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Anti-Inflammatory Activity Assay Using the Human Red Blood Cell Membrane Stabilization Method for Pasote Leaf Extract (Dysphania ambrosioides L.)

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Abstract **Original Research Article**

Dysphania ambrosioides L., or pasote leaves, is a herbal plant widely used in traditional medicine in various parts of the world, including in Indonesia. This plant is known to have multiple properties, especially in overcoming health problems related to inflammation. Inflammation is a complex biological response to injury or infection. Therefore, searching for compounds that can reduce inflammation is very important in developing new therapies. A useful technique for assessing a compound's anti-inflammatory properties is the red blood cell membrane stabilization method. This technique measures the harm that inflammatory agents cause to the membrane of red blood cells. This study aims to evaluate the anti-inflammatory activity of pasote leaf extract using the red blood cell membrane stabilization method. This study used a post-test-only control design method and a pure experimental research design. This research was conducted at the Pharmacy Laboratory, Faculty of Mathematics and Natural Sciences (FMIPA). The sample used was rat blood taken through the retroorbital sinus of the eye. The criteria for selecting rats were rats weighing 200-250 grams and healthy as indicated by active movement. Purposive sampling separated the samples into seven groups. The number of subjects in this study was 35 samples. Diclofenac sodium is a non-steroidal anti-inflammatory drug used to reduce inflammation and pain. At the highest concentration tested, which was 250 ppm, sodium diclofenac showed very high inhibitory activity with an average of 92.57%. Pasote leaf extract (Dysphania ambrosioides L.) is known to have various pharmacological potentials, including anti-inflammatory activity. At the highest concentration tested, which was 250 ppm, the pasote leaf extract showed very high inhibitory activity with an average of 78.72%. The erythrocyte membrane stabilization test results demonstrated the strong anti-inflammatory properties of pasote leaf extract, as the percentage of inhibition increased with concentration. The conclusion that can be drawn is the Pasote Leaf extract with a concentration of 250 ppm has ability to stabilize red blood cell membranes, which is 90.476%.

Keywords: Anti-inflammatory, Pasote Leaf Extract (Dysphania ambrosioides L.), Human Red Blood Cell Membrane Stabilization Method.

INTRODUCTION

Dysphania ambrosioides L., or pasote leaves, is a herbal plant widely used in traditional medicine in various parts of the world, including Indonesia. This plant is known to have multiple properties, especially in overcoming health problems related to inflammation. Inflammation is a complex biological response to injury or infection; if not managed properly, it can contribute to the development of various chronic diseases, such as arthritis, heart disease, and cancer. Therefore, searching for compounds that can reduce inflammation is very important in the development of new therapies [1-3].

Flavonoids, alkaloids, and terpenoids are among the numerous bioactive substances in pasote leaf extract that may have anti-inflammatory properties [4]. Previous studies have shown that methanol extracts of pasote leaves have significant antioxidant activity, which can contribute to anti-inflammatory effects. This antioxidant activity is crucial because oxidative stress often contributes to inflammation [5, 6].

The red blood cell membrane stabilization method is a useful technique for assessing the anti-inflammatory properties of a compound [7]. This technique measures the harm that inflammatory agents cause to the membrane of red blood cells. Anti-

inflammatory compounds are supposed to shield red blood cell membranes from this kind of harm. Research conducted by Damiti *et al.,* (2021) shows that the use of this method can provide a clear picture of the anti-inflammatory potential of herbal extracts [8, 9].

In addition, other studies have also shown that various plant extracts, including those from Lantana camara and Eleutherine bulbosa, exhibit significant anti-inflammatory activity through a similar mechanism, namely red blood cell membrane stabilization [8]. This suggests that this approach can be widely applied to evaluate the anti-inflammatory potential of various plant extracts [10, 11].

Thus, this study aims to evaluate the anti-inflammatory activity of pasote leaf extract using the red blood cell membrane stabilization method. It is hoped that the results of this study can contribute to the development of more effective and safe herbal medicines for the treatment of inflammatory conditions, as well as enrich the scientific literature on the health benefits of Dysphania ambrosioides L [12, 13].

MATERIALS AND METHODS

This study used a post-test-only control design method and a pure experimental research design. This research was conducted at the Pharmacy Laboratory, Faculty of Mathematics and Natural Sciences (FMIPA). The sample used was rat blood taken through the retroorbital sinus of the eye. The criteria for selecting rats were rats weighing 200-250 grams and healthy as indicated by active movement. Purposive sampling was used to separate the samples into seven groups (K1, K2, K3, K4, K5, KP, and KN). The number of subjects in this study was 35 samples.

Table 1: Research Variables, Operational Definitions, and Measurement Scales

Variable	Definition	Measurement Tool	Indicator	Measurement Scale
Pasote Leaf Extract	Pasote leaf extract is the result of pasote leaf extraction obtained by maceration using a 96% ethanol solvent. 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm - Ratio (numeric)	-	50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm	Rasio (numeric)
Anti-inflammatory activity	Anti-inflammatory activity is reviewed on the stability of red blood cell membranes so that they do not lyse (rupture)	Spectrophotometry UVVis	x test solution ≥ x sodium diclofenac x test solution < x sodium diclofenac	Nominal (categorical)

RESULTS AND DISCUSSION

1. Phytochemical Screening Results

Phytochemical screening results of Pasote Leaf Extract (Dysphania ambrosioides L.) including alkaloids (Dragendorf, Wagner, Meyer), flavonoids, tannins, saponins, steroids, triterpenoids, and phenolic tests, can be seen in Table 2.

Table 2: Phytochemical Screening Results of Pasote Leaf Extract (Dysphania ambrosioides L.)

Compound Group	Results	Results
Alkaloids (Dragendorf,Wagner, Meyer)	+++	Dragendorf : Orange Wagner : Brown Meyer : White sediment
Flavonoids	+	Red
Tannins	+	Green
Saponins	+	bubbles/foam
steroids	-	No color change
Triterpenoids	-	No color change
Phenolics	+	Brown orange

Table 2 shows the results of the phytochemical screening of Pasote leaf extract (Dysphania ambrosioides L.) based on the compounds tested. This screening is important to determine the presence of bioactive compound groups that can contribute to the therapeutic effects of this extract.

The presence of alkaloids in Pasote leaf extract is strongly positive. Alkaloids are known to have various biological activities, including antipyretic, analgesic, and anti-inflammatory effects. These compounds generally have significant pharmacological activity. Flavonoids were detected with positive results. These compounds

have many health benefits, including as antioxidants and anti-inflammatories. The presence of flavonoids can help prevent various degenerative diseases. Tannins were also detected in the extract. These compounds have an astringent effect and are often associated with therapeutic effects, such as reducing inflammation and healing wounds. Tannins can help bind proteins and prevent inflammation.

The presence of saponins indicates that Pasote leaf extract has the potential to be an antimicrobial and immunomodulator. Saponins are also often considered to have emulsifying properties and increase nutrient absorption. The absence of steroids indicates that this group of compounds is not present in Pasote leaf extract. Steroids often play a role in hormonal and anti-inflammatory systems. Like steroids, triterpenoids were also not detected. However, triterpenoids usually have bioactive activities, so their absence may limit the potential for certain effects in the extract. The presence of phenolic compounds indicates good antioxidant potential. These compounds can help fight free radicals and have protective effects against various diseases. From the results of this phytochemical screening, Pasote leaf extract (Dysphania ambrosioides L.) contains several groups of significant bioactive compounds, such as alkaloids, flavonoids, tannins, saponins, and phenolics. The presence of these compounds indicates the potential of the extract in the health sector, especially in anti-inflammatory and antioxidant effects. Conversely, the absence of steroids and triterpenoids may limit the classification of certain pharmacological properties. The compounds present, however, have enough potential to warrant additional study.

2. Results of Erythrocyte Membrane Stabilization Test on Sodium Diclofenac and Pasote Leaf Extract (Dysphania ambrosioides L.)

a. Results of Anti-Inflammatory Activity Test of Sodium Diclofenac

Diclofenac sodium is a non-steroidal anti-inflammatory drug used to reduce inflammation and pain. One method for testing anti-inflammatory activity is through erythrocyte membrane stabilization. This test measures the ability of a compound to inhibit red blood cell lysis, which is an indication of anti-inflammatory activity.

The results of the anti-inflammatory activity test of sodium diclofenac in 3 replications with concentrations of 50, 100, 150, 200, and 250 ppm, along with the average, can be seen in Table 3.

Table 3: Sodium Diclofenac's Anti-Inflammatory Activity Test Results

Concentration (ppm)	% Inhibition			
	U1	U2	U3	Average
50	47.35	48.39	47.91	47.88
100	63.72	63.59	65.58	64.30
150	72.12	70.05	71.63	71.27
200	73.89	74.65	77.21	75.25
250	91.59	92.17	93.95	92.57

At a concentration of 50 ppm, sodium diclofenac showed moderate inhibitory activity with an average inhibition of 47.88%. This shows that at low concentrations, sodium diclofenac has begun to show a membrane-stabilizing effect. At a concentration of 100 ppm, there was a significant increase in inhibitory activity, with an average inhibition reaching 64.30%. This shows an increase in the effectiveness of sodium diclofenac in stabilizing erythrocyte membranes as the concentration increases. So, diclofenac sodium begins to show its ability to relieve inflammation. When the concentration increases to 150 ppm, the average inhibition increases to 71.27%, which means that this compound is increasingly effective in suppressing inflammation. At a concentration of 200 ppm, the anti-inflammatory activity of sodium diclofenac is greater than 70%, which averages 75.25%, indicating that this compound is increasingly effective and approaching maximum results. At the highest concentration tested, which was 250 ppm, sodium diclofenac showed very high inhibitory activity with an average of 92.57%. This shows the maximum potential of sodium diclofenac in stabilizing erythrocyte membranes at this concentration. This indicates that the higher the concentration, the better the effectiveness.

The results of the erythrocyte membrane stabilization test showed that sodium diclofenac had significant anti-inflammatory activity, with an increase in the percentage of inhibition along with increasing concentration. At the highest concentration tested, sodium diclofenac showed maximum potential in stabilizing erythrocyte membranes, indicating its effectiveness as an anti-inflammatory agent.

b. Results of Inhibitory Concentration 50 (IC50) of Sodium Diclofenac

Table 4 displays the average results of the sodium diclofenac Inhibitory Concentration 50 (IC50) Test.

Table 4: Sodium Diclofenac Inhibitory Concentration 50 (IC50) Test Results

IC50 Repeat			Average (ppm)
U1	U2	U3	
49.97973	49.77698	47.52652	49.97973

Inhibitory Concentration 50 (IC50) is the concentration of a compound needed to inhibit 50% of its biological activity. So, the lower the IC50 value, the stronger the compound is in suppressing activity, such as inflammatory activity. The IC50 test on sodium diclofenac is important for measuring how effective this compound is in reducing inflammation. The IC50 results can be a reference for determining the right dosage for its use. The IC50 value of 49.97973 ppm indicates that sodium diclofenac is effective in inhibiting 50% of the target activity (for example, certain enzymes or cells) at that concentration. This means that at a concentration of 49.97973 ppm, sodium diclofenac can reduce the biological activity of the target by 50%. The lower the IC50 value, the more potent or stronger a compound is in inhibiting its biological target.

In the discussion above, the results of the IC50 test of sodium diclofenac provide a clear picture of the effectiveness of this compound in overcoming inflammation. By understanding the IC50 value, we can determine the optimal dosage to maintain effectiveness and minimize side effects.

The IC50 test results of sodium diclofenac from each repetition were quite consistent. The average IC50 was around 49.97973 ppm, indicating that sodium diclofenac has a fairly strong anti-inflammatory effect. This means that at a concentration of around 50 ppm, this compound can inhibit 50% of the inflammatory activity tested. This makes sodium diclofenac a fairly effective drug for use in the treatment of inflammation because this concentration is relatively low and easy to achieve in therapy.

c. Results of the Anti-Inflammatory Activity Test of Pasote Leaf Extract (Dysphania ambrosioides L.)

Pasote leaf extract (Dysphania ambrosioides L.) is known to have various pharmacological potentials, including anti-inflammatory activity. One method to evaluate anti-inflammatory activity is through the erythrocyte membrane stabilization test, which measures the ability of the extract to inhibit red blood cell lysis.Table 5 displays the findings and average of the Anti-Inflammatory Activity Test of Pasote Leaf Extract (Dysphania ambrosioides L.) in three replications at concentrations of 50, 100, 150, 200, and 250 ppm.

Table 5: Anti-inflammatory Activity Test Results of Pasote Leaf Extract (Dysphania ambrosioides L.)

Concentration (ppm)	% Inhibition			
	U1	U2	U3	Average
50	44.69	44.24	43.26	44.06
100	60.62	60.83	61.4	60.95
150	68.58	66.36	64.65	66.53
200	70.35	70.05	70.7	70.37
250	78.76	78.34	79.07	78.72

At a concentration of 50 ppm, pasote leaf extract showed moderate inhibitory activity with an average inhibition of 44.06%. This indicates that at low concentrations, the extract has begun to show membrane stabilization effects. At a concentration of 100 ppm, there was a significant increase in inhibitory activity, with an average inhibition reaching 60.95%. This indicates an increase in the effectiveness of the extract in stabilizing erythrocyte membranes as the concentration increases. With this increase, it can be seen that the pasote leaf extract begins to show a stronger ability to inhibit inflammation. At a concentration of 150 ppm, the average inhibition increased to 66.53%. This shows that the extract is increasingly effective in stabilizing erythrocyte membranes at higher concentrations. At a concentration of 200 ppm, the average inhibition reached 70.37%, indicating that the extract continues to increase membrane stabilization activity with increasing concentration. At the highest concentration tested, which was 250 ppm, the pasote leaf extract showed very high inhibitory activity with an average of 78.72%. This shows the maximum potential of the extract in stabilizing erythrocyte membranes at this concentration. This figure is an indication that the compounds in this extract have significant potential to relieve inflammation.

The results of the erythrocyte membrane stabilization test showed that pasote leaf extract had significant anti-inflammatory activity, with an increase in the percentage of inhibition along with increasing concentration. At the highest concentration tested, the extract showed maximum potential in stabilizing erythrocyte membranes, indicating its effectiveness as an anti-inflammatory agent.

d. IC50 Results of Pasote Leaf Extract (Dysphania ambrosioides L.)

The IC50 test results of Pasote leaf extract (Dysphania ambrosioides L.) and the average can be seen in Table 6.

Table 6: IC50 Test Results of Pasote Leaf Extract (Dysphania ambrosioides L.)

Repeat IC50			Average (ppm)
U1	U2	U3	
56.26847	58.68489	59.80802	58.25379

The average IC50 value of Pasote leaf extract is around 58.25379 ppm. This shows that at a concentration of around 58.25 ppm, this extract can inhibit 50% of the tested activity. This relatively low IC50 number shows that Pasote leaf extract has great potential to provide therapeutic effects. In other words, the active compounds in this extract are quite effective in suppressing inflammation or other biological activities.

Looking at the three replications of the IC50 results, it can be said that the variation between the results is not too far. U1, U2, and U3 are each in the range of 56 to 59 ppm. This shows that the results of this test are consistent and reliable and indicate that the effectiveness of this compound does not fluctuate.

The results of the IC50 test will be extremely helpful in the creation of medicinal plants or herbs. By knowing the IC50, a more appropriate dose can be determined for use in therapy, and side effects that may arise if the dose is too high can be considered. The results of the anti-inflammatory activity test, which were previously discussed, are consistent with the IC50 test results, which demonstrate a fairly high level of effectiveness. This implies that the higher the concentration of the extract, the greater its ability to relieve inflammation. From the analysis above, the IC50 test results of the Pasote leaf extract (Dysphania ambrosioides L.) show that this compound has good potential in inhibiting biological activity with an average IC50 of 58.25379 ppm. This suggests that Pasote leaf extract could be a promising option in anti-inflammatory treatment.

CONCLUSION

Based on the results of this study, the conclusions that can be drawn are:

1. The results of phytochemical screening, the compounds contained in Pasote Leaf Extract (Dysphania ambrosioides L.) are alkaloids, flavonoids, saponins, tannins, and phenolics.
2. The extract with a concentration of 250 ppm has the highest anti-inflammatory activity. This result is seen from its ability to stabilize red blood cell membranes, which is 90.476%.
3. The ability to stabilize red blood cell membranes increases with increasing concentration in the anti-inflammatory activity test.
4. Pasote Leaf Extract (Dysphania ambrosioides L.) has anti-inflammatory activity. The IC50 values of the Na diclofenac standard and Pasote Leaf Extract (Dysphania ambrosioides L.) are 49.97973 and 58.25379 ppm, respectively.

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