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In Vivo Antihyperuricemia Activity of Kelubut Leaf Ethyl Acetate Extract (Passiflora foetida L.) From Samarinda City

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Abstract

The development of the times creates shifts in lifestyle and eating patterns that trigger hyperuricemia. Hyperuricemia is generally treated with synthetic drugs, but on the other hand, it causes side effects. So using natural ingredients such as kelubut leaves can be an alternative treatment. This study aimed to determine whether the ethyl acetate extract of kelubut leaves has activity as an anti-hyperuricemia. The research uses experimental research methods with a pretest and posttest design. Mice were used and divided into five groups, namely the positive control group, the negative control and the group given the extract with three different doses, including doses of 250 mg/Kg BW, 125 mg/Kg BW, and 62.5 mg/Kg BW. Mice will be conditioned by hyperuricemia and given different treatments in each group. Kruskal-Wallis and Mann-Whitney analyzed data on uric acid levels. The study's results showed that administration of the extract at a concentration of 250 mg/Kg BW reduced uric acid levels as much as the positive control group at 120 minutes but was not statistically different ($p>0.05$) from the positive control. This study concludes that the ethyl acetate extract of kelubut leaves has anti-hyperuricemic activity at a dose of 250 mg/Kg BW, showing the best-reducing activity in reducing uric acid levels in mice.

Keywords: Uric acid; Anti-hyperuricemic; Kelubut leaves

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INTRODUCTION

Along with the development of the times and the entry of globalization, it has created shifts in lifestyle and eating patterns that trigger various health problems, including hyperuricemia.[1] The incidence of hyperuricemia in Indonesia is estimated to be the second largest joint disease after osteoarthritis.[2] Based on the 2018 Riskesdas, reported from 34 provinces in Indonesia, East Kalimantan ranks 10th with the highest incidence of joint disease, with a prevalence of 8.12%.[3]

Limited excretion of uric acid but a high synthesis of the acid is linked to the increased prevalence of hyperuricemia.[4] It is possible to lower uric acid levels by taking synthetic drugs. Long-term usage of synthetic medications can lead to new health issues even when they are helpful. Encourages people to treat diseases with natural ingredients. Plants are safer than

other therapeutic methods because they minimize side effects.[2]

One of the plants utilized as medicine is kelubut, a wild plant that grows on vines.[5] Scientifically known as *Passiflora foetida* L., these plants are utilized as larvicidal, antimicrobials, cancer treatments, diabetes, stress, blood pressure, anaemia, and renal problems.[6] In addition, according to an ethnopharmacological study conducted on a community in Tanta Sub-District, South Kalimantan, by Yani Mulyani et al. (2019) reported that all parts of *Passiflora foetida* L. help reduce uric acid.[7]

Pharmacological activity in plants is thought to be due to metabolite compounds, one of which is flavonoids. Based on earlier studies, flavonoid metabolites found in the ethyl acetate extract of kelubut leaves[8] have the potential to be an anti-hyperuricemia agent by blocking the activity of the enzyme xanthine oxidase, which is involved in the formation of uric acid.[9] Thus, this study aims to determine whether the ethyl acetate extract of kelubut leaves has activity as an anti-hyperuricemia.

Method

Materials and Tools

The tools and material used in this study included easy touch test kits and uric acid strips, surgical scissors, oral sonde, injection syringe 1 mL (OneMed), kelubut leaves, ethyl acetate solvent (Smart-Lab), distilled water (Onelab waterone), potassium oxonate obtained from Mulawarman University pharmacy lab, Na CMC (Smart-Lab), allopurinol 100 mg (Bernofarm), dried melinjo (*Gnetum gnemon* L.) seeds, tissue, alcohol swabs (OneMed), and male white mice which had been declared ethically worthy by the Health Research Ethics Commission State Islamic University of Maulana Malik Ibrahim Malang with ethical clearance No. 04/EC/KEPK-FKIK/40/2023.

Extraction

The kelubut plants obtained from Samarinda City were collected and determined at the Laboratory of the Faculty of Forestry, Mulawarman University. The part used in this study is the leaves. Wet kelubut leaves are sorted wet, washed with running water, then dried, sorted, dry blended, and sieved to obtain Simplicia in powder form.[10] As much as 500 mg of simplicia powder was put into a glass jar, moistened with ethyl acetate solvent in the ratio (1:3) and then stirred until homogeneous. Soaking was carried out for five days, repeated maceration twice, and stirred every 48 hours. The resulting macerate solution is filtered and concentrated using a rotary evaporator at 70-110 rpm with a temperature of 40 – 45°C until a thick extract is obtained.[11]

Preparation of Solution

Preparation of 1% Na CMC Solution was done by a total of 1 gram of Na CMC is sprinkled on the surface of 20 ml of hot water until it swells. Then, it is stirred until a thick mass is formed, and water is added to make up a volume of 100 ml.[12] Meanwhile the standar allopurinol solution was prepared Referring to Masruroh's research (2016). Allopurinol was used at a dose of 10 mg/Kg BW, which was made by suspending 24 mg of allopurinol powder into 10 mL of 1% Na CMC.[12]

Preparation of hyperuricemia induction

Melinjo (*Gnetum gnemon* L.) and potassium oxonate are used to treat hyperuricemia. Melinjo (*Gnetum gnemon* L.) suspension was prepared by dissolving 1 gram of melinjo (*Gnetum gnemon* L.) seed powder in 3 mL of CMC Na 1% , which was then given to mice orally by administering 0.5 mL per mice and for potassium oxonate used a dose of 250 mg/kg BW weighing 500 mg and put into a 25 mL volumetric flask, then added 0.9% NaCl solution to the limit

mark. The volume of potassium oxonate solution administered to the experimental animals was 0.25 mL/20g BW intraperitoneally.[13,14]

Antihyperuricemic Activity Test

Mice are first adapted for 7 to 14 days in the laboratory by being given standard food and drink. Then, the mice were weighed and marked, and the initial uric acid level was measured as the initial level.[15] Then, the mice were conditioned to become hyperuricemia by giving feed mixed with melinjo (*Gnetum gnemon* L.) seed powder 40 mg/20 g BW for five days [16] before the Induction of potassium oxonate and melinjo (*Gnetum gnemon* L.) suspension. Then, potassium oxonate solution was inducted intraperitoneally and 1 hour after being given melinjo (*Gnetum gnemon* L.) suspension orally. Then, the uric acid levels of the mice were measured after 2 hours of being induced by potassium oxonate. After, the mice experienced hyperuricemia, characterized by uric acid levels reaching >3 mg/dL.[15] Then, each group was given the following treatment.

1. The positive control group of 3 mice was given allopurinol suspension orally;
2. The negative control group of 3 mice was given 1% Na CMC suspension;
3. Group A, as many as three mice were given ethyl acetate extract at a dose of 250 mg/kg BW;
4. Group B, as many as three mice were given ethyl acetate extract at a dose of 125 mg/kg BW;
5. Group C, as many as three mice were given ethyl acetate extract at a dose of 62.5 mg/kg BW.

Measurement of uric acid levels using easy touch GCU by cutting the tip of the end tail approximately 0.2 cm so that the blood comes out, then dripped on the tip of the strip that has been attached to the reader monitor and will be displayed after 20 seconds.[17] In this research, uric acid in mice was checked five times: pre-induction, post-induction, and immediately after treatment at three different times with a range of 60 minutes, 90 minutes and 120 minutes after treatment. In addition, data on uric acid levels is calculated using the reduction percentage in the following formula.[18]

$$\frac{Average\ levels\ after\ (Induction - treatment)}{Average\ levels\ after\ Induction} x100\%$$

Data on uric acid examination results were analyzed statistically using the Kruskal-Wallis test, followed by the Mann-Whitney test on SPSS.

Results and Discussion

The kelubut plant used in this study was obtained from Samarinda City. The determination was determined at the Laboratory of Ecology and Biodiversity Conversion of Tropical Forests, Mulawarman University. The determination results show that the kelubut plant samples are species *Passiflora foetida* L. The part of the plant used for the sample in the research is the leaves. Fresh leaf samples are then processed into simplicia powder to make dissolving the compounds in simplicia easier.[19] The powder used was 500 mg, then soaked in ethyl acetate solvent with a ratio of 1:3 and macerated twice. Ethyl acetate was chosen as the maceration solvent because it is not hygroscopic, has low toxicity, and is semi-polar, attracting both polar and nonpolar molecules.[20]

Maceration was carried out for five days in this study. Because the material used is leaves, which have a soft consistency and thin cell walls, the maceration process was chosen to extract the chemical components without heating.[21] In addition, the maceration method, in general, can extract most of the metabolites because it does not use heat in the process, so it does not damage the active metabolites contained in kelubut leaves. According to

Voight in the research of Pangestu et al. (2019), maceration is generally carried out within five days, after which equilibrium is reached between the material extracted on the inside and the material extracted on the outside of the cell.[22] Furthermore, re-maceration is needed to increase the removal of compounds not previously attracted by maceration.[23] Furthermore, the results of the macerate are collected and concentrated with a vacuum rotary evaporator and in a water bath to obtain a thick extract.

The viscous extract obtained in this study was 96.45 grams, and the extract yield was 6.43%. Yield is a parameter of extract quality calculated by comparing the viscous extract obtained with the simplicial used.[24] In this research, the yield does not meet the <10% requirement; this can be due to various factors, one of which is the polarity of the solvent used. The polarity of the solvent has a strong influence on the extract yield. The stronger the polarity of the solvent, the better the extraction power and, therefore, the higher the yield.[25]

Male mice were used as a test animal model to test the anti-hyperuricemic activity of kelubut leaves. Male mice were chosen because they were considered more stable in providing research data, able to metabolize drugs more quickly, and their body condition was biologically more stable compared to female mice, which had hormonal cycles caused by the production of the hormones estrogen and progesterone during the ovulation process. These hormones are released to help remove waste products from the body and produce products without physiological function.[26–28]

Mice were adapted for a week before testing, then fasted for 18 hours with only drinking water to ensure that the digestive tract was empty so that drug absorption was not disturbed and uric acid levels did not change.[29] The uric acid levels of the mice were then checked using a uric acid test strip. The strip test cannot identify uric acid concentrations below 3 mg/dL, making it a weak tool for assessing uric acid.[30] In this study, the uric acid value below 3 mg/dL is indicated by the number 2.94 mg/dL to facilitate statistical analysis.

The initial measurement results presented in Table 1 show that in each group, uric acid levels were <3 mg/dL after fasting. In other words, the mice's uric acid levels were normal after fasting, and the mice used in this study did not experience hyperuricemia before the test. These

Table 1. Average of The Measured Level of Uric Acid (mg/dL)

Group	to	t1	t60	t90	t120
Negative Control	2.94±0.00	5.97±0.55	5.53±0.55	5.23±0.51	4.87±0.47
Positive Control	2.94±0.00	4.43±0.20	4.00±0.43	3.13±0.15	2.96±0.03
Group A	2.94±0.00	4.37±0.51	3.25±0.33	2.94±0.00	2.94±0.00
Group B	2.94±0.00	4.2±0.55	3.50±0.50	3.18±0.36	2.94±0.00
Group C	2.94±0.00	4.47±0.51	3.80±0.50	3.45±0.53	3.09±0.26

Information:
to : Initial uric acid level
t1 : Uric acid levels after Induction
t60 : Uric acid level at 60 minutes after being treated
t90 : Uric acid level at 90 minutes after being treated
t120 : Uric acid level at 120 minutes after being treated

results are the same as the results of research on anti-hyperuricemia activity by Fadila and Susanti (2020), using nettle extract on mice where uric acid levels in each group were reported at hour zero to be below 3 mg/dL.[30]

causing it to be unable to be excreted through the urine. The time needed for potassium oxonate to reach peak uric acid levels in the blood ranges from 1.5 to 2 hours.[31] Based on the results of examining uric acid levels in Table 1, all test groups

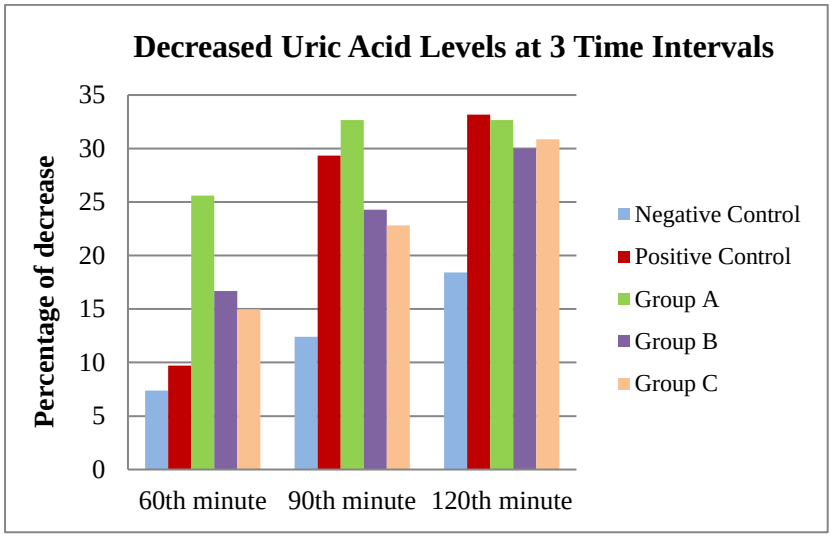

Figure 1. Diagram of Decreased Uric Acid Levels at Three-Time Intervals

The mice were then conditioned to hyperuricemia by being induced by a high purine diet and a uricase enzyme inhibitor. Melinjo (*Gnetum gnemon* L.) seeds have a high purine content, ranging from 50 to 150 mg/100 grams. According to previous studies, mice given melinjo (*Gnetum gnemon* L.) seeds and tested with a spectrophotometer increased blood uric acid levels in male mice up to 81.2% on the 10th day compared to the standard control group.[13]

This research uses potassium oxonate as an inducer by administering an intraperitoneal injection. Various studies have reported potassium oxonate as a component that can increase animal uric acid levels. In its mechanism, potassium oxonate will inhibit the uricase enzyme so that the conversion of uric acid to allantoin does not occur. Allantoin is a water-soluble molecule that aids in the elimination of uric acid in rodent urine. This inhibition impacts uric acid, which is retained and then accumulates, experienced hyperuricemia after being given potassium oxonate and melinjo (*Gnetum gnemon* L.) inducers with uric acid levels > 3 mg/dL.[15]

When the uric acid levels of the mice increased, each group received a different treatment: the positive control group was given Allopurinol, the negative control was given 1% Na CMC, and the other three groups received the extract at a dose of 250 mg/KgBW, 125 mg/KgBW, and 62.5 mg/KgBW. The uric acid levels of the mice were then measured at three different time intervals. Based on Figure 1, the negative control group experienced a lower decline than the other treatment groups; this is because the negative control group was not given drugs or extracts, only CMC Na, which did not have an anti-hyperuricemia effect.[32]

Unlike the case with the group given extracts and drugs, there was a significant decrease. Allopurinol was used as a positive control because it is widely used in the

community. Allopurinol is a selective inhibitor and substrate for the enzyme xanthine oxidase, which can permanently inhibit its activity. This drug also functions as an analogue substrate (purine), which occupies the active site of the xanthine oxidase enzyme, thereby preventing the formation of uric acid.[33] Based on Figure 1, Allopurinol decreased at 90 minutes, showing a significant decrease when compared to 60 minutes and 120 minutes; this is due to the peak effect of Allopurinol at 1.5 hours, which means the drug has experienced a maximum decreasing effect at 90 minutes. This result is the same as the research by Kusuma et al. (2019), in which the maximum working effect of Allopurinol is 1.5 hours.[34] Meanwhile, according to Jumain et al. (2018), Allopurinol has a half-life of 120 minutes, meaning that the drug has lost half of its initial level, and its effect begins to decrease.[35]

In the resulting study, the group that was given ethyl acetate extract of kelubut leaves within a period of 60 minutes to 120 minutes showed an effect of reducing uric acid levels, or in other words, ethyl acetate extract of kelubut leaves had an anti-hyperuricemic effect. In this case, the group given the extract at a dose of 250 mg/KgBW showed the most effective dose in reducing uric acid levels in mice when compared to the group given ethyl acetate extract of kelubut leaves at a dose of 125 mg/KgBW and a dose of 62.5 mg/Kg BW. This result is the same as the study of Jumain et al. (2018), who reported that the anti-hyperuricemic effect of the ethanol extract of African leaves resulted in decreased uric acid levels in mice in line with increasing doses of the extract given. [35]

However, the mechanism of the ethyl acetate extract of kelubut leaves in reducing uric acid levels is unknown. Based on previous studies, the ethyl acetate extract of kelubut leaves contains flavonoids that can potentially promote hyperuricemia. Flavonoid compounds work by blocking xanthine oxidase, thereby reducing uric acid levels.[9] Nindiasari's research (2015), where the ethyl acetate extract of kelubut leaves was separated using column chromatography, then the peak wavelength was determined using a TLC scanner, indicated that flavonoid of the flavon type had peak wavelengths at 281 nm and 311 nm.[36] According to Fadillah et al. (2017), the flavonoid content of kelubut leaves was highest in the ethyl acetate fraction (5.44 mg QE/g) when compared to the butanol fraction, n-hexane fraction and methanol extract.[37] Flavonoids, besides being anti-hyperuricemia, also function as anti-inflammatories by blocking cyclooxygenase and lipoxygenase enzymes, relieving pain associated with hyperuricemia.[38]

Based on the elimination half-life of Allopurinol, the data at 120 minutes was used for statistical analysis. Statistical results show that the data is not homogeneous or normally distributed, so the test used Kruskal-Wallis and Mann-Whitney. The Kruskal-Wallis test evaluated the significant differences in uric acid levels in all groups at 120 minutes. The test results $0.034<0.05$ showed that all treatment groups had significantly different uric acid levels at 120 minutes.

Mann-Whitney carries out a test to see which groups are different. The results of the Mann-Whitney test showed a significant difference in average uric acid levels between the positive control group and the negative control group and between the negative control group and the group given the extract. However, the positive control group in this study was statistically not significantly different from the treatment group given kelubut leaf ethyl acetate extract. This result is the same as the study of Wijaya et al. (2015),

who conducted a soursop leaf test, which significantly lowered uric acid levels compared to the negative control. However, the lowering effect was not significantly different from the positive control.[39]

Based on the research, administration of ethyl acetate extract of kelubut leaves at a dose of 250 mg/Kg BW can reduce uric acid levels as much as the positive control group at 120 minutes compared to group giving of ethyl acetate extract of kelubut leaves at a dose of 125 mg/mL and 62.5 mg/mL. Still, statistically, these results were not significantly different from the positive control.

Conclusion

Based on the results, the three test dosages of ethyl acetate extract from kelubut (Passiflora foetida L.) leaves in this study had an anti-hyperuricemia effect, with the 250 mg/kg BW dose showing the most efficient reduction in uric acid levels in mice.

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Conflict of interest

According to all authors, there are no potential conflicts of interest with the article's research, authorship, or publication.

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