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Abstract

Hyperuricemia is an abnormally high level of uric acid. Binahong leaves (*Anredera cordifolia* (Ten.) Steenis) is one of the plants traditionally used as an antihyperuricemia. This study aimed to determine the ethanol 70% subfraction activity of binahong leaves to uric acid level of male white mice. Antihyperuricemia assay was conducted for 36 days by dividing 24 mice into six groups, normal control was given standard feed and Na CMC 0.5%, positive control was given purine and allopurinol 0.8 mg/20 g BB. The assay group was given a purine feed and ethanol 70% subfraction of SF 3 binahong leaves with a dose of 1.83; 3.60; and 5.405 mg/20 g BB. Blood sampling was conducted by a 2 hours orbital sinus after the induction of potassium oxonate. Blood sampling was measured by enzymatic method using a clinical spectrophotometer. Data were analyzed statistically with ANOVA one way and Tukey. The result of Tukey Pos Hoc statistic showed the third dose with sig value 0.071 ($p > 0.05$) no significant difference to positive control of percentage of decrease equal to 56.6%. So it can be stated that the activity of decrease uric acid level dose 3 (5,405 mg / 20gBB) is proportional to positive control.

Keywords: Uric acid, *Anredera cordifolia* (Ten.) Steenis, antihyperuricemia, ethanol 70% subfraction leaf binahong.

INTRODUCTION

Indonesia is currently facing health problems of Non-Transmitted Diseases (NTD), which tend to increase every year. According to the Ministry of Health RI (2016) reported that deaths due to NTD rose from 37% in 1990 to 57% in 2015. The increased deaths due to this disease can be caused by changes in diet with unbalanced nutrition (Ministry of Health RI 2011). One sign of NTD is due to dietary changes that lead to increased levels of purine in the body causing hyperuricemia (Purwaningsih 2009).

The amount of side effect that arises from the use of synthetic drugs and the long duration of therapy becomes a problem in the health field. Development of herbal medicine can be a solution of the problem related to the number of medicinal plants in Indonesia. Plants that can be used as herbal remedies such as leaf binahong (*Anredera cordifolia* (Ten.) Steenis) traditionally to treat gout, heart, diabetes, stroke, asthma, acne, influenza, stiff, burn and so on (Susetya 2012). According to Lin (2002) reported that flavonoid compounds are suspected to inhibit the enzyme xanthine oxidase, which can inhibit the formation of uric acid and can cure hyperuricemia caused by accumulation of uric acid in the body or plasma. Flavonoid compounds found in the leaves of binahong allegedly can reduce uric acid levels.

Based on research conducted by Lidinilla (2014) reported that ethanol extract 70% of binahong leaf proven to decrease uric acid-induced rat rats at dose 200mg / kgBB 91,83% with decrease of hyperuricemia 3,56 mg / dl become 1,78 mg / dl. Based on research conducted by Mutiari (2015) reported that ethanol fraction of 70% of binahong leaf was proven to decrease uric acid level of male mice induced by potassium oxonate with a fraction dose of 3.66mg / 20gBB can decrease hyperuricemia from 4.048 mg / dl to 1.403 mg / dl. Based on the results of previous research, it is necessary to further research into the subfraction stage in order to produce a purer and cleaner compound of impure compounds by column chromatography method.

MATERIAL AND METHODS

Binahong leaf taken from BALITRO and mice obtained from Lippi Farm Hall. The experimental animals used were 24 tails divided into 6 treatment groups, each group consisting of 4 tails.

The division of animal groups:

- Group I : Normal control (0.5% Na-cmc solution)
- Group II : Negative control (High purine feed + 0.5% Na-cmc solution)
- Group III : Positive control (High purine feed + Allopurinol dose 0.8 mg / 20gBB)
- Group IV : Dose 1 SF 3 (Purine high feed + Subfraction dose 1.83 mg / 20gBB)
- Group V : Dose 2 SF 3 (High purine feed + Dose of 3.60 mg / 20gBB subfraction)
- Group VI : Dose 3 SF 3 (High purine feed + Dose of subfraction 5.405 mg / 20gBB)

Extraction

The simplisia powder is macerated by using 70% ethanol solvent and then evaporated with a vacuum rotary evaporator until a viscous extract was obtained. The condensed extract was fractionated with n-hexane, ethyl acetate and solvent ethanol in a separating funnel. A ethanol fraction were evaporated with a vacuum rotary evaporator and used as much as 20 g by making wet column chromatography using a mixture of n-hexane gradient solvent: ethyl acetate and ethyl acetate: methanol in a ratio of 10: 0 to 0:10.

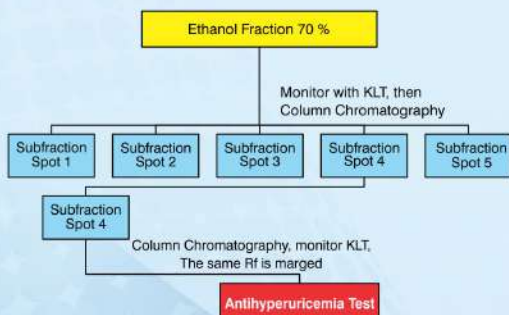


Figure 2. Scheme Extract Method

Antihyperuricemia Test

Prior to the experimental treatment, the test animals were acclimatized in a cage and fed and fed standard for 14 days. Day 15 to day 28, all test groups were induced high purine feeds of chicken liver juice per oral 200 g / 100 ml except normal group given standard feed and 0.5% Na CMC. Day 29 and day 36, all test groups were taken of their blood and measured uric acid levels 2 h after administration of potassium oxonate induction at 6 mg / 20 g intraperitoneal mice (group II, III, IV, V, VI) except group normal without being given potassium oxonate. On the 29th day until the 36th day, the purine high feed continues and all groups are treated according to the established group. Provision of the test material was performed by mouth and suspended using Na CMC 0.5%. Serum was taken as much as 20 μ l, enzyme reagent (1000 μ l of uric acid kit reagent), then in vortex and incubated for 5 min at 37 $^{\circ}$ C. The values of uric acid levels were read by microlab clinical spectrophotometer 300

RESULTS AND DISCUSSION

Table I. Results of sample preparation

No.	Jenis	Hasil
1.	Weight of simplisia powder	1,75 kg
2.	Viscouss extract of Binahong leaves	186,93 g
3.	Fraction of Binahong leaves	46,206 g
4.	Subfraction of Binahong leaves SF 3	5,35 g
5.	Subfraction Rendemen of Binahong leaves SF 3	26,76 %
6.	Subfraction dry loss of Binahong SF 3	5,8600 %

The results of flavonoid checking of the 5 subfraction stains can be seen in Figure 1 which given a green circle expressed positive containing flavonoids. It can be seen that the results of these UV-irradiated TLC 366 contained strong absorption so it can be said that 4 of the 5 subspecified stains were positive for flavonoids. The ethanol subfraction result of 70% of the SF 3 binahong leaves has the most flavonoid stain and is dominant among the others. The Rf values obtained from SF 3 are 0.41; 0.87; and 0.94. Thus, SF 3 was selected to continue testing of antihyperuricemia activity because it had the dominant flavonoids among other stains.



Table III. Percentage of Uric Acid Decline

Group	Baseline \pm SD (mg/dl)	Final Level \pm SD (mg/dl)	Percentage (%)
Normal control	1,08 \pm 0,06	1,07 \pm 0,06	0,09
Negative control	2,36 \pm 0,34	3,15 \pm 0,54	24,12
Positive control	3,14 \pm 0,27	1,15 \pm 0,17	62,44
Dose 1	3,42 \pm 0,55	2,48 \pm 0,37	27,44
Dose 2	3,81 \pm 0,35	2,03 \pm 0,23	27,68
Dose 3	3,67 \pm 0,23	1,59 \pm 0,08	56,6

Figure 1. Thin Layer Chromatography results

The percentage decrease in uric acid levels can be calculated from the results of uric acid levels in each group by comparing initial uric acid levels and final uric acid levels. The results of the percentage can be seen in Figure 3 at dose 3 subfraction showed the greatest decrease compared with doses 1 and 2.

In the percentage data of decreased mean uric acid levels obtained from each group showed that allopurinol has the ability to decrease the highest uric acid level of 62,44%. The second decrease percentage is owned by group of dose 3 with value 56,60%, followed by dose 2 which has value decrease equal to 27,68% then dose 1 with value 27,44%. From this percentage it is seen that this intergroup has a varying percentage of decline, this is influenced by the dosing of different sizes in each group. Based on the number of doses administered, the dose group 3 gives greater activity than the dose 2 and dose group 1. This indicates that dose 3 has similar activity or is proportional to the positive control group to decrease uric acid levels in male white mice.

CONCLUSION

Based on the results of this research, ethanol subfraction of 70% of binahong leaf (*Anredera cordifolia* (Ten.) Steenis) SF 3 obtained has antihyperuricemia activity. Result of statistic of Pos Hoc Tukey Dose 3 ethanol subfraction 70% leaf binahong (*Anredera cordifolia* (Ten.) Steenis) SF 3 equal to 5,405 mg / 20gBB can decrease uric acid level with sig value 0,071 ($p > 0,05$) positive control allopurinol dose 0.8 mg / 20gBB with percentage decrease 56.6%. So it can be stated that the activity of decreasing uric acid level dose 3 is proportional to positive control

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