

The Potency of Binahong Leaves (*Anredera cordifolia* (Ten.) Steenis) Subfraction with Ethanol 70% as an Antihyperuricemic Agent

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Abstract: Hyperuricemia is an abnormally high level of uric acid in a blood. Binahong leaves (*Anredera cordifolia* (Ten.) Steenis) is one of the plants traditionally used as an antihyperuricemic remedy. This study aims to determine the ethanol 70% subfraction activity of binahong leaves on the uric acid level of male white mice. Antihyperuricemia assay was conducted for 36 days by dividing 24 mice into six groups. There are normal control who was given standard feed and Na CMC 0.5%, positive control was given purine and allopurinol 0.8 mg/20 g BW and the assay group was given a purine feed and binahong leaves subfraction SF 3 with a dose of 1.83 mg/20gBW, 3.60 mg/20gBW, and 5.40 mg/20gBW. Blood sampling was conducted by orbital sinus after 2 hours from induction of potassium oxonate. Blood sampling was measured with an enzymatic method using a clinical spectrophotometer. The result showed that the third dose had no significant difference to the positive control with a percentage of decrease of 56.6%. The conclusion is that binahong leaves subfraction has the same activity as an antihyperuricemic agent with allopurinol at dose 5.40 mg/20gBW.

1 INTRODUCTION

Indonesia is currently facing health problems of Non-Communicable Diseases (NCDs), which tend to increase every year. The Ministry of Health RI (2016) reported that the number of death from NCDs rose from 37% in 1990 to 57% in 2015. The increased deaths can be caused by changes in diet with imbalance nutrition (Kemenkes RI, 2011). One sign of NCDs is due to dietary changes that lead to increased levels of purine in the body causing hyperuricemia (Purwaningsih, 2009). According to an epidemiological survey conducted in Bandungan, Central Java in a WHO-COPCORD collaboration (2015) 4,683 samples aged between 15-45 years the prevalence of hyperuricemia was 24.3% in males and 11.7% in women. This disease can be grouped into primary gout that commonly occurs (90% of cases) and which cause is unknown clearly, and secondary gout (10% of cases) that is experienced by women after menopause due to hormone imbalance (Daniati, 2015).

The number of side effects that arise from the use of synthetic drugs and the long duration of therapy has become a problem in the health field. The development of herbal medicine can be a

solution to the problems considering the widespread existence of medicinal plants in Indonesia. Binahong leaves (*Anredera cordifolia* (Ten.) Steenis) are traditionally used to treat gout, heart, diabetes, stroke, asthma, acne, influenza, stiff, burn and so on (Susetya, 2012). Binahong leaves contains active compounds such as saponins, polyphenols, flavonoids and polysaccharides (Rachmawati, 2008). Flavonoid compounds are suspected to inhibit the enzyme xanthine oxidase, which can inhibit the formation of uric acid (Lin, 2002).

Binahong leaves extracted with ethanol 70% has proven to decrease the level of uric acid at dose 200 mg/kgBW 91,83% from 3,56 mg/dl to 178 mg/dl (Lidinilla, 2014). Fraction binahong leaves with ethanol 70% at dose of 3,66 mg/20gBW has been proven to decrease the level of uric acid from 4.048 mg/dl to 1.403 mg/dl. Based on the results of previous research (Mutiarini, 2015), 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.

2 MATERIAL AND METHODS

2.1 Material

Mice, Vacuum Rotary Evaporator, Microcentrifuge, column chromatography, Vortex Mixer, micropipet, Clinical Spectrophotometer (Microlab 300), TLC plate (silica gel GF 254), binahong leaves (*Anredera cordifolia* (Ten.) Steenis), potassium oxonate (Sigma Aldrich Chemical), allopurinol, ketamine.

2.2 Methods

2.2.1 Sample Preparation

Binahong leaves taken from BALITTRO was dried by sun and covered with black cloth. Binahong leaves were then powdered and sieved with mesh number 40.

2.2.2 Mice Preparation

Twenty-four mice (Indonesian Institute of Sciences, region Indonesia) were acclimatized and were fed with standard feed. They were then divided into six test groups of four mice for antihyperuricemic test.

2.2.3 Extraction

The simplicia powder was macerated with ethanol 70% for three times, then filtered. The resulting mixture was collected and was evaporated with a vacuum rotary evaporator until a viscous extract was obtained.

2.2.4 Fractionation

A total of 170 g of binahong extract was fractionated with n-hexane and ethanol - water in a separating funnel – and was shaken for 15 minutes. After that it was allowed to stand to form 2 layers (n-hexane at the top and ethanol-water at the bottom). The ethanol coating: water was fractionated back with an ethyl acetate solvent, and then was rehydrated for 15 minutes. After that, it was allowed to stand to form two layers (ethyl acetate at the top and ethanol: water at the bottom). Each treatment was repeated until the top layer was clear then all the fractions of n-hexane, ethyl acetate and ethanol were evaporated with a vacuum rotary evaporator.

2.2.5 Subfraction Process

An ethanol fraction was 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: to 0:10.

2.2.6 Phytochemical Screening

Phytochemical screening was performed to test the presence of groups of alkaloids, saponins, tannins, flavonoids, and terpenoids with TLC method. The stationary phase employed was a GF254 silica gel plate with a mobile phase system and a detection reagent adjusted to each of the detected compounds. Silica Gel GF245 as a stationary phase and ethyl acetate – methanol - ammonia (4-1-1) as a mobile phase. The principle of separation on TLC based on absorption and partition. TLC method was chosen because can describe a chromatographic pattern of samples, has a simple procedure and diverse motion phases (Hanani, 2014).

2.2.7 Antihyperuricemic Test

From Day 15 to Day 28, all test group were induced orally with high purine feeds of chicken liver juice (200 g/100 ml) while the normal control was given standard feed and 0.5 ml of Na-CMC. On Day 29 and Day 36, blood samples were taken. Uric acid level was measured 2 hours after intraperitoneal administration of potassium oxonate induction at 6 mg/20 g to all groups except Group I. From Day 29 to Day 36, the feed was continued to be given orally according to the treatment group and was suspended using Na-CMC. Serum was taken as much as 20 µl, 1000 µl of uric acid kit reagent (Human), then was mixed in the vortex and was incubated for 5 minutes at 37 ° C. The values of uric acid levels were read by clinical spectrophotometer.

The following is the division of animal groups:

- Group I as a normal control (standard feed with Na-CMC solution).
- Group II as a negative control (high purine feed with Na-CMC solution).
- Group III as a positive control (high purine feed with allopurinol at dose 0.8 mg/20gBW).
- Group IV as Dose 1 assay (high Purine feed with binahong leaves subfraction at dose 1.83 mg/20gBW).
- Group V as a Dose 2 assay (high purine feed with binahong leaves subfraction at dose of 3.60 mg/20gBW).

Table 1. The results of sample preparation

| No. | Data | Results |
|-----|--|----------|
| 1. | The weight of simplicia powder | 1,75 kg |
| 2. | Thick extract of binahong leaves | 186,93 g |
| 4. | Fraction of binahong leaves | 46,21 g |
| 5. | Subfraction of binahong leaves SF 3 | 5,35 g |
| 6. | Subfraction of yield of binahong leaves SF 3 | 26,76 % |
| 7. | Subfraction of dry loss of binahong SF 3 | 5,86 % |

- Group VI as a Dose 3 assay (high purine feed with binahong leaves subfraction at dose 5.40 mg/20gBW).

2.2.8 Statistical Analysis

Data was analyzed by one-way ANOVA which have previously tested for normality and homogeneity. The data then continued with Pos Hoc Tukey test to know the differences between groups.

3 RESULTS AND DISCUSSION

One of the plants that can be used as herbal remedies is the leaf of binahong (*Anredera cordifolia* (Ten.) Steenis) which traditionally treats gout, heart disease, diabetes, stroke, asthma, acne, influenza, stiff, burn and so on (Susetya, 2012). Binahong contains active compounds such as saponins, polyphenols, flavonoids and polysaccharides (Rachmawati, 2008).

Table 1 shows the results of the extraction process. The percentage of subfraction yields is 26.76%. The percentage of yields can show the effectiveness in determining the appropriate method for the process. The calculation of yield aims to find out how much recovery of secondary metabolite compounds in the subfraction. In the ethanol subfraction results, 70% of the SF 3 binahong leaf was carried out with a drying rate of 5.86 % in order to see the quality of the obtained subfraction. The compound may be a volatile oil residue, organic solvent or water contained in there. The dry shrinkage percentage of the binahong leaves was less than 10 %. Thus, the result of drying drift is <10%, it can be said the quality of the good subfraction results.

The preparation of subfraction using column chromatography resulted from 20 g ethanol fraction obtained 5 stain which then continued checking of

Tabel 2. The result of phytochemical screening of binahong leaves SF 3 subfraction yield using TLC method.

| No. | Secondary Metabolite | Result |
|-----|----------------------|--------|
| 1. | Alkaloid | - |
| 2. | Flavonoid | + |
| 3. | Saponin | - |
| 4. | Tanin | - |
| 5. | Steroid | - |

Note: (+) Positive, (-) Negative

flavonoid compound. There are four spot were confirmed positive of containing flavonoids. Flavonoids are phenolic compounds because their color will change to purple light color if they are added with bases or ammonia. In addition, flavonoids contain conjugated aromatic compounds because they exhibit strong absorption bands in UV light (Harborne, 1987).

The results of flavonoid checking of the 5 subfraction stains. The positive results, which contain flavonoids, are given a yellow circle (figure 1). The results of binahong leaves subfraction with ethanol 70 % has the most flavonoid stain which dominant among the other. 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 has the dominant flavonoids among other stains. Flavonoid compound are suspected to inhibit xanthine oxidase which can inhibit the formation of uric acid.

The next step is phytochemical screening. As shown in Table 2, the binahong leaves contain flavonoids compound only. Due to the process of column chromatography with mixed motion phases and variations in the phase comparison of motion.

The measurements of uric acid levels in white

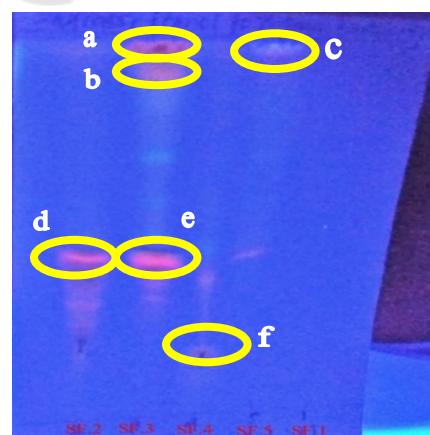


Figure 1. Thin layer chromatography results with Rf value (a) 0,94; (b) 0,87; (c) 0,90; (d) 0,43; (e) 0,41; (f) 0,20.

Tabel 3. The percentage of uric acid decline

| Group | Baseline ± SD (mg/dl)* | Final Level ± SD (mg/dl)* | Percentage (%) |
|------------------|------------------------|---------------------------|----------------|
| Normal control | 1,08 ± 0,06 | 1,07 ± 0,09 | 0,09 |
| Positive control | 3,14 ± 0,27 | 1,18 ± 0,17 | 62,44 |
| Dose 1 | 3,42 ± 0,55 | 2,48 ± 0,37 | 27,44 |
| Dose 2 | 2,81 ± 0,35 | 2,03 ± 0,23 | 27,68 |
| Dose 3 | 3,67 ± 0,23 | 1,59 ± 0,08 | 56,6 |

*Note: average of 5 experimental mice

male mice induced high purine feed in the form of chicken liver juice can be seen in Table 3 the significance value of homogeneity test was 0.122 ($p>0.05$) which show that the data is homogeneously distributed. The Shapiro-Wilk test indicates that the data is normally distributed. The significant values was less than 0.05 which stated a significant difference in the treatment.

The result of the Tukey test showed no significant differences between negative control, dose 1 dan dose 2 groups which is presumably because the dose given is too small to affect the small activity at this dose. There was also no significant difference between positive control group and dose 3 group. Binahong leaves subfraction with dose 5.40 mg/20 gBW have equal lowering uric acid activity with allopurinol group (table 3).

The binahong leaves subfraction has a flavonoid compounds that have antihyperuricemic activity. Allopurinol has the same mechanism of action with flavonoids in reducing uric acid level. It contains oxipurinol, the main metabolite, as xanthine oxidase inhibitors that the conversion of hypoxanthine to xanthine, and xanthine to uric acid (Sukandar *et al.*, 2009)

4 CONCLUSIONS

Based on the results of this research, ethanol subfraction of 70% of binahong leaves (*Anredera cordifolia* (Ten.) Steenis) SF 3 obtained has antihyperuricemic activity. The activity of decreasing uric acid level in dose 3 (5.40 mg/20 gBW) is proportional to allopurinol.

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