NASKAH PUBLIKASI

AKTIVITAS ANTIINFLAMASI SECARA IN-VIVO EKSTRAK ETIL ASETAT DAUN KELUBUT (*Passiflora foetida* L.) DARI KOTA SAMARINDA

IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF KELUBUT LEAF ETHYL ACETATE EXTRACT (*Passiflora foetida* L.) FROM SAMARINDA CITY

Chaerul Fadly Mochtar[1] ; Reni Selviana Devi[1*] ; Hasyrul Hamzah[1] ; Ayu Faradillah[1] ; Elva Hafidzah[1] ; Fathiah Putri Varizza[1] ; Novia Misnawati Aisyiyah[1] ; Qur'anni Akhwatun Husna[1]

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2023

Naskah Publikasi

Aktivitas Antiinflamasi Secara In-Vivo Ekstrak Etil Asetat Daun Kelubut (*Passiflora Foetida* L.) dari Kota Samarinda

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

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LEMBAR PERSETUJUAN

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IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF KELUBUT LEAF ETHYL ACETATE EXTRACT (Passiflora foetida L.) FROM SAMARINDA CITY

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Abstract

Anti-inflammatory namely Kelubut (Passiflora foetida L.). Kelubut plants are widely found in various regions in Indonesia, including in Central Kalimantan. The composition of the main chemical compounds includes 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 leaves extract (Passiflora foetda L.) against mice (Mus musculus).

This research was carried out by The Pretest and Posttest Control Group Design with 5 treatment groups, namely negative control, positive control, and ethyl acetate extract of kelubut leaves with 3 dose namely 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 edema develops rapidly and persists for 6 hours. After being induced by carrageenin, wait for 30 minutes and measure the volume of edema every 30 minutes to 120 minutes.

Based on the results of the percentage inhibition of all groups of ethyl acetate extract of kelubut leaves, they have anti-inflammatory activity, but the resulting abilities are different. The percentage of inflammation inhibition by ethyl acetate extract at 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, it can be seen that the ethyl acetate extract group of kelubut leaves at a dose of 250 mg/KgBW had the greatest inflammatory inhibition activity compared to 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 the appearance of 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 with a percentage of 1.8%, asthma at 2.4%, diabetes mellitus 2 .0% and in joints 7.3%. [2]

One of the medicinal plants that are believed to be an anti-inflammatory 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] The composition of the main chemical compounds includes 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 cyclosignase or lipooxygenase and inhibit leukocyte accumulation so that they can be anti-inflammatory.[9] From the description above, this research was carried out to determine the anti-inflammatory activity of the Kelubut leaves (Passiflora foetida, L.) against carrageenin-induced mice (Mus Musculus).

Method

Materials and tools

Aquadest, 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.

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Research sites

This research was conducted at the Laboratory of the Faculty of Pharmacy, Muhammadiyah University, East Kalimantan.

Research procedure

a. Plant Determination

Determination of kelubut leaves was carried out at the Laboratory of the Faculty of Forestry, University of 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 results of the determination showed that the kelubut leaf samples in this study were declared correct.

b. Material Preparation

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

c. 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 to treatment (not eating but still being given water) to equalize the condition of the mice and prevent the effects of the food consumed. [11]

d. 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

e. Extract Yield Calculation

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

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$$\%\ Yield = \frac{Extract\ weight\ obtained\ (g)}{Dry\ powder\ weight\ before\ extraction\ (g)} x100\%$$

f. 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 stirred until it becomes a homogeneous mass and diluted in a volumetric flask with distilled water to a volume of 100 ml.

g. Preparation of Carrageenin 1%

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

h. Preparation of Diclofenac Na Suspension

Diclofenac sodium 50 mg suspended with CMC Na 0.5%. CMC is 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.

i. Calculation of Dose for Test Animals

Diclofenac Sodium Dose

Dose for humans = 50 mg (positive control)

The conversion value of 20-grams 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)

j. Anti-inflammatory Test

The test animals that had been prepared were 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 measure the volume of each hind leg with a plethysmometer. [14] The measurement results are recorded as the initial

volume. Mice were divided into several groups and given the following treatments:

1. Group negative control consisted of 3 mice treated with CMC Na 0.5%
2. Group positive control consisted of 3 mice treated with Diclofenac Na.
3. Group 1 consisted of 3 mice treated with kelubut leaf extract at a dose of 250 mg/KgBW
4. Group 2 consisted of 3 mice treated with kelubut leaf extract at a dose of 125 mg/KgBW
5. 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 which indicated that the data was homogeneous and normally distributed. [15]

Results and Discussion

Testing the anti-inflammatory activity of kelubut leaves extract is a test 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 with the aim that kelubut leaves 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 with the preparation of 1500 grams of simplicia powder, then macerated with ethyl acetate for 5 days and re-macerated 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 ± 40 ^{o} C until a thick extract was obtained, then 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]

It can be seen in Table 1. Below is 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 carried out 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 used the method of forming artificial edema on the soles of mice induced by carrageenin. The use of 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 carrying out the treatment, each mouse fasted for 8 hours to avoid the influence of the food content on the treatment given. Then the body weight was weighed, to determine the appropriate drug administration, then 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 develops rapidly and persists

for 6 hours. After being induced by carrageenin, wait for 30 minutes. This is because after giving carrageenin there is 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, given extract doses of 1, 2 and 3 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 Udema Volume Measurement Results

Group	t0	t1	t30	t60	t90	t120
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

It can be seen in Figure 1. Below, the research results obtained show that the administration of CMC-Na colloidal solution did not affect the decrease in the volume of inflammation on the soles of mice. In the CMC-Na group, the volume of inflammation produced increased and persisted for up to 120 minutes. This was because CMC-Na was only a solvent for drug media so there was no stimulation in the form of drugs to reduce edema so that edema would increase and the process of removing mediators inflammation in the body of mice only occurs naturally.

In the positive control group, there was 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.

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For treatment with ethyl acetate extract of kelubut leaves dose 1 which is 250 mg/KgBB, dose 2 which is 125 mg/KgBB, and dose 3 which is 62.5 mg/KgBB, experienced various processes of reducing swelling. The test group with ethyl acetate extract from kelubut leaves showed anti-inflammatory activity resulting from all doses.

This is due to the presence of secondary metabolites in kelubut leaves. Based on the results of a phytochemical screening conducted by Raden Roro and Antoni (2022) it is known that kelubut leaves and stem extracts contain secondary metabolites from the 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]

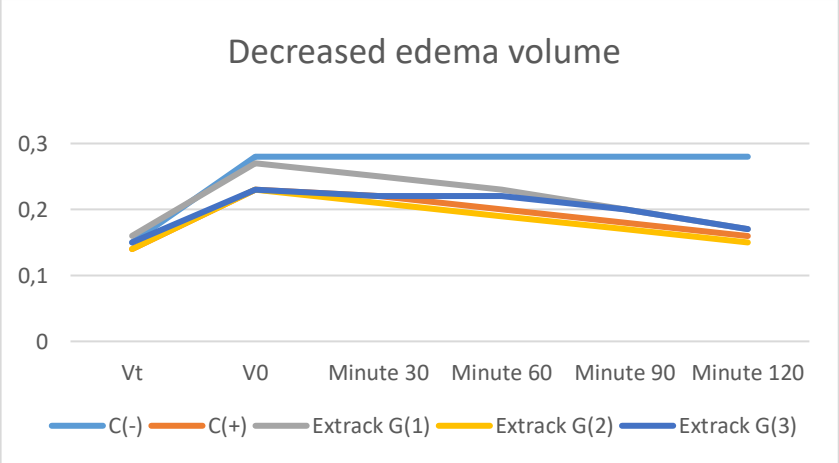

Figure 1. Graph of Decreased Udem Volume at 4 Time Intervals

To find out whether there were significant differences in the test animals between treatment groups, a statistical analysis of the One-way ANOVA test was carried out. The results of the One-way Anova test showed that there was a significant difference in the percentage change in edema at each observation time. This corresponds to a significance value of 0.001 <0.05. Based on the results of the ANOVA test, it is continued with the LSD (Least Significance Different) test to

find out which treatment is significantly different if the null hypothesis is rejected. (26)

Conclusion

Based on the results of the research that has been done, it can be concluded that the ethyl acetate extract of kelubut leaves (Passiflora foetida L.) with dose groups 1, 2, and 3 has anti-inflammatory activity in reducing the volume of edema on the legs of mice.

Thank-you note

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Conflict of Interest

The authors declare that there is no potential conflict of interest with the research, authorship, or publication of the article.

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LAMPIRAN

SURAT KETERANGAN ARTIKEL PUBLIKASI

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

Assalamu'alaikum Warahmatullahi wabarakatuh

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Menyatakan bahwa artikel yang berjudul "In-Vivo Anti-Inflammatory Activity Of Kelubut Leaf Ethyl Acetate Extract (*Passiflora foetida* L.) From Samarinda City" telah di submit pada jurnal Fundamental and Applied Pharmaceutical Science pada tahun 2023.

Demikian surat keterangan ini dibuat untuk dapat dipergunakan sebagai mana mestinya.

Wassalamu'alaikum Warahmatullahi wabarakatuh

Samarinda, Senin 21 Agustus 2023

Mahasiswa/i	Dosen Pembimbing Skripsi
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Letter of Acceptance

To Whom It May Concern,

We are pleased to inform that the manuscript, entitled:

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

Author [S]: Chaerul Fadly Mochtar, Reni Selviana Devi, Hasyrul Hamzah, Ayu Faradillah, Elva Hafidzah, Fathiah Putri Varizza, Novia Misnawati Aisyiyah, Qur'anni Akhwatun Husna

has been reviewed with some revision by authors and will be published in Journal of Fundamental and Applied Pharmaceutical Science, Vol 3, No 1 (2023): August.

Thank you.

Best regards,

Sabtanti Harimurti, S.SI.,M.Sc.,Apt.,Ph.D