Original Research Report

THE EFFECT OF *BINAHONG* (*Anredera cordifolia* (Ten.) Steenis) LEAF ETHANOLIC EXTRACT ON THE REDUCTION OF BLOOD URIC ACID LEVELS IN HYPERURICEMIC MALE WHITE WISTAR RATS (*Rattus norvegicus*)

Panji Anugerah[1*], Safrizal Rahman[2]

[1]Orthopedic and Traumatology Specialist Medical Education Program, Faculty of Medicine, Syiah Kuala University, Banda Aceh, Indonesia
[2]Faculty of Medicine, Syiah Kuala University, Banda Aceh, Indonesia

ABSTRACT

Binahong (*Anredera cordifolia* (Ten.) Steenis) is empirically used to treat burns, rheumatism, gout, typhoid, and stroke. *Binahong* leaves contain flavonoids that have an antioxidant effect. This study aimed to identify and test *binahong* leaf flavonoid compounds' antioxidant properties in reducing uric acid levels. This study began by producing *binahong* leaf extract and then identifying the flavonoid content with a phytochemical test. The results indicated that the *binahong* leaf extract contained antioxidant flavonoid compounds. The data were followed by testing flavonoid activity in lowering uric acid levels. This study used male white Wistar rats, of which the uric acid was induced by chicken liver juice. The animals used in the test were 25 rats divided into five groups (i.e., negative control group, positive control group, and treatment groups with dose I, dose II, and dose III). In each group, there were five male white rats. This study was a laboratory experiment using a pre-test post-test design with control groups (pre-test post-test control group design), where the grouping was done based on a randomized group design. By using statistical tests, the results obtained showed that there was a significant decrease of uric acid levels in the positive control group and the treatment group. In conclusion, *binahong* leaf ethanolic extract can reduce blood uric acid levels induced by chicken liver juice in male white Wistar rats.

Keywords: *Binahong*; blood uric acid; hyperuricemia; gout; rats; medicine

***Correspondence:** Panji Anugerah, Orthopedic and Traumatology Specialist Medical Education Program, Faculty of Medicine, Syiah Kuala University, Banda Aceh, Indonesia. Email: panji_a@mhs.unsyiah.ac.id

Article history
●Submitted 2 Dec 2022 ● Revised 10 Jan 2023 ● Accepted 18 Feb 2023 ● Published 10 Mar 2023

How to cite: Anugerah P & Rahman S (2023). The effect of *binahong* (*Anredera cordifolia* (Ten.) Steenis) leaf ethanolic extract on the reduction of blood uric acid levels in hyperuricemic male white Wistar rats (*Rattus Norvegicus*). Folia Medica Indonesiana, 59 (1), 20-25. https://doi.org/10.20473/fmi.v59i1.36052

pISSN: 2355-8393, eISSN: 2599-056x

Highlights:

1. Antioxidant flavonoid compounds can be found in *binahong* leaf extract.
2. *Binahong* leaf extract is as effective as allupurinol in reducing uric acid levels.

INTRODUCTION

Gout arthritis is an inflammatory disease caused by the accumulation of uric acid crystals in synovial fluid and other tissues. Generally, the clinical manifestations of gout are pain, stiffness, and swelling in the joints. Gout usually occurs in the big toe, ankle, heel, elbow, wrist, and finger joints. Hyperuricemia is an early occurrence of gout, with increased blood uric acid levels reaching more than 7.0 mg/dl. The higher the uric acid level, the higher the risk of gout. Uric acid levels are influenced by age, sex, weight, and serum creatinine levels (Ragab et al. 2017, Centers for Disease Control and Prevention 2020).

The hyperuricemia incidence varies in each country. The asymptomatic hyperuricemia prevalence rate is 5% in the United States, 6.6% in England, and 8% in Scotland. In New Zealand, hyperuricemia is more common among Maori men (27.1%) compared to European men (9.4%). In a study conducted among Atayal people in Taiwan, it was found that 41.4% of 342 residents over 18 years old had hyperuricemia (George & Minter 2022).

The specific prevalence of hyperuricemia in Indonesia is unknown, but the data from several

studies in various regions showed a high incidence. The incidence of hyperuricemia in Sinjai, South Sulawesi, was 10% in men and 4% in women (Manampiring 2010). A survey conducted on 4,683 samples aged between 15-45 years in Bandungan, Central Java, found that the prevalence of hyperuricemia was 24.3% in men and 11.7% in women (Ladeska et al. 2018). The proportion of hyperuricemia in Tegal increased from 5.7% in 2007 to 8.7% in 2008. In the 2008 medical record data from Kardinah General Hospital, 40% of 1,068 patients who had their uric acid levels checked suffered from hyperuricemia (Prabawa 2019).

Hyperuricemia is an increase in blood uric acid above normal levels. Increased synthesis of purines into uric acid (overproduction) or decreased elimination of uric acid by the kidneys (underexcretion) can lead to hyperuricemia (Maiuolo et al. 2016). Unfavorable lifestyles, such as high purine intake and alcohol consumption, affect the incidence of gout. Genetic disorders of metabolism also play a role in causing excess uric acid production or decreasing uric acid excretion (Kanbay et al. 2016).

Treatment of gout is divided into acute attack management and chronic attack treatment. There are mainly three stages in the treatment of this disease: 1) overcoming acute attacks, 2) reducing uric acid levels to prevent the accumulation of uric acid crystals in the tissues and joints, and 3) using hypouricemic drugs to prevent hyperuricemia and gout from worsening. Patients must comprehend the fundamentals of therapy in order for gout treatment to be effective. Avoiding risk factors that can trigger attacks is an essential part in the management of this disease (Engel et al. 2017).

Allopurinol is the hypouricemic drug of choice for treating chronic gout caused by the overproduction of uric acid. Whereas, probenecid is the uricosuric drug of choice for treating gout caused by underexcretion of uric acid. Allopurinol functions as an inhibitor of xanthine oxidoreductase, an enzyme that prevents the formation of uric acid crystals by xanthine oxidase (Seth et al. 2014). However, allopurinol has various side effects, including hepatitis, gastrointestinal intolerance, and allergic reactions. Therefore, a new inhibitor of xanthine oxidoreductase as an alternative to allopurinol is needed (Qurie et al. 2022).

The main objective of this research was to find a new xanthine oxidoreductase inhibitor, with the same inhibitory activity as allopurinol and fewer side effects. The idea used was that utilizing natural ingredients is known to be safer for human consumption (Astini et al. 2017, Asari & Sugiyanta 2021). Indonesia's abundant biodiversity, if applied with the proper technology, can provide a competitive advantage in the global competition, particularly in the use of traditional medicinal plants (Hamdan et al. 2019, Astuti et al. 2020).

Binahong, also known as Madeira vine or mignonette vine, is one of Indonesia's abundant medicinal plants. Empirically almost all parts of *binahong* plant can be used for treatment, including lung disease, diabetes mellitus, hemorrhoids, dysentery, burns, and gout (Sakti et al. 2019). The plants contain many active compounds such as flavonoids (Dadiono & Andayani 2022). These compounds are abundant in *binahong* leaves, where leaf fertility is advantageous for obtaining high levels of active compounds (Rohani 2021). Antioxidant flavonoids can inhibit xanthine oxidase activity, thereby preventing the formation of uric acid. In addition to flavonoids, oleanolic acid has anti-inflammatory properties (Serrano et al. 2020). The active compounds in *binahong* are believed to be an alternative hypouricemic drug that reduce uric acid levels with fewer side effects than synthetic drugs like allopurinol (Engel et al. 2017).

MATERIALS AND METHODS

This study was a laboratory experiment employing a pre-test post-test design with control groups, where the grouping was determined by a randomized block design and the sample size was determined by a completely randomized design. This study employed male Wistar-strain white rats (*Rattus norvegicus*). The rats were randomly divided into five groups. All groups received the same diet for seven days to induce an increase in uric acid levels. Each group was fed chicken liver juice, pellets, and drinking water during the study (Sakti et al. 2019).

The minimum sample size was determined using the Federer formula as follows: $(n-1)(t-1)>15$. The number of the treatment groups is symbolized by “n”, while the number of replications or number of samples per group is symbolized by “t”. In this experiment, the minimum number of samples required per group was as follows: $(n-1)(5-1)>15$; $t=5$. It produced a result of $n>4.75$, with $t=5$. Based on the calculation, the number of samples per group must exceed 4.75. Therefore, five rats per group were utilized in this study. A total of 25 rats were required for the five groups studied. The rats were considered hyperuricemic when their blood uric acid levels exceeded 3.0 mg/dl (Engel et al. 2017).

The administration of *binahong* leaf extracts is classified into low, medium, and high doses. The dosage for the extract administration in humans was 50 mg/kg bw for the low dose, 100 mg/kg bw for the medium dose, and 200 mg/kg bw for the high dose.

These doses were converted for animal administration using the following method: converted dose for the test animal=absolute dose x conversion value. The conversion value for the rats in this study was 0.01836. The following are the results of the formula that served as a reference for conducting this experiment: 0.9 mg for the low dose (dose I), 1.8 mg for the medium dose (dose II), and 3.6 mg for the high dose (dose III). Distilled water was used to liquify the extracts, which were then administered orally using a gastric probe (Sakti et al. 2019).

The standard therapeutic dose of allopurinol for hyperuricemia in the general population is 300 mg/50 kg bw. Therefore, the allopurinol dose for humans as a reference in this research was 300 mg per day. The conversion value for dose conversion between human and rats was 0.01836. The converted allopurinol dose for the rats was 5.4 mg/200 g bw (Sakti et al. 2019).

Twenty-five rats, previously divided into five groups with each group consisted of five rats, were induced by chicken liver juice. Chicken liver contains purines of 150-1,000 mg/100 g. The chicken liver juice dose was 3 ml/200 g bw, adjusted to the maximum fluid intake capacity of the rats, which was 5 ml/200 g bw. Group I (positive control group) was fed chicken liver juice, distilled water, and allopurinol with a dose of 5.4 mg/kg bw. Group II (negative control group) was fed chicken liver juice and distilled water. Group III (treatment group I) was fed chicken liver juice, distilled water, and *binahong* leaf extract with a dose of 0.9 mg. Group IV (treatment group II) was fed chicken liver juice, distilled water, and *binahong* leaf extract with a dose of 1.8 mg. Group V (treatment group III) was fed chicken liver juice, distilled water, and *binahong* leaf extract with a dose of 3.6 mg (Ladeska et al. 2018).

The normal distribution of the data obtained was assessed using one-sample Kolmogorov-Smirnov test and the Shapiro-Wilk test, while the homogeneity of the data was assessed using using the Levene test. If these two conditions were met, a one-way ANOVA test would be performed to determine whether or not there was a significant difference among the treatment groups (Ladeska et al. 2018). If there was a significant difference, then a difference test using the Duncan method would be conducted. However, if one or both of these tests were not met, the analysis would be carried out using the Kruskal-Wallis test. Povidone-iodine was used to close and dry the wound from a previous blood draw on the tail. After blood samples were taken, the experimental animals were returned to their cages (Sakti et al. 2019).

RESULTS

Data on the blood uric acid levels before and after the experiment from all groups were tested using the Smirnov test for data normality and the Levene test for homogeneity. The data are normally distributed and equal in variances if p=0.05 in each test. One-way ANOVA test can be conducted on normally distributed and homogeneous data. If p=0.05 in the ANOVA test, then a difference test must be carried out using the Duncan method.

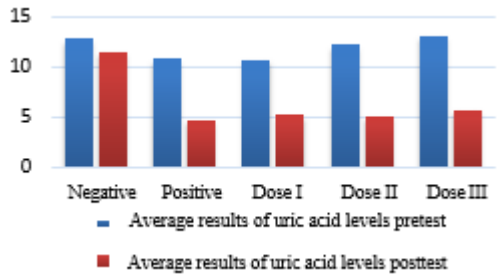

Figure 1. Average results of uric acid levels in all groups.

The analysis of the data resulted in a significance value of 0.05, so the data were considered homogenous. The decrease in uric acid levels in the rats from all groups was normally distributed. Therefore, the data can be continued to be analyzed in the ANOVA test.

Table 1. Results of the ANOVA test on data of the rats' uric acid levels.

F Count	F table 0.05	F table 0.01	p-value
3.846	2.87	4.43	0.018

The results of the ANOVA test ($p<0.05$) indicated that uric acid levels in the control and treatment groups decreased significantly after treatment, with the calculated F value exceeding the F table value. The data showed a significant decrease, so a difference test using the Duncan method was conducted. The purpose of the difference test was to compare the differences among the animal groups.

The conclusion from the analysis results was that the decrease in uric acid levels among the negative control group, the positive control group, and the treatment groups differed significantly. However, the decrease in uric acid levels between the positive control group and the treatment groups did not differ significantly ($p>0.05$). The treatment group I with the highest dose of *binahong* leaf extract demonstrated

the greatest reduction in uric acid levels. The positive control group, treatment group II, and treatment group III followed suit.

Table 2. Results of the least significant difference test using the Duncan method.

Group	N	1	2
Negative control	5	1.300	
Treatment I	5		5.140
Treatment II	5		5.680
Positive control	5		6.000
Treatment III	5		7.400
P-value		1.000	0.223

DISCUSSION

The results of this study showed an increase in uric acid levels among the rats in the control and treatment groups after being fed chicken liver juice for seven days. It was because chicken liver juice contains high levels of purines, which triggered xanthine oxidase to catalyze the formation of uric acid. *Binahong* leaf extract administration could reduce uric acid levels of experimental animals, i.e., male white rats (*Rattus norvegicus*) induced by chicken liver juice. The results of the ANOVA test ($p<0.05$) proved that there was a significant average difference between the control and treatment groups. The presence of essential compounds in *binahong*, such as flavonoids, was assumed to play a role in lowering uric acid levels (Darmawan & Hidayati 2020).

This research is also supported by several other studies. Ablat & Mohamad (2018) reported in their study that flavonoids reduce uric acid levels by inhibiting the enzyme xanthine oxidase, which is responsible for the formation of uric acid. The types of flavonoids that can inhibit the activity of xanthine oxidase are quercetin, myricetin, kaempferol, luteolin, apigenin, and chrysin. Nadinah, as cited by Martha & Zummah (2018), stated that luteolin and apigenin can work as xanthine oxidase inhibitors with similar performance as allopurinol.

Allopurinol was used as a comparison drug because it is a modern medication commonly used to reduce uric acid levels. It is believed that this nucleic acid derivative can inhibit the production of uric acid. This inhibitory mechanism of allopurinol is used to maintain stable uric acid synthesis. With *binahong* leaf extract, the flavonoids and xanthine oxidase interact to make the bonds loose, then stabilize the xanthine oxidase (Martha & Zummah 2018).

The normality test (one-sample Kolmogorov-Smirnov test) showed that the data on the blood uric acid levels of all groups were normally distributed ($p\geq0.05$). The homogeneity test (Levene test) showed homogeneous variance ($p\geq0.05$). The analysis can be continued with an ANOVA test. If $p\leq0.05$, a difference test can be carried out using the least significant difference and Duncan method.

In the difference test for all groups, the positive control group and treatment groups showed a significant difference ($p\leq0.05$) from the negative control group. All treatment groups showed no significant difference ($p\geq0.05$) from the positive control group. Even though the blood uric acid levels in all treatment groups and the positive control group were not normal, there was a decrease in uric acid levels compared to the negative control, and the the results in all treatment groups were comparable to that of the positive control group. Based on the findings of this study, it can be concluded that increasing the dose concentration of the *binahong* leaf ethanolic extract correlates significantly with the extract's efficacy in lowering total cholesterol levels. The treatment groups I, II, and III showed a significant difference in the reduction of total cholesterol levels. Comparing the decrease in uric acid levels between the treatment and control groups illustrated this point.

Strength and limitations

The study provides an insight on the identification and testing the of antioxidant activity of *binahong* leaf flavonoid compounds in reducing uric acid levels and discovering a novel xanthine oxidoreductase inhibitor that has the same inhibitory effect as allopurinol but with fewer adverse effects. The study about *binahong* has not been widely explored in a lot of other studies.

CONCLUSION

The administration of *binahong* (*Anredera cordifolia* (Ten.) Steenis.) leaf extract for three days can decrease uric acid levels. *Binahong* extract's effectiveness in reducing uric acid levels is comparable to that of allopurinol, and it is even more effective at the third dose. The higher the *binahong* leaf extract dose administered, the bigger the reduction in uric acid levels.

Acknowledgment

We would like to thank the Orthopedic and Traumatology Laboratory, as well as the Orthopedic and Traumatology Specialist Medical Education Program, Faculty of Medicine, Syiah Kuala

University, Banda Aceh, Indonesia, for their assistance in carrying out this study.

Conflict of interest

None.

Ethical consideration

An ethical approval was obtained from the Research Ethics Committee, Syiah Kuala University, Banda Aceh, Indonesia (No. 225/KE/FK/2014 on 23/09/2014).

Funding disclosure

None.

Author contribution

PA was in charge of the study's design, data collection, and data analysis. SR made necessary intellectual revisions to the work and gave the final draft his approval for publication.

REFERENCES

Ablat A, Mohamad J (2018). The antioxidant and xanthine oxidase inhibitory activity of plumeria rubra flowers. Molecules 23**,** 400. doi: 10.3390/molecules23020400.

Asari H, Sugiyanta S (2021). Influence of administering watermelon rind water extract (*Citrullus vulgaris* Schard) on glucose level of male white rats (*Rattus norvegicus*) induced with streptozotosin. Folia Medica Indonesiana 56**,** 174. doi: 10.20473/fmi.v56i3.24528.

Astini DAAAS, Gunawan HA, Santoso RMWA, et al (2017). The effect of soursop leaf extract on pancreatic beta cell count and fasting blood glucose in male Wistar rats exposed to a high-fat diet and streptozotocin. Folia Medica Indonesiana 53**,** 12. doi: 10.20473/fmi.v53i1. 5484.

Astuti SD, Tirtana RD, Mahmud AF, et al. (2020). Ultraviolet (UV) activation effect on antibacterial agents of red betel (*Piper crocatum*) extract to Streptococcus mutans. Journal of Physics: Conference Series 1445**,** 012004. doi: 10.1088/1742- 6596/1445/1/012004.

Centers for Disease Control and Prevention (2020). Gout. CDC. [Web Page]

Dadiono MS, Andayani S (2022). Potensi tanaman *binahong* (*Anredera cordifolia*) sebagai obat alternatif pada bidang akuakultur. Jurnal Perikanan Pantura 5**,** 156. doi: 10.30587/jpp. v5i1.3769.

Darmawan E, Hidayati N (2020). The effect of celery and phyllantus nirury combination on uric acid level in hyperuricemic rats (thesis). Universitas Ahmad Dahlan. [Thesis]

Engel B, Just J, Bleckwenn M, et al (2017). Treatment options for gout. Deutsches Arzteblatt International 114, 215-22. doi: 10.3238/arztebl. 2017.0215.

George C, Minter DA (2022). Hyperuricemia. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2022PMID: 29083565. [Web Page]

Hamdan M, AS N, Rahayu M, et al (2019). Influence of *Ginkgo biloba* (Egb) extracts in apoptosis index and number of neurons at *Rattus novergicus* with lead (Pb) exposure. Research Journal of Pharmacy and Technology 12**,** 5883. doi: 10.5958/0974- 360X.2019.01020.5.

Kanbay M, Jensen T, Solak Y, et al (2016). Uric acid in metabolic syndrome: From an innocent bystander to a central player. European Journal of Internal Medicine 29**,** 3–8. doi: 10.1016/j.ejim. 2015.11.026.

Ladeska V, Pahriyani A, Gunawijaya MS (2018). The potency of *binahong* leaves (*Anredera cordifolia* (Ten.) Steenis) subfraction with ethanol 70% as an antihyperuricemic agent. In Proceedings of the 1st Muhammadiyah International Conference on Health and Pharmaceutical Development, pp. 143–6. SCITEPRESS - Science and Technology Publications. ISBN 978-989-758-349-0. doi: 10.5220/ 0008240801430146.

Maiuolo J, Oppedisano F, Gratteri S, et al. (2016). Regulation of uric acid metabolism and excretion. International Journal of Cardiology 213**,** 8–14. doi: 10.1016/j.ijcard.2015.08.109.

Manampiring AE (2010). Prevalensi hiperurisemia pada remaja obese di Kota Tomohon. Laporan Penelitian IPTEK dan Seni. Manado [Journal].

Prabawa AAGW (2019). Hubungan rasio lingkar pinggang panggul dengan kadar asam urat pada wanita menopause di GKJ Gondokusuman Yogyakarta (thesis). Universitas Kristen Duta Wacana. [Thesis]

Qurie A, Preuss CV, Musa R (2022). Allopurinol. In: StatPearls. Treasure Island (FL): StatPearls Publishing. PMID: 29763117. [Web Page]

Ragab G, Elshahaly M, Bardin T (2017). Gout: An old disease in new perspective – A review. Journal of Advanced Research 8**,** 495–511. doi: 10.1016/j.jare.2017.04.008.

Rohani S (2021). Isolation and characterization of wound healing compounds from chloroform extract of *binahong* leaves (*Anredera cordifolia* (Ten.) Steenis). Magna Medica Berkala Ilmu Kedokteran dan Kesehatan 8**,** 40. doi: 10.26714/magnamed.8.1.2021.40-59.

Sakti DS, Haresmita PP, Yuniarti N, et al (2019). Phagocytosis activity of *binahong* (*Anredera cordifolia* (Tenore.) Steenis) from Secang, Magelang, Central Java, Indonesia. Journal of Pharmaceutical Sciences and Community 16**,** 7–

13. doi: 10.24071/jpsc.001693.

Serrano JL, Figueiredo J, Almeida P, et al (2020). From xanthine oxidase inhibition to in vivo hypouricemic effect: An integrated overview of in vitro and in vivo studies with focus on natural molecules and analogues. Evidence-Based Complementary and Alternative Medicine 2020**,** 1–17. doi: 10.1155/2020/9531725.

Seth R, Kydd AS, Buchbinder R, et al (2014). Allopurinol for chronic gout. Cochrane Database of Systematic Reviews. doi: 10.1002/ 14651858. cd006077.pub3.