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Antihyperuricemia Potential in Ethanol Extract of Siamese Bamboo *(Thyrsostachys siamensis)* Leaves on Male White House Mice (*Mus musculus* L.)

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Abstract

Hyperuricemia is commonly treated with the synthesis drug allopurinol. Allopurinol lowers uric acid by inhibiting the enzyme xanthine oxidase but provides side effects on the skin and kidneys. In addition, it can be overcome with secondary metabolite compounds in plants. This compound is found in bamboo leaves that have long been used by Chinese people for treatment. This study aims to find out the ability of siamese bamboo leaf extract (Thyrsostachys siamensis) in lowering the uric acid levels of white house mice. Fine siamese bamboo leaves are extracted using 70% ethanol and then evaporated with a rotary evaporator. Obtained a viscous extract with a yield of 55.42% which was further analyzed the phytochemical content and obtained that siamese bamboo leaf extract contains alkaloids, flavonoids, saponins, tannins, and triterpenoids. The ability to reduce uric acid levels was tested in male white house mice (Mus musculus L) with six treatment groups, namely group I (without treatment), group II (negative control), group III (positive control), group IV (extract 5 mg / 20 g WW), group V (extract 10 mg / 20 g WW, group VI (extract 20 mg / 20 g WW) which had previously been adapted in the test cage environment. Tests of the activity of siamese bamboo leaf ethanol extract (Thyrsostachys siamensis) against the reduction of white house mouse uric acid showed the best percentage reduction at an extract dose of 5 mg / 20 g BB of 65.85%. This value does not differ much and is comparable to the decrease by allopurinol 0.78 mg / 20 g BB of 58.97%. Therefore, siamese bamboo leaves (Thyrsostachys siamensis) proved effective in lowering uric acid levels. This proves that siamese bamboo leaves can lower the uric acid levels of white house mice and have the potential to be used as a treatment material for hyperuricemia and is expected to provide information on the use of bamboo plants (Thyrsostachys siamensis) as a lowering of uric acid levels. The results of this uric acid reduction test can be used as a reference to determine how to consume bamboo leaf extract (Thyrsostachys siamensis) for the treatment or prevention of gout.

Keywords: *Hyperuricemia; Mus musculus L.; Siamese Bamboo; Thyrsostachys siamensis; Uric Acid*

Introduction

Hyperuricemia (uric acid disease), often known as gout, is still an issue in the medical community. Kidney failure and other gout-related problems, such as kidney stones, are still rather common. In Indonesia, the prevalence of gout is 6-13.6/100,000 individuals, and it is predicted to rise (Khasanah dkk., 2019). According to Riskesdas data in 2013, the prevalence of joint disease at the age of 75 years was 54.8%, age 65-74 years was 51.9%, and age 55-64 years was 45.0% (Fauzan & Yuli Kusumawati, 2017; Rusman, 2021).

Hyperuricemia is a condition where the level of uric acid in the blood exceeds the normal limit, with levels of 6.0 mg/dL for women and 7.0 mg/dL for men (Latief et al., 2021).). Consuming foods or fruits with a high-fat content influences how much uric acid is expended, which can contribute to hyperuricemia (Dwityanti et al., 2021). If left untreated, the accumulation of uric acid can lead to kidney stones and chronic renal disease, which can harm joints and soft tissues (Fitrya & Muharni, 2014; Hardian et al., 2014; Wahyuningsih et al., 2016).

The medication allopurinol is typically used to treat an increase in uric acid levels. This medication works by preventing the xanthine oxidase enzyme from doing its job, which lowers uric acid production. Allopurinol has side effects in the form of allergies to the skin, stomach, and intestines; blood disorders (Susanti, 2019), and acute interstitial nephritis (Florencia, 2020). Additionally, allopurinol can worsen kidney injury by raising the toxicity of several cytotoxic medications (Syfridiana, 2017). Numerous studies have been conducted to look for and identify alternative antihyperuricemic medications with minimum side effects.

According to Rasyad and Erjon, pineapple extract can reduce uric acid levels in male white house mice by 34.2% on a percentage basis. When compared to the 38.16% drop in the use of the medicine allopurinol, the percentage decrease is stated to be less effective (Rasyad et al., 2019). According to Aini and Marwan's research, onion extract may be able to reduce uric acid levels, however, it does so less effectively than the allopurinol drug. In a group test using onion extract, the average blood uric acid level in test animals was 10.60±1.12; in a group test using allopurinol, it was 11.32±1.46 (Aini et al., 2020). Both of these natural ingredients contain flavonoid compounds that can inhibit the work of the xanthine oxidase enzyme. Xanthine oxidase has a role in catalyzing hypoxanthine into xanthine. Xanthine will then become uric acid so that in the presence of flavonoid compounds it can inhibit uric acid production (Gunarti et al., 2021). Both of these natural ingredients have the potential to reduce uric acid levels, but their effectiveness is below that of allopurinol. Therefore, it is necessary to research natural ingredients that are more effective than allopurinol in reducing uric acid.

According to research by Klinjapo (2020), the leaves of Pai Ruak bamboo (Thrsostachys siamensis GAMBLE), which is still in the same genus as Siamese bamboo *(Thyrsostachys siamensis)*, contain active substances in the form of flavonoids (orientin, isoorientin, vitexin, and isovitexin). Flavonoids, lactones, and phenolic acids are present in the active chemicals found in Pai Ruak bamboo leaves. The substances in bamboo leaves have effects that can reduce the body's uric acid levels (Kurniawati, 2017). according to Kamonwannasit et al (2019), Siamese bamboo leaves include secondary metabolites, including flavonoids, saponins, and tannins, and have a DPPH radical scavenging action with a value of 503 µg/mL. However, there has been no research that has tested the activity of Siamese bamboo leaf *(Thyrsostachys siamensis)* extract in decreasing uric acid levels. Therefore, researchers were interested in researching the potential of Siamese bamboo leaf *(Thyrsostachys siamensis)* extract to decrease uric acid levels in white house mice *(Mus musculus L)*.

Research methods

Materials and tools

The materials used in this study included 70% ethanol, MERCK glacial acetic acid for analysis, 98% H_2SO_4 MERCK

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for analysis, 30% MERCK methanol for analysis, MERCK concentrated HCl for analysis, MERCK chloroform for analysis, ammonia, Mayer's reagent, Na-CMC SIGMA-ALDRIG, chicken liver, allopurinol 300 mg HEXPHARM, fine Siamese bamboo leaves taken from the Tanah Sareal District, Bogor City, as much as 80.3 grams, Siam bamboo leaf extract, house mouse food, and a strip measuring uric acid. The tools used were an electric heater, drip plate, cage for white house mice *(Mus musculus L)* with Pure Line DDY strain male 4 weeks old and weighing ± 22–24 grams, as many as 18 house mice, glass utensils, cholesterol measuring device Easy Touch GCU brand, heating coat, scissors, syringe, and gloves.

Identification and Determination of Siamese Bamboo (Thyrostachys siamensis)

Fine Siamese bamboo leaves were taken from the Tanah Sareal District, Bogor City. The determination of them was carried out to ensure that the sample was used as research material. This determination is carried out by sending parts of leaves, stems, branches, leaf shoots, and plant roots to the Biological Research Center of the Indonesian Institute of Sciences (LIPI) (Puspitaningrum et al., 2017).

Extract Making

Fresh Siamese bamboo leaves had been cleaned and dried in an oven at 70ºC for 4 hours. It gained 80.3 grams of dried Siamese bamboo leaves then were mashed using a blender and sieved through a 100-mesh sieve. Fine Siamese bamboo leaves were ready for extraction.

Extracts were made by the maceration method. The sample was macerated in 485 mL of 70% ethanol for 48 hours. The extracts were separated by filtration and the resulting filtrate was concentrated with a rotary evaporator at a temperature of 50ºC. Then it was proceeded with evaporation in a bath to produce a thick crude extract

Preliminary Analysis (Harborne, 2013)

Preliminary analysis was in the form of qualitative phytochemical analysis. This analysis was conducted to determine the active compounds contained in the extract of Siamese bamboo leaves. Phytochemical analysis was carried out based on the method (Harborne, 2013). The compounds identified were alkaloids, flavonoids, saponins, steroids/triterpenoids, and tannins.

Identification of flavonoids was carried out using the cyanidin test. 0.1 grams of extract sample were heated for 5 minutes in 5 mL of 30% methanol. The filtrate was added along with a few drops of concentrated HCl and magnesium powder. The formation of a red color indicated the presence of flavonoid compounds.

Identification of alkaloids and steroids/ triterpenoids was carried out using the Culvenor-Fitzgerald method. A total of 4 grams of extract samples were finely chopped and then ground with the addition of chloroform solvent to form a paste. After forming a paste, 10 mL of 0.05 N ammonia was added and ground again. Then it was filtered into a test tube. H_2SO_4 2 N was added and shaken vigorously. The filtrate was allowed to stand until two layers were formed (the chloroform and the sulfuric acid layers). The two layers were separated using a dropper. The sulfuric acid layer was then used for the identification of alkaloids by adding Wagner's reagent. If the addition of Wagner's reagent forms a brown precipitate or the solution became turbid, it was positive containing alkaloid compounds. The chloroform layer was used to identify the presence of steroid and triterpenoid compounds. The chloroform layer formed was transferred to a drip plate, and five drops of anhydrous acetic acid were added and waited to dry. After drying, three drops of H_2SO_4 were added. If a red or purple color was formed, it was positive containing a triterpenoid. If it was blue, it was positive containing a steroid.

An alkaline test was carried out to confirm the identification of Saponin. The dried sample of fine bamboo leaves was put into a test tube and added to distilled water. Then it was heated at 100°C for three minutes and cooled. After that, shake

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vigorously for a minute. The foam formed as high as not less than 1 cm and remained stable after being allowed to stand for 15 minutes. It was indicating the presence of saponins.

Identification of tannins was done by adding 0.1 grams of extract to 5 mL of distilled water in a test tube. Then simmer for 5 minutes. After boiling, the residue and the filtrate were separated by filtration. A 1% $FeCl_3$ solution was added to the resulting filtrate. If a dark blue or blackish green color were formed, it indicated the positive presence of tannins.

Test Solution Making

Making a Na-CMC 0,5% b/v suspension was done by sprinkling 0.5 grams of Na-CMC on 7.5 mL warm distilled water, then ground until homogeneous and diluted to 100 mL with distilled water (Hidayah et al., 2018).

Calculation of the dose of allopurinol with a conversion dose for humans weighing 70 kg in white house mice weighing 20 g was 0.0026. The dose of uric acid for humans was 300 mg/day. The dose was converted to body weight (WB) of 20 g because the white house mice used weighed 22–24 g. the dose conversion from human to the white rat:

White house mouse dosage = 300 mg x 0.0026
= 0.78 mg/20g BW

The volume of adding 0.001% allopurinol suspension to white house mice was 20 g per mL of suspension containing 1 mg of allopurinol.

Volume= (0.78 mg)/(1 mg) ×1 mL = 0.78 mL

The making of 0.001% b/v allopurinol suspension was done by grinding 1 allopurinol tablet until smooth. Meanwhile, 0.5 grams of Na-CMC were sprinkled on 7.5 mL of warm distilled water, and crushed until homogeneous. Then 1 mg of allopurinol was added little by little and ground again until homogeneous. It was diluted and calibrated by using distilled water until the volume of the solution was 100 mL.

Induction Solution Making. The induction solution used was chicken liver juice. Chicken liver juice was made by mashing 0.2 grams of chicken liver and adding 100 mL of distilled water.

Making a 5% b/v extract solution was carried out by sprinkling as much as 0.5 grams of Na-CMC over 7.5 mL of warm distilled water, then adding 5 grams of thick extract. crushed them until homogeneous, then added 50 mL of distilled water, moved it to a 100 mL volumetric flask, and calibrated with distilled water (Latief, et al., 2021).

Uric Acid Testing on White House mice (Latief, et al., 2021)

This study used white house mice *(Mus musculus L)* with a male DDY Pure Line strain aged 4 weeks and a body weight of ± 22–24 grams as many as 18 house mice. White house mice were grouped randomly into 6 groups. Each group consisted of 3 white house mice. White house mice were conditioned in glass plastic cages measuring 13 cm x 7.5 cm x 12 cm for 7 days. Then they fasted for 12 hours, but the white house mice were still given water. Initial blood was taken from the tail vein in each house mouse as a pre-test measurement of uric acid levels by using a glucometer and a uric acid strip test.

Measurement of uric acid levels was carried out after treatment (posttest) with three repetitions. The breakdown of the groups was as follows:

Group I : No treatment.
Group II : Negative controls were given Na-CMC suspension and chicken liver juice.
Group III : Positive controls were given chicken liver juice as an inducer of uric acid and then treated with allopurinol.
Group IV : extract group with a dose of 5 mg/20 g BW. White house mice were given chicken liver juice as a uric acid inducer and then treated with bamboo leaf extract at a dose of 5 mg/20 g BW.

Group V : Extract group with a dose of 10 mg/20 g BW. White house mice were given chicken liver juice as an inducer of uric acid and then treated with bamboo leaf extract at a dose of 10 mg/20 g BW.

Group VI : Extract group with a dose of 20 mg/20 g BW. White house mice were given chicken liver juice as an inducer of uric acid and then treated with bamboo leaf extract at a dose of 20 mg/20 g BW

Results and Discussion

Table 1. Results of Phytochemical Analysis of Ethanol Extract of Siamese Bamboo Leaves

No.	Tests	Observation	Rseult
1.	Alkaloids	A brown precipitate was formed when Wagner's reagent is added.	+
2.	Flavonoids	There was a red color change after the addition of magnesium powder.	+
3.	Steroids	A purplish-red color was formed after the addition of concentrated sulfuric acid.	-
4.	Triterpenoids	A purplish-red color was formed after the addition of concentrated sulfuric acid.	+
5.	Saponins	Formed a stable foam for 5 minutes	+
6.	Tannins	formed a blackish-green color after the addition of 1% $FeCl_3$	+

Table 2. Average of Decrease Uric Acid Level (mg/dL) on white house mice

Treatment Group	Number of House mice	Average Initial Uric Acid Level (mg/dL)	Average Induced Uric Acid Level (mg/dL)	Average Final Uric Acid Level (mg/dL)	Percentage of Decreased Uric Acid Level (%)
No treatment	3	4.3±0.47	3.5±0.31	4.4±0.42	-
Negative control (Na-CMC)	3	3.2±0.30	6.5±0.52	8.5±0.4A5	-
Positive Control (Allopurinol)	3	LO	11.1±0.40	4.5±0.15	58.97±2.83[a]
Ethanol extract of bamboo leaves 5 mg/20 g BB	3	3.5±0.51	9.2±0.31	3.1±0.25	65.85±1.61[ab]
Ethanol extract of bamboo leaves 10 mg/20 g BB	3	LO	10.9±0.15	4.2±0.25	61.67±1.96[b]
Ethanol extract of bamboo leaves 20 mg/20 g BB	3	3.1±0.31	8.8±0.26	LO	>

Siamese bamboo leaves *(Thyrsostachys siamensis)* were determined to find out whether the sample used was actual Siamese bamboo leaves. The determination of Siamese bamboo leaves was carried out at the Herbarium Bogoriense for Botany, Biology Research Center, LIPI Bogor with the number 22.59/IPH.1.01/If.07/IX/2018. The results showed that the sample of Siamese bamboo leaves used was real and actual

Siamese bamboo leaves (Thyrsostachys siamensis Gamble) from the Poaceae family.

The extract of Siam bamboo leaves *(Thyrsostachys siamensis)* was obtained from maceration for 24 hours with 70% ethanol as the solvent. 70% ethanol solvent is a magic solvent because it can react with polar, semipolar, and non-polar materials. This solvent was expected to attract all the compounds contained in Siamese bamboo leaves, especially the content of flavonoid compounds, which are known to act as antihyperuricemia. The use of ethanol as a solvent is known to be safe enough to extract a material that will be used as a drug ingredient (Latief et al., 2021). The thick extract obtained was 44.5 g, with the yield value of the Siam bamboo leaf extract obtained at 55.42%.

The thick extract obtained was then analyzed for the content of secondary metabolites through a phytochemical analysis test. Phytochemical analysis of the ethanolic extract of Siamese bamboo leaves *(Thyrsostachys siamensis)* included six types of tests, namely alkaloids, flavonoids, steroids, triterpenoids, saponins, and tannins. Based on the test results, it was found that the ethanolic extract of bamboo leaves contained alkaloids, flavonoids, triterpenoids, saponins, and tannins (Table 1).

The presence of alkaloid compounds was indicated by the formation of a white precipitate during the test. Flavonoids showed positive results because during the test there was a color change to red in the test solution after adding magnesium powder. The presence of triterpenoids was indicated by the appearance of red color in the chloroform phase as a result of the separation of the alkaloid test when concentrated sulfuric acid was added. The presence of a stable foam for approximately 5 minutes indicated the presence of saponins. The results of the phytochemical analysis of the ethanol extract of Siamese bamboo leaves *(Thyrsostachys siamensis)* can be seen in Table 1.

Siamese bamboo leaf extract *(Thyrsostachys siamensis)* as an antihyperuricemia was able to reduce blood uric acid levels in white house mice. The secondary metabolites contained in the Siamese bamboo leaves combined to inhibit the oxidation reaction by xanthine oxidase. Alkaloids, especially indole, were able to stop radical chain reactions. Flavonoids, which are polyhydroxy compounds, have properties that are very easily oxidized so that they can prevent oxidation by xanthine oxidase. Prevention of oxidation reactions occurred by transferring an electron (Anggraito et al., 2018).

Protection by tannins occurred because of the ability of tannins to donate hydrogen in the neutralization reaction that initiated the oxidation process by xanthine oxidase or at the termination of the chain reaction that occurred. Tannin is an antioxidant reserve that can increase the antioxidant capacity of other tissues. While saponins have an antioxidant effect by forming hydroperoxides as secondary antioxidants, thereby increasing the antioxidant ability of other components (Zuraida et al., 2017). The results of decreased uric acid levels in white house mice can be seen in Table 2.

The determination of initial uric acid levels was carried out to know the uric acid levels of male white house mice before the process of induction of uric acid levels with chicken liver juice. In that treatment, there was LO data in treatments III and V. LO means the initial uric acid level <3 mg/dL. Based on the induction treatment, it was shown that giving chicken liver juice for 14 days could increase blood uric acid levels in male white house mice. Chicken liver has a high purine content, which can be used as raw material for uric acid formation. Giving ethanol extract from Siamese bamboo leaves for 7 days can decrease uric acid levels in hyperuricemic house mice. The best percentage decrease in uric acid levels was in the treatment IV group (65.85%).

The value of the percentage decrease in uric acid levels was analyzed using the One-Way ANOVA test, which had previously been tested for homogeneity and normality tests. Data on the percentage decrease in uric acid levels showed normal and homogeneous results, where the normality test data

obtained sig values of 0.928 > 0.05. And the homogeneity test showed the value of sig. 0.699 > 0.05.

The results of the Anova table on the percentage of decrease in uric acid levels indicated that there was a significant difference between each treatment group because of the sig. value obtained 0.000 < 0.05. It can be concluded that there was an effect of giving ethanol extract from Siamese bamboo leaves to decrease uric acid levels.

The data analysis was continued through the Tukey test. The results obtained showed that there was a significant difference between the positive control group and the treatment group V (10mg/20g BW). Treatment group IV (5mg/20g BW) did not differ significantly from the allopurinol-positive control group, and treatment group IV (10mg/20g BW) did not differ significantly from the treatment group V (10mg/20g BW). Therefore, it can be concluded that treatment groups IV and V ethanol extract from Siamese bamboo leaves can decrease uric acid levels to levels that are comparable to allopurinol-positive control groups. But, the highest concentration of decrease was in group IV with a percentage of 65.85%.

Allopurinol is a drug used to treat gout. Allopurinol reduces uric acid levels by inhibiting the xanthine oxidase enzyme (Brunton et al., 2006; Trevor et al., 2010).

Based on the data in Table 2 above, the untreated group showed unstable uric acid levels. The initial measurement to the final measurement did not show a clear increase or decrease. The negative control group showed an increase in the average level of uric acid. It showed that the addition of Na-CMC did not affect uric acid levels in house mice. The increase in uric acid levels occurred due to the addition of an induction solution using chicken liver juice. Chicken liver juice supplies purines into the rat's body, which is then metabolized into uric acid.

The Group VI ethanol extract of Siamese bamboo leaves at a dose of 20 mg/20 g BW resulted in a decrease in uric acid levels that could not be calculated because the final measurement obtained LO (Low) data. The results of the LO measurement showed that the uric acid level in the house mouse blood was below the tolerance level that could be measured by the Easy Touch GCU tool. Although the decrease could not be calculated, it can still be concluded that there was a decrease in uric acid levels because the induced uric acid level was 8.75 mg/dL, while at the final measurement after being given the extract, the LO results were obtained.

The average uric acid level in the untreated group gave unstable results. The possibility of this instability was due to the amount of consumption of different house mice (uncontrolled feeding). The results obtained indicated that the use of chicken liver juice as a solution to induce uric acid was effective in giving the effect of hyperuricemia. It was shown by an increase in uric acid levels in the negative control. Apart from giving chicken liver juice, the increase in uric acid may also be influenced by the food given containing substances that can affect uric acid levels. Apriansyah (2011), Wardatun & Rahmi (2012), and Restina & Effendi (2020) stated that there was one compound that can induce an increase in uric acid better than chicken liver juice. The compound is potassium oxonate. Potassium oxonate is a compound that can inhibit the uricase enzyme or oxidase. Uricase enzymes play a role in breaking down uric acid into allantoin, which is soluble in water so that it is easily excreted through urine (Restina & Effendi, 2020). Inhibition of the uricase enzyme causes the breakdown of uric acid into allantoin, which accumulates in the body, causing hyperuricemia.

The ethanol extract group of Siamese bamboo leaves at a dose of 5 mg/20 g BW and a dose of 10 mg/20 g BW resulted in a decrease that was not much different when compared to the decrease that occurred in the positive control group. While the extract dose of 20 mg/20 g BW showed a decrease that could not be calculated, the resulting decrease was quite high. It showed that the decrease in uric acid levels in house mice

was not directly proportional to the dose given. Based on the results of the study, it can be concluded that the ethanol extract from Siamese bamboo leaves is effective in decreasing uric acid levels in house mice and Siamese bamboo leaves can be used as an ingredient to decrease uric acid levels. The activity test of the ethanol extract of Siamese bamboo *(Thyrsostachys siamensis)* leaves on decreasing house mouse uric acid showed the best percentage at a dose of 5 mg/20 g BW of 65.85%. This value was not much different and comparable to the decrease by allopurinol of 0.78 mg/20 g BW of 58.97%. Therefore, the leaves of Siamese bamboo *(Thyrsostachys siamensis)* have been proven to be effective in decreasing uric acid levels and have the potential as an antihyperuricemia

Conclusions and suggestions

Conclusion

Based on the phytochemical analysis, it was found that the ethanol extract of Siamese bamboo leaves *(Thyrsostachys siamensis)* contained secondary metabolites in the form of alkaloids, flavonoids, triterpenoids, saponins, and tannins. The activity test of the ethanol extract of Siamese bamboo *(Thyrsostachys siamensis)* leaves on decreased the house mice uric acid showed the best percentage at a dose of 5 mg/20 g BW of 65.85%. This value was not much different and comparable to the decrease by allopurinol of 0.78 mg/20 g BW of 58.97%. Therefore, the leaves of Siamese bamboo *(Thyrsostachys siamensis)* have been proven to be effective in decreasing uric acid levels and have the potential as an antihyperuricemia.

Suggestion

This research is a preliminary study limited to knowing the ability of Siamese bamboo leaf extract as an antihyperuricemia. Hopefully, further research can be carried out in the future to be able to isolate and identify compounds that have an important role in antihyperuricemia.

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