and the temperature in the cage was set at room temperature. Every day the rabbits were fed in the form of standard feed/standard food (carrots, kale, pellets), and drinking water was provided ad libitum. 6.) Assessment of eosinophils, basophils,

and neutrophils before and after treatment. Assess the number of eosinophils, basophils, and neutrophils of the nasal mucosa by scraping the nasal mucosa of rabbits and staining with Giemsa and viewing using an electron microscope with 400x magnification as much as 100x field of view at histology laboratory, Faculty of Veterinary Medicine Unsyiah Banda Aceh. 7.) Sensitization of Experimental Animals with Allergens In experimental animals, rabbits were sensitized with ovalbumin. Experimental animals were given 0.4% ovalbumin solution emulsified in Tween 80 with $\text{Al}(\text{OH})_3$ in 100 mL saline. Experimental animals were sensitized by intraperitoneal administration of 5mL ovalbumin on days "14, 16, 18, and 20". Then 10 μL of a similar solution was administered intranasally once a day for seven days, until day 27.¹⁸ 8). Animal Treatment Experiments Rabbits were divided into five groups, each group consisting of 5 rabbits, with three groups being treated with 5% kirinyuh leaf ethanol extract ointment, 10% and 15%, and 1 group as a positive control and 1 group as a negative control. The experimental animals were sensitized with ovalbumin, then given an ethanol extract of kirinyuh leaves (*Chromolaena odorata* (L)) ointment with three preparations. Group 1 was given an ethanol extract of kirinyuh leaves 5 %, group 2 was given 10%, group 3 was assigned 15%, 1 group was a positive control, and 1 group was a negative control. The ointment was applied to the nasal mucosa of the experimental animals as much as 0.1 g each p the next day, carried out for seven days. All data is processed using one-way ANOVA.

RESULTS

The study's results reported that the ethanol extract of kirinyuh leaves (*Chromolaena odorata* (L)) ointment with concentrations of 5%, 10%, and 15% could reduce inflammatory cells for seven days of treatment. The decrease in inflammatory cells was positively correlated with the allergic response elicited during the administration of kirinyuh leaf ethanol extract ointment. A reduction in basophil cell response marks a decrease in allergies. Basophil cells have been reported as precursors of the IgE response in the pathogenesis of the allergic reaction.

The study's results described the phenomenon that kirinyuh leaf ethanol extract ointment can be involved in reducing the response of immune cells involved in allergies, especially in the mucosal area. Thus preventing the interaction of allergen responses with the immune system as well as functioning as immunoprotection.

The 5% concentration has a better ability to reduce inflammatory cells. A decrease in inflammatory cells correlates with a reduction in allergies, characterized by a decline in basophil cells (Figure 1).

Figure 1 shows that all concentrations of the ethanol extract of kirinyuh leaf ointment used in this study had a response to reduce allergic infections in the nasal mucosa of rabbits as animal models in this study. The concentration of 5% has a better ability than the concentration of 10% and 15%. Basophil cells and neutrophil cells experienced a dominant decrease compared to eosinophil cells. This indicates that allergic and inflammatory responses have decreased because these two cells predominantly work on allergic and inflammatory reactions by the host immune system.

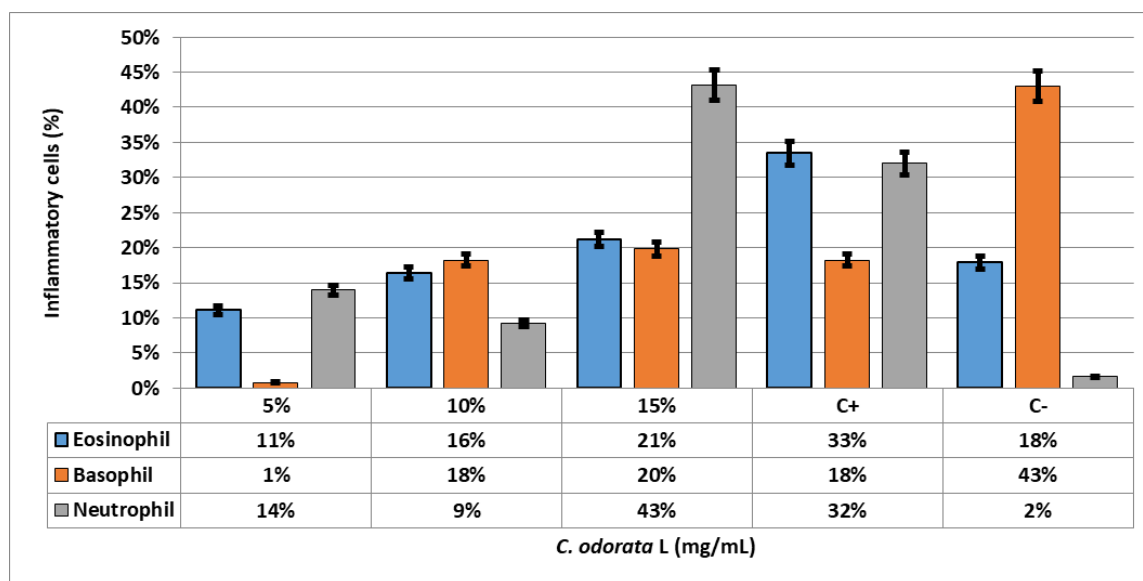


Figure 1. Inflammatory cell response in rabbits induced by allergens after being given kirinyuh leaf ethanol extract ointment for seven days.

Kirinyuh leaf ethanol extract ointment gave a maximum response to basophil cells and neutrophil cells by 300%. It decreased to 400% response to respond to allergens that trigger allergies in the nasal mucosa. Meanwhile, neutrophil cells are still increasing (Figure 2). Figure 2 shows A scatter diagram showing the role of the ethanol extract of kirinyuh leaf ointment with various concentrations during the immune system's response for protection

against allergens. Basophils and neutrophils have an operational phase (spread) reaching 300% peak phase and decreasing again at an operational level of 400%. The seven-day treatment time is considered the maximum response time of inflammatory cells to decrease allergen response in modeling nasal mucosal allergy. The morphology of inflammatory cells can be seen in Figure 3.

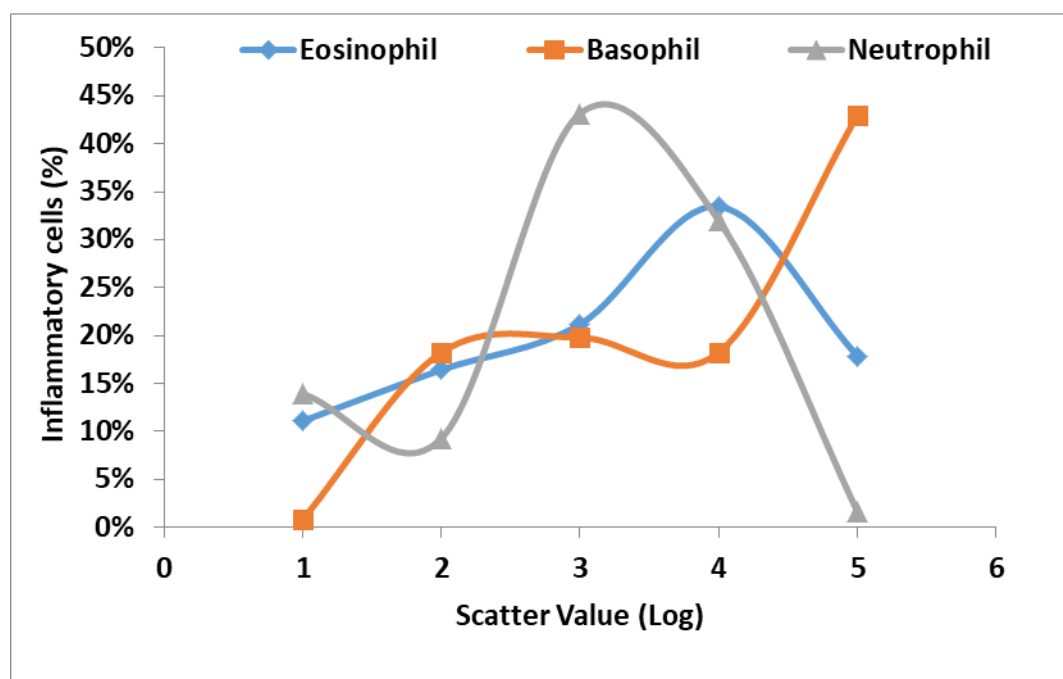


Figure 5.2. Scatter diagram of the relationship between inflammatory cells during the inflammatory cell response in the host's body for 7 days.

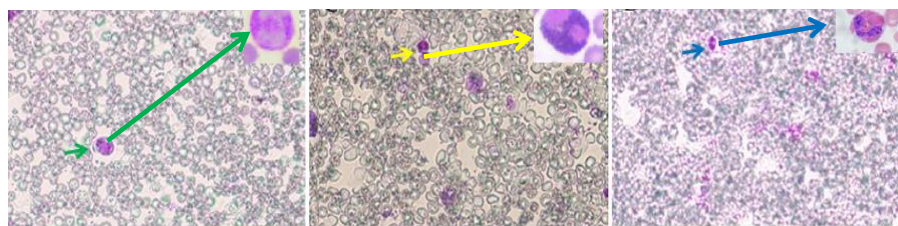


Figure 3. Inflammatory cell histopathology. Green arrows (basophils), yellow arrows (neutrophils), and blue arrows (eosinophils). Images were taken at 400x magnification using a light microscope

Table 1 shows the total distribution and concentration of the ethanol extract of kirinyuh leaf frequency of inflammatory cells affected by the (*Chromolaena odorata* (L)) ointment.

Table 1. Frequency and distribution of inflammatory cells from mucosal scraping preparations

Cons	N	Eosinophil				Basophil				Neutrophil			
		Cell	SDV	Freq	Scale	Cell	SDV	Freq	Scale	Cell	SDV	Freq	Scale
5%	5	132	37.26	20%	Moderate	0.2	0.45	2%	Low	3.4	5.94	28%	Low
10%	5	132	30.30	20%	Moderate	0.4	0.55	3%	Low	1	1.73	8%	Low
15%	5	124	15.17	19%	Low	0.8	0.45	6%	High	3.8	1.30	31%	Moderate
C+	5	55.8	21.00	8%	Low	0.4	0.55	3%	Low	3.6	1.52	30%	Moderate
C-	5	224.6	237.26	34%	High	11	14.70	86%	High	0.4	0.89	3%	Very Low

Table 2 describes the presence of inflammatory cells after being induced with various concentrations of ethanol extract of kirinyuh leaf ointment. This value will be used as a reference for the response of inflammatory cells to allergens. Value 40% (high), 30-39% (moderate), and value <30% (low). This value was used to measure the distribution of inflammatory cell activity affected by the ethanol

extract of kirinyuh leaf ointment from each concentration for seven days of animal model treatment. Values on a low scale indicate a better role for the test material in reducing the frequency of inflammation and allergies. While the large scale shows the response to reduce inflammation and allergies is not maximal.

Table 2. Total distribution and frequency influenced by the concentration of (*Chromolaena odorata* (L))

Cons	N	Eosinophil	Basophil	Neutrophil	r	p
5%	5	11%	1%	14%	0.907	0.024
10%	5	16%	18%	9%		
15%	5	21%	20%	43%		
C+	5	33%	18%	32%		
C-	5	18%	43%	2%		

Oneway ANOVA statistical analysis showed no significant difference in the number of inflammatory cells between basophils, eosinophils, and neutrophils

$p > 0.05; 1.00$). Furthermore, the Kruskal Wallis Test analysis showed differences in the number of inflammatory cells based on the concentration of the

test material, related to allergen response ($p < 0.05$; 0.024). with a strong relationship ($r=0.907$). Based on the analysis of the Independent Samples T Test, it showed that there was no significant difference

between the activity of eosinophil cells and neutrophils ($p > 0.05$; 1.00), as well as the activity of basophil cells with neutrophils and basophil cells with eosinophils ($p > 0.05$; 1.00)

DISCUSSION

This study assessed the effect of giving kirinyuh leaf ethanol extract (*Chromolaena odorata* (L)) ointment on eosinophil, basophil, and neutrophil levels of rabbits induced by allergens in the nasal mucosa. The results of this study reported that the ethanol extract of kirinyuh leaves provided benefits for reducing inflammatory cells, especially eosinophil cells and basophil cells, as indicators of decreased allergies, while neutrophils as indicators of reduced inflammation during seven days of treatment.

Basophils play an essential role in allergic inflammation related to IgE activity. This process occurs because basophils migrate to sites of inflammation and induce the secretion of various mediators, including cytokines, chemokines, and proteases. Upon encountering an antigen, IL-3-stimulated basophils release several effector molecules that may contribute to allergic inflammation.¹⁹ Eosinophils and basophils are reported to be involved in allergic inflammation and circulate at relatively low levels in the blood, forming 0.1-1 % and 1-5% of white blood cells.²⁰ Studies in humans using allergens in vivo have shown the ability of basophils to induce increased expression of CD63 associated with allergic responses. However, there are no clinical symptoms of an allergic reaction.

An increase in the number of eosinophils is associated with histamine release. In contrast, neutrophils have been reported as essential histamine producers in allergic responses. Neutrophils are estimated to store 0.29 pg/cell and release 50% of the histamine content. The release of these allergic response substances highly depends on the allergen response to stimulation with other neutrophil agonists.

Like mast cells, basophils activated due to cross-linking between IgE and FcεRI receptors will rapidly degranulate and release their cellular contents. In addition, inflammatory mediators such as complement factors C5a and C3a, MBP, PAF, and chemokines can start basophils without cross-linking IgE.²³ Also, basophils contain the anticoagulant heparin, which prevents blood from clotting too quickly, and the vasodilator histamine, which increases blood flow to the network. Neutrophils are reported to be important effector

cells of the immune system. These cells work to prevent the development of pathogens in the body through phagocytosis to trap and kill pathogens that attack the host.

Results of the study reported that, in general, the ethanol extract of kirinyuh leaf ointment with various concentrations reduced inflammatory cells for seven days of treatment. This decrease in inflammatory cells is associated with reduced allergic reactions in animal models. Pathophysiologically, an allergy reduction is characterized by a decline in the response of eosinophil and basophil cells, which these two cells are reported to trigger the IgE response to allergies. In comparison, the decrease in neutrophils is associated with the production of histamine as a protein in allergic reactions. Specifically, Figure 1 shows a concentration of 5% has a better ability than concentrations of 10% and 15%.

According to Warrington (2012), this ability is related to the drug absorption response during an immune response to allergens.²⁵ This means that this test material can reduce the immune response and increase the action of the active compounds contained in the ethanol extract ointment of kirinyuh leaves while working to inhibit allergen activity. Specifically, kirinyuh leaves contain flavonoid compounds that function as antioxidants

Flavonoids are reported to inhibit histamine release, IL-4, IL-13 synthesis, and CD40 ligand expression by basophils.²⁷ Flavonoids indirectly work to reduce basophil activity. This study is in line with the research findings reported by Kawai (2007). In addition, Park (2008) explained that flavonoids could suppress the release of inflammatory mediators such as histamine and proinflammatory cytokines through their function as antioxidants, cytoprotective and anti-inflammatory mechanisms. Pharmacologically, flavonoids show their potential activity for treating allergic inflammatory diseases through the down-regulation of mast cell activation.²⁸ So, it can be assumed that the flavonoids in the ethanol extract of kirinyuh leaves can interact with the immune response that binds to mast cells to reduce the release of inflammatory mediators. Such as histamine, thereby suppressing allergic activity.

In this study, the concentration of the ethanol extract of kirinyuh leaf ointment at a concentration of 5% could better suppress the development of allergies than other concentrations. As a comparison, Alexandrakis (2003) reported that Flavones and kaempferol at 100 mL could inhibit mast cell proliferation by more than 80% on days 3, 4, or 5 of treatment. These results indicate that flavonoids can inhibit the proliferation of Molecule-histo Compatibility (MHC)-1 in inducing the development of secretory granules and the accumulation of inflammatory mediators.²⁹ In this study, a decrease in basophil cell activity was found, possibly because the flavonoids from the ethanol extract of kirinyuh leaf ointment can inhibit the expression of IL-4 and CD40 ligands mediated through their inhibitory action on activated T cell nuclear factor activation.

The results of this study also describe neutrophils have increased because one of the functions of plant flavonoids is to modulate increased neutrophil work to prevent infection or inflammation. Busse (1984) has previously reported that flavonoids are natural plant compounds that have been shown to have various anti-inflammatory

effects. The role of flavonoids in the inflammatory response are lysosomal enzyme release, chemiluminescence (CL) response, and superoxide anion production.³¹

In this study, the ethanol extract ointment of kirinyuh leaves succeeded in suppressing the development of eosinophil and basophil cells and activating the intensity of neutrophil work. The ability to stop the growth of these three cells is possible because the flavonoid compounds in the ethanol extract ointment from kirinyuh leaves can inhibit factor mediators from activating IgE and mast cell responses by increasing the role of neutrophil cells.

This study has reported the role of ethanol extract ointment from kirinyuh leaves (*Chromolaena odorata* (L)) as an anti-inflammatory agent in the allergic response of the nasal mucosa. In general, it can accelerate inflammatory healing and reduce allergic reactions based on eosinophil, basophil, and neutrophil cell profiles. This potential can be made possible by using this plant as a therapeutic ingredient in healing allergies and inflammatory reactions.

CONCLUSION

Based on the research objectives, this study can be concluded that the ethanol extract ointment of kirinyuh leaves (*Chromolaena odorata* (L)) applied topically can reduce allergic reactions and reduce inflammation. Kirinyuh leaf ethanol extract ointment ((*Chromolaena odorata* (L)) can reduce eosinophil, basophil, and neutrophil inflammatory cells in the

nasal mucosa in rabbit allergy modeling for seven days. A 5% concentration of kirinyuh leaf ethanol extract ointment (*Chromolaena odorata* (L)) has an effect that is better in responding to the activity of eosinophil, basophil, and neutrophil inflammatory cells in the nasal mucosa in rabbit allergy modeling for 7 days.

ETHICAL STATEMENTS

Several ethical contributions KEPPKN Registration Number: 1171012P. Description Of

Ethical Exempted "Ethical Exempted" Number: 004/EA/FK-RSUDZA

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