

Research Article

Anti-inflammatory potential of african leaf water extract

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Abstract

Inflammation is the immune system's main response to infection and irritation. African leaf (*Gymnanthemum amygdalinum*) is one of the medicinal plants that can be used as anti-inflammatory because of the presence of flavonoid compounds. Flavonoid compounds have an anti-inflammatory effect that can regulate arachidonic acid metabolism by inhibiting cyclooxygenase (COX) and lipooksigenase activities. This study aims to obtain scientific information and the potential of African leaf extract as an anti-inflammatory in vitro and in vivo test. In this study, in vitro and in vivo anti-inflammatory activity tests were carried out. The in vitro anti-inflammatory activity was evaluated by the ability of African leaf extracts to prevent protein denaturation and in vivo anti-inflammatory activity by observing the reduction of edema in the soles of rats induced by carrageenan by giving three levels of doses of African leaf extract. The results showed that the African leaf extract at a concentration of 8273.91 mg L⁻¹ was able to inhibit 50% denatured protein (IC₅₀), while in the in vivo test African leaf had the potential as an anti-inflammatory with the most effective percentage of inhibition at a dose of 200 mg kg⁻¹ which is 85.20%. African leaf have the potential of approximately 2 times greater than the positive control of diclofenac sodium which is 45.70%.

Introduction

Inflammation is one of the main responses of the immune system to injury, infection and cell damage characterized by heat, redness, pain, swelling and impaired physiological function. The drugs commonly used to control inflammation are non-steroidal anti-inflammatory drugs, which have several side effects, especially gastric irritation (Drini, 2017).

One of the medicinal plants that can be used as anti-inflammatory is African leaf (*Gymnanthemum amygdalinum*) (Georgewill and Georgewill, 2010). African leaf, commonly known as bitter leaf, is a shrub that grows up to

3 meters high in the African tropics and other parts of Africa, particularly, Nigeria, Cameroon and Zimbabwe. It is reputed to have several health benefits (Farombi et al., 2011) African contain vernoniosides A1, A2, A3, B1, B2, B3, sesquiterpenes, lactones, vernolides, vernodalol, vernolepin, vernodalin, vernomygdin, hydroxylvernolide, tannins, luteolin, and xanthan (Ijeh & Ejeh 2011).

The infusion method can only search for polar substances where one of these compounds is a flavonoid and the anti-inflammatory activity of various plants is thought to be derived from flavonoids (Ramadhani, 2013).

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The mechanism of flavonoids is to inhibit lipid peroxidation, so that will reduce the adhesion of inflammatory cells to the endothelium and generally reduce the inflammatory response (Nijveldt et al., 2012).

The in vitro anti-inflammatory activity was evaluated by the ability of African leaf extracts to prevent protein denaturation. In vivo anti-inflammatory activity was carried out by observing the reduction of edema in the soles of the white rats induced by carrageenan with three levels of doses of African leaf extract. This study aims to obtain scientific information and the potential of African leaf extract anti-inflammatory activity.

Materials and Methods

Materials and apparatus

The tools used in this study include rat cages, pletismometer, analytical scales, animal scales, glassware, Uv-Vis spectrophotometer and a water bath. The ingredients used are Bitter leaf 2% of carrageenan, 0.5% of CMC-Na, aquadestilata, 50 mg diclofenac sodium tablets, sodium potassium tartrate, copper sulfate, NaOH, NaCl, KCl, Na₂HPO₄, KH₂PO₄, Bovine Albumin Serum and methanol. The test animals used in the study were 30 male white rats *Sprague-Dawley* strain aged 2-3 months with a weight of ± 200 gram.

Time and site

This research was conducted from May to July 2020 at the Pharmacy Laboratory, Mathematics and Natural Science Faculty, University of Pakuan, Bogor and the Tropical Biofarmaka Study Center, Research and Community Services Program, Bogor Agricultural Institute.

Methods of experiment

African leaf collection (Gymnanthemum amygdalinum)

African leaf are obtained from Cariu Village, Bogor Regency. Plant determination is carried out at the Plant Conservation Center of the Indonesian Institute of Sciences (LIPI). Cibinong, Bogor. Air-drying, dry bitter leaf is sorted, grinded and sieved with a 30 mesh sieve. The water content and ash content were determined (Hanani, 2015).

Preparation of african leaf water extract

An extract of African leaf is made by weigh 200 g of the powder of African leaf and added 2 L of aquadest in the infusion pan. Heated on a water bath for 15 minutes starting from the temperature reaching 90 °C while stirring occasionally. The water extract is filtered using filter paper, then adding hot water through the dregs up to a volume of 2 L. Then performed a phytochemical test (alkaloid, flavonoid, saponin, and tannin) (Hanani, 2015).

In vitro anti-inflammatory activity test

The in vitro anti-inflammatory activity was evaluated by the ability of African leaf extracts to prevent protein denaturation (Hildebrandt et al., 2008).

Preparation of protein reagents

2 g of sodium potassium tartrate, 1 g of copper sulfate are weighed and dissolved in 90 mL of aquabides, then 10 mL of 1 N NaOH are added (Hildebrandt et al., 2008).

Preparation of phosphate buffer pH 7.4

In 800 mL of aquabidest, 8 g of NaCl, 200 mg of KCl, 1.44 g of Na₂HPO₄ and 245 mg of KH₂PO₄ are dissolved. Measured the pH with adjusting using Phosphoric Acid or NaOH (Bertholf, 2014).

Preparation of 0.6% BSA solution

The 0.6% BSA solution was prepared by weighing 0.6 g of BSA and then dissolving it in 100 mL of aquabidest.

Preparation of test solutions

The test solution consisted of 2, namely diclofenac sodium solution and African leaf extract solution. The diclofenac sodium test solution was prepared by weighing 500 mg of standard diclofenac sodium, dissolving 10 mL of methanol, then adding up to 100 mL using aquabidest, so that the main solution of diclofenac sodium with a concentration of 5000 mg L⁻¹ was obtained. Extract solution of African leaf is made by weighing 12 g of powder of Bitter leaf, then boiling it in 100 mL of aquabidest at 90 °C for 15 minutes. After cooling the extract was filtered and added to 100 mL using

aquabidest, so that the extract solution was obtained with a concentration of 120000 mg L⁻¹ (Hildebrandt et al., 2008).

Analysis

Positive control solutions were prepared by mixing 1.5 mL of 0.6% BSA solution, 1.5 mL of phosphate buffer solution pH 6.4 and 6 mL of protein reagent solution. The absorption of the mixture was directly measured using a spectrophotometer at a wavelength of 540 nm (without treatment) (Bertholf, 2014; Hildebrandt et al., 2008).

Negative control solution was prepared by mixing 1.5 mL of 0.6% BSA solution, 1.5 mL of phosphate buffer solution pH 6.4 and 6 mL of protein reagent solution. The mixture was then heated at 37 °C for 30 minutes, then heated at 70 °C for 10 minutes. After cooling, the absorption of each solution was measured using a spectrophotometer at a wavelength of 540 nm (with treatment) (Bertholf, 2014; Hildebrandt et al., 2008).

The difference between negative and positive controls is that in the negative control, heating is carried out while in the positive control it is not, this is to illustrate the occurrence of protein denaturation due to heating.

The test solution for diclofenac sodium was made into a series of test solutions with 0.06 pipettes; 0.12; 0.36; 0.6 and 0.12 mL of the 5,000 mg L⁻¹ test solution into 5 test tubes, then add up to 1.5 mL of each tube using a phosphate buffer. To each tube, 1.5 mL of 0.6% BSA solution was added. This mixture was then heated at 37 °C for 30 minutes, then heated at 70 °C for 10 minutes. After cooling, the absorption of each solution was measured using a spectrophotometer at a wavelength of 540 nm (Bertholf, 2014).

Extract solution of African leaf is made into a series of test solutions with 50 pipettes; 100; 150; 200 and 250 µL of 120,000 mg L⁻¹ mother liquor into 5 test tubes, then add up to 1.5 mL of each tube using phosphate buffer (Bertholf, 2014; Hildebrandt et al., 2008).

To each tube, 1.5 mL of 0.6% BSA solution was added. This mixture was then heated at 37 °C for 30 minutes, then heated at 70 °C for 10 minutes. After cooling, the absorption of each

solution was measured using a spectrophotometer at a wavelength of 540 nm. Protein denaturation inhibitory activity is measured using the formula (Bertholf, 2014; Hildebrandt et al., 2008; Quasie, 2016) :

Inhibition Ability

$$(IC) = \frac{A_{\text{sample}} - A_{\text{negative control}}}{A_{\text{positive control}} - A_{\text{negative control}}} \times 100\%$$

Explanation :

A_{sample} : diclofenac sodium solution and sample absorption

$A_{\text{negative control}}$: negative control solution absorption

$A_{\text{positive control}}$: positive control solution absorption

Furthermore, a graph of the relationship between concentration (x-axis) and drag (y-axis) is drawn, then the linear regression equation is determined. The linear regression equation is used to calculate the concentration that can inhibit the denaturation of 50% protein (IC₅₀).

In vivo anti-inflammatory activity test

In vivo anti-inflammatory testing in this study was by subplantar injection of carrageenan solution, then measuring the volume of edema on the soles of the rats' feet was measured using a pletismometer. The anti-inflammatory activity was shown by the ability of the test material to reduce edema caused by carrageenan induction on the soles of the feet of the test animals (Mishra et al., 2011).

Ethics review

This research has been approved by the Animal Ethics Commission Team, Faculty of Mathematics and Natural Sciences, Pakuan University with the Ethics Committee Decree No. 102/KEPHP-UNPAK/05-2020.

Research design

Rats were randomly divided into five treatment groups, each group consisting of 6 rats. Group I as a negative control was given CMC-Na solution, group II as a positive control was given diclofenac sodium, groups III, IV, and V

were given water extract of African leaf with three dose ratings, namely 100 mg kg⁻¹, 150 mg kg⁻¹, 200 mg kg⁻¹.

Preparation the dosage

The extract dosage used in this study was 100 mg kg⁻¹ for dose I, 150 mg kg⁻¹ for dose II, 200 mg kg⁻¹ for dose III, 5.3 mg kg⁻¹ diclofenac sodium as positive control.

Preparation of diclofenac sodium suspension

Diclofenac sodium tablet 50 mg finely crushed, then sieved and taken as much as 4.5 mg. After that this diclofenac sodium powder was suspended in 10 mL of 0.5% CMC-Na until it was homogeneous. The dosage of diclofenac sodium is 5.3 mg kg⁻¹.

Preparation of extract suspensions

The water extract of African leaves was weighed as much as 20.30 and 40 mg. Each was crushed with the addition of 0.5% CMC-Na suspension until homogeneous, put into a 10 mL volumetric flask, stirred to the mark with 0.5% CMC-Na suspension (Mishra et al., 2011).

Inflammation inductor preparation

Carrageenan is weighed as much as 200 mg, then dissolved in 10 mL of physiological NaCl solution and stirred to obtain a 2% concentration of carrageenan suspension (Mishra et al., 2011).

Inflammation testing

The test was started by fasting the mice for 18 hours while still giving them drinking. Rats were grouped into 6 treatment groups. The rat's left leg was inserted into a plestimometer containing liquid mercury until the liquid rose to the upper boundary line, the number was recorded on the instrument as the initial volume (Vo), namely the volume of the leg before being given the drug and induced with a carrageenan solution (Mishra et al., 2011).

Each rats were given a suspension of the test material orally according to its group. 15 minutes later, each of the rats' feet was injected subplantar with 0.2 mL of 2% carrageenan solution and 30 minutes after induction the measurements were taken by immersing the rat's left leg in a plestimometer until the solution

reached the upper limit line of the rat's left foot and the numbers were recorded obtained. Changes in fluid volume were recorded as rat foot volume (Vt). Measurements were taken every 60 minutes for 360 minutes.

Calculation and data analysis

Data obtained in the form of the volume of rat feet, then used to calculate the inflammation that occurs with the following calculation equation (Adedapo et al., 2014):

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

Explanation :

Vt: Mean volume of rat feet after 2% carrageenan induction

Vo: The average volume of rat feet before being induced by carrageenan 2%

The % inflammation data obtained is then used to calculate the % inhibition of edema by comparing it with a negative control at the same hour with the following equation (Adedapo et al., 2014):

$$\% \text{ Edema Inhibition} = \frac{a-b}{a} \times 100\%$$

Explanation :

a: % inflammation negative control (at the same hour)

b: % inflammation control for each comparison dose (at the same hour)

The % edema inhibition data obtained were then used to see the percentage of anti-inflammatory potential, by comparing with diclofenac sodium as a positive control with the following calculation equation (Adedapo et al., 2014):

$$\% \text{ Anti-inflammatory potentiation} = \frac{\% \text{ edema inhibition average dose}}{\% \text{ positive control inhibition rate}} \times 100\%$$

Observation data were collected and presented in the form of tables, graphs and one-way ANOVA (Analysis of Variance) statistical analysis. The AUC data between the volume of edema against the time was carried out by the Kolmogorov-Smirnov test to determine whether the data were normally distributed or

not. Levene Statistic test to determine the homogeneity of the variants.

Data that has been normally distributed and homogeneous is followed by one-way analysis of variants (Oneway Difference) with a 95% confidence level and followed by the LSD (Least Significant Difference) test to determine whether or not there is a significant difference. Data analysis was carried out with the SPSS program.

Results and Discussion

African leaf simplicia is made by harvesting fresh in Cariu Village, Bogor Regency. African leaf used in this research process have been determined at the Center for Utilization and Innovation of Science and Technology, Indonesian Institute of Sciences (LIPI), Cibinong, Bogor. The results of the determination stated that the plants used in this study were the Asteraceae family of *Gymnanthemum amygdalinum*.

The number of fresh African leaf used was 11.5 kg. Then done several stages of making simplicia until finally it became a simplicia

powder and the yield was 1.386 kg, so that the yield was 15.965%.

The African leaf powder was extracted using the infusion method as much as 200 g with 2 L of water as a solvent, so that 660 mL of liquid extract was obtained and then made into a thick extract, the yield was 10.6 g so that the extract yield was 5.3%.

Moisture content and ash content of african leaf powder

The gravimetric method was used to determine the water content of the leaves. The obtained water content of 8.94% Determination of the ash content of African leaf was carried out by annealing using a furnace, and obtained an ash content of 4.615%.

Phytochemical test results powder african leaf

Phytochemical test results can be seen in Table 1 and it is found that African leaf powder contains alkaloids, flavonoids, tannins, and saponins.

Table 1. African leaf simplicia phytochemical test results

Phytochemical Test	Observation result	+/-
<i>Saponin</i>	Formed foam 4 cm 20 minutes	+
<i>Tanin</i>		
FeCl ₃	Greenish blue	+
<i>Alkaloid</i>		
Mayer	White sediment	+
Dragendroff	Red	+
Boucharlat	Brown	+
<i>Flavonoid</i>	Yellow	+

In vitro anti-inflammatory activity test results

Protein denaturation is known to cause inflammation and rheumatoid arthritis. Several anti-inflammatory drugs such as phenylbutazone, indometazine and diclofenac sodium have shown activity in inhibiting temperature-induced protein denaturation (Phanse et al., 2012). Testing of protein denaturation inhibition activity was carried out using the protein denaturation inhibition test method conducted

by (Heendeniya, 2018) by making slight modifications to the procedures and formulas to calculate inhibitory power.

Using a modified method, it is known that the inhibitory power of 50% denatured protein (IC₅₀) from diclofenac sodium is 1041.61 ppm or equivalent to the consumption of 520.80 mg of the active substance diclofenac sodium. The IC₅₀ value is obtained from the linear regression equation (Figure 1.A).

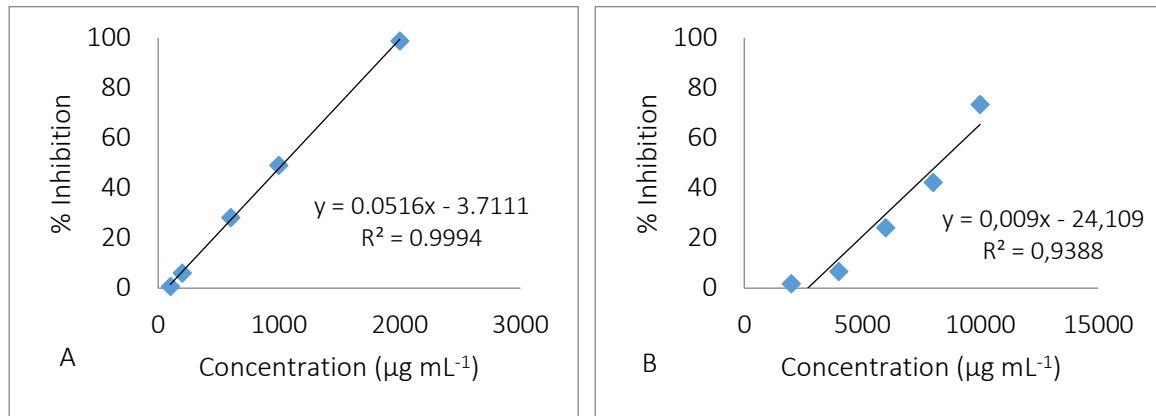


Figure 1. Inhibition of diclofenac sodium (A) and African leaf water extract (B)

The same method was used to test the inhibition of African leaf against 50% denatured protein. The results showed that African leaf had 50% protein denaturation (IC_{50}) inhibitory activity at the concentration 3751.88 ppm or equivalent to 0.8274 g African leaf simplicia (Figure 1.B). The inhibition of the protein denaturation process from African leaf extract is caused by the presence of flavonoids (Verma, 2011).

In vivo anti-inflammatory activity test results

In the anti-inflammatory activity test, data were obtained in the form of edema volume in rats' feet. The measurement of the volume of inflammation uses a pletismometer which has a principle based on Archimedes' law.

Table 2. Rats' feet volume by treatment according to group at time t

Group	Volume of foot edema at hour (mL) ± SD						
	0	1	2	3	4	5	6
I	0.775 ± 0.060	0.820 ± 0.058	0.850 ± 0.054	0.875 ± 0.050	0.856 ± 0.052	0.841 ± 0.054	0.835 ± 0.050
II	0.753 ± 0.052	0.783 ± 0.057	0.805 ± 0.059	0.825 ± 0.062	0.811 ± 0.063	0.798 ± 0.062	0.785 ± 0.058
III	0.771 ± 0.063	0.798 ± 0.058	0.826 ± 0.062	0.845 ± 0.062	0.835 ± 0.062	0.820 ± 0.062	0.801 ± 0.065
IV	0.758 ± 0.052	0.780 ± 0.054	0.808 ± 0.054	0.830 ± 0.050	0.815 ± 0.054	0.801 ± 0.058	0.786 ± 0.052
V	0.760 ± 0.051	0.785 ± 0.047	0.805 ± 0.048	0.828 ± 0.048	0.818 ± 0.048	0.805 ± 0.043	0.786 ± 0.045

Explanation:

- I: Negative control, CMC-Na solution
- II: Positive control, diclofenac sodium dose of 5.3 mg kg⁻¹
- III: Dose I, a dose 100 mg kg⁻¹ of African leaf water extract
- IV: Dose II, a dose 150 mg kg⁻¹ of African leaf water extract
- V: Dose III, a dose 200 mg kg⁻¹ of African leaf water extract

The increase in edema volume was caused by the release of inflammatory mediators such as prostaglandins, histamine, bradykinin, and serotonin in the tissue after being induced by

carrageenan. The decrease in edema volume occurred at 4 to 6 hours. This decrease occurred due to inhibition of prostaglandin synthesis into the tissue. This volume data is then

used to determine the percentage of edema inhibition.

The percentage of edema inhibition was obtained by comparing the difference between the volume of treatment and negative control. The results of percent inhibition in four treatments, namely positive control, water extract at a dose of 100 mg kg⁻¹, 150 mg kg⁻¹ and 200 mg kg⁻¹ are shown in Table 3.

The analysis showed that the anti-inflammatory effect of the aqueous extracts of African

leaves was dose-dependent. The greater the dose of water extract used by African leaves, the inhibition of edema in the rats' feet was also greater.

The results obtained the greatest percentage of inhibition is treatment IV, namely with a dose of 200 mg kg⁻¹ of 38.9394%. This result is close to the percent inhibition of the positive control for diclofenac sodium which is 45.7029%.

Table 3. Percent of edema inhibition according to treatment group at time

Group	Percent Inhibition of average edema (hour)						\bar{x}
	1	2	3	4	5	6	
I	44.76	39.36	40.15	45.53	46.00	58.40	45.7029
II	38.06	24.64	22.96	21.96	25.89	43.46	29.4985
III	40.59	25.19	24.58	24.35	29.62	48.46	32.1365
IV	40.85	34.73	26.55	26.69	35.27	51.95	38.9394

Explanation :

I: Positive control, Dosage of diclofenac sodium is 5.3 mg kg⁻¹

II: Dosage I, a dose 100 mg kg⁻¹ of African leaf water extract

III: Dosage II, a dose 150 mg kg⁻¹ of African leaf water extract

IV: Dosage III, a dose 200mg kg⁻¹ of African leaf water extract

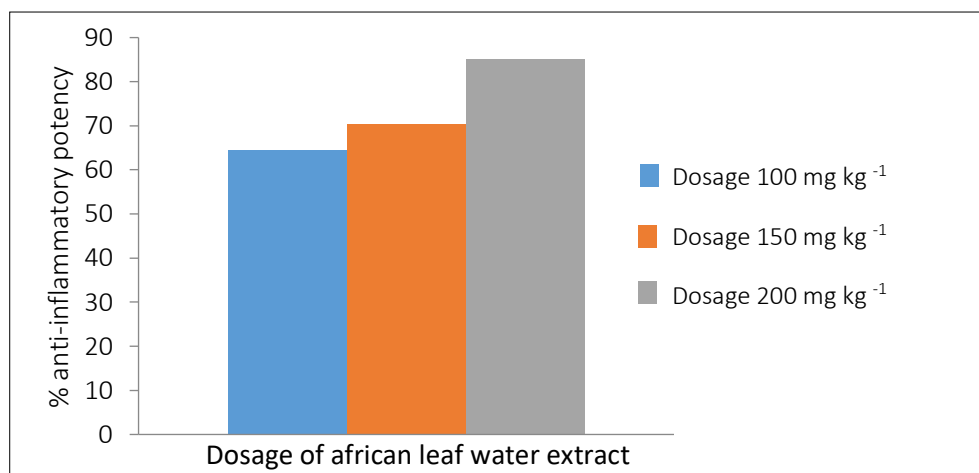


Figure 2. Percentage of african leaf water extract anti-inflammatory potential

The percentage of anti-inflammatory potency was calculated by comparing the percentage inhibition of each dose with the percentage inhibition of diclofenac sodium as a positive control. The results were obtained at a dose of 100 mg kg⁻¹ is 64.54%, a dose of 150 mg kg⁻¹ is 70.31% and a dose of 200 mg kg⁻¹ is 85.2% compared with diclofenac sodium as a positive control (Figure 2). So that the water extract treatment group of African leaf which has the

potential as the most effective anti-inflammatory is in the 200 mg kg⁻¹ dose group.

Conclusion

The water extract of African leaf (*Gymnanthemum amygdalinum*) in the in vitro test with a concentration of 3751.88 ppm was able to inhibit 50% denatured protein (IC₅₀), while in vivo tests has potential as anti-inflammatory

by the percentage of the most effective inhibitory power at a dose of 200 mg kg⁻¹ which is 85.20%. African leaf have the potential of approximately 2 times greater than the positive control of diclofenac sodium.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Authors Contribution

L.A.S: Conducted field experiments and recorded field observations. L.A.S: Conceived the idea and supervised the experiment and written the concept and discussion. L.A.S and Z.R: written the draft, data analysis and references.

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