

In-Vivo Anti-Inflammatory Activity of *Kelubut* Leaf Ethyl Acetate Extract (*Passiflora foetida* L.) from Samarinda City

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Abstract

Kelubut (*Passiflora foetida* L.), as an anti-inflammatory, is widely found in various regions in Indonesia, including in Central Kalimantan. Its main chemical compounds include alkaloids, phenols, glycosides, flavonoids, and cyanogenic compounds. Flavonoids show more than a hundred kinds of bioactivity, including antipyretic, analgesic, and anti-inflammatory. This study aims to determine the anti-inflammatory activity of *kelubut* leaf extract (*Passiflora foetida* L.) against mice (*Mus musculus*). This research went through a Pretest and Posttest Control Group Design with 5 treatment groups, negative control, positive control, and ethyl acetate extract of *kelubut* leaves with 3 doses of 250 mg/KgBW, 125mg/KgBW and 62.5 mg/KgBB. Before being given treatment, each treatment group was induced by carrageenin by sub-planar injection into the sole of the left leg of the mouse. Then, the edema developed rapidly and persisted for 6 hours. After being induced by carrageenin, the researchers waited for 30 minutes and measured the volume of edema every 30 minutes to 120 minutes. The inhibition of all groups of ethyl acetate extract of *kelubut* leaves showed anti-inflammatory activity, but the resulting abilities were different. Inflammation inhibition by ethyl acetate extract at a dose of 250 mg/KgBW was 92.78%, at a dose of 125 mg/KgBW, was 91.76%, and at a dose of 62.5 mg/KgBW was 84.61%. From the results obtained, the ethyl acetate extract group of *kelubut* leaves at a dose of 250 mg/KgBW had the greatest inflammatory inhibition activity compared to a dose of 125 mg/KgBW and 62.5 mg/KgBW.

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INTRODUCTION

Inflammation is a disorder that often occurs in humans and animals and is characterized by redness, heat, swelling, and disturbing pain.¹ According to the 2018 Basic Health Research of the Republic of Indonesia in Rahayu's research

(2022), diseases involving inflammatory processes in the human body in Indonesia still have quite high rates, such as cancer at 1.8%, asthma at 2.4%, diabetes mellitus at 2.0% and in joints at 7.3%.²

One of the medicinal plants that are believed to be an anti-inflammatory

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treatment is *kelubut* (*Passiflora foetida* L.). *Kelubut* leaves are widely found in various regions in Indonesia, including in Central Kalimantan.³ *Kelubut* leaves are an alternative treatment for several diseases, such as inflammation, rheumatism, diarrhea, and abdominal pain.⁴ Its main chemical compounds include alkaloids, saponins, phenols, glycosides, flavonoids, steroids, and cyanogenic compounds.⁵⁻⁸

From the various research results reported, the chemical compounds that have anti-inflammatory properties are flavonoids. Flavonoids can inhibit cyclooxygenase or lipoxygenase and inhibit leukocyte accumulation to be anti-inflammatory.⁹ From the description above, this research was conducted to determine the anti-inflammatory activity of the *Kelubut* leaves (*Passiflora foetida*, L.) against carrageenin-induced mice (*Mus Musculus*).

METHOD

Materials and Tools

The materials and tools needed for this research included distilled water, 0.5% CMC Na, 1% carrageenin, diclofenac Na, ethyl acetate solvent, *Kelubut* leaves extract (*Passiflora foetida* L.), mice (*Mus musculus*), mouse cages, sonde, gloves, weight balance, injection syringe, rotary evaporator, mercury plethysmometer, markers, beaker glass, stopwatch,

Research design

This research is pure experimental research with the Pretest and Posttest Control Group Design conducted in the laboratory to obtain the desired data and results.

Research sites

This research was conducted at the Laboratory of the Faculty of Pharmacy,

Universitas Muhammadiyah Kalimantan Timur.

Research procedure

Plant Determination

Determination of *kelubut* leaves was carried out at the Laboratory of the Faculty of Forestry, Universitas Mulawarman Samarinda, East Kalimantan. *Kelubut* leaves (*Passiflora foetida* L.) used in this study were obtained from Loa Janan Ilir District, Samarinda City, East Kalimantan Province. The determination results showed that the *kelubut* leaf samples were declared correct.

Material Preparation

Kelubut leaves collected were then washed clean, dried in the open air, and protected from direct sunlight. After drying, the leaves were crushed to obtain simplicia powder.¹⁰

Preparation of Test Animals

The test animal used in this study was white mice (*Mus musculus*). Before the study, the mice were adapted to the cage for 1 week to make the mice adapt. Mice were fasted for 12-18 hours before treatment (not eating but still being given water) to equalize the condition of the mice and prevent the effects of the food consumed.¹¹

Preparation of *Kelubut* Leaves Ethyl Acetate Extract

The extract was prepared by maceration. The sample was put into a glass jar, added with ethyl acetate in a ratio (1:3), which was soaked for 5 days and stirred 2 times on the 2nd and 4th day. After that, re-maceration was carried out with a new solvent 2 times. Furthermore, the extract was concentrated with a Rotary Evaporator to obtain a thick extract

Extract Yield Calculation

Yield calculations were performed to determine the percentage of extract produced from each gram of dry powder calculated by formula.¹²

$$\% \text{ Yield} = \frac{\text{Extract weight obtained (g)}}{\text{Dry powder weight before extraction (g)}} \times 100\%$$

Preparation of 0.5% CMC Na Solution

A 0.5% CMC Na solution was prepared by weighing 500 mg of CMC Na into 10 ml of hot distilled water and then allowed to stand for less than 15 minutes until it was clear and gel-like. Then, it was stirred until it became a homogeneous mass and

diluted in a volumetric flask with distilled water to a volume of 100 ml.

Preparation of Carrageenin 1%

Carrageenin weighed accurately 0.1 gram and then dissolved in 10 mL of physiological saline (0.9% NaCl).¹³

Preparation of Diclofenac Na Suspension

Diclofenac sodium 50 mg suspended with CMC Na 0.5%. CMC was sprinkled into hot water until dissolved and homogeneous. Then, diclofenac sodium was added to the CMC mixture until it was evenly dispersed, and the remaining hot water was added to the desired volume.

Calculation of Dose for Test Animals

Diclofenac Sodium Dose

Dose for humans = 50 mg (positive control)

The conversion value of 20-gram mice = 0.0026

Dose for mice 20 grams = 0.0026 x 50 mg = 0.13 mg

Dose in mice 20 grams = (0.13 mg)/(20 grams) = 6.5 10⁻³ mg/g BW mice
= 6.5 mg/kg body weight of mice

Dose CMC Na 0.5%

Na CMC 0.5% (negative control) 0.5 ml = 20 grams (BB mice)

Anti-inflammatory Test

The animal test prepared was marked with a marker on one of the mice's hind legs so that when the legs were placed in mercury, they were always the same. Then, the researchers measured the volume of each hind leg with a plethysmometer.¹⁴ The measurement results were recorded as the initial volume. Mice were divided into several groups and given the following treatments: Group negative control consisted of 3 mice treated with CMC Na

0.5%; Group positive control consisted of 3 mice treated with Diclofenac Na; Group 1 consisted of 3 mice treated with *kelubut* leaf extract at a dose of 250 mg/KgBW; Group 2 consisted of 3 mice treated with *kelubut* leaf extract at a dose of 125

mg/KgBW; and Group 3 consisted of 3 mice treated with *kelubut* leaf extract at a dose of 62.5 mg/KgBW.

Data Analysis Techniques

Data were analyzed statistically using the Analysis of Variance (ANOVA) method in SPSS Statistic Viewer 26 software. Previously, the data were tested for normality with the Shapiro-Wilk and Homogeneity tests, where both tests had a requirement of $P > 0.05$, indicating that the data was homogeneous and normally distributed.¹⁵

RESULTS AND DISCUSSION

Testing the anti-inflammatory activity of *kelubut* leaf extract is to determine the

ability of the extract to reduce inflammation by looking at the decrease in inflammation diameter against the dilution level of the extract, which is then measured to see whether *kelubut* leaf extract can reduce inflammation in white male mice and can have a different effect on anti-inflammatory activity and variations in doses of *kelubut* leaf extract.

This research began by preparing 1500 grams of simplicia powder, then macerating ethyl acetate for 5 days and re-macerating 2 times. Maceration was chosen because this method is easy to do with simple equipment and is safe to use

for heat-resistant compounds such as flavonoids. Then, the extract was filtered and evaporated with a rotary evaporator at a temperature of $\pm 40^{\circ}\text{C}$ until a thick extract was obtained. The thick extract was heated with a water bath.¹⁶⁻¹⁸ Ethyl acetate solvent was chosen as a maceration solvent because it is not hygroscopic, has low toxicity, and is semi-polar, attracting both polar and non-polar molecules.¹⁹

Table 1 shows the yield calculation obtained from the maceration of the ethyl acetate extract of *kelubut* leaves.

Table 1. Extraction results of *Kelubut* leaves

Sample Weight	Solvent volume	Extract Weight	Yield (%)
1.500 gr	4.5 liters	96.45 gr	6.43 %

The anti-inflammatory test was done using male mice (*Mus musculus*) as test animals because male mice have a stable biological condition when compared to female minutes whose biological condition is influenced by their cycle period (estrus). The mice used in this study were 15 mice weighing about 20-30 grams and aged about 2-3 months.²⁰⁻²¹ The treatment group was divided into 5 groups by grouping 3 mice for the group negative control (CMC-Na), 3 mice for the group positive control (Diclofenac Na), 3 mice for group 1 with an extract dose of 250 mg/KgBW, 3 mice for group 2 with an extract dose of 125 mg/KgBW and 3 mice for group 3 with an extract dose of 62.5 Kg/BW.

The method used in this anti-inflammatory test was forming artificial edema on the soles of mice induced by carrageenin. Carrageenin as an inducer solution has several advantages, including not leaving scars, not causing permanent tissue damage, and providing a more sensitive response to anti-inflammatory drugs.²²

Before the treatment, each mouse fasted for 8 hours to avoid the influence of the food content on the treatment given. Then, the body was weighed to determine the appropriate drug administration. The initial volume of the left leg of the mouse was measured using a Digital Plethysmometer to determine the volume of the leg before being given further treatment.²³ After that, each treatment group was induced by carrageenin by injecting it sub-planarly into the sole of the left leg of the mice. Then, edema developed rapidly and persisted for 6 hours. After being induced by carrageenin, the treatment stopped for 30 minutes because after giving carrageenin, there was a release of inflammatory mediators such as histamine and serotonin. Then, the volume of the left leg of the induced mice was measured. After that, extract doses 1, 2 and 3 showed positive control and negative control according to the treatment group. The volume of edema was measured every 30 minutes to 120 minutes and observed to see the volume of edema decreased from each group.

Table 2. Average Edema Volume Measurement Results (mL)

Group	t ₀	t ₁	t ₃₀	t ₆₀	t ₉₀	t ₁₂₀
Negative Control	0.15±0.01	0.28±0.01	0.28±0.01	0.28±0.02	0.28±0.02	0.28±0.02
Positive Control	0.14±0.01	0.23±0.02	0.22±0.02	0.20±0.02	0.18±0.02	0.16±0.02
Group 1	0.16±0.02	0.27±0.01	0.25±0.01	0.23±0.01	0.20±0.01	0.17±0.02
Group 2	0.14±0.01	0.23±0.01	0.21±0.01	0.19±0.01	0.17±0.01	0.15±0.01
Group 3	0.15±0.02	0.23±0.01	0.22±0.02	0.22±0.02	0.20±0.02	0.17±0.01

Information:

- t₀ : Initial volume
- t₁ : Volume after carrageenin induced
- t₃₀ : Volume of edema in 30 minutes after being treated
- t₆₀ : Volume of edema in 60 minutes after being treated
- t₉₀ : Volume of edema in 90 minutes after being treated
- t₁₂₀ : Volume of edema in 120 minutes after being treated

Figure 1 illustrates that the administration of CMC-Na colloidal solution does not affect the decrease in the volume of inflammation on the soles of mice. In the CMC-Na group, the volume of inflammation produced increases and persists for up to 120 minutes. CMC-Na is only a solvent for drug media, so there is no stimulation of drugs to reduce edema so that edema will increase. The process of removing mediators inflammation in the body of mice only occurs naturally.

In the positive control group, there is a very significant decrease. A significant decrease occurred because the positive control was treated with Na-diclofenac. Diclofenac Na is an NSAID class of drugs that works to inhibit cycloignase through antagonism with arachidonic acid to bind to the cyclooxygenase enzyme. Treatment with ethyl acetate extract from *kelubut* leaves, dose 1, which is 250 Kg/BW,

dose 2, which is 125 Kg/BW, and dose 3, which is 62.5 Kg/BW, experienced various processes of reducing swelling. The test group with ethyl acetate extract from *kelubut* leaves showed anti-inflammatory activity resulting from all doses due to secondary metabolites in *kelubut* leaves. Based on a phytochemical screening conducted by Raden Roro and Antoni (2022), *kelubut* leaves and stem extracts contain secondary metabolites from alkaloids, flavonoids, tannins/polyphenols, steroids, and saponins.²⁴ The mechanism of action of flavonoids as an anti-inflammatory through inhibition of cyclooxygenase (COX) and lipooxygenase activity, inhibiting the accumulation of white blood cells, inhibiting neutrophil degranulation, and inhibiting histamine.²⁵

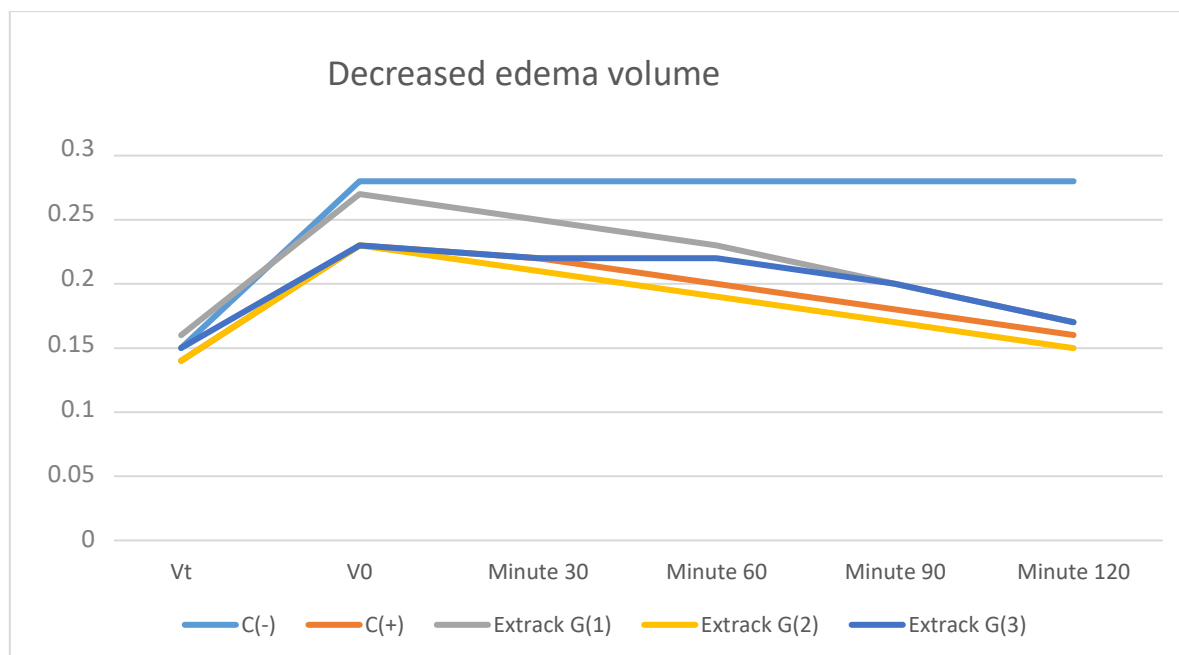


Figure 1. Graph of Decreased Edema Volume at 4 Time Intervals

To find out any significant differences in the test animals between treatment groups, a statistical analysis of the One-way ANOVA test was carried out. The One-way ANOVA test showed a significant difference in the percentage change in edema at each observation time. This finding corresponded to a significance value of $0.001 < 0.05$. Based on the ANOVA test, the LSD (Least Significance Different) test was continued to find out which treatment was significantly different if the null hypothesis was rejected.²⁶

CONCLUSION

Based on the research done, the ethyl acetate extract of *kelubut* leaves (*Passiflora foetida L.*) with dose groups 1, 2, and 3 had anti-inflammatory activity in reducing the volume of edema on the legs of mice.

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- 21 Chaerul Fadly Mochtar Luthfi M, Reni Selviana Devi, Hasyrul Hamzah, Ayu Faradillah, Elva Hafidzah, Fathiah Putri Varizza, et al | In-Vivo Anti-Inflammatory Activity of *Kelubut* Leaf Ethyl Acetate Extract (*Passiflora foetida* L.) from Samarinda City
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