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Effect of leaf ethyl acetate fraction Binahong (Anredera Cordifolia (Ten.) Steenis) on Uric Acid Levels of Hyperuricemia Male Mice

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Abstract: A study on the effect of the ethyl acetate fraction of binahong leaves (*Anredera cordifolia* (Ten.) Steenis) on uric acid levels in hyperuricemia male mice has been carried out. A total of 25 male mice were first induced with chicken liver juice 25 g/kg orally for 14 days to get hyperuricemia test animals. The test animals were divided into six groups, namely those that received 12.5 mg/kg, 25 mg/kg and 50 mg/kg fractions, positive control, negative control and 10 mg/kg allopurinol as a comparison. Data analysis used two-way analysis of variance (ANOVA) followed by Duncan's Multiple Range T-test with 95% confidence intervals. This study stated that the fraction used lowered blood uric acid levels. The best reducing effect of the fractions was shown by a dose of 50 mg/kg. This study shows that the ethyl acetate fraction of *A. cordifolia* exhibits antihyperuricemia activity which has the potential for drug development in the future.

Keyword: Anredera Cordifolia, Ethyl Acetate Fraction, Hyperuricemia, Uric Acid .

INTRODUCTION

Gout is a term used for a condition that leads to a group of diseases caused by monosodium urate deposits in the tissues due to prolonged hyperuricemia (Ferri, 2018). Hyperuricemia is an increase in serum uric acid levels above normal values, in men above 7 mg/dl and in women above 6 mg/dl. Hyperuricemia can be hereditary, namely the presence of metabolic defects (abnormalities) so that the synthesis of uric acid becomes excessive and abnormal (Dalimartha, 2008).

Gout clinical manifestations include acute and chronic arthritis, soft tissue inflammation, topus formation, gouty neuropathy and nephrolithiasis. Untreated hyperuricemia in patients with gout can cause chronic damage to arthritis (Ferri, 2018).

The prevalence of hyperuricemia has increased rapidly in recent decades (Guan *et al* ., 2016) and is the most common cause of inflammatory arthritis in men over 40 years of age and women over 60 years of age (Kuo et al., *2015*) . Increased uric acid levels are indirectly related to gout which can increase the risk of hypertension, obesity, stroke and premature death (Guan *et al* ., 2016). The average serum uric acid level is 0.5-1.0 mg/dl in men higher

than women, this causes male sex as a risk factor for hyperuricemia and gout. Lower serum urate levels in women are associated with the presence of estrogen, which acts as an antihyperuricemia (Wahjuni *et al* ., 2012).

Gout in Indonesia based on Basic Health Research (Riskesdas) in 2013 was 11.9% based on the diagnosis of health workers and 24.7% based on diagnosis and symptoms. The prevalence of joint disease in West Sumatra in 2013 was 12.7% based on the diagnosis of health workers and 21.8% based on the diagnosis and symptoms (Ministry of Health of the Republic of Indonesia, 2013).

The standard and recommended treatment therapy for gout is *allopurinol* , which lowers total uric acid levels in the body by inhibiting *xanthine oxidase* . The use of *allopurinol* can cause side effects of nausea, vomiting and diarrhea, but it can also cause peripheral neuritis, depression of the spinal cord elements and sometimes aplastic anemia. Liver toxicity and intestinal nephritis have also been reported. *Allopurinol* can also bind to the eye lens which will cause cataracts (Katzung, 2007).

Binahong or *Madeira vine* (*Anredera cordifolia* (Ten.) Steenis) is a herbal plant that is most often used to cure various diseases in several countries in Asia such as Vietnam, Taiwan, China and Korea. The leaves are often used as natural medicine (Yuniarti & Lukiswanto, 2017). Binahong is used traditionally to treat various diseases such as skin diseases, hypertension, inflammation and gout (Sukandar *et al* ., 2011). The ability of binahong to cure various types of diseases is closely related to the active compounds contained in it. Binahong plants contain saponins, alkaloids, polyphenols, flavonoids and mono polysaccharides which belong to the L-arabinose, D-galactose, L-rhamnose, D-glucose groups (Rachmawati, 2008). Binahong leaves have pharmacological effects such as: antibacterial, antiobesity, antihyperglycemic, cytotoxic, antimutagenic, antiviral, antidiabetic, antiulcer and anti-inflammatory (Kottaimuthu *et al* ., 2012). Other activities of this plant are as a hepatoprotector, anti-obesity, increasing breast milk and lowering blood pressure (Yuniarti & Lukiswanto, 2017)

Several researchers in Indonesia have proven that this plant can treat diabetes mellitus, tuberculosis, rheumatism, gout, asthma, typhoid, hypertension, hemorrhoids, but it is also used as a diuretic, postpartum recovery, wound healing, post-circumcision surgery, gastritis, colitis. , cancer (Yuniarti & Lukiswanto, 2017). Other studies also prove that the ethanol extract of binahong leaves can also reduce uric acid levels (Meilian *et al* ., 2014).

The use of traditional medicine is increasing among the people along with the development of *back to nature* . The use of traditional medicine is considered safer because it has relatively low side effects compared to modern medicine. So this research was conducted to see the effect of the ethyl acetate fraction of binahong leaves (*Anredera cordifolia* (Ten.) Steenis) on uric acid levels in hyperuricemia male mice.

This study was conducted to determine the optimal dose of ethyl acetate fraction of binahong leaves to reduce uric acid levels in hyperuricemia male mice induced by chicken liver juice. The results of this study are expected to provide knowledge to the public that binahong leaves are a medicinal plant that can be used as an alternative treatment, as well as provide scientific information about the effect of the ethyl acetate fraction of binahong leaves in reducing uric acid levels in hyperuricemia male white mice induced by liver juice chicken. Furthermore, to be able to increase knowledge in the field of health sciences regarding research and development of new drugs.

METHOD

This research was conducted in several stages, namely sampling, sample identification, preparation of binahong leaf ethanol extract, preparation of extract fractions, preparation of experimental animals, dose planning and grouping of experimental animals, preparation of

test preparations, treatment of experimental animals, measurement of uric acid levels, processing. and data analysis.

The research data will be analyzed statistically using a two-way *Analysis of Variance* (ANOVA) which is used to compare differences in average uric acid levels based on the treatment group factor and the length of observation factor, then proceed with Duncan's Multiple Range T- test) (Arifin, 2017).

RESULTS AND DISCUSSION

Results

Binahong Leaf Fraction

The dry sample obtained from 13 kg of fresh sample is 580 gram. The total extract obtained was 53.4 grams and the fractionation results obtained were 1.0267 grams of n-hexane fraction and 3.8350 grams of ethyl acetate fraction.

Characterization and Results of Phytochemical Tests

1. The results of determining the yield of the n-hexane fraction of binahong leaves obtained a yield value of 0.177%.
2. The results of determining the yield of the ethyl acetate fraction of binahong leaves obtained a yield value of 0.661%.
3. The organoleptic test results of the ethyl acetate fraction of binahong leaves were blackish green in color, characteristic aromatic odor and thick consistency.
4. The results of the phytochemical screening of the ethyl acetate fraction of binahong leaves showed that they were positive for alkaloids, flavonoids, triterpenoids and steroids.

The average uric acid level of mice at the 7th, 14th and 21st day of observation in each group

1. The mean uric acid levels of the negative control group mice on days 7, 14 and 21 were 1.64 ± 0.241 mg/dL, 1.7 ± 0.399 mg/dL and 1.68 ± 0.217 mg/dL respectively.
2. The mean uric acid levels of the positive control group mice on days 7, 14 and 21 respectively were 7.2 ± 1.093 mg/dL, 6.02 ± 0.759 mg/dL and 4.98 ± 0.370 mg/dL.
3. The average uric acid levels of the mice at the dose of 12.5 mg/kg on days 7, 14 and 21 respectively were 5.48 ± 0.482 mg/dL, 3.96 ± 0.619 mg/dL and 2.88 ± 0.526 mg/dL.
4. The average uric acid levels of the mice at the dose of 25 mg/kg on days 7, 14 and 21 respectively were 5.06 ± 1.076 mg/dL, 3.56 ± 2.458 mg/dL and 2.58 ± 1.462 mg /dL.
5. The average uric acid levels of the 50 mg/kg BW group mice on days 7, 14 and 21 respectively were 3.66 ± 0.550 mg/dL, 2.64 ± 0.297 mg/dL and 1.56 ± 0.279 mg /dL.
6. The average uric acid levels of the comparison group mice on days 7, 14 and 21 were 3.46 ± 0.971 mg/dL, 2.62 ± 0.545 mg/dL and 1.5 ± 0.245 mg/dL respectively.

Analysis Of Variant (ANOVA) Statistical Calculation Results

analysis of variance (ANOVA) statistical calculations on uric acid levels for the treatment group and the time of observation obtained the following data:

1. Based on the results of statistical data processing using a two-way analysis of variant (ANOVA) for the treatment group and the time of observation on uric acid levels there was a significant effect ($P<0.05$), the treatment group had a significant effect on uric acid levels in the test animals ($P < 0.05$), while the time of observation also had a significant effect on the uric acid levels of the test animals ($P<0.05$). Thus a further test was carried out using Duncan's test to see the effect of each of these factors.
2. Duncan's advanced test results
   a. Treatment group

The results obtained after Duncan's further test on group factors obtained 4 subsets.

b. Observation time

The results of Duncan's further test of observation time obtained 3 subsets.

Discussion

This study was conducted to determine the effect of giving the ethyl acetate fraction of binahong leaves on reducing uric acid levels in hyperuricemia male mice. The sample used in this study was binahong leaves and the animals used in this study were normal male mice. Binahong leaves were chosen as the sample in this study because the results of previous studies using binahong leaf extract proved to have activity in reducing uric acid levels in hyperuricemia male mice (Meilian, 2014). This research is different from the research that has been done, because this study uses the ethyl acetate fraction of binahong leaves and the inductor used to make hyperuricemia is chicken liver juice while the equation with previous research is to reduce uric acid levels in hyperuricemia male mice.

Binahong leaf samples (*Anredera cordifolia* (Ten.) Steenis) were obtained from the Payakumbuh area. After that identification was carried out at the Herbarium for the Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang. The results of the identification carried out, the binahong leaves used in this study were the same as the collections in the Herbarium. Sample preparation begins with washing the sample to separate dirt or other foreign materials from the sample using clean water. The washed binahong leaves are dried to reduce the water content and prevent enzymatic reactions that can decompose the active substance content in the sample. After the binahong leaves are dry, the process of grinding and sifting is then carried out, which aims to obtain a homogeneous powder. Refinement and sieving aims to increase the surface area of the sample so that during the extraction stage the interaction between the solvent and the sample becomes more effective, thereby facilitating the solubility of the bioactive components and increasing the extraction yield.

Binahong leaves are extracted by maceration method. Maceration is the process of soaking the sample using a solvent with several times of shaking or stirring at room temperature. Soaking causes a difference in pressure inside and outside the cell resulting in breakdown of the cell wall and membrane which will dissolve the secondary metabolites in the solvent. The use of ethanol as a solvent is because ethanol is a suitable solvent for isolating polar organic compounds and ethanol has a polarity close to that of methanol. The advantages of ethanol compared to methanol are economical, non-toxic, safe, relatively non-toxic and can maintain the stability of substances by inhibiting the action of enzymes (Rostagno *et al* ., 2004). Binahong leaves were soaked in 70% ethanol for 3 days with 3 repetitions. During immersion, stirring was carried out several times. The purpose of stirring is to homogenize the solution during the immersion process and accelerate the contact between the sample and the solvent. After 3 days, the filtrate was collected and concentrated using *a rotary evaporator.* The purpose of concentration is to concentrate the extract and separate the solvent and active compounds from binahong leaves. The concentration process is carried out at a temperature of 50ºC, so that the components of the secondary metabolites are not damaged and to obtain a thick extract (Khunaifi, 2010).

After obtaining a thick extract of binahong leaves, it is followed by a fractionation process from the extract that has been obtained before. Fractionation is carried out using organic solvents based on polarity levels that do not mix and can be separated using a separatory funnel. Fractionation starts from non-polar, semi-polar and polar solvents. The non-polar solvent used is n-hexane, the semi-polar solvent uses ethyl acetate and the polar solvent uses aquadest. The fraction obtained was concentrated again using *a rotary evaporator* . The purpose of fractionation itself is to simplify the chemical groups of the

extracts obtained. The use of *a rotary evaporator* will expand the evaporation area as well as a heat source that helps the evaporation process. The solvent evaporation process will be accelerated by reducing air pressure which causes a decrease in the vapor pressure of the solvent so that the solvent will boil at a temperature much lower than its boiling point. The ethyl acetate viscous fraction was obtained as much as 3.8350 g (0.661%). The ethyl acetate fraction was chosen in this study because the viscous ethyl acetate fraction of binahong leaves contains flavonoid compounds, where the benefits of flavonoids are antioxidants, can treat inflammatory conditions and can repair cells damaged by free radicals. The results of Astuti's research (2011) stated that the part of the binahong leaf extracted with ethanol contained flavonoids ranging from 20-70 mg/L and had activity as an antioxidant.

After obtaining the viscous fraction, characterization was carried out which included organoleptic and yield. The viscous fraction obtained is green-black in color, has a distinctive aromatic odor and forms a thick consistency. The yield obtained from 580 grams of dry sample obtained the n-hexane fraction of 1.0267 grams (0.177%) and the ethyl acetate fraction of 3.8350 grams (9.207%). The results of the phytochemical test of the ethyl acetate fraction of binahong leaves showed that it was positive for alkaloids, flavonoids, triterpenoids and steroids.

This ethyl acetate fraction was studied further about the effect of giving ethyl acetate fraction from binahong leaves (*Anredera cordifolia* (Ten.) Steenis) on uric acid levels in hyperuricemia male mice. The ethyl acetate fraction of binahong leaves was dissolved in 1% Na CMC suspension to make the test preparations in the study and used distilled water as a solvent. The test preparations were administered orally because the oral route is the route of drug administration that is commonly used, easy to administer, safe and does not hurt (Loomis, 1987).

The experimental animals used were male mice weighing between 20-30 grams and 2-3 months old. Before mice were used for research, mice were acclimatized for 7 days. The purpose of acclimatization itself is to accustom mice to experimental conditions and determine the feasibility of mice to be used. The mice used were mice that did not experience a body weight deviation of 10% during acclimatization (Vogel, 2002).

After acclimatization, the animals were grouped into 6 groups consisting of a negative control group, a positive control group, a 12.5 mg/kg dose group, a 25 mg/kg dose group, a 50 mg/kg dose group and a comparison group. The negative group was given standard mouse food and standard drink and Na CMC suspension, while the other five groups were given chicken liver juice as an inducer to induce hyperuricemia for 14 days. After 14 days of administration of chicken liver juice, the uric acid levels of the test animals were measured. After the uric acid levels of the test animals were high (hyperuricemia), the test animals were given the ethyl acetate fraction once a day orally for 7, 14 and 21 days according to the group that had been planned. After being given treatment, the mice's uric acid levels were measured on days 7, 14 and 21 by cutting the mice's tails and wiping them until they bled, the blood that came out was dripped on a uric acid test strip that had been attached to the *Easy Touch* ® GCU tool, Mice uric acid levels will be seen on the Multicheck monitor in mg/dL. All mice were sacrificed after all groups of test animals were given treatment.

Examination of uric acid levels using the glucotest and *Easy Touch* ® GCU striptest. This tool is a tool used to monitor the level of uric acid levels in the blood. The *Easy Touch ® GCU* strip test kit is designed for quantitative measurement of uric acid levels in the blood. The technology used is *an electrode-based biosensor* . This measurement is based on determining the change in current caused by the reaction of uric acid with the reagent at the electrodes of the strip. When the blood sample touches the sample target area on the strip, the blood will automatically be drawn into the reaction zone on the strip. The test result will be shown on the screen after 20 seconds (Bioptics techologi Inc).

Measurement of uric acid levels was carried out on the 7th, 14th and 21st days, the parameters observed were uric acid levels which were affected by the type of treatment and the length of observation during the administration of the test preparations. The type of treatment observed was the test group which had the effect of reducing uric acid levels, while for the duration of the observation, it was expected that with the duration of administration of the test preparation, the condition of the test animals was getting better based on the value of uric acid levels approaching the normal value.

The results of statistical calculations using a two-way analysis of variant (ANOVA) according to the treatment group factor and the time of observation showed a significance value of 0.000 (P <0.05), which means that there was a significant difference between the treatment group and the time of observation on uric acid levels, in the *group* treatment showed a significance value of 0.000 (P<0.05), which meant that there was a significant difference in uric acid levels, and at the time of observation showed a significance value of 0.000 (P<0.05) which meant that there was a significant difference in uric acid levels (P< 0.05), thus followed by Duncan's test of the independent effect of each of these factors.

In Duncan's further test, based on the treatment group factor, the uric acid levels of the test animals showed a significant difference in the treatment group because they were in different subsets. In subset 1 there is a negative control group, in subset 2 there is a 50 mg/kg dose group and a comparison group, in subset 3 there is a 25 mg/kg dose group and a 12.5 mg/kg dose group, while in subset 4 there is a positive control group . The results of Duncan's further test showed that the negative control group and the positive control group had significant differences because they were in different subsets (P<0.05) with the average uric acid level of mice in the positive control group (6.067) greater than the value the average uric acid level of mice in the negative control group (1.673). The positive control group and the 12.5 mg/kg dose group had significant differences because. were in a different subset with the average uric acid level of mice in the positive control group (6.067) greater than the average uric acid level of mice in the 12.5 mg/kg dose group (4.107). The 12.5 mg/kg dose group and the 25 mg/kg dose group had no significant difference because they were in the same subset with a significance value of 0.252 (P>0.05). The 50 mg/kg dose group and the comparison group had no significant difference because they were in the same subset, meaning that the 50 mg/kg dose group and the comparison group could reduce uric acid levels to near normal values.

In the results of Duncan's follow-up test based on the treatment group factor for uric acid levels in Appendix 2 Table 8, these results are not good because the doses of 12.5 mg/kg and 25 mg/kg are in the same subset due to inaccurate selection of doses. a dose of 25 mg/kg is better at reducing it than a dose of 12.5 mg/kg, whereas for the differences in the negative control and positive control groups the results were as expected . Furthermore, Duncan's follow-up test based on the time factor of observation of the uric acid levels of the test animals showed that between the 7th day of testing was significantly different from the 14th and 21st days of testing because each uric acid level was in a different subset .

Reducing uric acid levels in the blood of mice by administering the ethyl acetate fraction of binahong leaves due to the presence of flavonoid group compounds which have a mechanism as *xanthin oxidase inhibitors* , thereby inhibiting the formation of uric acid, can reduce uric acid levels in the body and can cure hyperuricemia caused by accumulation uric acid in the body/plasma. Quercetin and myrisetin are compounds belonging to the flavonoid class which are thought to be present in binahong leaves. Quercetin and myrisetin form a 3-hydroxyl group in the benzopyran ring which will reduce the affinity of the *xanthin oxidase enzyme bond* (Lin, 2002). Binahong leaves are known to contain oleanolic acid (Hammond *et al* ., 2006). Oleanolic acid is an anti-inflammatory agent that will inhibit swelling and prevent tissue damage in gout by inhibiting *nitric oxide production* (Mo *et al.*, 2007). Oleanolic acid is a triterpenoid group which is an antioxidant in plants (Liu, 1995).

From the results of the comparison of uric acid levels in mice to the length of observation, it was seen that there was a decrease in uric acid levels in mice and the average uric acid levels in mice for 21 days of administration of the ethyl acetate fraction of binahong leaf test preparations also showed a decrease in uric acid levels in mice. It was proven on the 21st day of testing that there was a decrease in uric acid levels in each dose group compared to the comparison group.

The comparison group in this study aimed to see the effect of giving the ethyl acetate fraction of binahong leaves with the largest dose in reducing uric acid levels in mice that were close to the reduction in uric acid levels in the comparison group. The comparison group used *allopurinol* at a dose of 10 mg/kg. In contrast to the research conducted by Meilian (2014) where the dose used was 13 mg/kg. *Allopurinol* is useful for treating gout because it lowers uric acid levels. This drug works by inhibiting *xanthine oxidase* , an enzyme that converts *hypoxanthine* to *xanthine* and converts *xanthine* to uric acid. Through a feedback mechanism *allopurinol* inhibits the synthesis of purines which are *xanthine precursors* . *Allopurinol* undergoes biotransformation by the enzyme *xanthine oxidase* into *alloxantine* , which has a longer half-life than *allopurinol* (Sulistia GG et *al* ., 2007). The results obtained in the administration of the ethyl acetate fraction at a dose of 50 mg/kg showed a decreasing effect almost the same as *allopurinol* at a dose of 10 mg/kg in male mice.

CONCLUSION

From the study of the effect of the ethyl acetate fraction of binahong leaves (*Anredera cordifolia* (Ten.) Steenis) on uric acid levels in hyperuricemia male mice, the following conclusions can be drawn: 1. Ethyl acetate fraction of binahong leaves at a dose of 12.5 mg/kg, a dose of 25 mg /kg and a dose of 50 mg/kg can reduce uric acid levels in hyperuricemia male mice ($P<0.05$). 2. Administration of the ethyl acetate fraction at a dose of 50 mg/kg showed a uric acid reduction effect almost the same as that of *allopurinol* at a dose of 10 mg/kg. 3. The results of a two-way ANOVA showed that there was a significant effect on the treatment group with the time of testing for uric acid levels in mice.

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