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Anti-Inflammatory Activity Test of Tapak Liman (*Elephantopus scaber* L.) Leaf Extract Using Red Blood Cell Membrane Stabilization Method

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ABSTRACT

Inflammation is the reaction of living tissue to trauma or infection, both in acute and chronic conditions. One plant with anti-inflammatory potential is the tapak liman leaf (*Elephantopus scaber* L.), which contains secondary metabolites such as phenolics, flavonoids, tannins, alkaloids, steroids/treponoids, and saponins. The purpose of this study was to determine the anti-inflammatory activity of *Elephantopus scaber* L. leaf extract at concentrations of 0.005%, 0.01%, 0.02%, 0.04%, and 0.08% using the red blood cell membrane stability method. Anti-inflammatory activity showed that 70% ethanol extract of tapak liman leaves had low anti-inflammatory activity at a concentration of 0.005% with a stability of 98.00%, while the highest activity was found at a concentration of 0.08% with a stability of 99.65%. Microscopic examination showed that at concentrations of 0.005%, 0.01%, 0.02%, 0.04%, and 0.08%, the administration of tapak liman extract could restore the normal shape of erythrocytes. The Kruskal-Wallis test yielded a significance value of 0.000, which is less than 0.05, meaning that H_a is accepted. It can be concluded that there are differences in the anti-inflammatory activity of tapak liman leaf extract (*Elephantopus scaber* L.) at concentrations of 0.005%, 0.01%, 0.02%, 0.04%, and 0.08%.

Keywords: Anti-inflammatory; Tapak Liman Leaves; Red Blood Cells; Membrane stability

ABSTRAK

Inflamasi merupakan reaksi dari jaringan hidup terhadap trauma atau infeksi, baik dalam kondisi akut maupun kondisi kronik. Salah satu tanaman yang berpotensi sebagai antiinflamasi adalah daun tapak liman (*Elephantopus scaber L.*) yang mengandung senyawa metabolit sekunder berupa fenolik, flavonoid, tanin, alkaloid, steroid/treponoid dan saponin. Tujuan dari penelitian ini adalah untuk mengetahui aktivitas antiinflamasi ekstrak daun tapak liman (*Elephantopus scaber L.*) dengan konsentrasi 0,005%, 0,01%, 0,02%, 0,04% dan 0,08% metode stabilitas membran sel darah merah. Aktivitas antiinflamasi menunjukkan bahwa ekstrak etanol 70% daun tapak liman yang memiliki aktivitas antiinflamasi yang kecil pada konsentrasi 0,005% stabilitas sebesar 98,00% sedangkan untuk aktivitas yang tertinggi terdapat pada konsentrasi 0,08% stabilitas sebesar 99,65%. Pemeriksaan mikroskopis menunjukkan bahwa pada konsentrasi 0,005%, 0,01%, 0,02%, 0,04% dan 0,08% dengan pemberian ekstrak tapak liman dapat mengembalikan bentuk normal sel eritrosit. Uji Kruskal-Wallis didapatkan nilai signifikansi yaitu 0,000 lebih kecil dari 0,05 yang artinya H_a diterima. Dapat disimpulkan bahwa ada perbedaan aktivitas antiinflamasi ekstrak daun tapak liman (*Elephantopus scaber L.*) pada konsentrasi 0,005%, 0,01%, 0,02%, 0,04% dan 0,08%.

Kata kunci: Antiinflamasi; Daun Tapak Liman; Sel Darah Merah; Stabilitas Membran

INTRODUCTION

Inflammation is an immune response that functions as a defense mechanism for the body. Inflammation is the biological response of body tissues to physical injury or microorganisms, whether long-term or short-term. Clinically, inflammation is characterized by increased temperature (warmth or heat), redness in the affected area, swelling, pain, and decreased or impaired function of the inflamed organ or tissue(1). The anti-inflammatory pathway begins with the development of an immune response aimed at removing antigens from the body. This process continues until the antigen is removed from the body. After the antigen is removed, acute inflammation occurs, lasting from several hours to several days. Repeated or continuous exposure to inflammatory antigens can cause chronic inflammation, a condition that can lead to tissue structure damage and decreased body function. The inflammatory mechanism can occur in a specific area or spread throughout the body(2).

Indonesia is a country with a relatively high incidence of anti-inflammatory diseases. Based on data obtained from an Indonesian health survey conducted by the Ministry of Health, the prevalence of diseases involving inflammation includes cancer at 1.2%, hepatitis at 0.12%, asthma at 1.6%, and pneumonia at 10.8%. Rheumatoid arthritis(3). is a chronic inflammatory disease with an estimated number of sufferers in Indonesia of (<) 1.3 million people. Based on global prevalence figures, this is 1.5-1% of Indonesia's population of 268 million in 2020(4).

The treatment commonly used to treat inflammatory diseases is the administration of steroids or nonsteroidal anti-inflammatory drugs (NSAIDs), which are widely used. However, long-term use or excessive consumption of these drugs can cause side effects because they contain chemicals or synthetic substances that can lead to drug dependence. Meanwhile, nonsteroidal anti-inflammatory drugs (NSAIDs) can cause hypertension, ulcers, and bleeding. They work by suppressing signs of inflammation and providing antipyretic (fever-reducing) and analgesic (pain-relieving) effects(5). Therefore, it is necessary to develop traditional medicines that can be passed down from generation to generation and have high efficacy and value. Traditional medicines made from plants can be used to maintain health by strengthening the immune system and

reducing various diseases. One type of plant that can be used in traditional medicine is Tapak liman leaves (*Eleptophantopus scaber* L.)(6).

Tapak liman, also known as *Eleptophantopus scaber* L., is a plant widely known by the public as easy to grow and having many benefits as a traditional medicine. Scientific studies on the use of medicinal plants have proven that medicinal plants do contain substances or compounds that are beneficial to health. Tapak liman leaves (*Eleptophantopus scaber* L.) contain substances and compounds that are beneficial to health. This plant often grows wild and is commonly found in grass fields, roadsides, and around houseyards(7). It has various uses, including as an analgesic, anti-inflammatory, and laxative. Additionally, it can treat various diseases such as insomnia, diabetes, hepatitis, rheumatism, bronchitis, and arthralgia (joint pain)(8).

Research on tapak liman leaves (*Eleptophantopus scaber* L.) conducted by(9)(10) reported that phytochemical screening results showed the presence of secondary metabolites such as phenolic compounds, flavonoids, tannins, alkaloids, steroids/treponoids, saponins, and essential oils. Flavonoids are metabolites that have anti-inflammatory properties. The anti-inflammatory mechanism of flavonoids works by inhibiting the activity of cyclooxygenase and lipoxygenase enzymes, which can directly cause the inhibition of prostaglandin biosynthesis (compounds in the body made from fat with hormone-like effects) and leukotrienes, which are the end products of the COX and lipoxygenase pathways(11). Flavonoids are secondary metabolic compounds responsible for antioxidant, antibacterial, anticancer, and anti-inflammatory effects(8). Based on research conducted(9), it was found that the metabolites contained in Tapak liman leaves (*Eleptophantopus scaber* L.) have the potential to be anti-inflammatory compounds.

According to Kumar in Hardy et al., (2018) Erythrocyte membrane stability testing can be used to assess anti-inflammatory activity potential, because the structure of erythrocyte membranes resembles that of lysosomal membranes, which play a role in inflammatory mechanisms. Stable lysosomal membranes have an important function in regulating inflammatory reactions, namely by preventing the release of enzymes from active neutrophil granules during the inflammatory process. When neutrophils are activated, the enzymes released can trigger various damages and exacerbate inflammation. Therefore, erythrocyte membrane stability testing using hypotonic solutions can also be used as an indicator to evaluate lysosomal membrane stability.

Based on the above explanation, this study was conducted to determine the “Anti-inflammatory activity of 70% ethanol extract of tapak liman leaves (*Eleptophantopus scaber* L.) using the red blood cell membrane stability method.” The method used by the researchers is the red blood cell membrane stability method, in which red blood cell membranes have a lipid composition similar to lysosomal membranes, which can be used as a model to assess membrane stability in inflammatory activity. This method does not require test animals, uses few samples, is quick and easy, and can provide a general picture of membrane protection or damage caused by certain compounds. This method is one of the most frequently used approaches in research as a biochemical parameter(12).

METHOD

This study used the maceration extraction method with 70% ethanol solvent, using 234.4 grams of dried simplisia at room temperature, which was then sieved with a mesh size of 80/100. The maceration process was then carried out by soaking the crude drug in 1700 ml of 70% ethanol for 3x24 hours, with the solvent being replaced every 1x24 hours. The maceration results were evaporated at a temperature of 40°C. This quasi-experimental study design used tapak liman leaf extract as the population, 70% ethanol as the solvent, and sodium diclofenac as the positive control. This study was conducted from May 29 to 31, 2025, at the Hematology Laboratory of the Pontianak Health Polytechnic with ethics code No. 169/KEPK-PK-PKP/V/2025. The sampling technique used was purposive sampling with 5 replications and a total sample size of 25. The method used to measure the stability of red blood cell membranes was a UV-Vis

spectrophotometer. The number of replications or repetitions in each treatment group can be determined using Federer's formula, namely:

$$(t - 1) (r - 1) \geq 15$$

Explanation:

t = number of treatment groups

r = number of replications of each treatment group

The number of treatment groups (t) in this study was 5 treatments plus 2 control groups. Therefore, the replication value (r) in this study was:

$$\begin{aligned}(t - 1) (r - 1) &\geq 15 \\(5 - 1) (r - 1) &\geq 15 \\4r - 4 &\geq 15 \\4r &\geq 19 \\r &\geq 4,75 \\r &= 5\end{aligned}$$

From the above formula, it is known that the number of replications for each treatment is 5 times, with a sample size of 25 samples.

1. Preparation of Test Solution

- a. pipette 1 mL of phosphate buffer pH 7.4 (0.15 M), add 0.5 mL of red blood cell suspension.
- b. Then add 1 mL of sample solution and 2 mL of hyposaline.
- c. Homogenize and incubate at 37°C for 30 minutes.

2. Test Control Solution (Negative Control)

- a. Pipette 1 mL of phosphate buffer pH 7.4 (0.15 M), add 0.5 mL of isosaline solution as a substitute for red blood cell suspension.
- b. Then add 1 mL of sample solution and 2 mL of hyposaline.

3. Negative Control Solution

- a. Pipette 1 mL of phosphate buffer pH 7.4 (0.15 M), then add 0.5 mL of red blood cell suspension.
- b. Add 1 mL of isosaline solution as a substitute for the sample solution and 2 mL of hyposaline.

4. Measurement of Red Blood Cell Membrane Stability

- a. Each test solution is incubated at 37°C for 30 minutes and centrifuged at 5000 rpm for 10 minutes.
- b. The supernatant obtained is collected and its hemoglobin content is calculated using a UV-vis spectrophotometer at a maximum wavelength of 550-560 nm.
- c. The percentage of red blood cell membrane stability can be calculated using the following formula:

% Test Solution Stability

$$= 100 - \left(\frac{\text{Abs test solution} - \text{Abs test control solution}}{\text{Abs Negative Control Solution}} \right)$$

% Diclofenac Sodium Stability

$$= 100 - \left(\frac{\text{Abs sodium diclofenac solution} - \text{Abs test control solution}}{\text{Abs negative control solution}} \right)$$

5. Preparation of red blood cell suspension

The red blood cell suspension was prepared from 5 ml of venous blood from a 21-year-old male subject.

- Mix 5 mL of venous blood and sterile Alsever solution in a 1:1 ratio into two centrifuge tubes, then homogenize.
- Then centrifuge at a speed of 3,000 rpm for 10 minutes.
- Separate the supernatant formed using a micropipette.
- Wash the blood cell sediment with isosaline solution and centrifuge again.
- Repeat the process several times until the supernatant is clear.
- Then make a 10% red blood cell suspension by mixing 2 mL of red blood cells with 18 mL of isosaline solution.

6. Normality Test and Kruskal-Wallis Test Results

Based on the data results, it shows that data at concentrations of 0.005%, 0.01%, 0.02%, 0.04% with significance values of (0.723, 0.283, 0.319, 0.789) respectively are normally distributed. then at a concentration of 0.08%, the significance value is ($0.000 < 0.05$), the data is not normally distributed. This can be seen from the significance value (< 0.05) at a concentration of 0.08% with a significance value of ($0.000 < 0.05$). Because the data is not normally distributed, it is followed by a non-parametric data test, namely the Kruskal-Wallis test. Berdasarkan hasil uji kruskal wallis yang diperoleh nilai signifikansi pada ekstrak yaitu 0,000 lebih kecil dari nilai signifikansi 0,05 yang artinya ada perbedaan aktivitas antiinflamasi ekstrak daun tapak liman pada konsentrasi 0,005%, 0,01%, 0,02%, 0,04% dan 0,08%.

RESULTS

Table 1. Phytochemical Screening

No.	Secondary Metabolite	Result	Unit	Conclusion
1.	Alkaloid	Green	(-)	Negative
2.	Flavonoid	Yellow	(+)	Positive
3.	Tannin	Greenish	(+)	Positive
4.	Saponin	Foamy	(+)	Positive
5.	Phenol	Blue-black	(+)	Positive
6.	Steroid	blue	(-)	Negative
7.	Terponoid	Yellow-green	(-)	Negative

Table 1, tapak liman leaf extract contains several secondary metabolites, namely flavonoids, tannins, saponins, and phenols.

Table 2. Extract Characteristics

Jenis Karakteristik Ekstrak	Hasil
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Organoleptis	Shape	Thick
	Smell	Characteristic leaves
	Color	Blackish green
Water Content		8.08%

Table 3, the results of the organoleptic test of the thick extract of tapak liman leaves showed that the extract was thick, had a typical leafy smell, was blackish green in color, and had a low moisture content.

Table 3. RBC Membrane Stability Assay

No.	Solution	Concentration (%)	Average Stability (%)
1.	Limnan Leaf Extract	0.005	98.00
		0.01	98.50
		0.02	99.07
		0.04	99.15
		0.08	99.65
2.	Positive Control (Sodium Diclofenac)	0.005	99.80
		0.01	99.81
		0.02	99.83
		0.04	99.98
		0.08	100.09

Table 3, the average percentage stability of tapak liman leaf extract at a concentration of 0.005% was 98.00%, at a concentration of 0.01% was 98.50%, 99.07% at a concentration of 0.02%, 99.15% at a concentration of 0.04%, and 99.65% at a concentration of 0.08%. Meanwhile, for the positive control (sodium diclofenac), the stability at a concentration of 0.005% was 99.80%, at a concentration of 0.01% it was 99.81%, 0.02% concentration at 99.83%, 0.04% concentration at 99.98%, and 0.08% concentration at 100.09%.

Table 4. Univariate Analysis

	N	Minimum	Maximum	Mean	Std. Deviation
Stabilitas Valid (Listwise)	25	97,78	99,72	98,8736	0,59479

Based on the table above, the univariate analysis has a mean value of 98.8736%, a minimum value of 97.78%, and a maximum value of 99.72%.

Table 5. Normality Test

Treatment		Shapiro-Wilk		
		Statistic	df	Sig.
Stability	Test solution concentration 0.005%	.952	5	.723
	Test solution concentration 0.01%	.871	5	.283
	Test solution concentration 0.02%	.883	5	.319
	Test solution concentration 0.04%	.956	5	.798
	Test solution concentration 0.08%	.552	5	.000

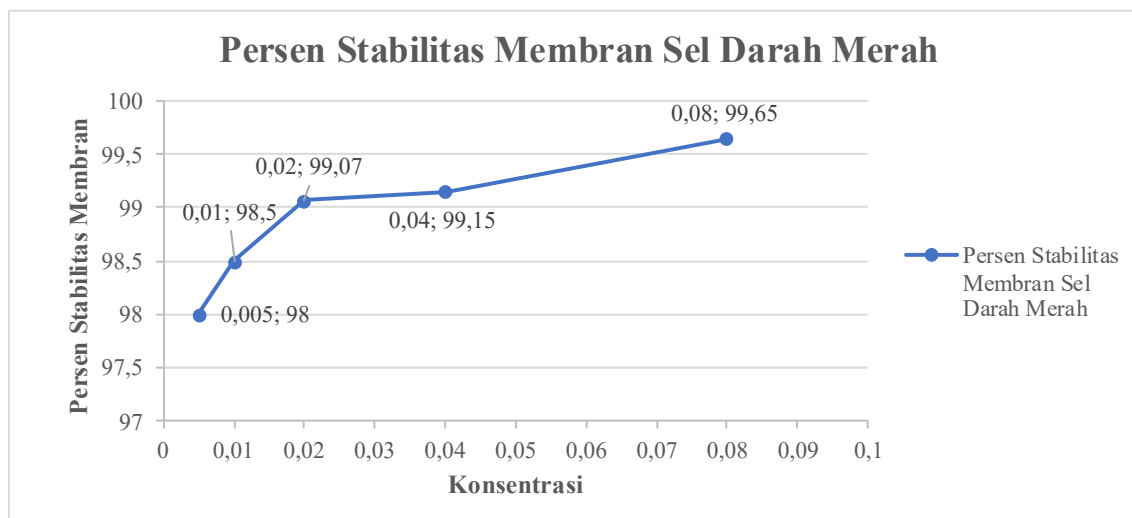
Based on the results of the data above, it shows that the data is not normally distributed. This can be seen from the significance value (<0.05) at a concentration of 0.08% with a significance value ($0.000 < 0.05$). Because the data is not normally distributed, it is followed by a non-parametric data test, namely the Kruskal-Wallis test.

Table 6. Kruskal-Wallis Test Results

	Stability of Test Solutions
Kruskal-Wallis H	22.221
df	4
Asymp. Sig.	.000

The table results show that the significance value obtained for the extract is $0.000 < 0.05$. This means that there is a difference in the anti-inflammatory activity of tapak liman leaf extract at concentrations of 0.005%, 0.01%, 0.02%, 0.04%, and 0.08%.

Tabel 7. Omparison graph of anti-inflammatory effects between concentrations



DISCUSSION

Screening tests have been conducted on extracts from tapak liman leaves. Phytochemical analysis has identified several secondary metabolites, including flavonoids, tannins, saponins, and phenols. The compounds contained in tapak liman leaf extracts play a role in anti-inflammatory activity. Flavonoids such as quercetin are known to have good ability in reducing acute inflammation. Certain flavonoids show strong inhibitory ability against various enzymes such as protein kinase C, protein tyrosine kinase, phospholipase A2, phosphodiesterase, and others (13). Flavonoids provide an effect that can reduce tissue damage due to inflammation. Inflammation is a physiological protective response of the body to tissue injury and inflammation (14).

Tapak liman leaves function as anti-inflammatories because they contain compounds such as flavonoids. Flavonoid anti-inflammatory compounds show potential as anti-inflammatories through antioxidant activity mechanisms and synergy with other bioactive compounds, making flavonoids compounds that play a role in anti-inflammation. This is in line with the ability of flavonoids to stabilize cell membranes, which has an anti-inflammatory effect by preventing the release of inflammatory mediators from lysosomes (15). Flavonoid compounds function to protect red blood cell membranes from hypotonic solutions. The impact of these hypotonic solutions is related to the amount of fluid that flows into the red blood cell membrane, which can cause the membrane to rupture, known as hemolysis. In this case, the flavonoid compounds contained in

the extract will interact with the applied hypotonic solution, thereby reducing the activity that damages the membrane. The amount of secondary metabolites contained in the extract reacts in proportion to the hypotonic solution added to the red blood cell suspension, so that it does not damage the erythrocyte cell membrane. Meanwhile, tannin and saponin compounds work to stabilize the membrane by binding cations, thereby strengthening the red blood cell membrane and other biological macromolecules(16). Saponins act like detergents and are thought to interact with many lipid membranes such as phospholipids, which are precursors to prostaglandins and other inflammatory mediators(17).

Previous research by (18) has shown that Tapak Liman leaves have antioxidant activity. Anti-inflammatory activity is closely related to antioxidant activity. These antioxidants can prevent oxidative stress that affects the stability of red blood cells. Red blood cells are very vulnerable to free radicals (reactive oxygen species/ROS), therefore antioxidant and anti-inflammatory compounds are needed to protect the cells. Antioxidant compounds inhibit or slow down oxidation by capturing free radicals, while anti-inflammatory compounds stabilize cell membranes.

Red blood cell membrane stabilization is used to determine anti-inflammatory activity in vitro. The red blood cells used in this study were normal red blood cells mixed with a hypotonic solution to cause hemolysis(19). They were then mixed with tapak liman leaf extract to determine the extract's ability to stabilize red blood cell membranes. The results of the study showed that the average concentration of tapak liman leaf extract was 98.8736%. The lowest percentage of tapak liman leaf extract stability was found at a concentration of 0.005%, which was 98.00%, while the highest result was at a concentration of 0.08%, which was 99.65%. When compared to sodium diclofenac (%), the stability of tapak liman leaf extract is close to sodium diclofenac but does not exceed the high (%) stability of sodium diclofenac.

From the results of the research data that has been conducted, the average percentage of stability of tapak liman leaf extract at a concentration of 0.005% is 98.00%, a concentration of 0.01% is 98.50, a concentration of 0.02% is 99.07%, a concentration of 0.04 is 99.15%, and 99.65% at a concentration of 0.08%. The results of the anti-inflammatory activity test (%) show that the extract has anti-inflammatory activity. Based on the results of the Kruskal-Wallis test, the significance of the extract was 0.000, which is smaller than the significance value of 0.05, meaning that there are differences in concentrations of 0.005%, 0.01%, 0.02%, 0.04%, and 0.08%. This effect becomes stronger with increasing concentration, reaching a peak of 99.65% at a concentration of 0.08%.

CONCLUSION

Elephantopus scaber L. leaf extract showed the highest anti-inflammatory activity at a concentration of 0.08% (99.65%), demonstrating erythrocyte membrane stabilization capabilities close to those of sodium diclofenac. The extract showed a significant effect ($p < 0.05$) in stabilizing red blood cell membranes compared to lower concentrations.

Further research is recommended using in vivo methods in animal models and identification of secondary metabolites that play an active role in the anti-inflammatory activity of Elephantopus scaber L. In addition, this study should be reinforced with preclinical toxicity tests to evaluate safety and studies on molecular mechanisms of action to understand how the active compounds work.

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