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Anti-Inflammatory and Analgesic Effect: an Experimental Study of Purple Leaves (*Graptophyllum pictum* (L.) Griff) Decoction

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ABSTRACT: Purple leaves (*Graptophyllum pictum* (L.) Griff) are known to contain flavonoid compounds as anti-inflammatory and analgesic properties. This study aims to obtain the efficacy of purple leaf decoction as anti-inflammatory and analgesic. The anti-inflammatory study used the Winter method with Sprague Dawley rat test, were divided into 5 groups, negative control was only given aquadest, positive control with a dose of diclofenac sodium 8.0208 mg/200g BW, and 3 groups of decoction with doses of 5.4 mg/200g BW, 10.8 mg/200g BW and 21.6 mg/200g BW. Anti-inflammatory testing was carried out by measuring the decrease in the volume of edema on the soles of the rat's feet. Analgesic testing using the Siegmund method with Deutche Denken Yoken mice. The mice were divided into 5 groups, negative control was only given aquadest, positive control with a dose of diclofenac sodium 1.1586 mg/20g BW, and 3 groups of decoction with doses of 1 mg/20g BW, 2 mg/20g BW and 4 mg/20g BW. The analgesic study was carried out by calculating the decrease in the number of writhing mice. The results of the percentage of anti-inflammatory effectiveness of purple leaf decoction doses (5.4 mg/200g BW, 10.8 mg/200g BW and 21.6 mg/200g BW) were 51.77%, 68.77% and 79.46%, respectively. The results of the percentage of analgesic effectiveness of purple leaf decoction doses (1 mg/20g BW, 2 mg/20g BW and 4 mg/20g BW) were 54.03%, 71.62% and 79.73%, respectively. Based on the test results, it can be concluded that purple leaf decoction has an anti-inflammatory and analgesic effect.

KEYWORDS: Anti-inflammatory, analgesics, purple leaves (*Graptophyllum pictum* (L.) Griff).

1. INTRODUCTION

Inflammation is an expression of tissue damage characterised by symptoms of rubor (redness), calor (heat), dolor (pain) and tumour (swelling) [1]. Inflammation occurs as a result of the release of chemical mediators from the damaged tissue and cell migration. The specific chemical mediators vary with the type of inflammatory process and include amines such as histamine and 5-hydroxytryptamine, lipids such as prostaglandins, small peptides such as bradykinin and large peptides such as interleukin-1 [2].

Pain is a common symptom, often associated with one or more diseases. Most diseases have pain symptoms that manifest as pain in the body's organs or tissues. Pain can also be used as a sign of poor body condition and can help diagnose disease by looking at the type and frequency of pain [3]. Anti-inflammatory and analgesic drugs are widely used, but these drugs often have serious side effects. It is estimated that among long-term users of anti-inflammatory and analgesic drugs, 15-40% experience upper gastrointestinal symptoms, 10-25% suffer from peptic ulcers, particularly stomach ulcers, and 1-4% experience life-threatening ulcer complications such as gastric bleeding. In addition, long-term use of anti-inflammatory and analgesic drugs can cause damage to the liver and kidneys [4].

Purple leaves in the community are used for the treatment of wounds, swelling, haemorrhoids, diarrhoea, ulcers, skin diseases, experimentally purple leaf extract has the efficacy of inhibiting swelling and reducing membrane permeability [5]. Purple leaves contain alcohol, pectin, formic acid, flavonoids, alkaloids, saponins, and triterpenoids. The content of essential oil is not less than 0.4%, and flavonoids 0.4%; with a marker active ingredient from the triterpenoid group, namely vomifoliol [6]. Purple leaf has long been known by Indonesians as a remedy for haemorrhoids [7], which works by helping to reduce swelling of the anal skin folds often referred to as haemorrhoids. In Manokwari, West Papua, people know purple leaves to treat

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haemorrhoids by pounding and drinking the boiled water [8]. However, the efficacy to treat inflammation has not been widely studied [9].

Ethanol extract of purple leaves (*Graptophyllum pictum*) is known to have anti-inflammatory and analgesic properties [10]. The compounds that have anti-inflammatory and analgesic properties are flavonoids [7]. Based on this, the author is interested in further research on the anti-inflammatory and analgesic activity of purple leaves which will be made in the form of a decoction.

2. MATERIALS AND METHODS

2.1. Materials

The determination of Purple leaves (*Graptophyllum pictum*) results from Indonesian Institute of Sciences (LIPI) number B-186/IPH.3/KS/I/2019 is carried out to ensure the correct of the simplisia used in research. In the animal study, male *Sprague Dawley* (SD) rats aged 2-3 months with a body weight of 150-200 g and male Deutche Denken Yoken (DDY) mice aged 2-3 months with a body weight of 25-30 g were used, diclofenac sodium as positive control, CMC sodium 0.5%, carrageenan 1%, acetic acid 3%, distilled water. All animal experiments were approved and permitted by the Health Research Ethics Committee of the Faculty of Pharmacy, Universitas Pancasila (ethical No. 105/KEPK-FFUP/XII/2020). In addition, efforts were made to minimize suffering and pain in animals experimental.

2.2. Methods

2.2.1 Preparation of the Purple leaves decoction

Weigh 3 grams of Purple leaves for anti-inflammatory and 1 gram for analgesic and add 200 mL of water. Then heated until a volume of half of the initial volume is obtained with occasional stirring and then filtered with a filter while hot enough to obtain the desired 100 mL decoction volume.

2.2.2 Anti-inflamatory activity test

Before the experiment was carried out on rats, rats were first fed for ± 16 hours while still given water. On the day of the test, the rats were weighed, then marked on the tail of the rat to differentiate one rat from another, a total of 25 rats were taken randomly and divided into five groups, with each as many as 5 rats are control negative given aquadest, control positive fiven natrium diclofenac dose 8.0208 mg, dose I given decocta purple leaves 5.4 mg, dose II given decocta purple leaves 10.8 mg, dose III given decocta purple leaves 21.6 mg.

Prior to treatment, the initial volume of the rat's paw was measured by immersing the rat's paw in the pletismometer device. For each anti-inflammatory test group, the rats were given the test substance preparation orally according to the treatment dose of each group. Thirty minutes later, the rat's paw was induced with 1% carrageenan up to 0.2 mL intraplantar. After carrageenan administration, the volume of rat paw edema was measured every 1 hour for 5 hours [11,12].

2.2.3 Analgesic activity test

As in the anti-inflammatory test, the test animals were first fed and then given a drink. the difference is that the animals used are mice and the doses used are also different, namely control negative given aquadest, control positive fiven natrium diclofenac dose 1.1586 mg, dose I given decocta purple leaves 1 mg, dose II given decocta purple leaves 2 mg, dose III given decocta purple leaves 4 mg.

For each analgesic test group, mice were given the test compound preparation orally according to the treatment dose of each group. Thirty minutes later, the mice were induced with 3% acetic acid up to 0.2 mL/20 g BW intraperitoneally. After acetic acid administration, the mice will move their front and back legs and rub their abdomens against the bottom of the cage [11,12].

2.2.5 Statistical analysis

Data analysis was performed using descriptive analysis with calculation of the area under the curve (AUC) with percentage inhibition of rat udm and mouse writhing, and using SPSS analysis of Kruskal-Wallis data and continued testing between groups of the Mann-Whitney test.

3. RESULTS

3.1 Anti-inflammatory test

Data from the average volume of urea in rat paws showed that in all test groups the greatest increase in urea occurred at the 3rd hour and began to decline from the 4th hour. The greatest increase in urea occurred in the negative control group. The increase in urea in the three test compounds was less than in the negative control. This indicates that the test compounds can inhibit the carrageenan-induced increase in edema on rat paws (Figure 1).

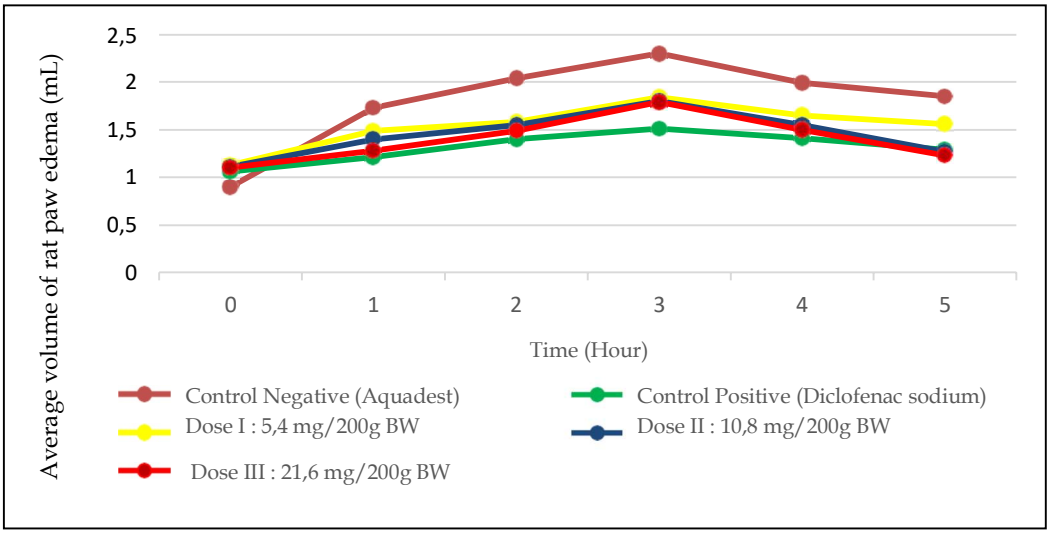

Figure 1. Average volume of edema (mL) of rat paw after giving purple leaves decoction

The effectiveness of anti-inflammatory drugs can be assessed not only by looking at the increase and decrease in the volume of edema on the paw of the rat feet, but also by calculating the AUC (area under the curve). The higher the AUC value, the lower the ability to reduce edema and, conversely, the lower the AUC value, the higher the ability to reduce edema on the soles of the rat's paw [13]. The information is shown in the Table 1 below.

Table 1. Value AUC anti-inflammatory

AUC Group	AUC1	AUC2	AUC3	AUC4	AUC5	Average AUC
Control negative	9.69	9.11	8.35	9.46	9.54	9.23 ± 0.54
Control positive	7.10	6.84	6.73	6.44	6.38	6.70 ± 0.30
Dose I	8.14	7.84	7.40	8.25	7.95	7.92 ± 0.33
Dose II	7.41	7.39	7.47	7.57	7.60	7.49 ± 0.09
Dose III	7.01	7.18	7.30	7.36	6.26	7.22 ± 0.14

The research sample showed that the samples were normally distributed and not homogeneous. Krusskal Wallis test showed at Table 2, that there were significant differences between the test groups.

Table 2. Mann-Whitney test results of anti-inflammatory group

Group test	AUC	Control (-)	Control (+)	Dose I	Dose II	Dose III
Control negative	9,23					
Control positive	6,70	*				
Dose I	7,92	*	*			
Dose II	7,49	*	*			
Dose III	7,22	*	*	*	*	

Notes: * there is a significant difference at the level of <0.05

3.3 Analgesic test

Data on the average number of mice squirming in all test groups showed that the number of mice squirming began to increase at 20 min and to decrease at 25 min after induction with 3% acetic acid. Most wriggles were observed in negative control group, with three test preparations showing less wriggles than negative control. This indicates that the compound tested can inhibit the amount of writhing in mice. (Figure 2)

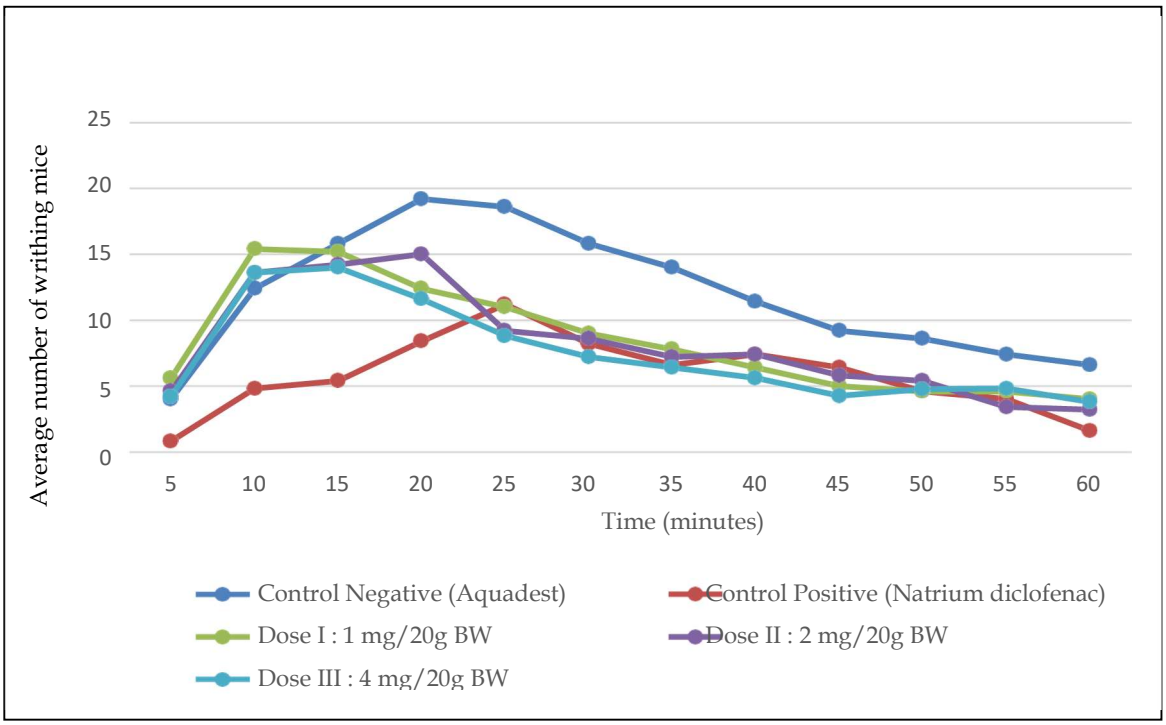

Figure 2. Average number of writhes of mice after administration of Purple leaves decoction

In addition to looking at the increase and decrease in the number of mice writhing, the effectiveness of analgesics can also be assessed by looking at the value of the AUC (area under the curve). The higher the AUC value, the lower the ability to reduce the number of writhing mice and, conversely, the lower the AUC value (Table 3), the higher the ability to reduce the number of writhing mice and Table 4 shows Mann-Whitney test results, obtained significant differences between each group.

Table 3. AUC analgesic value

AUC Group	AUC1	AUC2	AUC3	AUC4	AUC5	Average AUC
Control negative	640	697.5	762.5	725	687.5	702.5 ± 45.45
Control positive	322.5	360	342.5	314	380	343.8 ± 27.00
Dose I	500	425	570	578.5	470	508.7 ± 65.59
Dose II	385	390	425	476	552	445.6 ± 69.70
Dose III	352.5	385	412.5	470	462.5	416.5 ± 50.21

Table 4. Mann-Whitney test results of analgesic group

Group test	AUC	Control (-)	Control (+)	Dose I	Dose II	Dose III
Control negative	702,5					
Control positive	343,8	*				
Dose I	508,7	*	*			
Dose II	445,6	*	*			
Dose III	426,5	*	*	*	*	

Notes: * there is a significant difference at the level of <0.05

Figure 3 explains the percentage of inhibiting paw edema of rats or effective as anti-inflammatory dan inhibiting writhing of mice or effective as analgesic.

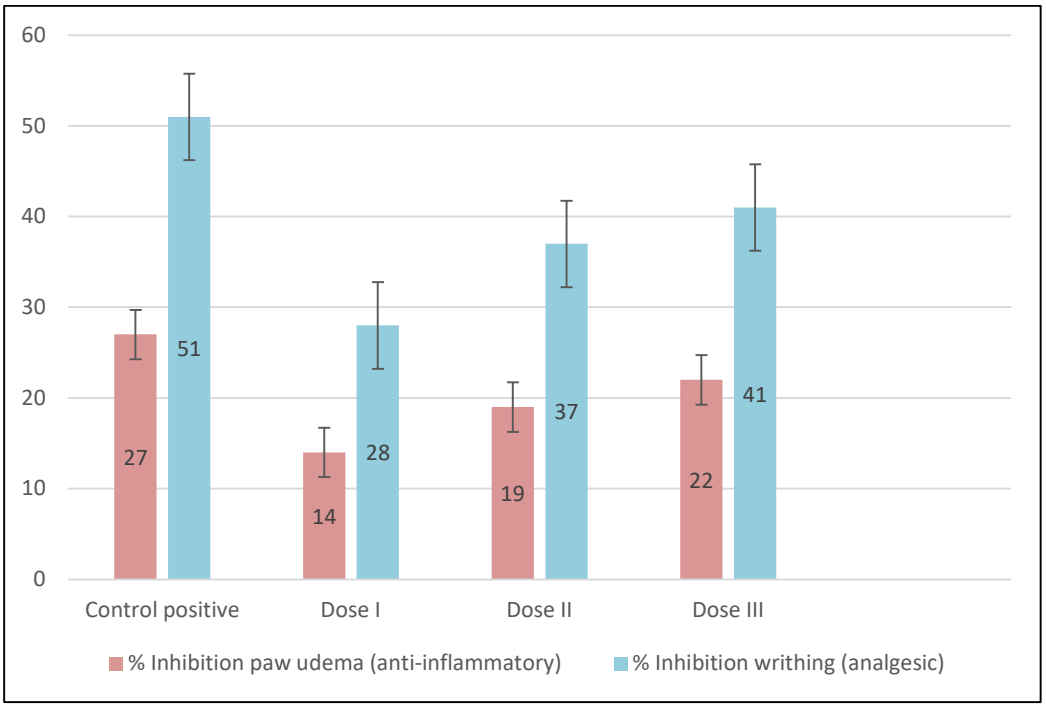

Figure 3. Percentage inhibition Purple leaves decoction

4. DISCUSSION

Anti-inflammatory and analgesic research on purple leaves decoction (*Graptophyllum pictum*) was carried out with the aim of scientifically proving that purple leaves have activity as anti-inflammatories and analgesics, because purple leaves have been widely used by the community for treatment, one of which is to treat haemorrhoids. This study used male mice and mice because male animals do not have an estrus period which can affect the physiological condition of the test animals so that it does not affect the test results [13]. The method used for anti-inflammatory testing is the Winter Method. This method was chosen because it is simple and commonly used. Research on anti-inflammatory effects was carried out using a mercury pletismometer with measurements based on Archimedes' law, which is when an object inserted into a liquid will exert an upward force or pressure equal to the volume moved. Chemical induction of inflammation using 1% carrageenan solution as much as 0.2 ml injected on the soles of rat feet intraplantar [14].

The anti-inflammatory study of purple leaf decoction used 3 different dose groups, namely doses of 5.4 mg/200g BW, 10.8 mg/200g BW and 21.6 mg/200g BW. The dose selection was based on previous research conducted by Melly Rohani who tested the anti-inflammatory effect of ethanol extract of purple leaves. The research conducted used the Winter Method and Indomethacin as a positive control while the test preparation used ethanol extract of purple leaves [5]. Based on this, the preparation used in the study is a decoction using water solvents, because it is a group of polar compounds such as ethanol. The choice of polar solvents is because the compounds expected to be extracted are flavonoids which have an anti-inflammatory effect [15].

The results of anti-inflammatory research obtained data on the average volume of rat paw edema in all test groups decreased in the 4th hour. The negative control group showed the greatest increase in edema volume compared to the other test groups, this indicates that the positive control and the three test preparations have anti-inflammatory effects. The anti-inflammatory effect can also be seen from the statistical test results obtained that the positive control group and purple leaf decoction doses of 5.4 mg/200g BW, 10.8 mg/200g BW and 21.6 mg/200g BW showed a significant difference to the negative control group. Purple leaf decoction doses of 5.4 mg/200g BW, 10.8 mg/200g BW and 21.6 mg/200g BW showed a significant difference to diclofenac sodium, with the AUC value of the diclofenac sodium group smaller than the three decoction preparations. This indicates that the anti-inflammatory activity of diclofenac sodium is better than the three doses of purple leaf decoction. Increasing the dose of 5.4 mg/200g BW with a dose of 10.8 mg/200g BW showed no significant difference indicating that the administration of purple leaf decoction both doses showed the same anti-inflammatory effect. The dose of 5.4 mg/200g BW with a dose of 21.6 mg/200g BW and a dose of 10.8 mg/200g BW with a dose of 21.6 mg/200g BW showed a significant difference indicating that the administration of different doses showed different anti-inflammatory effects. This indicates that increasing the dose does not provide an increase in the anti-inflammatory effect because not every increase in dose shows a significant difference.

Analgesic research using the Siegmund Method. This method is in the form of chemical stimulation and is the most commonly used method. Testing is done by looking at the decrease in mice writhing or the ability to relieve pain due to intraperitoneal administration of acetic acid. Symptoms of pain in mice as a result of acetic acid administration are characterised by contraction of the abdominal wall which results in the legs being pulled backwards, stretched and the abdomen touching the base of the abdomen, stretching and the abdomen touches the bottom of the space it occupies, this symptom is called writhing [13]. The preparation is given 30 minutes before being given acetic acid (inducing compound) which aims to see the work of the test preparation in providing a protective effect against pain that will be caused by the inducer [8].

The analgesic study of purple leaf decoction used 3 different dose groups, namely doses of 1 mg/20g BW, 2 mg/20g BW and 4 mg/20g BW. The dose selection was based on previous research conducted by Madya Soekarno who tested the analgesic effect of ethanol extract of purple leaves. The research used the method of calculating the pain response reaction time (in seconds) and Acetosal as a positive control and the test preparation used purple leaf ethanol extract preparation [16]. Based on this, the preparation used in this study is a decoction that uses water solvents, because it is a group of polar compounds such as ethanol. The choice of polar solvents is because the compounds expected to be extracted are flavonoids which have an effect as analgesics [4].

The results of analgesic research obtained data on the average number of writhing mice every 5 minutes in all test groups decreased the number of writhing at the 25 minute with the peak number of writhing occurring at the 20 minute. The negative control group showed the greatest increase in the number of writhes compared to the other test groups, this indicates that the positive control and the three test preparations have an analgesic effect. The analgesic effect can also be seen from the results of statistical tests that show a significant difference between the negative control group with the positive group and the purple leaf decoction

group. Purple leaf decoction doses of 1 mg/20g BW, 2 mg/20g BW, and 4 mg/20g BW showed significant differences with the negative control. Purple leaf decoction doses of 1 mg/20g BW, 2 mg/20g BW, and 4 mg/20g BW showed significant differences with the positive control, where the AUC value of the positive control group was smaller, indicating that the analgesic ability of the positive control was better than the test preparation.

The increase in the dose of 1 mg/200g BW with a dose of 4 mg/200g BW showed a significant difference indicating that the administration of purple leaf decoction dose of 4 mg/20g BW showed a better analgesic effect than the dose of 1 mg/20g BW. The dose of 1 mg/200g BW with a dose of 2 mg/200g BW showed no significant difference and the dose of 2 mg/200g BW with a dose of 4 mg/200g BW showed no significant difference indicating that the administration of purple leaf decoction of both doses showed the same analgesic effect. This indicates that increasing the dose does not provide an increase in analgesic effect because not every increase in dose shows a significant difference. Anti-inflammatory and analgesic studies use two different animals, namely rats for anti-inflammatory tests and mice for analgesic tests. Anti-inflammatory research uses mice because the method used will cause edema in the legs of mice, so mice are used so that observation of edema is easier than mice which have smaller leg sizes.

Analgesic research using mice because what will be observed is the response in the form of writhing due to the induction of irritants intraperitoneally, mice are used because the observation is easier because the response to be given is only writhing, whereas when using rat test animals, the pain response given will be more complex not only in the form of writhing, but rats can also scratch their bodies so that observations cannot be done properly. The results of anti-inflammatory and analgesic research from purple leaf decoction found that purple leaf decoction has an effect as anti-inflammatory and analgesic. Anti-inflammatory research shows that purple leaf decoction doses of 5.4 mg/200g BW, 10.8 mg/200g BW and 21.6 mg/200g BW have a percent inhibition of negative control successively by 14.19%, 18.85% dab 21.78%, when compared with previous research conducted by Melly Rohani who tested the anti-inflammatory effect of ethanol extract of purple leaves getting the results of a dose of 5.4 mg/200g BW has a percent inhibition of 58% [5]. The difference in these results can be caused by the use of different solvents, preparations with ethanol solvents provide a greater percent inhibition value against negative controls which indicates that the ability to inhibit edema is greater. This can be due to the phenolic compounds (flavonoids) contained in the extract are more soluble in ethanol solvents. This is because the solubility of ethanol is the same as the solubility of phenolic compounds, so ethanol extracts more phenolic compounds (flavonoids) than water.

Analgesic research shows that purple leaf decoction doses of 1 mg/20g BW, 2 mg/20g BW, and 4 mg/20g BW have a percent inhibition of negative control consecutively by 27.59%, 36.57% and 40.71%, when compared to previous research conducted by Madya Soekarno who tested the analgesic effect of ethanol extract of purple leaves getting the results of a dose of 200 mg/kg BW able to inhibit the reaction of pain response best compared to doses of 50 mg/kg BW and doses of 100 mg/kg BW, namely causing pain response reactions within 5.16 seconds [16]. The use of different methods makes comparison of results impossible, but it can be seen that both preparations in the form of decoctions and preparations in the form of ethanol extracts of purple leaves can provide the best analgesic effect with the largest dose. The dose used for anti-inflammatory and analgesic testing is better for choosing the same dose. The selection of the same dose aims so that the results obtained can be compared properly. In addition, it can also be done by converting one of the doses (inflammation for rats into analgesic doses for mice) so that the results obtained can describe the effect of the preparation at the same dose with different test animals.

The anti-inflammatory and analgesic effects of purple leaf decoction are thought to be because purple leaves contain flavonoids. Flavonoids are secondary metabolites of a plant that dissolve in polar solvents. Flavonoids are able to inhibit the formation of pain and inflammation. The anti-inflammatory mechanism carried out by flavonoids can be through several pathways, namely inhibiting the activity of cyclooxygenase and lipooxygenase enzymes directly which causes inhibition of prostaglandin and leukotriene biosynthesis which are the end products of the cyclooxygenase and lipooxygenase pathways. This can inhibit leucocyte accumulation and netrophil degranulation thereby directly reducing the release of arachidonic acid by netrophils, as well as inhibiting histamine release [15].

Inhibition of leukocyte accumulation during the inflammatory process will lead to a decrease in the body's response to inflammation, inhibition of leukocyte accumulation is due to inhibition of the enzyme cyclooxygenase so that thromboxane will be inhibited where thromboxane causes modulation of leukocytes. Inhibition of netrophil degranulation will reduce the release of arachidonic acid by netrophils. Inhibition of histamine release occurs because flavonoids can inhibit histamine release from mast cells [14]. The largest percent inhibition is produced by diclofenac sodium for both anti-inflammatory and analgesic with a value of

27.41% for anti-inflammatory and 51.06% for analgesic because diclofenac sodium is a chemical compound that has activity as an anti-inflammatory and analgesic, with the mechanism of action inhibiting the process of prostaglandin release by inhibiting through the cyclooxygenase I and cyclooxygenase II pathways [12].

5. CONCLUSION

The conclusion of this study is that the anti-inflammatory test dose III 21.6 mg/200 g BW decocta Purple leaves has the highest anti-inflammatory inhibitory effect of 21.78% on the feet of mice. Meanwhile, the analgesic test dose III 4 mg/20 g BW decocta Purple leaves has the best effect in inhibiting writhing in mice, namely 40.71%.

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