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Antioxidant, analgetic, and anti-inflammatory activity test of purple leaf ethanol extract (*Graptophyllum pictum* L. Griff) *in vitro* and *in vivo*

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ABSTRACT

Purple leaves (*Graptophyllum pictum* L. Griff) are one plant that is widely used as a medicine. Purple leaves have pharmacological activities that can be used as medicine, including antioxidants, anti-inflammatory, antidiabetic, analgesics, immunomodulators, antihemorrhoids, and antibacterial. The objective of this study was to determine the antioxidant properties of ethanol extract from purple leaves as well as its properties as an analgesic and anti-inflammatory against Wistar rats. Antioxidant activity was measured using the DPPH method with quercetin as a comperation in vitro the in vivo study of 25 Wistar rats was divided into 5 groups consisting of 5 animals each, namely normal control, positive control, negative control, treatment group with a dose of 50 mg/kg BW and treatment group with a dose of 100 mg/kg BW. The analgetic activity test was conducted by the writhing test method, while anti-inflammatory activity was measured by measuring the percentage of inflammatory volume using a mercury plethysmometer. The data was analyzed using the One-way ANOVA SPSS software program. The results showed that antioxidant activity showed an IC_{50} value of 72.312 ± 24.406 μg/mL (strong antioxidant). Meanwhile, in the analgetic test, the highest percentage of analgesic effectiveness was found at a dose of 100 mg/kg BW with a value of 129.64% and showed anti-inflammatory activity at a dose of 100 mg/kg BW with a percentage decrease in edem volume of 28.73% at the sixth hour.

Keywords: antioxidant, analgesic, anti-inflammatory, *Grapthophyllum pictum* (L.) Griff

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INTRODUCTION

Antioxidants are compounds that have the ability to neutralize free radicals, which harm body cells and result in many health issues, including inflammation (Kurniawati et al., 2020). While Inflammation is the complex biological response of blood vessel tissue to stimuli that cause damage. Furthermore, there is a correlation between inflammation and pain, which is linked to alterations in membranes, vascular permeability, and protein denaturation (Gunathilake et al., 2018). Pain is an unpleasant sensory and emotional experience for the sufferer, which is related to (threat) tissue damage. Mechanical, chemical, or physical stimulation that injure tissues can result in pain. Additionally, several chemicals known as pain mediators, including prostaglandins, histamine, bradykinin, and leukotriene, can be released in response to stimulation (Yane & Handayani, 2019).

Traditional medicine is a medicine made from ingredients or a combination of ingredients obtained from plants or minerals that have not been processed in the form of pure substances. People use traditional medicine for prevention, recovery, and health improvement. Traditional medicine is an alternative treatment that has been accepted in various countries. Traditional medicine in general uses plants, one of which is purple leaves (*Graptophyllum pictum* (L). Griff) (Triyandi et al., 2021).

Purple leaves are a plant that can be used for medicine. Purple leaves have 3 types of plant varieties, namely purple leaves, green leaves, and white mottled leaves. Purple leaves have pharmacological activities that can be used as medicines, including antioxidants (improving the deterioration of cells in the body), anti-inflammatory (reducing or suppressing inflammation), antidiabetic (lowering blood glucose levels), analgesics (relieving pain), immunomodulators (increasing the work of the immune system in the body), antihemorrhoids (treating symptoms and complaints of hemorrhoids), and antibacterial (as an antiseptic) (Sartika & Indradi, 2021). Flavonoids, tannins, alkaloids, steroids, saponins, alcohols, and calcium oxalate are the secondary metabolite compounds of purple leaves. This content has many benefits so it is widely used as medicine by the community (Rachim et al., 2021). In previous research, purple leaf infusion contained moderate antioxidants with an IC_{50} value of 125.09 µg/mL (Salim, 2018). In other research, purple leaves at a dose of 4.5 mg/200 g BW had a greater ability than a dose of 1.125 mg/kg BW to provide anti-inflammatory effects (Triyandi et al., 2021). In line with other research, purple leaf extract has the greatest analgesic activity at a dose of 12 mg/kg BW (Nhadira et al, 2019).

The purpose of this study was to determine the antioxidant activity of purple leaf ethanol extract (*Graphtophyllum pictum* (L.) Griff) in vitro using the DPPH method and to determine its effectiveness as an analgesic and anti-inflammatory against Wistar rats.

MATERIALS AND METHOD

Collection and authentication of plant material

Graphtophyllum pictum (L.) Griff was collected in Gunung Sari Village, Pasangkayu Regency, and authenticated by botanists from UPT. Suber of Sulawesi Biodiversity (Herbarium Celebense) with number 98/UN.28.UPT-SDHS/LK/2023 at Tadulako University.

Methods

Extract preparation

The preparation of purple leaf ethanol extract was carried out by maceration method using 96% ethanol solvent. Simplicia powder was weighed 1,000 grams and then extracted using a 96% ethanol solvent of 6 liters divided into 3 vessels. Maceration is carried out for 3x24 hours in a room that is protected from direct sunlight and is at room temperature with several times stirring. After obtaining the results of maceration, it is filtered on filter paper and then filtrate is obtained. After obtaining the filtra, it is then concentrated using rotavapor at a temperature of 70ºC and in a waterbath at 60ºC until a thick extract is obtained, then the yield is calculated (Elmitra et al., 2019).

$$\%\ Ectract = \frac{\text{weight of ectract obtained (g)}}{\text{weight of ectracted material (g)}} x\ 100\% \quad \text{.....(1)}$$

DPPH solution preparation

Antioxidant activity is determined by the DPPH free radical method. DPPH 0.004% b/v solution is made by dissolving 0.004 gram DPPH Crystals in 100 mL ethanol Pro Analyst (Antarti & Lisnasari, 2018).

Preparation of quartz comparative solution

The raw solution was made at a concentration of 100 ppm of quartz by weighing 10 mg of quartz in 100 mL of ethanol (for the calibration curve) with concentrations of 2,4,6,8 and 12 ppm (Sari et al., 2021).

Determining of maximum wavelength

A total of 1 mL of quartz cetin solution is pipetted into the cuvette then 1 mL of DPPH 0.004% b/v solution is added and homogenized After that, the absorption of the solution is read using a UV-Vis spectrophotometer at the maximum wavelength (400-800 nm) (Maulydya et al., 2023).

OT (operating time) determination

The determination of the Operating Time for the test material was carried out by pipetting 1000 µL of quartz cetin solution into the cuvette, then adding 1000 µL of DPPH 0.004% b/v solution. After that, read the absorption of the solution using a UV-Vis spectrophotometer every 5 minutes until the 30th minute with the maximum wavelength (Agustiarini & Wijaya, 2022).

Control solution absorbance measurement

Take 1 mL of DPPH 0.004% b/v solution then add 1 mL of ethanol solution pro Analyst and then homogenize. Then read the absorption using a UV-Vis spectrophotometer at a maximum wavelength with ethanol as a blank (Agustiarini & Wijaya, 2022).

Measurement of antioxidant activity of quartz comparator

Dilution was carried out from the 1000 ppm quartz master solution into 20, 40, 60, 80, and 100 ppm, each taking 1 mL then added with DPPH 0.004% b/v as much as 1 mL, then left to sit during the OT obtained and then read the absorption with a UV-VIS spectrophotometer at a maximum wavelength repeated 3 times (Agustiarini & Wijaya, 2022).

Measurement of antioxidant activity of purple leaf ethanol extract

Sample testing uses several concentrations, namely 20; 40; 60; and 80 ppm. Each sample was taken as much as 1000 µL then 200 µL DPPH 0.004% b/v was added, then incubated during the operating time of each sample. Then measure absorbance at the maximum wavelength and then record the absorbance value obtained at each concentration. Then observe the comparison with quartz as a comparison. The percentage of inhibition or inhibitor can be calculated using the Formula 2: (Prasetyo et al., 2021).

$$\%\ inhibition = \frac{\text{abs.control} - \text{abs.sample}}{\text{abs.control}} x\ 100\% \text{.....(2)}$$

Preparation of CMC Na colloidal solution

Sodium carboxymethyl cellulose (Na CMC) was weighed as much as 0.5 grams of Na.CMC was sprinkled into a mortar filled with hot water as much as 10 mL. Let stand for a homogeneous period, then pour into a 100 mL measuring flask, and add aquadest to the limit mark (Triyandi et al., 2021).

Purple leaf extract suspension manufacturing

Purple leaf extract (*Graptophyllum pictum* (L) Griff) was weighed to make a suspension with 0.2 grams (dose 50 mg/kg BW) and 1 gram (dose 100 mg/kg BW) respectively. Furthermore, 0.5% Na CMC is added to each extract and the volume is sufficient to 25 mL, then beaten until homogeneous (Suryandari et al., 2021).

Manufacturing of mefenamic acid suspension

Mefenamic Acid tablets as many as 1 tablet were crushed until smooth using a lump and weighed as much as 0.0036 g. Then it is suspended with Na CMC that has been made and the volume is enough to 100 mL with aqua dest (Wardani et al., 2020).

Preparation of diclofenac sodium suspension

Weigh 100 mg of diclofenac sodium, then add aquadest to a volume of 25 mL. Take the solution as much as 11.81 mL, put it in a jar then add 0.5% Na CMC and homogenize, and put it in a measuring flask enough with aquadest until the volume is 25 mL. Volume of each 2.5 mL/200 g BW rat (Triyandi et al., 2021).

Carrageenan suspension preparation

1% carrageenan is obtained by suspending 1 gram of carrageenan and then homogenizing it in 0.9% sodium chloride then putting it into a 10 mL measuring flask to be sufficient to the limit mark on the measuring flask (Triyandi et al., 2021).

Test animals

This study uses an experimental method with a group random design, using 50 white rats have been approved by the ethics committee of Tadulako University with an approval number of 1795/UN28.1.30/KL/2024. The test animals used had inclusion criteria, namely male sex, rat weight of 100-200 grams, about 2-3 months old, healthy, active movement, normal behavior and activities, and macroscopic no anatomical and morphological abnormalities (BPOM RI, 2020).

Analgesic testing

This study used 25 rats and was grouped into 5, namely; normal control (no treatment), negative control group (Na CMC 0.5%), positive control group (mefenamic acid), and treatment group (purple leaf ethanol extract at a dose of 50 mg/kg BW and purple leaf ethanol extract at a dose of 100 mg/kg BW, each group was filled with 5 rats. Before the experiment was carried out for 18 hours, the rats were not fed. To stimulate the occurrence of squirting, acetic acid induction is carried out and then observed to find out the number of squirting every 5 minutes until the 30th minute. The number of squirms in each group was averaged and the percentage of analgetic protection in each group was calculated. The analysis was carried out to find out the differences in all treatment groups. The research data on the sigmun method in the form of the cumulative number of squirts in each treatment group was used to calculate the squirt protection and % of analgetic effectiveness with the formula (Sasongko et al., 2016).

$$\% \text{ writhing movement protection} = 100-(P/K \times 100\%)$$

Information:

P = Average cumulative number of experimental groups per individual

K = Number of cumulative squirms of the control group, mean cumulative number of squirms of rats, and percent. protection of squirms from all treatment groups

$$\% \text{ Analgetic activity} = \frac{\%protection\ of\ test\ material}{\%\text{acetic acid protection}} \times 100\% \quad \text{.....(3)}$$

Anti-inflammatory testing

This study used 25 rats and was grouped into 5, namely; normal control (no treatment), negative control group (Na CMC 0.5%), positive control group (sodium diclofenac), and treatment group (purple leaf ethanol extract at a dose of 50 mg/kg BW and purple leaf ethanol extract at a dose of 100 mg/kg BW, each group was filled with 5 rats. Before the experiment, the Wistar rats were not fed for 18 hours. Each mouse was weighed and the left leg was marked as the limit for measuring inflammation. Next, the leg was inserted into a plestimometer and the initial volume (Vo) of the rat's leg was recorded. Then, reagentation induction was carried out on the soles of the rats' feet to stimulate edema. After 1 hour of inflammation is measured as Inflammation volume after a certain time (Vt) and each test group is given treatment on an oral basis. Measurements are carried out for 6 hours and are measured once every 1 hour. Then the percentage of inflammation for each dose of the test substance is calculated with the formula percent of inflammation (Rikomah et al., 2019):

$$\% \text{ Inflammation} = \frac{(Vt-Vo)}{Vo} \times 100\%$$

Information:
Vt = Volume of inflammation after a certain time
Vo = Initial volume of the foot

Statistical analysis

The data from the test observation results will be statistically analyzed with One-way ANOVA with a confidence level of 95%, first, a normality test and a homogeneity test are carried out. If the results are not normally distributed and homogeneous, they will then be analyzed using the Kruskal Wallis nonparametric test to find out the significant difference with the $p<0.05$ value selected as the significance level. If there is a significant difference, the Man-Whitney test is carried out. Data classification was carried out using the SPSS V.29 software program.

RESULT AND DISCUSSION

Antioxidant activity

The antioxidant activity of purple leaf extract was carried out using the DPPH method. The concentration of antioxidant compounds that can inhibit the activity of DPPH free radicals by 50%. The smaller the IC_{50} value, the greater the antioxidant activity. $IC_{50\ was}$ obtained using the formula Y= bx+a based on linear regression (Maulydya et al., 2023).

Figure 1 shows the results of quercetin and purple leaf ethanol extract in inhibiting DPPH. The results of quercetin with a linear equation obtained an IC_{50} value of 66.26 ± 23.93 µg/mL, it can be said that quercetin has strong antioxidant activity and can inhibit DPPH free radicals. Meanwhile, in the testing of purple leaf ethanol extract, three repetitions were carried out for each concentration, and data on the standard value of the division was obtained by substituting in a linear equation IC_{50} value of 72.312 ± 24.406 µg/ml was obtained, this shows that purple leaf extract contains strong antioxidants so that the sample can inhibit of DPPH free radicals.

Based on the IC_{50} value, it can be stated that quercetin and purple-leaf ethanol extract both contain antioxidants, but the IC_{50} value in quercetin is lower than that of purple-leaf ethanol extract. This is because quercetin itself is a pure antioxidant that is often used as a comparison (Kammoda et al., 2021). In the meantime, secondary metabolites including alkaloids, sitosterols, glycosides, saponins, steroids, phenolic compounds, tannins, and flavonoids including anthocyanins and leucoanthocyanins have been found to be present in purple leaves (Disi et al., 2023). In the phenolic and flavonoid groups, it can donate or give hydrogen atoms to DPPH free radicals so that it can form a stable DPPH compound. The hydroxy group in phenol compounds is not the only one that affects the ability to dampen free radicals but is also influenced by the position or location of the group, and the presence of a 4-oxo group in its basic framework (Sartika & Indradi, 2021). The content of flavonoid compounds in purple leaves is a low-

molecular compound with high antioxidant properties. Flavonoids produce large amounts of simple phenolic acids, which can fight free radicals and enhance antioxidant effects (Rajeshwaran et al., 2021).

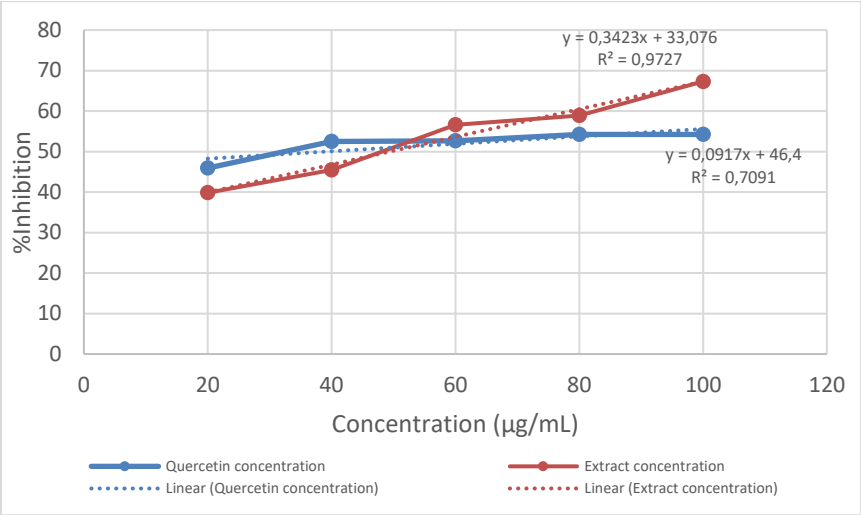

Figure 1. Antioxidant activity of quercetin and *Grapthophyllum* extract

Analgetic test results

In the analgetic activity test, the Writhing test method with chemical stimulation was used, namely Acetic Acid by injecting rats to stimulate pain. This method was chosen because it can trigger the release of arachidonic acid from phospholipid tissue through cyclooxygenase and form prostaglandin, causing pain. From the test, the recorded data included the accumulation of the activity of the Wistar rats observed every 5 minutes for 30 minutes. The data was used to calculate the percentage of analgetic power and the presentation of the protective power that appeared to determine the percentage of analgetic effects.

The 1% acetic acid inducer provides a painful effect, which is indicated by the presence of wriggling or contractions in the abdominal area of rats shown in Table 1. The results of the analgetic effect test showed a decrease in the average number of writhing in rats included in the positive control group by giving mefenamic acid of 3.6 mg/kg BW and the extract group with doses of 50 mg/kg BW and 100 mg/kg BW when compared to the negative control. This shows that both extract control (50 mg/kg BW and 100 mg/kg BW) and positive control (mefenamic acid) can reduce wriggling in rats. The smaller the average number of squirms shown by the group of rats, the better the analgesic effect (Darmayanti et al., 2020).

The percentage of analgetic power protection is used to determine the decrease in the number of squirming, the higher the amount of protection, the smaller the number of squirming that occurs. So that the *Grapthophyllum* extract given is able to withstand the pain stimulus of acetic acid in the pain response. Protection percentage data can be seen in Table 2, This test uses the Mann-Whitney further test because the data is not normally distributed and homogeneous. Based on the results obtained from the presentation of the largest analgesic power protection or close to the percentage of protection in the positive control group, there was a dose of 100 mg/kg BW in the extract group 80.23%, which means that at a dose of 100 mg/kg BW, it is effective in providing analgesic effects. Meanwhile, the comparison of doses of 50 mg/kg BW and 100 mg/kg BW was obtained without a statistically significant difference where 50 mg/kg BW extract had a greater variation than 100 mg/kg BW extract.

Table 1. The average number of writhing movements of animals induced 1% acetic acid and treated by *Graptophyllum* extract

Time (minute)	Writhing movement average ±					*p value*
	Normal group	Negative group	Mefenamic acid	Extract 50 mg/kg BW	Extract 100 mg/kg BW	
5	0 ± 0.00	3.4 ± 0.10	1 ± 0.70	1.2 ± 0.40	0.4 ± 0.50	0.001
10	0 ± 0.00	2.4 ± 0.50	1 ± 0.00	1.0 ± 0.70	0.4 ± 0.50	0.001
15	0 ± 0.00	2.2 ± 0.80	1.6 ± 0.50	1.4 ± 1.10	0.4 ± 0.50	0.004
20	0 ± 0.00	1.8 ± 0.40	0.4 ± 0.50	1.2 ± 0.80	0.6 ± 0.50	0.005
25	0 ± 0.00	1.6 ± 0.50	0.2 ± 0.40	0.6 ± 0.90	0.4 ± 0.50	0.012
30	0 ± 0.00	2.0 ± 0.70	0.8 ± 1.10	0.2 ± 0.40	0.4 ± 0.50	0.010

Exp: Value *p value* Obtained from *Kruskal-wallis Test* where the value *p<0,05* There is a significant difference

Table 2. The average percent of analgetic protection of animal induced by 1% acetic acid and treated with *Graptophyllum* extract

Group	% Analgetic protection ± SD	*P value*
Negative Group	0 ± 0,000^	0.003
Positive Group	61.89 ± 13.11	
Dosage extract 50 mg/Kg BW	56.38 ± 24.07^	
Dosage extract 100 mg/kg BW	80.23 ± 5.53^*	

Note : *= significant difference with negative control (p <0,05). ^ = not significantly different from the positive control. The p-value is obtained from the Kruskal-Wallis Test where the value p <0.05 is a significant difference

The percentage test results were carried out to prove that purple leaf ethanol extract has the ability as an analgetic drug, statistical data analysis can be seen in Table 3. The results of statistical analysis with the Kruskal-Wallis Test produced a significant value (P<0.05) which showed that the average percentage of analgetic was significantly different. The results showed that the positive control group and the 50 mg/kg BW extract group showed no significant difference in analgetic effects, while the extract group dose of 100 mg/kg BW had a significant difference. The larger the dose given, the greater the activity, so the dose of 100 mg/kg BW has a stronger analgetic activity than mefenamic acid.

Table 3. Analgetic activity percentage of *Grapthophyllum* extract compared to mefenamic acid as positive control

Group	%Analgetic activity ±	*P value*
Positive group	100 ± 0.000	0.044
Dosage extract 50 mg/Kg BW	91.11 ± 38.886	
Dosage extract 100 mg/kg BW	129.64 ± 89.40	

Exp: The p-value was obtained from the Kruskal-Wallis test where the p<0.05 value was significantly different

From the test results, it can be seen that the positive control and purple leaf ethanol extract (doses of 50 mg/kg BW and 100 mg/kg BW) both have analgetic effects. Mefenamic acid itself has the ability to inhibit the enzyme cyclooxygenase (COX) which plays a role in forming prostaglandins. Meanwhile, in ethanol extract, purple leaves have analgetic activity. Especially in alkaloid compounds and flavonoids in purple leaves have anti-inflammatory and analgetic content, this is related to the content of flavonoids whose mechanism of action inhibits the action of the cyclooxygenase enzyme (Nhadira et al., 2019). The enzyme cyclooxygenase functions in stimulating the release of pain mediator, prostagladin (Darmayanti et al., 2020). In line with reports stating that phytoconstituents such as flavonoids,

alkaloids, tannins, and steroids have significant analgetic activity after the process of separation of compounds in plants (Yimer et al., 2020).

Anti-inflammatory test results

Artificial inflammation was performed for anti-inflammatory testing, by injecting 1% Carrageenan into the soles of the rats' feet so it can cause swelling. Carrageenan is a sulfated polysaccharide derived from red seaweed plants and plays a role in the formation of udem in the acute inflammatory model. The volume of inflammation was measured before and after administration of the test material. The tool used to measure the volume of inflammation is a plestimometer.

The comparator used is sodium diclofenac where sodium diclofenac is one of the drugs that has an anti-inflammatory effect and is often used as a comparative control in studies regarding anti-inflammatory tests. In addition, diclofenac sodium has the ability to be absorbed quickly in the body and has low side effects compared to other anti-inflammatory drugs (Suryandari et al., 2021). NSAID are a bunch whose component of activity restrains the chemical cyclooxygenase 2 (COX-2) in creating prostagladin (an provocative arbiter) in tissues (Amran only et al., 2018).

In Figure 2, it can be seen that the positive control and extract control groups experienced a significant reduction in edema compared to the negative control. The largest decrease in edema volume occurred in the control of the extract at a dose of 100 mg/kg BW where the decrease occurred constantly from the first hour to the sixth hour. Meanwhile, in positive control, it was produced with a significant decrease in edema volume. In the control dose of 50 mg/kg BW experienced a lower decrease, while in the negative control, the increase in edema was quite long.

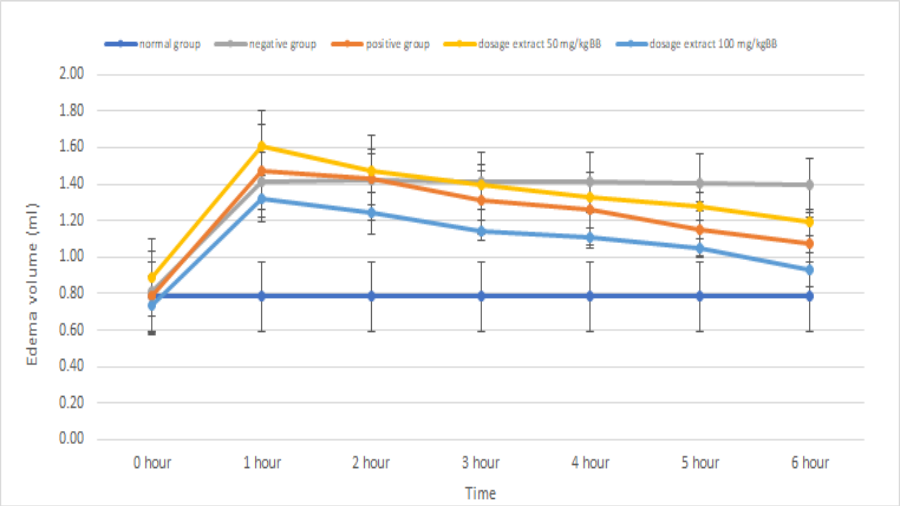

Figure 2. The average decrease in edema volume of carrageenan induced rat treated by *Grapthophyllum* extract

To determine the percentage of reduction in rat foot edema, it can be seen in Table 4 where the positive control and the dose of 50 mg/kg BW did not have a significant difference, while at the dose of 100 mg/kg BW, there was a significant difference and and it can be interpreted as a dose of 100 mg/kg BW had a fairly high anti-inflammatory activity.

From the analysis of statistical data using the ANOVA, it was shown that the positive control (sodium diclofenac) produced no significantly different anti-inflammatory effect with a noticeable volume of rat leg edema at the observation period ($P>0.05$) and this finding is in line with the general function of diclofenac sodium in reducing inflammation by blocking the enzyme cyclooxygenase by converting arachidonic acid into prostaglandins which are mediators of inflammation (Mandang et al., 2022). Purple

leaf ethanol extract with a dose of 50 mg/kg BW and a dose of 100 mg/kg BW also experienced a significant decrease in the volume of rat leg edema during the entire observation period (P>0.05). Alkaloids, flavonoids, tannins, saponins, steroids, alcohols, and calcium oxalate are the contents of purple leaf compounds. The complex and different substance causes purple to clear out to have an anti-inflammatory impact. Flavonoids are one of the compounds that are able to inhibit the cyclooxygenase and lipooxygenase pathways. Alkaloid compounds also have an anti-inflammatory role in preventing the formation of prostagladin (Rachim et al., 2021).

Table 4. Average percentage of decrease in edema volume of carrageenan induced rat treated by *Grapthophyllum* extract

Time (hour)	Average percent decrease in edema ± SD				*p value*
	Negative group	Positive group	Dosage extract 50 mg/kg BW	Dosage extract 100 mg/kg BW	
1	84.73 ± 48.065	90.52 ± 20.434	86.23 ± 28.660	86.53 ± 46.793	0.966
2	85.84 ± 47.443	85.62 ± 19.323	71.89 ± 33.548	74.76 ± 41.331	0.900
3	84.73 ± 48.065	70.27 ± 15.713	63.40 ± 35.790	60.87 ± 32.586	0.709
4	84.73 ± 48.065	64.35 ± 21.154	55.61 ± 32.631	55.24 ± 31.503	0.515
5	83.30 ± 45.687	50.11 ± 17.496	50.16 ± 32.467	46.94 ± 27.134	0.274
6	81.70 ± 46.989	39.94 ± 19.400	41.13 ± 37.703	28.73 ± 15.665	0.093

Exp: The *p-value* was obtained from the ANOVA One-way *test where the* p>0.05 *value* was no significant difference.

CONCLUSION

It can be stated that purple leaves (*Graptophyllum pictum* L Griff) have a strong antioxidant activity. The IC value was 72.312 ± 24.406 µg/ml. Purple leaf ethanol extract can also provide analgesic and anti-inflammatory effects with an effective dose of 100 mg/kg BW.

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