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Effectiveness of Anting-Anting Leaves (Acalypha indica L.) in Reducing Uric Acid Levels in Hyperuricemic Male White Rats (Rattus novergicus)

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ABSTRACT

Hyperuricemia is an elevated level of uric acid in the blood that induces uric acid needle shaped crystals saturation resulting in joint stiffness. Anting-anting leaf contains quercetin and kaempferol which are capable of decreasing uric acid in the body. The purpose of this study was to determine the effect of ethanol extract of anting-anting leaves (*Acalypha indica* L.) in decreasing uric acid levels in male rats Sprague Dawley induced by caffeine. This is an experimental research using 25 male rats, which were divided into 5 groups: negative, positive group, and three groups given the extract dose of 3.15 g/BW, 6.3 g/BW and 12.6 g/BW. Data were obtained from the examination of normal levels of uric acid, the level of uric after induction and the level after the treatment. Data were analyzed using one-way ANOVA. The test results showed that ethanol extracts of anting-anting leaf dose of 12.6 g/BW could significantly decrease uric acid levels in male rats' blood that was induced by caffeine dose of 5.29 mg/200gBW and was equivalent to the level of positive control (allopurinol 1.8 mg/200gBW). It is concluded that ethanol 96% extract of anting-anting leaf (Acalypha indica L.) has an effective effect in decreasing uric acid.

***Keywords**: Allopurinol, Gout, and Anting-Anting Leaves*

1. INTRODUCTION

Based on *Word Health Organization* data, Gout prevalence is 2 – 5 % of the total world population, especially among 40-50 aged men, and menopause women. This prevalence in Indonesian rural area reaches 1.7% and 4.8% in urban areas.

Uric acid is a waste product created from Adenin and guanin catabolism, which is produced by purin nucleotide breakdown [5]. High level of uric acid in the blood (hyperuricemia) is defined as an elevated uric acid > 7 mg/dl for male and > 6 mg/dl for female [6]. High level of uric acid is also known as pirai or gout, which is defined as acute inflammation with pain in articulation point of the body caused by crystal monosodium uric accumulation in joint and soft tissue [4].

Allopurinol is a medication used to decrease high blooduric acid levels, which is included as uricostatic group and a xantin oxidase inhibitor. It was proven to be effective in patients with hyperurisemic and will be transformed by liver into an active metabolite, oxypurinol. However allopurinol may have unwanted side effects, such as skin allergy, fever etc. [2]

Anting – anting leaf (*Acalypha indica* L.) is a plant with an efficacy to lower blood uric acid. This plant is classified as a shrub and is commonplace on roadsides, fields, and mountain slopes. It contains quercetin and kaempferol compound which are derivatives of flavonoid that functions as xantin oxidase inhibitor [1].

2. RESEARCH METHOD

2.1. Instruments

Digital measurement, rotary evaporator (Eyela), waterbath, blender, and uric acid test were used as the research instruments to measure the levels of the required compound.

2.2. *Materials*

This research also used the following materials: allopurinol (Nova), Na CMC 0,5% (Bratachem), Cafein (Bratachem), Ethanol 96%, Aquadest, anting-anting leaves obtained from KH. Hasyim Asyari farm (only the leaves were used), and 3-4 months aged male white Sprague dawley rats, weighing between 150-200 grams.

2.3 Method

2.3.1. Plant determination

Anting-anting leaves were obtained from KH. Hasyim Asyari farm and were examined in "*Herbarium Bogoriense*", Botanical Division of the Biological Research Center- LIPI Bogor with the aim of detecting the plant characteristics.

2.3.2. Materials Preparation

The collected Anting-anting leaves were sorted to to separate the dirt or foreign material left on the leaves. Then, Anting-anting leaves were rinsed under running water and then thinly chopped to speed up the drying process. The drying stage was done without a direct sunlight exposure by only utilizing the air. Afterwards, dry sorting was carried out to separate foreign materials or the remaining dirt. The

anting-anting leaves were then made into powdered form using blender and then stored in a clean container.

2.3.3. 96% Ethanol Extraction Process of Anting – anting leaf

The extract was made by maceration method, by soaking the simplicia in 96% ethanol for 5 x 24 hours with a 1 : 4 ratio of anting-anting leaf, then it was stirred every 8 hour. All the filtrated material obtained from maceration was evaporated using a rotary evaporator with 61^0C temperature until the volume decreased, then it was thickened on a waterbath with a temperature of 70^0C until a thick extract was formed.

2.4 Anting-anting leaf Extract Testing

2.4.1. Organoleptic Test

Organoleptic test was used to check the shape, color, smell and taste of Ethanol extract of Anting-anting leaf.

2.4.1. Water content test

To test the water content, an empty bowl was entered into the oven for at least 2 hours. Then, it was transfered it to a desiccator for 30 minutes to room temperature. Its empty weight was measured, and the powdered sample was also measured for 2-3 gram into cup measurement. This sample was put into the oven with temperature 95ºC-100ºC and the maximum 105 mmHg air pressure for 5 hours. Then, it was moved into the cup measurement using tongs into the desiccator for about 30 minutes. Afterwards, it was weighed again for at least two times.

2.4.3. Ash Content Analysis

To analyze the ash content, an empty porcelain crucible was placed in the temperature of 550ºC for 1 night. Afterwards, the temperature was set into 40ºC. It was taken out from the crucible and put on a desiccator for 30 minutes. It was measured then 2 grams of 86% ethanol extract from Anting-anting leaf was put into the porcelain crucible. It was mix until homogeneous, and was put into the oven with temperature of 100 ºC for 24 hours. The next step was moving it into the ash furnace until the temperature reached 550ºC for 8 hours, then the temperature was set into 40ºC.The crucible was taken with a clamp and was put it into desiccator for 30 minutes. Then its weight was measured as soon as it cooled down.

2.4.4. Solvent Residue Test

The ethanol extract of Anting-anting leaf was measured for 5 gram, then it was dissolved in 10 ml of aquadest., After that, it was vortexed with 3000 rpm speed for 10 minutes and centrifuged for 10 minutes with 13.000 rpm speed. Then its solvent residue was determined by HPLC (*High Performance Liquid Chromatograhy*).

2.4.5. Phytochemicals Screening Test

2.4.5.1. Flavonoid Test

0,5 gram of Anting-anting leaf extract was added into 10 ml of Ethanol which had been heated for 10 minutes. Next, it was filtered into a separating funnel while it was hot, added with 5 ml of petroleum gasolinde. The bottom layer was input to the vaporizer, and heated at temperature of 40ºC. Viscous solution then was added with ethyl acetate, and its filtrate was divided into 2. The first tube was added with Zn and 1 ml HCl 2N and concentrated HCl, then red color was formed. The second tube was added with Mg powder and concentrated HCl, and red color was formed, which indicated flavonoid.

2.4.5.2. Phenolic Test

0.5 gram of Anting-anting leaf extract was added with aquadest until it submerged, then heated. Filtrate was obtained then it was added with $FeCl_3$. The color would change into black, which indicated phenolic.

2.4.5.3. Tannin Test

Anting-anting leaf extract was weighed for as much as 1 gram and added with 10 ml of hot water that had been left for 1 hour, then it was filtered. The obtained filtrate was dripped with 1 % $FeCl_3$. If a dark blue or greenish black color was formed, it would positively contain tannins.

2.4.5.4. Saponin test

0.5 gram of Anting – anting leaf was added with 10 ml of hot water. It was waited until it cooled down. After that, it was mixed until the froth produced, then it was waited for 2 minutes and added with 1 drop of HCl 2 N. It was mixed again until froth produced and remained until 10 minutes.

2.4.5.5. Alkaloids test

0.5 gram of ethanol extract from Anting-anting leaf was added with 1 ml HCl 2N and 9 ml of hot aquadest. Then, it was cooled down for 2 minutes, and filtered. The obtained filtrate was then divided into 2 tubes. The first tube was added with Bauchardat reactant. and would form black-brownish sediment. The second tube was added with Dragendroff reactant that would form white sediment.

2.4.5.6. Triterpenoid and Steroid test

0.5 gram of ethanol extract of Anting-anting leaf was added with 2 ml of ethanol. It was waited it for a while before filtering. The obtained filtrate was evaporated until it turned viscous. After that, it was added with eter, 3 drops of acetate acid anhydrate, and 1 drop of concentrated H_2SO_4.

2.4.5.7. Glycosides test

3 gram of ethanol extract of Anting-anting , mleaf was put into 50 ml of colf flask, then added with 30 ml (mixture of 21 ml of ethanol and 9 ml of water). After boiling, it was waited for 10 minutes and the filtrate was strained. Then, it was added with 25 ml of 5% Pb acetate, and waited for 5 minutes. It was then filtered into a separating funnel. After that, it was added with chloroform and isopropanol of 3x3 ml. The bottom layer was evaporated at temperature of 40 ºC. After thickening, it was added with 2 ml of methanol and poured into test tube. The filtrate was evaporated until it dried and added with 2 ml of water, 5 drops of molish, and 2 ml of H_2SO_4. It would form a purple ring which indicated glycosides positive.

2.4.6. Dose Determination

The dose of Ethanol extract from Anting-anting leaf was 3.15 g/kgBW; 6.3 g/kgBW; and 12.6 g/kgBW. Allopurinol dose was determined at 3.6 mg/200 gBW. Cafein dose was determined at 5.29 mg/200 gBW.

2.5. Experimental Animal Test

The research used 25 male white *Sprague dawley* rats, which were divided into 5 following groups. Group 1 (negative control) was given Na CMC 0,5% suspension. Group 2 (positive control) was administered with oral allopurinol with the dose of 3.6 mg/200 gBW. Group 3 was provided with the extract of Anting – anting leaves with the dose of 3.15 g/kgBW. Group 4 was given extract of Anting – anting leaves with the dose of 6.3 g/kgBW. Group 5 was given extract of Anting – anting leaves at the dose of 12.6 g/kgBW.

Before receiving the treatment, the rats were fasted first. After that, 0.5% Na CMC was given to the negative control group and this group was given caffeine induction with the dose of 5.29 mg/200g BB rats for 6 days and the initial uric acid levels were measured. Furthermore, it was provided with 0.5% Na CMC allopurinol dose of 3.6mg/ 200 gBW and ethanol extract of the Anting-anting leaf for 9 days and uric acid levels were measured on day 16, 19 and 22.

2.6. Data analysis

The data obtained would be processed statistically using one way SPSS to see whether there was a significant difference. If it were significant , it would be continued with the LSD (*Least Significant Difference*).

3. RESULT AND DISCUSSION

3.1. Plant Determination

The result of plant determination that had been carried out at the Herbarium Bogoriense, Botanical Division of the Center for Biology Research and Development LIPI Bogor, showed that the plant used was the type of *Acalypha indica* L. with the *Euphorbiaceae tribe.*

3.2. Extract Results and Characteristics

Table 1. Extract Results and Characteristics

No.	Parameter	Resut
1	Shape	Sticky exctract
2	Color	Blackish dark green
3	Odor	Typical
4	Taste	Bitter
5	Yield	14,74%

3.3. Results of Extract Quality Parameters

Table 2. Results of Extract Quality Parameters

No.	Parameters	Results
1	Water content	13.90%
2	Ash content	13.90%
3	Remaining solvent	1.28%

3.4. Phytochemicals Screening Test

Table 3. Phytocemicals Screening Test Results of 96% Ethanol Extract from Anting-Anting leaf

No.	Secondary Metabolite	Results
1	Alkaloids	+
2	Saponin	+
3	Tannin	+
4	Phenolic	+
5	Flavonoid	+
6	Triterpenoid	+
7	Steroid	+
8	Glycoside	+

Description: (+) : present
(-) : none

Based on phytochemical screening test results, Table 3, showed that 96% Ethanol extracts of Anting - anting leaf positively contained Alkaloids, saponin, tannin, phenolic, flavonoid, triterpenoid, steroid and glycoside.

3.5. Uric Acid Level Test Results

Uric acid level test results from all of the group could be seen in **Table 4.**

Table 4. The average of uric acid level

Group	Treatment	Day- (mg/dl)				
		0	7	16	19	22
I	Control -	2.26	2.16	2.22	2.16	1.98
II	Control +	2.34	4.52	2.34	2.12	1.54
III	Dose I	2.4	4.02	2.96	2.42	1.92
IV	Dose II	2.54	4.02	3.02	2.58	2.0
V	Dose III	2.6	4.04	2.54	2.22	1.66

Table 4 presented that the average of uric acid level on the 7th day was the highest in the positive control group and

the lowest in the negative control group because it was not given induction. In contrast, after provided with the extract on days 16, 19 and 22, the highest average was in the 2nd dose group on day 16, and the lowest one was the positive control group on day 22.

Before administered with caffeine induction on day 0, the uric acid levels were measured to determine the normal levels of uric acid in the animal. Caffeine induction was given for 6 days in all groups, except for the negative group and its uric acid levels were continuously measured on the 7th day to ensure that the experimental animals had increased uric acid level. The results obtained showed that the positive group, dose I, dose II, dose III showed an increased uric acid levels. This increase was attributed to the fact that caffeine increases the purine in the blood for experimental animals.

The experimental animals were given a solution of Na CMC, allopurinol solution, and a solution of 96% ethanol extract from Anting-anting leaf with the dose of 3.15 g/kgBB rats, 6.3 g/kgBB rats for 9 days. On the day 16,19 and 22 after the treatment, uric acid levels were measured and it showed a decrease. Meanwhile, the group that was provided with 0,5% Na CMC suspension did not show a decreasing effect of uric acid levels due to the absence of any nutritious susbstances. The third dose on day 22 could reduce uric acid levels because it was close to the average result of the positive group and it could be seen as a whole., Thus, the dose of group III was able to reduce uric acid levels more optimally than to the dose of group I and group II.

This process was followed by data processing using SPSS with one way ANOVA analysis to determine whether there were significant differences or not between groups. The results of the normality test with one simpe Kolmogorov Smirnov Test showed that the data was normally distributed with a significant value of 0.355>0.05. Furthermore, the homogeneous test results showed that the data varied homogenously with the sig value 0.149 > 0.05. ANOVA test result showed a sig. The value of 0.333 < 0.05 indicated that there was a significant difference in each group. The data were continued with post hoc, namely with the positive group, with the dose of group III, and it was revealed that there was no significant difference with the value of p = 0.042 and p= 0.016, P < 0.05. This results can be seen in table 4, indicating the average decrease in uric acid levels in dose III which was greater than that of dose I and dose II.

There was no significant dfference between dose I group, with dose II, and dose III with P=0.653 and P=0.434 >0.05. This average decrease in uric acid levels showed an insignificant difference. The phytochemical components of Anting-anting leaf contained quercetin and kaempferol. This compound works by inhibiting the xantine oxidase enzyme which changes hypoxantine and xanthine and inhibits uric acid[3].

Based on the abovementioned description, it is conclusive that phytochemical content of the ethanol extract of Anting-anting leaf has may inhibit the work of enzyme of xanthine oxidase that reduces uric acid levels in male white rats, the same as the results showed by the positive group given allopurinol.

4. CONCLUSION

Based on the research, it is possible to conclude that the 96% Ethanol Extract of Anting-anting leaf, contains secondary metabolite, such as Alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroid and glycosides, which could reduce uric acid levels optimally at the third dose for 12. 6 g/kgBW.

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