

THE EFFECTIVENESS OF SALAM LEAF EXTRACT (*Syzygium polyanthum*) TEST IN REDUCE URIC ACID LEVELS IN MALE WHITE RATS (*Rattus novergicus*)

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ABSTRACT

This study aims to determine whether there is a therapeutic effect on bay leaf extract (*Syzygium polyanthum*) in reducing uric acid levels in male white rats (*Rattus novergicus*) and to determine what dose of bay leaf extract (*Syzygium polyanthum*) is effective in reducing uric acid levels in male white rat (*Rattus novergicus*).

The method in this study used Laboratory Experiments with Pretest-Posttest Control Group Design, the treatment groups were group I (negative control), group II (positive control), group III (bay leaf extract 0.4581 g), group IV (extract bay leaf 0.8181 g), and group V (bay leaf extract 1.1781 g).

The results showed that bay leaf extract (*Syzygium polyhantum*) was positive for flavonoid compounds. Statistical data analysis by Kruskal Walis showed that 0.003 p significance value 0.005 means that there is a significant difference between uric acid levels before administration of bay leaf extract and uric acid levels after administration of bay leaf extract and continued with Duncan's analysis to see differences in effective doses.

Keywords: Bay leaf extract (*Syzygium polyhantum*), Flavonoids, Uric Acid.

INTRODUCTION

Uric acid or known as (gout). Gout is a non-communicable disease caused by changes in life habits. Gout is a degenerative disease that attacks the joints, and is most often found in the community, especially experienced by the elderly (elderly). However, this disease is also often found among adolescents to adults [3].

Gout is a disorder that occurs in the joints due to the accumulation of purine substances in the body.

The solubility of uric acid is strongly influenced by the pH of the urine. At pH 5.0, urine can dissolve uric acid, at levels of 6-15 mg/dl. Uric acid will be ionized as urate and is abundant in blood plasma,

then becomes monosodium urate at pH 7.4. Blood plasma becomes saturated with monosodium urate concentration of 6.8 mg/dl at 37°C.

Foods that contain purines are found in green foods, for example, kale, green spinach, long beans, and other green plants.

The mechanism of uric acid formation can be initiated by the formation of uric acid from protein degradation into amino acids.

The formation of uric acid is influenced by the synthesis and breakdown of purine nucleotides that occur in the liver. Purines are proteins belonging to the nucleoprotein group, which come from food and the

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destruction of old body cells. Purines are the result of protein metabolism that can form uric acid.

Over time, the number of gout sufferers tends to increase. Gout can be found all over the world, in all human races. The prevalence of gout tends to enter a younger age, namely productive age which has an impact on decreasing work productivity. With the high prevalence of gout in the elderly, it causes gout to rank second after hypertension. In the city of Gorontalo, the prevalence of gout is divided by age. In 2014, cases of gout at the age of 45 years to age > 70 years were 394 cases. In 2015 there were 353, based on the results of the epidemiological survey, the prevalence of gout in men was 24.3% and women 11.7%.

Men do not have the hormone estrogen that can help remove uric acid, while women have the hormone estrogen which helps eliminate uric acid through urine [1].

Generally, to treat gout, synthetic drugs such as allopurinol are used. Allopurinol works by inhibiting the formation of uric acid through the formation of xanthine oxidation enzymes. This drug has side effects such as allergic skin reactions, headaches, liver, kidney damage, digestive tract disorders such as nausea, vomiting, and diarrhea. Given the many side effects caused by synthetic drugs, the tendency of people to use traditional medicinal plants [4].

The part of the bay plant that is always used is the leaf. The leaves are often used in the community as a spice in the kitchen as well as a complement to cooking bay leaves can also be used as a medicine for diarrhea, diabetes, itching, indigestion and stomach weakness.

The benefits of bay leaf for health are known as a plant that is often used by people as alternative medicine.

Bay leaves (*Syzygium polyanthum*) which have the potential to reduce uric acid levels are flavonoid compounds that can inhibit the enzyme xanthine oxidase in the formation of uric acid.

There are many traditional medicinal plants used as treatment. One of the plants used as traditional medicine to reduce uric acid levels is bay leaf (*Syzygium polyanthum* Wight). The part of the plant used is the leaves that are still fresh or that have been dried. Bay leaves have various medicinal properties that can be used in everyday life. In addition to treating gout, bay leaves can also be used as a medicine for high cholesterol, diabetes (diabetes mellitus), high blood pressure (hypertension), stomach ulcers (gastritis), and diarrhea. Salam plants contain tannins, flavonoids, alkaloids, and essential oils consisting of citrate and eugenol.

The mechanism of action of bay leaves can interfere with the work of the enzyme hypoxanthine, as a result, the formation of uric acid can be inhibited. The content of flavonoid secondary metabolite compounds serves to inhibit the work of xanthine oxidation enzymes. Flavonoids also have antioxidant activity, besides that flavonoids also function as anti-inflammatory, antiplatelet, and antitumor. Flavonoids are polar, because flavonoids bind to sugars, namely glycosides. So flavonoids are soluble in polar solvents [13].

Secondary metabolites such as flavonoid compounds are a class of secondary metabolites that are most commonly found in plants.

Flavonoid compounds are widely distributed in plant parts both in the roots, stems, leaves, fruit, and seeds, so that flavonoid compounds are also unconsciously found in daily food supplements.

Flavonoids are bound to the form of glycosides, so the homogeneity of the

above solvent using water is a good solvent for flavonoid glycosides.

While in the form of aglycones such as flavones, flavonols, flavanones are more homogeneous in chloroform and ether solvents. There are several derivatives based on flavonoid compounds such as flavanols, flavanones, flavones, isoflavones, anthocyanidins, and flavonols.

Flavonoids have a number of hydroxyl groups so that flavonoids are polar compounds. Flavonoids are polar compounds, so they will dissolve well in polar solvents such as ethanol, methanol, butanol, acetone, dimethylformamide and others.

The content of flavonoids which are believed to play a role in reducing uric acid levels in the blood caused by flavonoids can damage the action of the xanthine oxidase enzyme so that the formation of uric acid is inhibited.

The reason the researchers carried out this study was because the number of bay leaf (*Syzygium polyanthum*) plants that were not known to the public that its properties could reduce uric acid levels, the researchers were interested in conducting research "Test the effectiveness of bay leaf extract (*Syzygium polyanthum*) in lowering uric acid levels in male white rats. (*Rattus norvegicus*).

RESEARCH METHODS

The type and design used in this research is a type of laboratory experimental research, which is descriptive quantitative. The research design used is Pretest-Posttest Control Group Design,

The data collection techniques used in this study are analytical balance (Precisa®), a series of evaporator (Heidolf®), blender, 500 ml beaker (Pyrex), 1000 ml measuring cup (Pyrex), test tube (Pyrex)), oven, waterbath, NESCO (GCU), horn spoon, stir bar,

porcelain dish, mortar, stamper, and tube rack. Ingredients used Bay leaf (*Syzygium polyanthum*), Mask, Handsocon, Chicken liver juice, Allopurinol 100 mg, Ethanol 70%, Aquades, Uric acid test strip, Sonde rat, Aluminum foil, NaOH Pellet pet food, Watch glass, Alcohol sterile, mortar and pestle. Research preparation begins with preparing a complete rat cage along with a place for feeding and drinking, preparing experimental animals, namely 20 male white rats and adapting them to each treatment cage, Weighing the initial body weight of the animals, and preparing salan leaf extract for treatment rats. The test animals were divided into 5 groups, namely group 1 as a negative control, group 2 as a positive group, and groups 3, 4 and 5. in cages and given standard feed and adequate drinking water. Prior to treatment, the uric acid levels were first measured and considered as initial uric acid levels. Previously, adjustments were made in the cage for 7 days, placed in the cage and given standard feed and adequate drinking water. Prior to treatment, the uric acid levels were first measured and considered as initial uric acid levels. Previously, adjustments were made in the cage for 7 days, placed in the cage and given standard feed and adequate drinking water. Prior to treatment, the uric acid levels were first measured and considered as initial uric acid levels.

Sampling technique Samples in the form of bay leaves (*Syzygium polyanthum*) were obtained in Manunggal Karya Village, Randangan District, Pohuwato Regency, Gorontalo Indonesia. The sample is cleaned of dirt (wet sorting), a purification process is carried out to remove dirt, the sample is cut and then dried in the open with the help of sunlight. The dried and discolored samples were sorted dry and then mashed until the sample turned into a fine powder

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ready for extraction. Simplicia in the form of bay leaf powder (*Syzygium polyanthum*) was macerated using 70% ethanol solvent for 1 x 24 hours at room temperature. Bay leaf extract (*Syzygium polyanthum*) was obtained by filtering using a white cloth. The viscous extract was obtained by evaporating the ethanol extract using a rotary evaporator.

The dose of chicken liver which is considered to be able to increase uric acid levels based on the results of research conducted by (Nasrullah et al, 2015) is 5 ml/200 g body weight.

- In test sample 1 used bay leaf extract (*Syzygium polyanthum*) as much as 0.4581 gr.
- In test sample 2 used bay leaf extract (*Syzygium polyanthum*) as much as 0.8181 gr.
- In test sample 3 used bay leaf extract (*Syzygium polyanthum*) as much as 1.1781 gr.

The dose of allopurinol in humans is 100 mg / 70 kg of human body weight and the dose to be given to experimental animals is 1.8 mg for 200 gr male white rats.

Treatment of test animals A total of 20 tails were divided into 3 groups Induced with chicken liver juice as much as 5 ml/200 gr BW of male white rats of Wistar strain given 2 times to increase uric acid levels. Then the uric acid levels were measured after the 7th day, then on the 8th day the uric acid levels of the test animals were checked. In this study blood was taken through the tail vein (coccygeal vein). Rat tails were disinfected with 70% alcohol and allowed to dry. The tail vein was slashed with a small size, the first blood that came out of the rat's tail was removed then the blood was then dripped on the uric acid test strip with the NESCO GCU tool and waited for 20 seconds, then the uric acid level was measured.

Normal uric acid levels in white male rats of the Wistar strain were 4.37 mg/dl, while in female rats it was 2.92 mg/dl [7].

The data analysis technique used in this research is quantitative descriptive analysis. The data that has been obtained from the results of the study will then be analyzed statistically using SPSS version 16. The first data processing carried out is the prerequisite test, namely the normality test and homogeneity test to determine whether the data is normally distributed and homogeneous. The next test that was carried out was the 1-way ANOVA test or One Way Anova to determine whether there was an effect of the various treatments that had been given the One Way Anova further test, namely the Duncan test. If there is no effect in the ANOVA test, then it is continued with a non-parametric test, namely the KRUSKAL WALIS test. The further test carried out is the Mann-Whitney test to determine 2 samples or the two are not related.

RESEARCH RESULT

Phytochemical Screening

Flavonoid test on bay leaf extract (*Syzygium polyhantum*) using NaOH solvent, showed a color change to red, which can be seen in table 1.

Table 1. Phytochemical screening results

Compound	Method	Reactor	Test results	Note:
Flavonoids	1 ml NaOH	NaOH 12%	Red	(+)

Source: Processed data (2021)

Measurement of the initial level of uric acid

Based on the initial measurement of uric acid levels in white rats, the results shown in table 2 with different weights of white rats were obtained.

Table 2. Results of measurement before giving chicken liver juice

Group	Repetition	BB Mouse (g)	Results of Uric Acid Levels (mg/dL)
1	1	122	7.5

(Negative Control)	2	124	5.3
	3	128	12.3
	4	125	4.1
2 (Positive Control)	1	129	4.7
	2	136	9.7
	3	137	12.4
3 (Extract 0.4581 gr)	4	136	5.7
	1	141	7.1
	2	157	6.5
4 (Extract 0.8181 gr)	3	159	5.0
	4	160	6.0
	1	159	9.2
5 (Extract 1.1781 gr)	2	160	5.0
	3	172	14.0
	4	145	11.6
	1	176	4.8
	2	176	4.0
	3	165	10.0
	4	168	6.5

Source: Processed data (2021)

Giving Chicken Liver Juice

The provision of chicken liver juice was adjusted to the body weight of each white rat sample, so the results of uric acid levels showed that each white rat experienced an increase in uric acid levels. This can be seen in table 3.

Table 3. Results of measurement after giving chicken liver juice

Group	Repetition	BB Mouse (g)	Amount of Chicken Liver Juice (ml)	Results of Uric Acid Levels (mg/dL)
1 (Negative Control)	1	122	3.0	8.9
	2	124	3.1	5.6
	3	128	3.2	15.3
	4	125	3.1	6.0
2 (Positive Control)	1	129	3.2	6.6
	2	136	3.4	12.5
	3	137	3.4	14.0
	4	136	3.4	8.9
3 (Extract 0.4581 gr)	1	141	3.5	10.4
	2	157	3.9	11.1
	3	159	3.9	6.5
	4	160	4	7.9
4 (Extract 0.8181 gr)	1	159	3.9	10.8
	2	160	4	6.9
	3	172	4.3	16.1
	4	145	3.6	20.0
5 (Extract 1.1781 gr)	1	176	4.4	14.3
	2	176	4.4	6.2
	3	165	4.1	13.6
	4	168	4.2	8.5

Source: Processed data (2021)

Giving Bay Leaf Extract (*Syzygium polythantum*)

Based on Table 4, it can be concluded that there is a significant effect of bay leaf extract (*Syzygium polythantum*) on reducing uric acid levels. This is indicated by a significance value of $p < 0.005$, which means that there is a significant difference between uric acid levels before administration of bay leaf extract and uric acid levels after administration of bay leaf extract.

Table 4. Effect of bay leaf extract (*Syzygium polythantum*)

Group	Repetition	Results of Uric Acid Levels Before (mg/dL)	Results of Uric Acid Levels After (mg/dL)	Drop Amount Gout (mg/dL)	Sig (p)
1 (Negative Control)	1	8.9	7.0	↓	0.003
	2	5.6	11.4	↑	
	3	15.3	5.6	↓	
	4	6.0	4.2	↓	
2 (Positive Control)	1	6.6	5.2	↓	
	2	12.5	6.6	↓	
	3	14.0	6.5	↓	
	4	8.9	4.7	↓	
3 (Extract 0.4581 gr)	1	10.4	7.1	↓	
	2	11.1	6.6	↓	
	3	6.5	4.0	↓	
	4	7.9	5.3	↓	
4 (Extract 0.8181 gr)	1	10.8	6.6	↓	
	2	6.9	5.2	↓	
	3	16.1	11.6	↓	
	4	20.0	10.0	↓	
5 (Extract 1.1781 gr)	1	14.3	10.9	↓	
	2	6.2	4.0	↓	
	3	13.6	7.0	↓	
	4	8.5	6.6	↓	

Source: Processed data (2021)

DISCUSSION

Bay leaf plant (*Syzygium polythantum*), is one type of spice that is not foreign to the public. The bay leaf plant itself functions as a flavoring and complementary ingredient in cooking because it has a very distinctive aroma. However, apart from being used as a cooking ingredient, bay leaves are also believed to be a traditional medicine for health maintenance [1].

Secondary metabolites present in bay leaf plants are flavonoids, alkaloids, and essential oils.

Bay leaves are used as traditional medicine by the community, one of which is lowering uric acid levels.

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The sample used is bay leaf (*Syzygium polyhantum*) to get a dry sample, it must go through a drying process after which it is mashed using a blender, the result of the dry sample obtained is 2 kg. Dry samples were extracted using maceration extraction method with 70% ethanol as solvent.

Extraction is a method used in the process of separating a component according to a mixture using several solvents.

The principle of the extraction method is mass transfer of the substance components into the solvent, where the displacement begins to occur in the interfacial layer and then diffuses into the solvent.

The extraction process can be done by maceration, usually for fresh samples of 95% ethanol while the dry ones use 70% ethanol.

70% ethanol solvent is more effective to get the optimal amount of active substance, where molds and bacteria are very small to enter directly into the extraction liquid. The maceration extraction method has the advantage of ensuring that the active substance of the extracted sample is not damaged. The maceration process is carried out 1 x 24 hours to get the results of the liquid extract, the longer the maceration time, the contact between the solvent and the sample will increase the number of secondary metabolites and active ingredients that will be dissolved. The extraction yield obtained was 1.642 ml, the yield obtained in the liquid extract was 82.1%. After the maceration process is carried out, the evaporation process is carried out to obtain a thick extract. The result of the thick extract obtained is 12 grams [12].

The procedure is repeated once or twice using fresh solvent. This method obtains an imperfect extract according to the desired compound.

The content of bay leaves (*Syzygium polyhantum*) has properties, namely to treat gout disease by decoction of bay leaves (*Syzygium polyhantum*), ethanol extract from bay leaves (*Syzygium polyhantum*) can reduce uric acid levels in the blood [7].

Bay leaves contain alkaloids, saponins, steroids, phenolics, and flavonoids. The ethanol extract of bay leaves contains flavonoids and phenols. It is known that the content of flavonoid compounds is 14.87 mg equivalent to quercetin/100 g.

Examination by qualitative analysis with phytochemical screening test that in bay leaves (*Syzygium polyhantum*) positive contains flavonoid compounds. The main compounds found in bay leaves (*Syzygium polyhantum*) are flavonoid compounds.

The test animals used in this study were male white rats of healthy wistar strain aged 2-3 months with a body weight of 120-185 grams, a total of 20 rats were divided into 5 groups with different treatments. Each group consists of 4 tailsmouse.

Test animals that have similarities with humans ranging from anatomy, nutrition, pathology and metabolism to humans are white rats (*Rattus norvegicus*) [4].

Male white rats with the functional Wistar strain were able to give normal research results because they could not affect the estruss cycle and pregnancy. The rats were more stable in controlling their feed, and physical activity minimized the occurrence of errors during the study.

The rat strains that are often used for research are the Wistar and Sprague Dawley strains. The white rat (*Rattus norvegicus*) of the Sprague dawley strain was developed from the Wistar strain of white rat. Shows the characteristics of the Wistar strain, which is a long body with a

narrower head, thick and short ears with fine hair, red eyes, and the tail is never longer than the body.

Use male white rats with the Wistar strain because it can provide more stable research results because it is not influenced by the estrus cycle and pregnancy. The rats were easier to control their intake and physical activity, thereby reducing the occurrence of bias during the study. The hormone estrogen can reduce uric acid levels through the kidneys which are excreted in the urine so that researchers prefer male rats over female rats [8].

The white rat is an albino strain of *Rattus norvegicus*. Rats have several strains that are the result of same-sex reproduction or crossbreeding.

Male white rats with the functional Wistar strain were able to give normal research results because they could not affect the estrus cycle and pregnancy. Based on the conversion results, the rats were divided into 5 treatment groups with a total of 20 rats. Group 1 is a negative control using 4 rats with 3 ml of distilled water, group 2 is a positive control using 4 rats with the drug Allopurinol 100 mg/70 Kg BW which is converted, namely 1,8 mg BW, rats 200 grams. The drug dose of Allopurinol 100 mg is appropriate and the maximum dose of Allopurinol that is taken is 300 mg/day, because Allopurinol is the first-line drug to lower uric acid levels. Group 3 used 4 rats with a dose of bay leaf extract of 0.4581 g/ww 200 grams of rats, group 4 used 4 rats with a dose of bay leaf extract of 0.8181 g/ww of rats 200 grams, and group 5 used 4 rats with a dose of leaf extract. greetings 1.1781 g/BB rat 200 grams.

The goal of therapy is the termination of acute attacks, preventing future attacks, overcoming pain and inflammation very quickly and safely, preventing complications such as the

formation of thopi or toffus, kidney stones, and destructive asthropathies.

Meanwhile, for patients with acute rheumatism, allopurinol functions to inhibit the formation of tofus, and to reduce tofus that have already been formed. Tophus are hard lumps that contain hardened uric acid crystals covering lime, in the form of a powder.

Allopurinol aims to stop the production of uric acid in the body, before the metabolic process occurs. This drug is used for long-term treatment. For patients with chronic gout, allopurinol is useful in controlling gout symptoms, and protecting kidney function.

The chosen comparison is allopurinol because this drug is a synthetic drug that is generally used by people with hyperuricemia to reduce uric acid levels in the blood.

Uricosstatic mechanism of action is to inhibit the formation of uric acid, so that the uric acid produced is reduced. Chicken liver juice which was induced in 20 white rats was first adjusted in the cage for 7 days given standard feed and adequate drinking water. Induction was carried out for 7 days orally given 2 times/day at a dose of 5 ml/200 g BW/day. The purpose of induction is to condition rats to become uric acid before treatment.

Chicken liver is one of the foods that can trigger the accumulation of purine substances in the body.

Giving chicken liver to white rats is one of the ingredients used to trigger uric acid levels in male white rats.

Administration was done orally using an oral probe for 14 days of treatment. Induction of chicken liver juice was able to increase the concentration of uric acid to double the original uric acid concentration.

Uric acid levels in male white rats before being induced by chicken liver juice reached 5.0 mg/dL. After being

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induced, chicken liver juice is a solution that has the potential to cause gout because chicken liver juice is quickly excreted. If uric acid is greater than 7.0 mg / dL due to increased production of purines in the blood or can occur because the excretion of uric acid in the blood is reduced.

On the 7th day, uric acid levels in white rats experienced a high increase of 20.0 mg/dL.

On the 8th day the experimental animals were treated by inducing bay leaf extract (*Syzygium polyanthum*) until the 14th day the administration was adjusted according to the weight of the rats, namely the dose of 0.4581 g/bb 200 rats, the dose 0.8811gr/bb 200 rats. , and a dose of 1.1781 g/bb 200 rats.

Measurement of uric acid levels in white rats was carried out 3 times, namely before induced chicken liver juice, after chicken liver juice induced, and after induced bay leaf extract (*Syzygium polyanthum*). Based on the results of the prerequisite tests that have been carried out, it can be stated that the parametric test cannot be carried out because the homogeneity test has no significance with a result of 0.01 meaning that the results are not homogeneous. Based on the decision making of the homogeneity test where if the significant value is 0.05 then the value is homogeneous but if the value is 0.05 then the value is not homogeneous.

The results showed that bay leaf extract (*Syzygium polyanthum*) can be used to reduce uric acid levels, so it is recommended to use it. The decrease that occurred in groups 3, 4, and 5 showed the therapeutic effect of bay leaf extract (*Syzygium polyanthum*) as a drug to reduce uric acid levels. However, it can be seen from table 4 that the amount of uric acid decreased was higher in group 4 with an extract dose of 0.8181 g as much as 10 mg/dL. The increase that occurred

in the negative control group after administration of bay leaf extract (*Syzygium polyanthum*) was caused because each rat had different metabolic processes so that it could affect the working mechanism of the bay leaf extract,

Risk factors for increased uric acid occur due to genetic factors, high consumption of foods containing protein, and green vegetables [10].

The solubility of uric acid is strongly influenced by the pH of the urine. At pH 5.0, urine can dissolve uric acid, at levels of 6-15 mg/dl. Uric acid will be ionized as urate and is abundant in blood plasma, then becomes monosodium urate at pH 7.4. Blood plasma becomes saturated with monosodium urate concentration of 6.8 mg/dl at 37°C.

Gout partially disagree due to the presence of comorbidity, difficulty in achieving compliance, especially if there is a change in lifestyle on the effectiveness and safety of therapy with variations from patient to patient.

The decrease in uric acid levels occurs due to the content of secondary metabolites in the form of flavonoid compounds that are anti-inflammatory which provide a therapeutic effect to reduce uric acid levels [5].

The content of flavonoids in bay plants serves to inhibit the work of the xanthine oxidase enzyme, where uric acid is excreted through the urine.

In bay leaves (*Syzygium polyanthum*) which have the potential to reduce uric acid levels are flavonoid compounds that can inhibit the xanthine oxidase enzyme in the formation of uric acid [11].

Flavonoids are polar compounds, so they will dissolve well in polar solvents such as ethanol, methanol, butanol, acetone, dimethylformamide and others.

Flavonoids are bound to the form of glycosides, so the homogeneity of the above solvent using water is a good

solvent for flavonoid glycosides. While in the form of aglycones such as flavones, flavonols, flavanones are more homogeneous in chloroform and ether solvents.

In this study it was proven that bay leaf extract (*Syzygium polythantum*) can reduce uric acid levels in male white rats (*Rattus novergicus*), because bay leaves have flavonoid compounds that function to inhibit the work of the xanthine oxidase enzyme so that the formation of uric acid in the body is inhibited. The flesh of the crown of the gods also has almost the same properties. Where flavonoid compounds can inhibit the work of the xanthine oxidase enzyme so that the formation of uric acid can be inhibited [2].

CONCLUSION

Based on the results of the research and discussion, it can be concluded that there is a therapeutic effect of bay leaf extract (*Syzygium polythantum*) in reducing uric acid levels in male white rats (*Rattus noergicus*), and the effective dose in this study was treatment in group 4 with an extract dose of 0, 8181%, but according to the Duncan test results that there is no difference in each dose.

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