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Anti-inflammation Effect of *Kopasanda* Leaf (*Chromolaena odorata L.*) Extract on White Rats (*Rattus Norvegicus*) Feet Intraplantar Injection with Carrageenan

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This study aimed to investigate the anti-inflammatory effects of Kopasanda leaf (*Chromolaena odorata L.*) extracts against carrageenan-induced inflammation on white rats' (*Rattus novergicus*) feet through intraplantar injections. The inflammation volumes were measured using a plethysmometer over an eight-hour period. Active compounds known to have anti-inflammatory functions, such as flavonoids, saponins, rriterpenoids, alkaloids, and tannins, were found. This experimental study used white rats, which were divided into negative (no treatment), positive (treated with diclofenac sodium), and treatment (treated with extract) groups with three replications each. Three concentration variations of the extract were given. The data were analysed using ANOVA on the variables, concentration variations, and exposure times. The result shows that the average inflammation volume in rats with no treatment increased from 0.23 to 0.70, while inflammation volumes in rats treated with diclofenac sodium and extracts returned to their normal levels at the end of the treatment period. There was a significant difference in the time period of treatment exposure ($p = 0.000$), no significant difference in the extract concentration variations ($p = 0.298$), and no significant difference in the interaction between time of exposure and dosages ($p = 0.922$). The Duncan Multiple Range Test (DMRT) shows that the three concentration variations have the same effect. This study concluded that Kopasanda leaf (*Chromolaena odorata L.*) extract has an anti-inflammatory effect comparable with commercial agents such as diclofenac sodium.

Keywords: *inflammation, anti-inflammation, Kopasanda (Chromolaena odorata L.), white rats (Rattus norvegicus), carrageenan*

Inflammation is the body's immune response to harmful stimuli such as pathogens, cell damage, toxic compounds, and radiation. The inflammatory response in the body is essential to maintaining the condition of the tissues and homeostasis. Common symptoms of inflammation are swelling, redness, and vasodilation pain due to increased blood flow, increased cell metabolism, and the production of inflammatory mediators such as serotonin, bradykinin, prostaglandins, and inflammatory cytokinin of the body's prostaglandins (Prasetyana, 2022). Based on Riskesdas' (2013) data, nonsteroidal anti-inflammatory drugs (NSAID) have risen by 11.9% to 24.7% and are widely used due to the prevalence of joint diseases (Soleha et al., 2018).

As interest in the use of herbal remedies is increasing, the use of plants both for medicine and for other purposes is a present phenomenon. While medicinal plants contain components of active compounds and have various pharmacological effects, they still need to be scientifically proven (Sukmawati et al., 2015). In this context, Indonesians use plants as herbal medicines, as more than 60% of local medicines are derived from plants.

Kopasanda (*Chromolaena odorata L.*) is commonly known as Siam Weed, Christmas Bush or Floss Flower. The leaves have been used as medicine by people in Indonesia. This plant is also present in North America, Asia, West Africa, and Australia. The people in Indonesia's Makassar region use kopasanda leaf as a wound medicine and antioxidant (Nurhajanah et al., 2020; Rasyid et al., 2020; Sirinthipaporn & Jiraungkoorskul, 2017; Vijayaraghavan et al., 2017). Research conducted by Sumardi et al. (2018) identifies the active compounds found in kopasanda leaf as flavonoids, saponins, triterpenoids, alkaloids, and tannin compounds (Sumardi et al., 2018). These compounds have also been claimed to prevent the growth of cancer cells (Yu et al., 2015).

Kopasanda (*Chromolaena odorata L.*) leaves have anti-inflammatory activity, antibacterial, antioxidant and wound-healing effects, play an important role in wound healing, and have vitamin C. It also contains proteins, vitamin B1, vitamin B2, vitamin C, and carotene (Amina et al., 2023; Sukmawati et al., 2021; Sumardi et al., 2018). The content of vitamin C can accelerate healing and treat damaged skin.

This study determined the anti-inflammatory effect of the extract of Cromolaena odorata L. on white rats.

Methods and Materials

Research Sample

Kopasanda leaves were collected, dried, and powdered using a blender, and then sifted using a mesh sieve of 50. The powdered sample underwent maceration and soaking in 96% ethanol for 3 days. The maceration process was repeated three times, and the extracts were combined. The solvent was then evaporated and concentrated on a rotary evaporator to obtain a thick ethanolic extract.

Secondary Metabolite Compound Screening Results

Screening of secondary metabolite compounds was carried out to find an overview of the active compounds contained in the plant. The following procedures were employed to determine the presence of the different compounds:

To determine alkaloid compounds, the extract is diluted with Dragendroff reagent, turning it brick-red. The addition of Mayer reagent produces white precipitate, indicating that it contains alkaloid compounds.

To determine flavonoid compounds, concentrated hydrochloric acid is added to the extract, and as the reagent turns orange, it confirms the presence of flavonoid compounds.

To determine the content of tannins, if the extract is diluted with FeCl3 solution and it turns darkbrown, and with the addition of gelatin reagents produces a white precipitate, this indicates the presence of tannin compounds.

To determine the presence of steroid compounds, if concentrated sulfuric acid is added to the extract and turns purple, this will indicate that it contains steroid compounds.

To determine the content of saponin compounds, if the sample extract diluted with concentrated hydrochloric acid reagent produces foam, this will indicate it contains saponin compounds.

Carrageenan

In the process of swelling up the rats' feet, carrageenan solution was used. 1 g of carrageenan was dissolved in 100 mL of warm water, then 10 mL of 0.9% NaCl was added and mixed until evenly distributed. As much as 0.2 mL of the solution was used for intraplantar injection.

Treatment in Animals Test

The lab animals used are 18 male white rats (*Rattus norvegicus*) aged 2-3 months in a healthy and active state. The rats were acclimatized for two weeks, given food in the form of pellets, and given water.

The rats were divided into five groups. Three experimental groups were injected with 1 mg/200 grams of body weight, 2 mg/200 grams of body weight, and 3 mg/200 grams of body weight, respectively. One group was treated with 1 mg of diclofenac sodium per 200 grams of body weight, while another group was not treated with anything. This last group is the negative control group. All groups had three rats each.

Rats underwent an 18-hour fast prior to the testing but were still given water. Then the part of the leg (up to the ankle) was marked in each rat in order to uniformize the part that would be immersed in the plethysmometer. The initial volume of rat legs was measured before treatment and expressed as normal volume (T_0). A first injection of 0.2 mL of carrageenan solution was injected, followed by 2.0 mL of Kopasanda leaf extracts. Data gathering by measuring the rats legs was done at T_1 or after 1 hour to see the effect of the treatment. Another measurement was done at the second hour (T_2) until the 8th hour (T_8).

Results and Discussion

Sample Extraction

Extraction was carried out by maceration using 96% ethanol as a solvent. The macerated mixture was then concentrated using a rotary evaporator. For 200 grams of powdered sample, 15.31 grams of concentrated extract were obtained.

Screening Results of Secondary Metabolite Compounds

Secondary metabolite compound screening was carried out to obtain an overview of the active compounds contained in the plants. The following are the results of the procedures:

1. When the sample extract was diluted with Dragendroff's reagent, the color turned brick red, and when mixed with Mayer's reagent, it formed a white precipitate. This indicated that Kopasanda leaf contained alkaloid compounds.
2. When the sample extract was diluted with concentrated hydrochloric acid, the color turned orange. This indicates that Kopasanda leaf contained flavonoid compounds.
3. When the sample extract was diluted with the FeCl3 reagent, it did not turn dark brown, and when mixed with the gelatin reagent, it did not produce white precipitate. This indicated that Kopasanda leaves do not contain tannin compounds.
4. When the sample extract was diluted with concentrated sulfuric acid, it turned purple. This indicates that Kopasanda leaf contained steroid compounds.
5. When the sample extract was diluted with concentrated hydrochloric acid, it did not produce foam. This indicates that Kopasanda leaves do not contain saponin compounds.

Anti-inflammatory Test Results

The injection of the Kopasanda leaf extracts into the rats whose leg inflammation was artificially induced had positive results. Data from all three experimental groups showed that inflammation

subsided completely 8 hours after the injection of carrageenan, with the inflamed rats' legs returning to the original measurements obtained at the beginning of the procedures. This was in contrast with the negative control groups, which were injected with an inflammatory substance but were not given anything. The inflammation volume of this "no treatment group was as high as 0.80 mL, subsiding only to 70 mL at the end of the 8-hour period.

The inflammation among the rats in the positive control, or the group that was injected with diclofenac sodium, rose to 0.7 after the first hour but gradually declined, returning to the original pre-inflammation volume at T_8. Table 1 summarizes these results.

Table 1

Data on Inflammation for the Treatment Groups

Treatment Groups	No	Inflammation								
		T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
1mg/200gr BW	1	0,3	0,4	0,5	0,6	0,5	0,5	0,4	0,3	0,3
	2	0,3	0,4	0,4	0,6	0,5	0,4	0,3	0,3	0,3
	3	0,1	0,4	0,5	0,7	0,5	0,4	0,4	0,3	0,1
	4	0,1	0,4	0,5	0,6	0,5	0,4	0,4	0,2	0,1
	Mean	0,2	0,4	0,48	0,625	0,5	0,425	0,375	0,275	0,2
2mg/200gr BW	1	0,3	0,3	0,5	0,7	0,6	0,5	0,4	0,3	0,3
	2	0,2	0,2	0,5	0,7	0,5	0,5	0,4	0,4	0,2
	3	0,1	0,4	0,6	0,9	0,5	0,4	0,3	0,3	0,1
	4	0,3	0,3	0,5	0,6	0,525	0,4	0,4	0,3	0,3
	Mean	0,23	0,3	0,53	0,725	0,525	0,45	0,375	0,325	0,225
4mg/200gr BW	1	0,3	0,3	0,5	0,8	0,6	0,5	0,5	0,4	0,3
	2	0,2	0,4	0,5	0,6	0,5	0,5	0,4	0,3	0,2
	3	0,1	0,2	0,4	0,5	0,5	0,4	0,3	0,2	0,1
	4	0,3	0,5	0,6	0,6	0,5	0,4	0,3	0,3	0,3
	Mean	0,23	0,35	0,5	0,625	0,525	0,45	0,375	0,3	0,225
No Treatment	1	0,20	0,70	0,80	0,80	0,87	0,90	0,80	0,70	0,7
	2	0,30	0,70	0,80	0,80	0,8	0,80	0,70	0,70	0,70
	3	0,20	0,70	0,80	0,80	0,85	0,90	0,80	0,70	0,70
	Mean	0,23	0,70	0,80	0,80	0,84	0,87	0,77	0,70	0,7
Diclofenac Sodium	1	0,10	0,70	0,50	0,40	0,35	0,30	0,30	0,10	0,10
	2	0,30	0,70	0,60	0,40	0,37	0,40	0,30	0,30	0,30
	3	0,30	0,60	0,50	0,40	0,40	0,40	0,30	0,30	0,30
	Mean	0,23	0,67	0,53	0,40	0,37	0,37	0,30	0,23	0,23

The peak of swelling and inflammation for the experimental group was at 3 hours (T_3) after the first injection of carrageenan and the extract/treatment, with a volume range of 0.63–0.73 mL. At the end of data collection (T_8), the data shows that the inflammation volume of the feet is between 0.20 and 0.23 mL and is back to its normal size.

These results show that the three experimental groups had comparable anti-inflammatory action as the positive group, whereby all measurements returned to normal within the 8-hour period.

Statistical Analysis

The data was analyzed using a univariate ANOVA. the concentration variations of the extract and period time of treatment exposure (data collection) as the variables, with a level of significant value of α = 0.05.

Table 2

Statistical Analysis Results on Extract Concentrations and Time of Exposure

Source		Sum of Squares	DF	Mean Square	F	Sig.
Intercept	Hypothesis	1704.083	1	1704.083	65.911	.000
	Error	206.833	8	25.854[a]		
Conc. Variations	Hypothesis	.889	2	.444	1.306	.298
	Error	5.444	16	.340[b]		
Time period	Hypothesis	206.833	8	25.854	75.980	.000
	Error	5.444	16	.340[b]		
Conc, Variation Time period	Hypothesis	5.444	16	.340	.533	.922
	Error	51.750	81	.639[c]		

The analysis results show that the three concentration variations of the extract used in this study have or show the same antinflammatory effect, with p = 0.298 > α = 0.05. Thus, there is no difference in the anti-inflammatory effect of the different concentration variations of the extract of kopasanda leaf (*Chromolaena odorata L.*) extract.

The effect of time exposure on the extract shows a significant effect on reducing inflammation, with p = 0.000 < α = 0.05. Thus, there is no difference in the effect of the time length of extraction exposure of kopasanda leaf (*Chromolaena odorata L.*) extract as antiinflammation".

In the interaction of the two variables, namely the concentration variation of the extract and the time length of treatment exposure, the result is not significant with *p* = 0.922 > *α* = 0.05. Thus, the interaction of the concentration variation of the extract and the time length of extract exposure of Kopasanda leaf (*Chromolaena odorata L.*) extract as anti-inflammatory.

Table 3

Duncan Multiple Distance Test Results Based on Variations of Extract Concentration

Dosage	N	Subset 1
Conc. Variation of 1 mg	36	3.8611
Conc. Variation of 4 mg	36	3.9722
Conc. Variation of 2 mg	36	4.0833
Sig.		.271

The Duncan Multiple Range Test (DMRT) table shows that the three concentration variations of the extract have the same effect as anti-inflammation (in the same subset), although the concentration variation of 1 mg/200 g BW is higher than the 4 mg/200 g BW and 2 mg/200 g BW, respectively.

This study concludes that Kopasanda leaf (*Chromolaena odorata L.*) extract has an anti-inflammation effect on white rats (*Rattus novergicus*) feet intraplantar injected with carrageenan and that the three concentration variations of the extract have the same anti-inflammation effect as the positive control, which

is diclofenac sodium. The time period of kopasanda leaf (*Chromolaena odorata L.*) extract exposure significantly reduced the inflammation of the rat feet. At the 8th hour of exposure, the volume of the inflammation has returned to its normal size. The interaction between the concentration variation of the extract and the length of the treatment exposure does not significantly reduce the inflammation.

References

Amina, A., Soenandar, M., Tomponu, T., and Wahyu, H. (2023). Aktivitas antioksidan ekstrak etanol batang, daun dan akar Kopasanda (Chromolaena odorata L) dengan Metode ABTS (2,2'-azino-bis (3-etilbenzotiazolin-6-asam sulfonat). *Fullerene Journal of Chemistry. 7*, 2 (61-66).

Nurhajanah, M., Agussalim L., Iman S. Z., and Hajiriah, T. L. (2020). Analisis kandungan antiseptik daun kopasanda (Choromolaena odorata) sebagai dasar pembuatan gel pada luka. *Bioscientist: Jurnal Ilmiah Biologi. 8*(2); 284-93

Prasetyana, Vivi Atny. (2022). Uji Aktivitas Antiinflamasi Kombinasi Daun Kelor (*Moringa oleifera L.*) dan Natrium Diklofenak pada Tikus Putih (*Rattus norvegicus*) yang Diinduksi Karagenan. Skripsi Biologi. Repositori Universitas Kristen Duta Wacana

Rasyid S. A., Sugireng, Surya R. A., Sanatang, Rosdarni, Natalia WOR. (2020). The antibacterial activity of tembelekan leaf (Lantana camara L.) and kopasanda leaf (Chromolaena odorata L.) extracts against Staphylococcus aureus. *Infectious Disease Reports. 12* (Suppl 1); 8734-34

Sirinthipaporn, A., Jiraungkoorskul, W. (2017). Wound healing property review of siam weed, Chromolaena odorata. Pharmacognosy Reviews, 11(21); 35-38

Soleha, Maratu, Ani Isnawati, dan Rosa Winarsih. (2018). The Profile of Nonsteroid Antiinflammation Drugs Use in Indonesia. *Jurnal Kefarmasian Indonesia* vol. 8. No. 2.

Sukmawati, Rahmawati & Kenanga, Isyanda, A. 2021. Efek Antinefrotoksisitas Ekstrak Etanol Daun Pandan Wangi (pandanus amaryllifolius roxb) dengan Parameter Kadar Ureum Tikus Putih. *Journal As-Syifaa Farmasi*. Vol. 13, No. 2

Sukmawati, S., Yuliet Y., dan Ririen Hardani. (2015). Uji Aktivitas Antiinflamasi Ekstrak Etanol Daun Pisang Ambon (Musa Paradisiaca L.) terhadap Tikus Putih (Rattus Novergicus L) yang Diinduksi Karagenan. *Galenika Journal of Pharmacy* Vol. 1 (2).

Sumardi, Husori D., Julianto T., Fauza R., Eliska E. (2018). Toksisitas ekstrak daun kopasanda (Chromolaena odorata L.) terhadap larva Artemia salina Leach. *Jurnal Keperawatan dan Kebidanan Nersmid. 1*(1); 58- 65

Vijayaraghavan, K., Rajkumar J., Bukhari S. N. A., Al-Sayed B., Seyed M. A. (2017). Chromolaena odorata: A neglected weed with a wide spectrum of pharmacological activities (Review). *Molecular Medicine Reports. 7*:15(3); 1007-16

Yu, Z, Zhang T., Zhou F., Xiao X., Ding X., He H., Rang J., Quan M., Wang T., Zuo M., Xia L. (2015). Anticancer activity of saponins from Allium Chinese against the B16 melanoma and 4T1 breast carcinoma cell. *Evidence Based Complementary and Alternative Medicine.* e725023