Anti-inflammatory Activity Test of Red Pucuk Leaf Extract Against (Syzygium myrtifolium Walp) White Rats (Rattus norvegicus)

Ona Bugis Tehuayo[1], Angga Bayu Budiyanto[2], Irwandi[3]
Pharmacy Study Program, Faculty of Applied Science, Muhammadiyah University of Education

The plant S. myrtifolium Walp, also known as red shoots, is an ornamental plant with characteristic red easy leaves and green old leaves. This plant is known to contain active compounds that have potential as pharmacological agents, especially anti-inflammatory. The purpose of this study was to identify the anti-inflammatory activity of ethanol extract of red shoot leaves (S. myrtifolium Walp) against white rats (Rattus norvegicus) using several dose variations. The population in this study was the red shoots leaf plant (Syzygium myrtifolium Walp) obtained from Mariat Pantai Village, Sorong Regency. While the samples used in this study were red shoot leaves (Syzygium myrtifolium Walp) which were taken fresh green leaves. The results of phytochemical analysis identified the presence of alkaloid, flavonoid, tannin, saponin and phenol compounds. The test data were analyzed using ANOVA method followed by Paired Sample T test and LSD test to determine the presence and absence of differences from before treatment and after treatment. The test results showed that red shoot leaf extract has anti-inflammatory activity against white rats significantly at doses of 200 mg / kg BW, 300 mg / kgBB and 450 mg / kgBB.
Keywords: Anti-inflammatory, Red Pucuk Leaf , White Rat.

Corresponding Author:
Ona Bugis Tehuayo
Applied Science Pharmacy Study Program Muhammadiyah Sorong University of Education
bugistehuayoona@gmail.com

1. Introduction

Inflammation is a complex biological reaction of body tissues to injury that can be caused by physical trauma, toxic chemical agents, or microorganism infections. This response is characterized by clinical symptoms including erythema (redness), increased local temperature (heat), edema (swelling), pain (dolor) and impaired tissue function (fuction laesa) (Suryandari et al., 2021). Pathophysiologically, inflammation begins when tissue damage occurs which triggers the release of pro-inflammatory mediators such as prostaglandins and leukotrienes through the enzymatic pathways of cyclooxygenase (COX) and lipogenase (LOX). This activation produces unstable compounds such as hydroperoxides and endoperoxides, which are further metabolized into various inflammatory mediators such as prostaglandins, leukotrienes, prostacyclins and thromboxanes. Prostaglandins and leukotrienes play an important role in causing clinical signs of inflammation including pain swelling and increased vascular permeability (Nindia et al., 2021). Inflammatory treatment has activity in reducing inflammation, one of the drugs that can reduce inflammation is diclofenac sodium. Diclofenac sodium is a compound derived from phenylacetic acid which is included in the non-steroidal anti-inflammatory group (AINS), the selection of diclofenac sodium drugs as anti-inflammatory because it has the ability to block or inhibit strong cyclooxygenase with anti-inflammatory effects. This drug works by inhibiting the synthesis of prostaglandins which are mediators of pain and reducing the concentration of free arachidonic cells in leukocytes and can change the release of fatty acid absorption (Novika et al., 2021). The red shoot plant (S. myrtifolium Walp) is a type of ornamental plant that has red shoots and green old leaves (Karim et al., 2023) This plant contains useful chemical compounds that can be used as medicinal plants, this can be proven by research (Suryandari et al., 2021) which shows that the isolation of anthocyanins and

antioxidants can be used as a medicinal plant.) which shows that the isolation of anthocyanins from significant (Juwita et al., 2017) Utilization of red shoots (S. myrtifolium Walp) in the community is used as herbal tea tea from red shoot leaves has flavonoid compounds that are efficacious as natural dyes and antioxidants.

Red shoot leaves (S. myrtifolium Lin) contain chemical compounds such as flavonoids, phenolics, and terpenoids that work as antitumor and anti-angiogenesis. Based on results from other studies, the ethanol-water fraction of red shoot leaves (S. myrtifolium Walp) showed the highest antioxidant activity. In addition, several species in the Syzygium genus have been shown to have anti-inflammatory activities that support the pharmacological potential of these plants as teraupetic agents in the management of inflammatory conditions. Overall, the Syzygium genus contains various chemical compounds such as flavonoids, alkaloids, tannins and terpenoids that can be utilized in the field of medicine, one of which is inflammation (Endang et al., 2022). Flavonoids have activity as anti-inflammatory agents by inhibiting cyclooxygenase and lipogenase, reducing leukocyte accumulation and inhibiting the release of neutrophil mediators so that there is no release of inflammatory mediators such as prostaglandins and histamine (Audina et al., 2018). Acts as an immunomodulatory agent by inhibiting the degranulation of mast cells that release histamine and reducing the synthesis of interleukin-1 by monocyte cells (Yusrinie et al., 2023). Tannins have anti-inflammatory activity by accelerating the response of neutrophils and macrophages and stimulating the formation of phagocytosis in the body (Fitriyanti et al., 2020). Based on some of the research findings above, it can be the basis for testing the anti-inflammatory activity of ethanol extract of red shoot leaves (S. myrtifolium Walp) on reducing edema swelling in carrageenan-induced white mice.

2. Methods

This research is an experimental study conducted from November to December 2024 at the Pharmacy Laboratory, Faculty of Applied Science, University of Education Muhammadiyah (UNIMUDA) Sorong. This research design involves independent variables in the form of ethanol extract of red shoot leaves (Syzygium myrtifolium Walp) and dependent variables in the form of anti-inflammatory activity, with test animals in the form of white rats (Rattus norvegicus) as a controlled variable. The population in this study were red shoots (Syzygium myrtifolium Walp), with green leaves as the sample used. Red shoot leaves used are green leaves that have been cleaned of dirt, dried, The processed, powdered herbal extract was then extracted using a maceration method with 96% ethanol. Fifteen male white rats (Rattus norvegicus) aged 2–3 months, weighing approximately 311 grams, were randomly divided into five treatment groups. 3 treatment groups were given red shoot leaf extract at a dose of 200 mg / kg BB, 300 mg / kg BB, and 450 mg / kg BB. 2 Other groups are used as positive control and negative control. This test is carried out using the udem method, namely the method of swelling the soles of the feet of rats using a carrageenan solution and measuring the soles of the feet of rats using a plastimometer after that, rats that have been measured by the volume of udem are given oral treatment and observe the decrease in udem for 6 hours. The results obtained were analyzed using One Way Anova test and further tests, namely paired sample T-test and LSD test.

3. Results and Discussion

Table 1. Results of Simplisia Preparation

Data	Description
Sample weight after wet sorting	2.167 grams
Sample weight after drying	588 grams

Data	Description
Sample weight after pollination	504 grams

Ket: obtained the results of making simplisia in the sample after basic sorting, namely (2,163 grams) the weight of the sample after drying is (588 grams) and the weight of the sample after pollination (504 grams).

Tabel 2. Red Pucuk Leaf Yield

Simplisia	Weight of simplisia (g)	Extract weight (g)	Sample weight (kg)	Yield (%)
Red Pucuk Leaf	504 grams	108 grams	5 kg	21,42 %

Ket: 504 grams were extracted using the maceration method and produced an extract of (108) grams. The yield of red shoot leaf extract obtained was (21.42%). The red shoot leaves used in this study are green leaves that are still fresh and are used as much as 5 kg and then sorted. The purpose of sorting the base is to clean the dirt that is still attached, then dried using an oven with a temperature of 40-500 C, the dried sample is then mashed and the extraction process is carried out with 96% ethanol solvent. 96% ethanol is used because it is easy to obtain, has high solubility and is it exhibits greater ease of penetration through the cell wall compared to ethanol solvents of lower concentrations (Novira et al., 2021). The simplisia powder was macerated for 3x24 hours and continued with remeseration for 2x24 hours. Remeseration is a method of repeated extraction with the addition of solvents carried out after filtering the first meserat and so on. Remeseration aims to optimize the withdrawal of secondary metabolite compounds from red shoot leaf simplisia powder so as to maximize the extract of a compound (Fatmawati & Royani, 2023). Meserat from red shoot leaves is evaporated using a water bath at 400C with the aim of separating the solvent from the extract so that a thick extract is obtained (Rumondor & Komalig, 2019). The result of evaporation of the thick extract was 108 grams, from this amount, the calculation of the yield of ethanol extract of red shoot leaves (Syzygium myrtifolium Walp) was carried out, which was recorded at 21.42% as shown in table 4. This yield calculation aims to determine the amount of extract produced from fresh simplisia used in the extraction process (Eka & Dwi, 2022).

Tabel 3. Phytochemical Screening Test Results

No	Testing	Reagen	Observasi	Deskription
1	Alkaloid Test	Mayer	There is white precipitate	+
		Burchard	There is brown precipitate	+
		Dragendrof	There is a brick red precipitate	+
2	Flavonoid Test	PB II acetat	There is a yellow precipitate	+
3	Saponin Test	Aquadest	There is stable frothy sediment	+
4	Tanin Test	$FeCl_3$	Formed blue-black	+
5	Fenol Test	$FeCl_3$	Formed blackish green	+
6	Steroid Test	Liebarman Burchard	No blue color	_

Description:
(+) = Contains secondary metabolite compounds
(-) = Does not contain secondary metabolite compounds

This study identifies active compounds in red shoot leaf extract, which includes flavonoids, alkaloids, saponins, tannins, phenols and steroids. The purpose of this identification is to determine the presence of bioactive compounds in plants that can potentially be used as teraupetic agents in the treatment of various diseases (Saragih & Arsita, 2019). Data from the identification test results of bioactive compounds

in red shoot leaf extracts in table 3. Based on the results obtained, it is known that red shoots leaf extract contains flavonoids, alkaloids, saponins, tannins, phenols, but does not contain steroid compounds. Qualitative tests of alkaloid content were carried out using 3 types of reagents, namely mayer reagent, dragendrof, and burchardat. mayer reagent indicates the presence of alkaloids through the formation of a white to yellowish precipitate. Meanwhile, the use of dragendrof reagent will produce an orange color in alkaloid compounds. According to (Sulistyarini et al., 2016), if a compound contains alkaloids, then the test with dragendrof reagent will form a brown, orange, or orange precipitate. This event takes place the interaction between alkaloid compounds with tetraiodobismutat (III) ions contained in the reagent (Sulistyarini et al., 2016). A positive test of burchard reagent is characterized by the formation of a brown precipitate. This precipitate appears as a result of complex formation between alkaloids and potassium metal ions (K+), through coordination covalent bonds, which produce insoluble potassium-alkaloid complex compounds and precipitate. Flavonoid test using PB II acetate reagent where positive results were obtained. Red shoot leaf extract was positively identified as containing flavonoid compounds, indicated by the formation of a yellow precipitate. This is because flavonoids have a benzene ring that forms a yellow precipitate (Maulidie & Widia Astuti, 2019). The tannin test can be done with the addition of FeCl3. A color change to blackish green indicates a positive reaction which indicates the presence of tannin compounds. Tannins are polar compounds that contain hydroxyl groups (-OH), so they can react with iron ions (Fe3 =) in FeCl3 and form these distinctive colored complexes. (Sulistyarini et al., 2016). Phenol phytochemical test is marked by a blackish green color change. Phenol compounds have acidic properties characterized by the ease of hydroxyl groups (-OH) in releasing protons. In addition, phenols also have the ability to form complex compounds (chelates) with metal ions, are easily oxidized, and can undergo polymerization which produces dark colored compounds (Ikalinus et al., 2015). The saponin test shows positive if there is foam when shaken for 10 seconds and allowed to stand for 10 minutes. The results of the experiments carried out there is foam in the experiment of saponin compounds, this shows a positive result because the DPM extract contains saponin compounds (Nurjannah et al., 2022). Positive results in the Liberman burchad test are marked by the formation of a violet color which means that there are no steroid compounds in red shoots leaf extract (Ahlan Sangkal et al., 2020).

Tabel 4. Mean±SD Inflammatory Inhibitory Response

	Mean±SD (hours) inflammatory inhibition response						
Group	T0	T1	T2	T3	T4	T5	T6
Diklofek Sodium	0,08±0,01	0,07±0,01	0,06±0,01	0,05±0,01	0,04±0,01	0,04±0,01	0,03±0,01
Na CMC	0,08±0,02	0,08±0,02	0,08±0,01	0,07±0,01	0,06±0,01	0,06±0,01	0,05±0,01
200 mg dose	0,08±0,01	0,07±0,01	0,06±0,01	0,05±0	0,04±0,01	0,04±0	0,03±0,01
300 mg dose	0,08±0,01	0,08±0,01	0,06±0,01	0,05±0,01	0,04±0,01	0,04±0,01	0,03±0,01
450 mg dose	0,09±0,01	0,08±0,02	0,07±0,02	0,06±0,02	0,05±0,02	0,04±0,01	0,03±0,01

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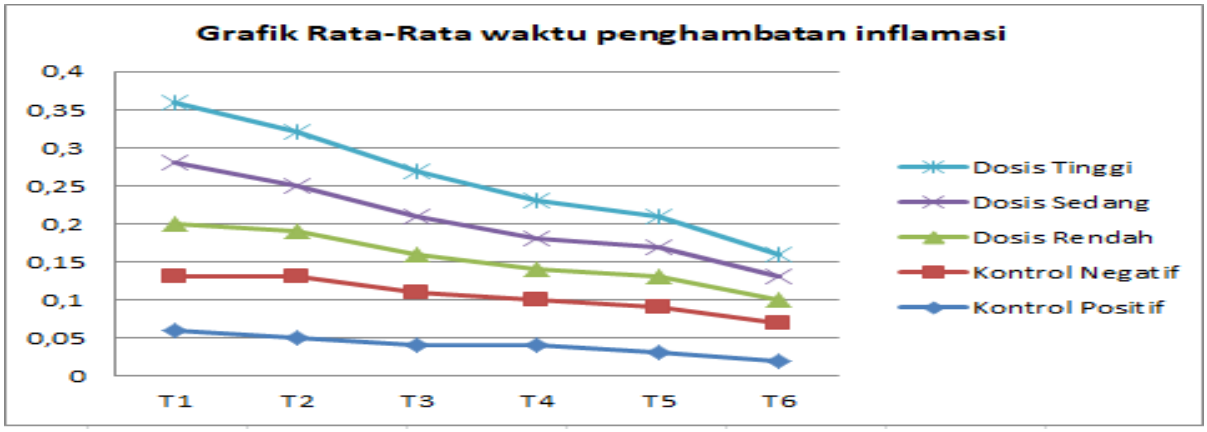

Figure 1. Graph of average udem volume

Ket: In the figure above, it can be explained that there is a decrease in udema every time (per hour) in each treatment, so it can be concluded that there is anti-inflammatory activity that occurs in each control group.

Table 5. Test Results of Anti-inflammatory Activity of Red Pucuk Leaf Before and After Treatment

Kelompok	T0-T1	T0-T2	T0-T3	T0-T4	T0-T5	T0-T6
Natrium diklofenak	0,423	0,057	0,035	0,023	0,020	0,008
Na CMC	0,423	0,423	0,414	0,396	0,380	0,380
200 mg does	0,423	0,057	0,020	0,010	0,006	0,013
300 mg does	0,423	0,038	0,015	0,032	0,006	0,004
450 mg does	-	-	0,020	0,008	0,005	0,003

Type: Paired Sample T test

a. If the sig value> 0.05 then there is no significant difference between the conditions before and after treatment.
b. If the sig value <0.05 then there is a significant difference before and after treatment

Tabel 6. Test Results of Differences in Anti-inflammatory Activity of Red Pucuk Leaf Between Each Treatment Group

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	6944.170	4	1736.042	4.267	.003
Within Groups	33766.206	83	406.822		
Total	40710.376	87			

Type: One Way ANOVA Test
If the value obtained is sig. <0.05 then there is a significant difference.
If the value obtained is sig. > 0.05 then there is no significant difference.

Tabel 7. LSD Test Results

Multiple Comparisons

(I) kelompok_uji	(J) kelompok_uji	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kontrol positif	kontrol negatif	21.57778*	6.72327	.002	8.2055	34.9501
	dosis 200 mg	1.23856	6.82143	.856	-12.3290	14.8061
	dosis 300 mg	-3.45056	6.72327	.609	-16.8229	9.9218

Multiple Comparisons						
(I) kelompok_uji	(J) kelompok_uji	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
	dosis 450 mg	3.61856	6.82143	.597	-9.9490	17.1861
kontrol negatif	kontrol positif	-21.57778*	6.72327	.002	-34.9501	-8.2055
	dosis 200 mg	-20.33922*	6.82143	.004	-33.9068	-6.7717
	dosis 300 mg	-25.02833*	6.72327	.000	-38.4007	-11.6560
	dosis 450 mg	-17.95922*	6.82143	.010	-31.5268	-4.3917
dosis 200 mg	kontrol positif	-1.23856	6.82143	.856	-14.8061	12.3290
	kontrol negatif	20.33922*	6.82143	.004	6.7717	33.9068
	dosis 300 mg	-4.68912	6.82143	.494	-18.2567	8.8784
	dosis 450 mg	2.38000	6.91819	.732	-11.3800	16.1400
dosis 300 mg	kontrol positif	3.45056	6.72327	.609	-9.9218	16.8229
	kontrol negatif	25.02833*	6.72327	.000	11.6560	38.4007
	dosis 200 mg	4.68912	6.82143	.494	-8.8784	18.2567
	dosis 450 mg	7.06912	6.82143	.303	-6.4984	20.6367
dosis 450 mg	kontrol positif	-3.61856	6.82143	.597	-17.1861	9.9490
	kontrol negatif	17.95922*	6.82143	.010	4.3917	31.5268
	dosis 200 mg	-2.38000	6.91819	.732	-16.1400	11.3800
	dosis 300 mg	-7.06912	6.82143	.303	-20.6367	6.4984

Remarks. If the value obtained is sig. <0.05 then there is a significant difference.If the value obtained is sig. > 0.05 then there is no significant difference Statistical analysis was performed using SPSS software based on the response data of the test animals after treatment. In the normality test obtained using spihiro wilk analysis to determine whether the data is normally distributed or not, the results found show that the P value is> 0.05, where in K+ there is (0.958), K- (0.609), P1 (0.772), P2 (0.308) and P3 (0.549). meaning that the overall data obtained is normally distributed so that further tests can be carried out, namely the Paired Sample T test and the One Way Anova test. Furthermore, the homogeneity test uses the Levene test and produces a p>0.05 value, this value meets the homogeneity test requirements, it can be concluded that the data obtained is homogeneously distributed and suitable for further analysis. Based on Figure 10, it can be seen that all treatment groups experienced a gradual decrease in edema. Meanwhile, the negative control group showed an inflammatory process that lasted until the 6th hour which was characterized by a high percentage of swelling that still occurred around (0.05 ± 0.01) while the positive control given diclofenac sodium showed faster inhibition of inflammation than the negative control. The 3 treatment groups that received red shoots leaf extract at doses of 200 mg/KgBB, 300 mg/KgBB, and 450 mg/Kg BB showed a significant decrease in inflammation when compared to the negative control group, meaning that diclofenac sodium and red shoots leaf extract have anti-inflammatory effects at the same time. The Paired Sample T test in this study was used to evaluate the existence of statistically significant differences between the values before and after treatment. The significance criterion is determined based on the p value where p<0.05 indicates a significant difference between pretest and treatment data. Conversely, if the p value is> 0.05, the difference is considered statistically insignificant (Ardhana & Rahman, 2024). Based on table 5. The results showed that the positive control, low dose of 200 mg, medium dose of 300 mg, and high dose of 450 mg experienced a decrease or inhibition of inflammation, where the positive control decreased at the 2nd hour with a value

of (0.057), the low dose of 200 mg decreased at the 2nd hour with a value of (0.057), the medium dose of 300 mg decreased at the 2nd hour with a value of (0.038), and the high dose of 400 mg experienced a decrease in inflammatory resistance at the 3rd hour with the value obtained, namely (0.020). While the negative control got a p>0.05 value, which means that it did not experience inflammatory inhibition from hours 1-6Then the results of statistical analysis tests were carried out using SPSS to evaluate the average response of test animals after being given treatment. The conclusion of the results obtained is that the three doses of red shoot leaf extract have anti-inflammatory activity against white rats at doses of 200 mg / kg BW, 300 mg / kgBB and 450 mg / kgBB. Analysis of one way ANOVA test in table 6 shows a significance value of P <0.05 the results obtained are 0.03 which reinforces that there are statistically significant differences between treatment groups. The results of the LSD (Least Significant Difference) follow-up test in Table 7 show that significant differences also occur among control groups. The statistical test results obtained are that the 200 mg, 300 mg and 450 mg extracts have the same activity as the positive control, this is because the three controls have a significant value> from 0.05 which indicates there is no difference, while the negative control has a difference from all controls, because the negative control has a sig value <0.05 which indicates a significant difference. So it can be concluded that the negative control has low activity compared to all groups.

4. Conclusion

Based on the results of the research that has been carried out, it can be concluded that ethanol extract of red shoot leaves (S. myrtifolium Walp) can provide anti-inflammatory effects on white rats (Rattus norvegicus) induced by carrageenan. The dose of ethanol extract of red shoot leaves (S. myrtifolium Walp) that can provide anti-inflammatory activity is the dose of 200 mg / kg BW, 300 mg / kg BW and 450 mg / kg BW. Further research is recommended to explore the potential of ethanol extract of red shoot leaves (S. myrtifolium Walp) for other pharmacological activities, in order to broaden the understanding of teraupetic properties in addition to the anti-inflammatory effects that have been proven previously.

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